IMMUNO-NUTRITIONAL STATUS AND INFECTIOUS DISEASE PREVALENCE AMONG COMMERCIAL SEX WORKERS OF BANGLADESH

A
DISSERTATION SUBMITTED
TO
THE UNIVERSITY OF DHAKA
FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

May 2016

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Overview, Objectives and Study Design

a. Overview

Bangladesh has upgraded from low income ($ ≤1045/year) country to lower-middle income country, with annual incomes of $1046 to $ 4125 according to World Bank's (2015)[1] latest estimates of Gross National Income (GNI) per capita. Thus Per capita income in Bangladesh rose from $ 1190 to $ 1314 (BBS, 2015). The country has also made significant progress in human development in the areas of literacy, gender parity in schooling and reduction of population growth[2]. Bangladesh has been cognizant of the importance of addressing differential needs of women and the existence of gender based discrimination since its independence, and commitment have been enshrined in the national constitution. The country has therefore taken measures to in promote gender equality (GE) and women's empowerment in a progressive manner over time. The Government formulated it first women's development policy (WDP) in 1997, updated it in 2011 and formulated two national action plans (NAP) for its implementation. The Government of Bangladesh is in the process of developing its seventh five year plan (7FYP) considering the identified national priorities in promoting gender equality for the next five years based on the review of the progress during the sixth five year plan (SFYP) (Begum, 2014). As the continuation of development, for the first time, Bangladesh Election Commission (EC) has recognized '**sex workers'** among the list of 15 professional groups in the country's new electoral database and national identity cards[3].

Bangladesh responded early and decisively to the potential threat of HIV despite economic and human development. In the period since the adoption of the first national strategy in 1985, services have been established and scaled up, particularly for Most At Risk Populations (MARPs). MARPs in Bangladesh are identified as sex workers, people who inject drugs (PWIDs), Males who have Sex with Males (MSMs) and Hijras (male to female transgender people). Clients of sex workers (bridging populations) are recognized as being at higher risks but are targeted through interventions for other populations (e.g. transport workers) and general population[4]. However, recorded HIV infection was found higher among international migrant workers who were not identified as MARP before. According to the 3rd National Strategic Plan[4] (2011-15), a range of emerging risks groups (international migrant workers, Heroin smokers, Transport workers, especially vulnerable adolescents and prisoners) were also identified for whom specifically targeted interventions will be implemented.

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[1] WB update says 10 countries move up in income Bracket (1st July 2015) (www.worldbank.org/en/news/press-release)

[2] United Nations Country profile: Bangladesh.(http://www.un-bd.org/countryprofile/html)

[3] Bangladesh EC recognizes sex workers as professionals (17th August, 2010). (http://www.deccanherald.com/content/89392/bangladesh-ec-recognise).

[4] 3RD National Strategic Plan (NSP) for HIV and AIDS Response, 2011-15. National AIDS/STD PROGRAM (NASP), MOHFW, GOB.

Prostitution was legalized in Bangladesh in 2000 even though the Bangladesh constitution provides that the “state shall endeavour to prevent gambling and prostitution”. As estimated 2, 00,000 women and teenage girls work in Bangladesh’s sex industry[5,6]. However, lower number of people in prostitution than present (60,000-100,000) was reported previously (Alam, 2010; Kanangara, 2003). Child Prostitution is widespread and a serious problem. The majority of Bangladeshi Prostituted girl children are based in brothels, with a smaller number of children exploited in hotel rooms, parks, railway and bus stations and rented flats. The UN children’ fund (UNICEF) estimated in 2004 that there were 10,000 underage girls used in commercial sexual exploitation in the country, but other estimates placed the figure as high as 29,000. Many girls involved in child labour, such as working in factories and as domestic workers are raped or sexually exploited as well victimized in violence, oppression (Decker and Miller, 2010) these girls are highly stigmatized and many of them flee to escape such abuse, but often they find that prostitution is the only option open to them for survival-once in prostitution they become even more marginalized, another recent study (Rahman MA, 2014) is in agreement with this finding. More than 20.000 children are born and live in the 18 registered red light areas of Bangladesh. Boys tend to become pimps once they grow up and girls continue in their mother’s profession. Most of these girls enter the profession before the age of twelve (Hansen, 2012).

Girls are often exploited and sold by family members (stepmoms/step-uncles/mock-husband) or others (lovers/pimps) to the brothels for a period of 2 or 3 years of bonded sex work[6]. The authorities generally ignore the minimum age of 18, often circumvented by false statement of age, for legal female prostitution, the government rarely prosecutes procurers of minors (Rahman MA, 2014; Hansen, 2012).

Girls[6] or women (poor, divorced/destitute/abandoned and young) from poor rural families migrate to cities in search of employment as domestic workers or in Bangladesh’s booming garment industry. With very little skills or education, these girls are extremely susceptible to danger and exploitation in unfamiliar cities. Against this backdrop, the situation of women in the sex trade is highly precarious (Ullah, 2005; Sadhana and Sanghamitra, 2008)

Rapid[7] economic growth and social change is creating new patterns of vulnerability. There is high population mobility within the country as well as people going overseas for employment. In this context, established support structure is weakened and people, especially women are more exposed to exploitation, including sexual exploitation.

Although Bangladesh is still a low HIV prevalence country, the Central City is clearly approaching a concentrated epidemic among IDUs, with prevalence close to 5%. The

[5] janmoellerhansen,smugmug.com/Bangladesh/sex-workers/

[6] Exploitation of CSWs in Bangladesh, 2015 http://www.ukessays.com/essays/sociology/exploitation-of-commercial-sex-workers retrieved December, 2015

[7] 3[RD] National Strategic Plan for HIV and AIDS Response , 2011-15, National AIDS/STD PROGRAM (NASP), MOHFW, GOB

increasing trend in HIV prevalence and its increasing geographic spread, the continued high-risk behaviors of IDUs, and substantial mixing of most-at-risk groups (MARPs) with the bridging populations, remind us that HIV may not remain confined to any specific sub-population or neighborhood. The considerable prevalence of risky sexual behaviors among almost all the sentinel groups is of grave concern. The negative effects of existing risk behaviors could potentially outweigh the positive effects of the slowly increasing trend in condom use and knowledge of HIV, unless further steps are swiftly taken (Islam M and conigrave, 2008).

Immune system and nutrition

Immunology is the host defense mechanisms those are brought into action when the body faces external (e.g. Bacteria, virus) or internal (such as tumor cells) forces of destruction. Thus our immune system is an efficient, complex defense system like shield against infection, disease and foreign substances.

The immune system has many different responsibilities. It does not only provide protection from infection through natural barriers, but also adapts itself to provide immunity against infection by “remembering” the infectious microorganism from a previous exposure.

The immune system is highly complex and depends on the normal functioning of a large number of immune cells. Levels of micro-nutrients in the diet are also vital for immune system. Due to the complexity of the immune system, it is extremely difficult to assess the effects of diet on our immune function. However, research results have identified dietary factors (both macro and micro nutrients in appropriate amount) for the integrity, coherence (messages sent between immune cells intended to bring about coordinated attack against diseases must be correctly created, sent, received and acted upon) and normal functioning of immune cells. Both under-nutrition (e.g. PEM) and over-nutrition (e.g. obesity) alter immune function and increase infections. If the level of threat increases and most deffences weaken, the chances of illness must increase. Thus disease only occurs when the immune system overwhelmed by the attacking organisms. A healthy balance of all nutrients helps support the immune system. Immune system maintenance requires a steady intake of all the necessary vitamins and minerals[8].

Immunocompetence is a sensitive and functional determinant of nutritional status because it has the unique quality of being altered even before the onset of clinical symptoms of malnutrition (Varela et al, 1997a). Infection causes such alterations in food habit often lead them to severe weight loss resulting in health deterioration and development of malignant or infectious complications (Moseson et al, 1989).

[8] http://www.medicalnewstoday.com/articles/13904.php

Role of different nutrients in immunity

Carbohydrate supply energy to the immune system so that it can work better and fight diseases. Protein makes enzymes, which help the immune cells killing germs, viruses and bacteria etc. They also help to keep the GI tract and immune system healthy. Fats are necessary for the body and immune system to work properly. They serve as fuel for the immune cells. Phytochemicals, vitamins and minerals are substances found in plant foods that help to boost the immune system[9]. However, now it is also known that almost all of nutrients given in quantities beyond a certain threshold will suppress immune responses (Chandra, 1997).

The antioxidants (β-carotene, vitamin-E, vitamin-C and micro-minerals e.g. Cu, Zn, Fe and Se) are called "immuno-active nutrients" those are naturally abundance in certain foods act as first line defense against lipid peroxidation in the body and have the capacities to modulate immune responses or functions by stabilizing highly reactive, potentially harmful "free radicals" or reactive oxygen intermediates (ROI) or reactive oxygen species (ROS) (Mayes, 2000a; 2000b; Park, 1994; Bendich, 1990). Free radicals (or ROI/ROS) have extreme tendency to acquire an electron for their unpaired electrons. Free radicals are generated during cellular metabolism (e.g. lipid peroxidation) or can be ingested or inhaled as environmental pollutants (Bendich, 1992; 1988). Production of free radicals in the body is an unavoidable consequence, for example, during phagocytosis (essential defense mechanism against infections) huge amount of superoxide ($o_2^{\bullet}$) free radicals are generated by neutrophils, monocytes, macrophages and eocinophils (Babior and woodman, 1999). Once a free radical is generated, it attacks whatever next to it resulting in unavoidable damages (Halliwell and Gutteridge, 1989; von Sonntag, 1987). Free radical attack causes an extensive DNA, membrane and enzyme damage producing a wide range of products, which are found present in high amount in cancer tissue DNA.

The major cause of excess ROS or ROI is the depletion of dietary antioxidants, inappropriate activation of phagocytic cells in chronic inflammatory diseases (Halliwell and Gutteridge, 1989). Alcohol drinking and cigarette smoking also induce antioxidant deficiencies and oxidative stress (Marangon et al, 1998). The free radicals, in excess, are immunosuppressive (Bendich, 1990). In human, lifestyle and clinical conditions that are associated with increased production of free radical impairs immune responses (Bendich, 1992). Thus antioxidant nutrients which minimize and control the production of free radical in humans are also potential immuno-enhancer.

In term of the mode of action, the antioxidants are of two types (i) chain breaking antioxidants (e.g. α-tocopherol, ascorbic acids and β-carotene) which interfere with chain

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[9] http://www.gmhc.org/health/nutrition/.factsheets/immunesystem.html)

propagation and (ii) preventive antioxidants (endogenous enzymatic antioxidants), which reduce the chain initiation. Micro-minerals or trace elements with carrier proteins such as superoxide dismutase, metallothianine (copper/zinc containing enzyme), and ferroxidase (iron containing enzyme) mainly produce the enzymatic antioxidants. Thus micronutrient deficiencies could impair immunity results in decreased lymphocytes proliferation, antibody production, phagocytic and other immune functions. It is also reported that HIV-positive patients have poor antioxidant defense and increased oxidative stress (Allard et al, 1998).

Malnutrition, Immunity and STIs

Malnutrition influences the malfunctioning of Immune system and invades infectious diseases. The sophisticated immune systems which protect against colonization and tissue invasion are remarkably effective in the well nourished host. Chandra (1991) has reviewed the ways in which the immune process is affected by malnutrition. Once the pathogen is established within the body there are variation in the severity, duration and extent of the infection which may be affected by the health care system, environment and cares.

Macronutrient deficiency directly reduces BMI and its long-term deficiency may result in CED as well as anemia. However, anemia is a single important determinant that reflects the nutritional status of a nation in the developing countries (Mackie and Ludlam, 1996). Serum total proteins represent non-visceral protein status in the body (Anderson et al, 1982). Serum albumin and transferrin are constructive proteins and have a direct relationship with nutritional status, and an inverse association with stress and inflammation.

Vitamins, minerals and trace elements constitute micronutrients profile (Way, 1999; Truswell, 1996). Among all micronutrients, three antioxidant vitamins (e.g. β-carotene, vitamin E and vitamin C) and three antioxidant micro-minerals (e.g. iron, copper and zinc) have potential immuno-modulating properties. These nutrients are essential in the maintenance of defense mechanism or immunity. It is also documented that both antioxidant vitamins and micro-minerals play key role on both humoral and cell mediated immunity.

Under-nutrition results in deficiencies of all those nutrients. Deficiency of the natural antioxidant vitamins (E,C, and β-carotene) are likely to induce oxidative stress and lipid peroxidation (Mayes, 2000a;2000b), and deficiency of micro-minerals (Cu, Zn, Fe) impair enzymatic function of endogenous antioxidants such as metallo-thiaonein (MT), ferroxidases (ceruloplasmin) and superoxide dismutase etc. in the body.

Disease and infection are often used synonymously, but there are important distinctions. In pure sense disease is only present when infection leads to abnormalities of organ function and host experiences clinical manifestations of infection. In addition a certain number of organisms must be present before an infection can be termed disease (Tomkins and Watson, 1989).

According to the Centers for Disease Control and Prevention (CDC), an infectious disease is caused either by the invasion of viruses or bacteria or parasites or fungi. Infectious diseases can range from common illness, such as the cold, to deadly illness, such as AIDS. Depending on the specific illness and community hygienic condition and practices, an infectious disease can spread in many ways. In developed countries by sexual transmission or airborne or blood–borne transmission or by direct skin contact specific illness occurs. Some infections also seem to occur in less-developed countries due to insect-borne or food-borne or water-borne transmission[10]

It is well evident that, malnutrition is synergistic with immunity and infection (Scrimshaw, Sangiovanni, Chandra, 1997). Malnutrition induces immunodeficiency (Chandra, 1997; 2001) that influences the susceptibility to infectious agents including HIV (Varela et al, 1997a, 1997b; Marcos et al, 1997 and Islam et at, 2001). It is also reported that frequent episodes of infections can cause malnutrition.

Nutrition is an important determinant for immunity. Malnutrition is the most common cause of immunodeficiency (Chandra, 2001). Nutritional deficiencies alter immunocompetence and increase risk of infections that further cause depletion of body's store micronutrients. Multiple malnutrition is one of the top public health problems in Bangladesh. Highest HIV prevalence and AIDS mortality in the developing or less developed countries focus its association with poverty and malnutrition (UNAIDS 2003).

Malnutrition is a global problem. Malnutrition restricts child growth, impairs immune development, renders susceptibility to infections, deficits cognitive functions, and undermines motivation and productivity. Malnutrition is increasing relentlessly among the socio-economically deprived people of the developing countries like Bangladesh where poverty, unemployment, underemployment, illiteracy and ignorance are overwhelming. The poverty and malnutrition are frequently prevalent in these less developed countries.

Our first line of defense against foreign organisms is the innate or non-specific immune system such as the barrier tissues like skin that stops the entry of organism into our bodies and also protective substances those are found on the surfaces of epithelia and in secretions e.g. in saliva and tears. If however, these barrier layers are penetrated, the body contains cell that responds rapidly to the presence of the invader. These cells include macrophages and neutrophils that engulf foreign organisms and kill them without the need for antibodies. Immediate challenge also comes from soluble molecules that deprive the invading organism of essential nutrients (e.g. iron). A second line of defense is the specific or adaptive immune system which includes (a) humoral immunity or production of antibodies

[10] http://www.healthcare.utah.edu/healthinfo/adult/infectious/immune.htm

(soluble protein that binds to foreign antigen) by "B" cells and (b) "T" cell-mediated responses in which specific cells recognize foreign pathogens and destroy them[11]

The organs involved with the immune system are called the lymphoid organs, which affect growth, development, and the release of lymphocytes--a type of infection-fighting white blood cell—are vital to an effective immune system. Lymphocytes "patrol" the body for infectious microorganisms. After the initial formation of lymphocytes in the bone marrow, some are also matured in the bone marrow and become "B" cells. Some other lymphocytes finished their maturation in the thymus and become "T" cells. "B" and "T" cells are the two major groups of lymphocytes which recognize and attack infectious microorganisms. Although each type of lymphocyte fights infection differently, the goal of protecting the body from infection remains the same. The B cells actually produce specific antibodies to specific infectious organisms, while the T cells kill infectious microorganisms by killing the body cells that are affected. In addition, T cells release chemicals, called lymphokines, which trigger an immune response to combat cancer/ tumorigenic cell or a virus. Besides "B" and "T" cells other types of white blood cells, such as phagocyte ("engulfing" cells) and cytotoxic cells (natural killer cells), actually kill the infectious microorganism by "devouring" it[12].

Commercial Sex workers (CSWs) – Bangladesh context

The term 'Commercial sex worker (CSW)' can be defined as an occupational group who earn their livelihood through providing sexual services.

'Woman commercial sex worker'-it may be the last option that a woman has in terms of work (Perez, 2006). The most misery of being a Commercial Sex worker (CSW) is, at the same time being mothers of illegitimate children, being the target of those children's frustration and anger [13]

The existence of selling female sex for the gratification of males is one of the oldest vices in society. The profession could not sustain unless there was any demand from the males. Among the reasons behind the beginning and sustaining of the profession, the subordination and weaker position of women in the socio-economic structure of society can be assumed as most significant (Hossain et al, 2004).

Sex work is considered as a social problem which is also the result of other problem i.e. poverty, dowry, exploitation, human trafficking etc. Despite the fact that biological need can hardly be avoided and some contradiction in law of our country doesn't mean absolute denial of the profession, sex work is illegal in Bangladesh like other Asia pacific countries (Taposh, 2006).

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[11] http://www.pathmicro.med.sc.edu/book/immunol-sta.htm
[12] http://www.healthcare.utah.edu/healthinfo/adult/infectious/immune.htm

[13] Prostitution: sex workers manifesto, 1997.

Approximately one million men buy sex from prostitute women and children in Bangladesh (Kanangara, 2003). Gradually, the practice of prostitution took various forms and arrangements which include places of availability, rate, glamour, and associated persons, pimps, shelter, and connection with law enforcing agency. Now, institutionalized prostitution is called “commercial sex”. With the advent of gay prostitute or male sex worker, so called prostitutes are called in a new name “female commercial sex worker” (Hossain et al, 2004).

About the trafficking of women and children, prostitution is alarming feature of the said globalization process. For instance, over the last decade 20, 00,000 were lured under false circumstances and sold into the sex industry in different countries including Pakistan, India, and the Middle East. Another source says about 40,000 children from Bangladesh are involved in prostitution in Pakistan and Bangladeshi girls are also trafficked to India for commercial sex trade (ILO-IPEC, 1996).

Economic and political changes over the past decades have apparently created financial hardship in Bangladesh, particularly for women, resulted in a dramatic increase in the number of women and girls in the sex work industry, either voluntary or against their will. Some have moved from villages fleeing hardship and poverty in search for better life; others are the victim of sexual exploitation (UNFPA 2002).

Commercial Sex workers (classifications, classes and the position)

The term ‘Commercial sex worker (CSW)’ can be defined as an occupational group who sell sex for livings. With the advent of gay prostitute or male sex worker, the so called female prostitutes are called as “female CSWs”. The sexual services were broadly categorized under four main groups- brothel based, hotel based, home based (sexual services carried on in rented houses by small groups of girls) and floating/ street/ slum based (Hossain et al, 2004)

There exists difference among sex workers, like rich and poor, upper and lower class. In fact there are more divisions among the sex workers than the class divisions in the society. The lowest rung starts with the homeless variety that totally depends on the street. Then come the ones who have homes but are very poor. These two haunt the street in the night. Next comes the section that comes from home and stands on the street in the daytime. This group is the major group among sex workers. These three are the known sex workers who undergo all the harassment described above, among them the night dwellers suffer the most. These three groups will end up in brothels if there is any. They are the ones who are being organized now. Then comes the variety that is either based in home or lodge. They are relatively safe. But succumb in the police raids. It is a jackpot for the police; they fetch lot of money. The clients will find enough money to save them and themselves. In fact they will be substituted by the street dwellers in the cases charged in the raid. Then the call girls who will visit the clients only on prior arrangements. They are sex workers out of pure choice. They make money more than any executive in the country and don't suffer any harassment or the stigma attached. In fact they move in the high society and are most sought after. Most of the celebrities, men or women, come under this category. This is the general picture, but it will find shades of differences in different states and locations. Here we have to understand that the first three categories need help. They have to pay heavily for the so-called morality, which actually is a dead value[14].

Among the four types of commercial sex workers (CSWs), floating/ street commercial sex workers (CSWs) are found visible especially to the experienced and/ or curious eyes. Also, eviction of brothels forced many CSWs to float around the country. Reports of studies conducted by NGOs in Bangladesh reveal that many evicted eligible sex workers did not quit their professions which prove they can never give up (Taposh, 2006).

In Bangladesh, there is a general perception that eviction of brothels might have reduced the bad practice in the country and new recruitment will become difficult. But report highlighted the opposite picture, indeed (Hossain et al, 2004).

Pressure from different sources to evict brothel areas has created threats to our normal social life. It is needless to say that unless effective measures are taken to solve other social problems, for instance poverty, dowry, exploitation, human trafficking etc, it is not possible to stop women to get involved in sex work. When brothels are evicted, number of floating or hotel or residence based sex worker will be increased. Eviction of brothels never terminates prostitution rather increased the number of street/ floating sex workers. Interviews with evicted sex workers reveal the fact that eviction cannot be the way to solve this social problem and without proper initiatives to support them financially, this will increase the number of floating sex workers or unprotected sex work outside brothel areas (Taposh, 2006).

Floating sex workers are physically “visible” but socially “hidden”. Different circumstances under which destitute people are forced to transform their bodies into sex objects and to peddle sexual services as an item of commercial transaction certainly seem to point towards a social existence devoid of basic human rights (Khan, CPD). Floating/ street sex workers are often regarded as occupying the lowest point in a status hierarchy of CSWs (Mckeganey and Barnard 1996). Amongst all types of CSWs, they are the most visible and most harassed and often referred to as ‘low classes’.

[14] FIRM: www. firm-kerala.org and Maitreya@asianetindia.com

History of the sex trade in Bangladesh

There is a belief that women from low social status, 'Dalit' are prone to prostitution, but it is not true. There are women from all social classes, caste and religions who are involved in sex trade. Women from low social classes are more visible and vulnerable to police and legal action[15]

Sex trade came into existence in all strata of society, but the sociological process was very complex. We cannot draw a simple connection between social determinants and entry of women in sex trade. Tracing out the reasons in a simplistic manner is not worthy. But the process can be understood as phenomena in a world of patriarchal values, marginalization of women from economically productive activity and under valuation of reproductive role of women. Hence, there is too much diversity in the life of women in prostitution[9].

In this subcontinent prostitution was recorded in both publicly and personally. Khan and Arefeen (1989) mentioned about report of the census of Bengal, 1901, census of India, 1911, the works of Joarder (1986), personal diary of Dewan Raja, etc. and found the inclusion of prostitutes as 'unskilled labour, not agriculture' or 'indefinite or disreputable' or in 'miscellaneous column'. After the partition of India and following the independence of Bangladesh many brothels were grown up over times. Gradually sex trade (prostitution) has got international dimension by its linkage to trafficking of women and children to India as destination or transit of Pakistan or also to some countries of Middle East.

The destitute women and children migrate to town and cities in search of works as housemaids or laborers without sufficient awareness of their vulnerable situations (insecure residence, livelihood etc). Some of them very often fall victims of the rapist or pimps when left by guardian, husband or adult sons. So, the process starts from rural areas with socio-economic and environmental disfavor that result in rural-urban migration sprouting huge numbers of slums in the cities. Then facing harder reality of survival some of slum dwellers are recruited in brothels. The garment industry attracts the young girls who also face harsh experience of low wage, insecure movement and sexual exploitation. Ultimately, some of them cannot get rid of the vicious circle of sex market mechanism. Also a growing trend of globalization of Bangladesh has an impact on the development of sex industry, thus the cash and materials inflow of foreign aid mostly benefits a new rich class who are involved in construction dealership, or commercial sector. This class along with the politically powerful class creates the demand of prostitution in the big cities (Hossain et al, 2004).

However, the factors that pushed women to enter into sex industry vary from person to person and also country to country. But economy is the major factor all over the world and, of course, in third world's country.

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[9] Jayasree@vsnl.com and www.thrani.com

Commercial sex and sex workers– global thinking (who, where, and why)

Commercial sex is an ancient and widespread phenomenon. Women, men and transgender sell sex all over the world and have done so forever. It's more useful to think about sex work as the sale of services and time rather than selling one's body[17].The funny thing about sex is that it was for sale even in Buddha's time and was called the oldest profession[16].

According to network of sex work projects (NSWP), the obvious answer of the question 'why do people sell sex' is "because people by sex". The sex industry is subject to the same economic rules as any other business or industry- demand drives supply[17].

Today, the trade in women's bodies is a global business grossing over six billion dollars for the traffickers, and it is growing fast. The UN estimates that about four million women are being trafficked as sex slaves. Some 50,000 women are brought into US every year, predominantly from the Ukraine, Albania, the Philippines, Thailand, Mexico and Nigeria (Wilson 2000).

The main thing here is to gain dignity of those who are involved. It is like the shift from the slave and laborer to the worker. Though the slave does the same labor like worker, it is a duty for hoer. Even though a worker's existence is expressed and known through hoer activity, it is not a duty. The labor is freed and is independent of the doer. Thus it can be sold. Before it was 'the slave' who was being sold. This difference gave the worker freedom from work and a different existence. S/he could move around in the society, have leisure and make merry even with the employers. This freedom gives dignity to work as well as the worker. And that is why the prostitutes stopped being prostitutes and turned sex workers. It is the dignity of works they are searching for[16].

Now the question arrives whether sex work can be recognized or considered as real work. It is usually called selling the body or considered as exploitation of women who are victims. Nobody sells the body (a pun, of course) rather they sell services. We have to distinguish rape and trafficking from sex work. Sex work is a conscious choice, while the other two is not.

Commercial sex takes place in many kinds of ways and involves many different types of people, many of whom are in no way stereotypical. People from various backgrounds and classes sell sex and they do so for a broad range of reasons. These reasons often change over time. According to global view some people sell sex as a full time occupation, while others sell sex only occasionally. Although some people are willing to be sex workers and others are pressured to sell sex. In fact, between the educated and resourceful woman who chooses commercial sex from a broad range of possibilities and the poor, uneducated woman who is physically forced to do it, there is a whole continuum of situations[17].

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[16] Maitreya@asianetindia.com retrieved in 2008

[17] www.nswp.org.htm.

Many other people (third parties) involved in commercial sex in addition to the sex workers and clients. These include business owners, bar tenders, cleaners, taxi drivers, maids, receptionist, touts, security staff, local vendors and also the people with whom sex workers share their private lives. Different sex work venues include streets, brothels, bars, saunas, massage parlors and other settings[18].

Because of the illegal status of sex work, prostitutes are subjected to a lot of violence, extortion, harassment, and even rape (Josephine Ho 2003). Sex workers all over the world share stigmatization and violation of human rights. The double standard of social justice makes their life miserable and exploitable[19].

Various aspects of commercial sex works were much well-organized and rooted in deep. An investigator (Hansen, 2012) reported well-organized sex industry as well as brothels in Bangladesh than his native country Denmark and similar societies.

Globally sex worker's organizations are fighting for their basic human rights, police harassments, abuses etc. No matter what the reason of entering sex industry –sex workers all over the world face social stigma and become the subject of violence as well as are at greater risk for HIV transmission.

Commercial sex and risk of HIV/AIDS

There are an estimated more than thirteen thousand adults living with HIV in Bangladesh. This is below 1 per 1,000 adults in the general population, and less than 1 percent among vulnerable groups. But despite the low incidence, Bangladesh is considered to be a high-risk country for several reasons, including:

•large sex industry and sustained high-risk sexual behaviour
• multiple partnership with PWID and CSWs and others and very low use of condoms
• high incidence of self-reported sexually transmitted infections among vulnerable groups
• high levels of HIV/AIDS in the two neighbouring countries, India and Myanmar
• the high proportion of young people, who constitute one third of the population — the highest in the region[20]

The awareness level of general people of Bangladesh for the risk of being attacked by HIV/AIDS is not satisfactory, let alone awareness of commercial sex workers. In fact, commercial sex workers are at the forefront of HIV/AIDS risk and at the same time they are

[18] (NSWP: www.nswp.org.htm) retrieved in 2009

[19] (Jayasree@vsnl.com and www.thrani.com). retrieved in 2009

[20] (www.unicef.org/bangladesh/health_nutrition_402.htm) retrieved in 2010

good media for transporting it to their clients and to their children. Thus, they are considered as vulnerable groups of HIV/AIDS.

It is quite natural that commercial sex workers are more susceptible to HIV/AIDS for their promiscuous sex behavior. It is more likely that their clients include those who are drug users, cross-border movers, and have sexual relation with a number of sex workers of varied brothels or places. The research reports suggest varied health hazards, and high risk of having HIV/AIDS among them. Moreover, CSWs do not find friendly behaviors and quality health services from doctors or health care clinics. Consequently, they rely on quacks for health matters that cause increased vulnerability (Hossain et al, 2004).

As a matter of fact, the issue of commercial sex workers and the high risk of contagious diseases through sexual practices are very poorly addressed in theory and practices in Bangladesh. Time has come to deal the matter with sufficient sociological data so that the problem related strategies could be appropriately formulated and implemented to achieve more effective results (Hossain et al, 2004).

Sex business, sex workers and sex users-all are risk factors in spreading the STDs as well as HIV epidemic among general population. The individuals with higher rate of STD are also at increased risk of HIV infection (Boyer and Ellen, 1994). Sex business is associated with infectious diseases including STDs and HIV that also impairs immunity and influences susceptibility to other Infectious diseases.

HIV and STD are quickly spreading by sexual contact and intravenous drug use (Bluthenthal et al, 2000). Worldwide it is estimated that 71% of HIV infection is transmitted through heterosexual contact (Ehrhardt, 1992). It has been recorded that most of the women were infected with HIV/ AIDS through heterosexual contact with their IDU sex partner. However unlike the developed world where HIV transmission occurs mostly through homosexual contact, unprotected risky heterosexual contact account for most of the HIV spreading in the developed country (WHO AIDS, 1998; NRC, 1997). Immunological dysfunctions associated with malnutrition may produce AIDS and it is called NAIDS (Beisel, 1996).

Infections with other STIs increase the chances of spreading or acquiring HIV. Effective treatment of STIs is one of the proven methods for preventing HIV. In Bangladesh, however, the HIV-prevention campaign seems to be more effective than the general STI treatment/prevention campaign. A study on 311 health service providers from both urban and rural areas found that they had good knowledge on HIV/AIDS but they lacked knowledge on other STIs (Hossain I et al, 2002).

Sexually Transmitted Infections[21] (STIs)

Sexually Transmitted Infections (STIs) other than HIV have shown to enhance the transmission of HIV and to be more common among female commercial sex workers (María et al, 2006). Until the 1990s, STIs were commonly known as *venereal diseases*, the word *venereal* being derived from the Latin word venereus, and meaning relating to sexual intercourse or desire, ultimately derived from Venus, the Roman goddess of love[22]. "Social disease" was a phrase used as a euphemism.

The World Health Organization (WHO) has recommended *sexually transmitted infection* as the preferred term since 1999. '*Sexually transmitted infection' or STI* is a broader term than '*sexually transmitted disease'.* An infection is colonization by a parasitic species, which may not cause any adverse effects. In a disease, the infection leads to impaired or abnormal function. In either case, the condition may not exhibit signs or symptoms. Sexually transmitted infections (STI), also referred to as sexually transmitted diseases (STD) and venereal diseases (VD), are infections that are commonly spread by sex, especially vaginal intercourse, anal sex and oral sex. Most STIs initially do not cause symptoms. This results in a greater risk of passing the disease on to others. Symptoms and signs of disease may include vaginal discharge, penile discharge, ulcers on or around the genitals, and pelvic pain. STIs acquired before or during birth may result in poor outcomes for the baby. Some STIs may cause problems with the ability to get pregnant.

More than 30 different bacteria, viruses, and parasites can cause STIs. Bacterial STIs include chlamydia, gonorrhea, and syphilis among others. Viral STIs include genital herpes, HIV/AIDS, and genital warts among others. Parasitic STIs include trichomoniasis among others. While usually spread by sex, some STIs can also be spread by non-sexual contact with contaminated blood and tissues, breastfeeding, or during childbirth. STI diagnostic tests are easily available in the developed world, but this is often not the case in the developing world.

The most effective way of preventing STIs is by not having sex. Some vaccinations may also decrease the risk of certain infections including hepatitis B and some types of HPV. Safer sex

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21 *"Sexually transmitted infections (STIs) Fact sheet N°110". who.int. November 201. Retrieved 30 November 2014.*

"How You Can Prevent Sexually Transmitted Diseases". cdc.gov. November 5, 2013. Retrieved 5 December 2014.

World Health Organization (2003). Guidelines for the management of sexually transmitted infections (PDF). Geneva: World Health Organization. p. vi. ISBN 9241546263. GBD 2013

*"Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013.". Lancet **385** (9963): 117–71. doi:10.1016/S0140-6736(14)61682-2. PMC 4340604. PMID 25530442.*

"STD Trends in the United States: 2010 National Data for Gonorrhea, Chlamydia, and Syphilis". Centers for Disease Control and Prevention. Retrieved 15 September 2012. Gerd Gross, Stephen K. Tyring (2011).

Sexually transmitted infections and sexually transmitted diseases. Heidelbergh: Springer Verlag. p. 20. ISBN 9783642146633.

22 *"Venereal". dictionary.reference.com. Retrieved June 18, 2013.*

practices such as use of condoms, having a smaller number of sexual partners, and being in a relationship where each person only has sex with the other also decreases the risk. Circumcision in males may be effective to prevent some infections. Most STIs are treatable or curable. Of the most common infections, syphilis, gonorrhea, chlamydia, trichomoniasis are curable, while herpes, hepatitis B, HIV/AIDS, and HPV are treatable but not curable. Resistance to certain antibiotics is developing among some organisms such as gonorrhea.

In 2008, it was estimated that 500 million people were infected with syphilis, gonorrhea, chlamydia or trichomoniasis. At least an additional 530 million people have genital herpes and 290 million women have human papillomavirus. STIs other than HIV resulted in 142,000 deaths in 2013. In the United States there were 19 million new cases of sexually transmitted infections in 2010. Historical documentation of STIs date back to at least the Ebers papyrus around 1550 BC and the Old Testament. There is often shame and stigma associated with these infections. The term '*sexually transmitted infection'* is generally preferred over the terms '*sexually transmitted disease'* and '*venereal disease'*, as it includes those who do not have symptomatic disease.

Human immunodeficiency virus (HIV)[23]: HIV is carried in body fluids and most commonly passed on through unprotected sex. It can also be transmitted by coming into contact with infected blood –for example, sharing needles to inject steroids or drugs. It can also be spread by blood transfusion, breast feeding and from mother to child during pregnancy (placental transfer). The HIV virus attacks, weakens and then damages the immune system, making it disable to fight against infections and diseases. The virus actually kills CD4 cells, which are white blood cells that help fight off various infections. There's no cure for HIV, but there are treatments that allow most people to live a long and otherwise healthy life. AIDS is the final stage of an HIV infection, when body can no longer fight life-threatening infections. Most people with HIV look and feel healthy and have no symptoms. When HIV develops first, one may experiences a flu-like illness with a fever, sore throat or rash. This is called a seroconversion illness. When HIV is at its most advanced stage, an individual is said to have AIDS (acquired immunodeficiency syndrome). There are different stages of the progression of and HIV infection. The stages include 'primary infection', 'asymptomatic infection', 'symptomatic infection', and 'AIDS'. In the primary infection stage, an individual will have flu like symptoms (headache, fatigue, fever, muscle aches) for about 2 weeks. In the asymptomatic stage, symptoms usually disappear, and the patient can remain asymptomatic for years. When HIV progresses to the symptomatic stage, the immune system is weakened, and has a low cell count of CD4+ T Cells. When the HIV infection becomes life-threatening, it is called AIDS. People with AIDS fall prey to opportunistic infections and die as a result. When the disease was first discovered in the 1980s, those

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[23] *"Hiv/Aids". Mayo Clinic.com. 2012-08-11. Retrieved 2013-06-30. "AIDS". Avert.org. Retrieved 2013-06-30.*
"HIV/AIDS Treatment". Niaid.nih.gov. 2009-06-03. Retrieved 2013-06-30.

who had AIDS were not likely to live longer than a few years. There are now antiretroviral drugs (ARVs) available to treat HIV infections. There is no known cure for HIV or AIDS but the drugs help suppress the virus. By suppressing the amount of virus in the body, people can lead longer and healthier lives. Even though their virus levels may be low they can still spread the virus to others.

Human immunodeficiency virus or HIV comprises HIV-1 and HIV-2, both of which are retro viruses and are responsible for acquired immunodeficiency syndrome (AIDS). Fundamentally they are similar in structure and lifestyle, but they have different pathogenicity. HIV-1 is strongly virulent and pathogenic in comparison to HIV-2. Prevalence of HIV-1 infection has been reported worldwide but HIV-2 infection is found only in West Africa (Rosen, 1996). Both the viruses show substantial antigenic cross reactivity in their core protein, but the envelope glycoproteins are less reactive (Denies et al 1988).

HIV may harbor in pre-ejaculated fluids and appears in semen (Fischl, 1988) and also in vaginal and cervical secretion during menstrual period (Vogt et al, 1986, 1987). It can even survive in ordinary water for extended period of time (Resnick et al, 1986). To ensure detection of antibodies against both of the viruses following infection, it is therefore, necessary to use epitops on the envelop region and major cross reacting core proteins of the viruses (Gurtler et al, 1994). A simple blood test is usually used to test for an HIV infection. Some clinics may also offer a rapid test using a finger-prick blood test or saliva sample. Following HIV infection, the earliest specific antibody response may be of IgM followed by IgG (Loussert-Ajaka, 1994).

Immunity in AIDS: HIV can remain latent for 10-20 years in human body (Boyer and Ellen, 1994). When the deadly opportunistic HIV virus could suppress the immunity, AIDS is produced that results in untreatable syndromes of different diseases. Following the primary infection by HIV, it destroys the signal system (T_H cells) of the immunity and thus inactivates the entire defense mechanism of the body.

***Cellular abnormalities of lymphocytes*:** The typical abnormalities that are evident in peripheral blood by primary HIV infection are (i) lymphopenia (ii) a decrease in the number of helper-inducer T_H cells (CD4), and relatively normal T_S cell (CD8) (consequently a reversal in $CD4^+/CD8^+$ T_S cell ratio) (iii) raised immunoglobulin levels (IgG and IgA) (iv) and increased γ-interferon (Roit, 1994). Natural killer cell number is within normal range but their activity is reduced.

Functional abnormalities seen in vivo: AIDS patients have an increased susceptibility to infections with opportunistic organisms (*Pneumocystis carinii*, *Toxoplasma gondii*, *Cryptococcus neoformans*, herpes simplex, herpes zoster, cytomegalovirus, *Mycobacterium avium-intracellular*, *etc*). These patients also have increased incidence of neoplasms (Kaposi's sarcoma). Delayed hypersensitivity response to common antigens (tetanus, diphtheria, streptococcal antigen, tuberculin, Candida antigen, trichophyton, etc.) is decreased in AIDS patients. They fail to produce antibody in response to various antigenic challenges (KLH, tetanus toxoid, pneumococcal polysaccharide).

Human papillomavirus (HPV): there are more than 40 different strands of HPV[24] and many do not cause any health problems. In 90% of cases the body's immune system clears the infection naturally within 2 years. Some cases may not be cleared and can lead to genital warts (bumps around the genitals that can be small or large, raised or flat, or shaped like cauliflower) or cervical cancer and other HPV related cancers. Symptoms might not show up until advanced stages. It is important for women to get pap smears in order to check for and treat cancers. There are also two vaccines available for women (Cervarix and Gardasil) that protect against the types of HPV that cause cervical cancer. HPV can be passed through genital-to-genital contact as well as during oral sex. It is important to remember that the infected partner might not have any symptoms. The human papillomavirus (HPV) is the most common STI in the United States.

Herpes simplex[25] **virus-1 and 2:** the two most common forms of herpes are caused by infection with herpes simplex virus (HSV). HSV-1 is typically acquired orally and causes cold sores, HSV-2 is usually acquired during sexual contact and affects the genitals. However, either strain may affect either site. Some people develop symptoms of HSV a few days after coming into contact with the virus. Small, painful blisters or sores usually develop, which may cause itching or tingling, or make it painful to urinate. After one infected, the virus remains dormant (inactive) most of the time. However, certain triggers can reactivate the virus, causing the blisters to develop again, although they're usually smaller and less painful. Some people are asymptomatic or have very mild symptoms. Those that do experience symptoms usually notice them 2 to 20 days after exposure which last 2 to 4 weeks. Symptoms can include small fluid-filled blisters, headaches, backaches, itching or tingling sensations in the genital or anal area, pain during urination, Flu like symptoms, swollen glands, or fever. Herpes is spread through skin contact with a person infected with the virus. The virus affects the areas where it entered the body. This can occur through kissing, vaginal intercourse, oral sex or anal sex. The virus is most infectious during times when there are visible symptoms; however those who are asymptomatic can still spread the virus through skin contact. The primary attack is the most severe because the body does not have any antibodies built up. After the primary attack, one might have recurring attacks that are milder or might not even have future attacks. There is no cure for the disease but there are antiviral medications that treat its symptoms and lower the risk of transmission (Valtrex). Although HSV-1 is typically the "oral" version of the virus, and HSV-2 is typically

[24] *"Human Papillomavirus (HPV) | Overview". FamilyDoctor.org. 2010-12-01; "STD Facts - Human papillomavirus (HPV)". Cdc.gov. Retrieved 2013-06-30.*

[25] http://www.webmd.com/genital-herpes/guide/genital-herpes-basics; *"Herpes". Avert.org. Retrieved 2013-06-30.*

the "genital" version of the virus, a person with HSV-1 orally can transmit that virus to their partner genitally. The virus, either type, will settle into a nerve bundle either at the top of the spine, producing the "oral" outbreak, or a second nerve bundle at the base of the spine, producing the genital outbreak.

Hepatitis B[26]: among the five known hepatitis viruses: A, B, C, D, and E 'Hepatitis B' is an infection caused by the hepatitis B virus (HBV) which affects the liver and can cause both acute and chronic infections, is also a major public health problem worldwide. According to recent estimates approximately 30% of the world's population i.e. about 2 billion persons have serologic evidence of current or past HBV infection. HBV infection can cause liver diseases including subclinical infection of the liver, acute self-limited hepatitis, and fulminant hepatitis. HBV is also a major human carcinogen and the sequelae of chronic HBV infection are cirrhosis and primary liver cancer (hepatocellular carcinoma, HCC). The hepatitis B virus can survive outside the body for at least 7 days. During this time, the virus can still cause infection if it enters the body of a person who is not protected by the vaccine. The incubation period of the hepatitis B virus is 75 days on average, but can vary from 30 to 180 days.

Hepatitis B is spread by percutaneous or mucosal exposure to infected blood and various body fluids, as well as through saliva, menstrual, vaginal, and seminal fluids. Sexual transmission of hepatitis B may occur, particularly in unvaccinated men who have sex with men and heterosexual persons with multiple sex partners or contact with sex workers. Infection in adulthood leads to chronic hepatitis in less than 5% of cases. Transmission of the virus may also occur through the reuse of needles and syringes either in health-care settings or among persons who inject drugs. In addition, infection can occur during medical, surgical and dental procedures, tattooing, or through the use of razors and similar objects that are contaminated with infected blood (Collier and Webster, 2010).

Most people do not experience any symptoms during the acute infection phase. However, some people have acute illness with symptoms that last several weeks, including yellowing of the skin and eyes (jaundice), dark urine, extreme fatigue, nausea, vomiting and abdominal pain. A small subset of persons with acute hepatitis can develop acute *liver failure which can lead to* death. In some people, the hepatitis B virus can also cause a chronic liver infection that can later develop into cirrhosis of the liver or liver cancer. More than 90% of healthy adults who are infected with the hepatitis B virus will recover naturally from the virus within the

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26 "Hepatitis B Fact sheet N°204". who.int. July 2014. *Retrieved* 4 November 201 http://apps.who.int/vaccine_research/proposals/global_prevalence_hepB/en/index.html; **http://en.wikipedia.org/wiki/Hepatitis_B. Retrieved April,** 2016.

first year. Diagnosis is typically by testing the blood for parts of the virus and for antibodies against the virus.

The infection has been preventable by vaccination since 1982. Vaccination is recommended by the World Health Organization in the first day of life if possible. Two or three more doses are required at a later time for full effect. This vaccine works about 95% of the time.

Syphilis[27]: syphilis is a chronic sexually transmitted bacterial infection caused by the spirochaete '*Treponema pallidum'*. It is usually acquired by sexual contact but may also be transmitted by transfusion of infected blood. The infection is systemic and the disease is characterized by periods of latency. These features, together with the fact that *Treponema pallidum* cannot be isolated in culture, mean that serologic techniques play a major role in the diagnosis and follow-up of treatment for syphilis.

An estimated 11.76 million cases of syphilis occurred worldwide in 1999. The incidence and prevalence of the disease varies widely in different parts of the world and may fluctuate considerably within any one region from year to year. The highest rates are seen in developing countries, particularly sub-Saharan Africa and South and Southeast Asia. If syphilis remains untreated, it can lead to complications and death. Syphilis has two stages (Scott, 2010), early syphilis (Primary, secondary and latent) and late syphilis (latent, benign tertiary, cardiovascular and neuro-syphilis). In the early stages causes a painless, but highly infectious, sore on genitals or around the mouth known as chancres. The sore can last up to six weeks before disappearing. The bacteria then move throughout the body, damaging many organs over time. Secondary symptoms such as a rash, flu-like illness or patchy hair loss may then develop. These may disappear within a few weeks, after which a symptom-free phase is appeared. The late or tertiary stage of syphilis usually occurs after many years, and can cause serious conditions such as heart problems, paralysis and blindness. Thus, the course of the disease is divided into 3 stages. An infected person who has not been treated may infect others during the first two stages, which usually last one to two years. In its late stages, untreated syphilis, although not contagious, can cause serious and irreversible cardiovascular and neurological problems.

The symptoms of syphilis can be difficult to recognize. Diagnosis usually depends on the demonstration of antibodies in the blood. Two types of antibody are produced in response to infection, non-specific anti-lipid reagin antibody and specific anti-treponemal antibody. These appear soon after infection; the specific anti-treponemal antibodies may persist for many years, whereas the reagin antibodies may disappear after treatment.

[27] *"STD Facts –Syphilis". Cdc.gov. Retrieved 2013-02-18***.** http://en.wikipedia.org/wiki/Hepatitis_B. **R**etrieved April, **2016**

A positive IgG treponemal test suggests infection with *Treponema pallidum* at some point in the past, but does not distinguish between treated and untreated infections. This is because treponemal tests (eg, EIA, multiplex flow immunoassay, or fluorescent treponemal antibody-absorbed) may remain reactive for life, even following adequate therapy. Therefore, the results of a non-treponemal assay, such as 'rapid plasma reagin' or RPR and VDRL are needed to provide information on a patient's disease state and history of therapy. The condition can be treated with antibiotics, usually penicillin injections. When syphilis is treated properly, the later stages can be prevented.

Gonorrhoea: gonorrhoea[28] is a bacterial STI easily passed on during sex. Gonorrhea is caused by bacterium that lives on moist mucous membranes in the urethra, vagina, rectum, mouth, throat, and eyes. Transmission is usually the result of vaginal, oral or anal sex (Scott, 2010). Symptoms of Gonorrhea usually appear 2 to 5 days after contact with an infected partner however, about 50% of women and 10% of men don't experience any symptoms and are unaware they're infected. In women, gonorrhoea can cause pain or a burning sensation when urinating, a vaginal discharge (often watery, yellow or green), painful sexual intercourse, bleeding during or after sex or between periods, sometimes causing heavy periods, severe pain in lower abdomen (if infection spreads to fallopian tubes), or fever (if infection spreads to fallopian tubes), however many women do not show any symptoms. In men, gonorrhoea can cause pain or a burning sensation when urinating, increased urinary frequency, a white, yellow or green discharge from the tip of the penis, red or swollen urethra, swollen or pain or tenderness in the testicles or sore throat. It's also possible to have a gonorrhoea infection in one's rectum, throat or eyes. Gonorrhoea is diagnosed using a urine test or by taking a swab of the affected area. There are some antibiotic resistant strains for Gonorrhea but most cases can be cured with antibiotics. Gonorrhoea can lead to serious long-term health problems if left untreated, including infertility.

Trichomoniasis[29]: trichomoniasis is an STI caused by a tiny protozoan parasite called ***Trichomonas vaginalis*** (TV). It can be easily passed on through sex and most people don't know they're infected. Trichomoniasis affects both women and men, but symptoms are more common in women. In women, trichomoniasis can cause a frothy yellow or watery vaginal discharge that has an unpleasant smell, soreness or itching around the vagina, and pain when passing urine. In men, trichomoniasis rarely causes symptoms and experienced pain or burning after passing urine, a whitish discharge, or an inflamed foreskin. Trichomoniasis can sometimes be difficult to diagnose and required urine test or swab test of the affected area.

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[28] *"Gonorrhea - National Library of Medicine - PubMed Health". Ncbi.nlm.nih.gov. Retrieved 2013-06-30.*

[29] "STD Facts – Trichomoniasis". Cdc.gov. Retrieved 2013-02-18.
"Trichomoniasis: MedlinePlus". Nlm.nih.gov. Retrieved 2013-02-18.
"Trichomoniasis – NHS Choices". Nhs.uk. 27 February 2012. Retrieved 2013-02-18.

Once diagnosed, it can usually be treated with antibiotics. Most patients are treated with an antibiotic called metronidazole, which is very effective.

Chlamydia[30]**:** chlamydiais a sexually transmitted infection caused by the bacterium ***Chlamydia trachomatis***. Chlamydiais is transmitted and presents in a similar way to gonorrhea (Scott, 2010). Most people don't experience any symptoms, so they are unaware they're infected. In women, symptoms may include abnormal vaginal discharge, burning sensation during urination, pain in the lower abdomen during or after sex, and bleeding during or after sex or between periods. It can also cause heavy periods, although most women do not experience any symptoms. Symptoms in men include pain or a burning sensation when urinating, and a white, cloudy or watery discharge from the tip of the penis, and pain or tenderness in the testicles. Its also possible to have a chlamydia infection rectum (bottom), throat or eyes. If left untreated in both men and women, Chlamydia can infect the urinary tract and potentially lead to pelvic inflammatory disease (PID). PID can cause serious problems during pregnancy and even has the potential to cause infertility. It can cause a woman to have a potentially deadly ectopic pregnancy, in which the child is born outside of the uterus. However, Chlamydia can be cured with antibiotics. Diagnosing chlamydia is done with a urine test or by taking a swab of the affected area.

Epidemiological picture of STIs in Bangladesh

HIV/AIDS: Till date[31] Bangladesh remains a low HIV prevalence country with less than 0.1% overall prevalence in general population over the years (World Bank and UNIAIDS, 2009) and <1% among key (CSWs, PWID/IDUs, MSM and Hijras) and bridging Population (multiple clients). However, the overall HIV/AIDS cases have increased more than 25% over a decade till 2012 which is also alarming (World AIDS Report, 2011; Samuals and Wagles, 2011).

Bangladesh is considered to be at risk for a large-scale HIV epidemic because of the variety and gravity of risk factors for the spread of HIV. Since detection of the first case in 1989, the prevalence of HIV positive cases has increased steadily. Although the exact number of HIV cases is not known, the last two rounds of national surveillance provide a picture of rising prevalence, a high prevalence of active syphilis, and a high prevalence of risky sexual and injecting behaviors among recognized high-risk groups. Also, study (Shirin et al, 2000) in Bangladesh showed that 61.54% of IDUs has history of sexual promiscuity and multiple partnerships with different categories of CSWs.

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[30] "Chlamydia Infections: MedlinePlus". Nlm.nih.gov. Retrieved 2013-06-30.

[31] Global AIDS Response Progress Report (GARPR), Annual Progress Report Bangladesh, 2015; 3[RD] National Strategic Plan for HIV and AIDS Response , 2011-15, National AIDS/STD PROGRAM (NASP), MOHFW, GOB ; The Round 9 surveillance, 2011 and The Round 8 surveillance, 2007. http://www.aidsdatahub.org/nation

Different researchers had shown that genital ulcer diseases act as a precipitating factor for HIV seroconversion in high risk groups. Several studies in various populations had revealed a strong association between syphilitic infection and sexual transmission of HIV (Hanson et al, 2005; Reynolds et al, 2006; Karumudi and Augenbraun, 2005).

Bangladesh has been undertaking periodic national surveillance since 1998 based on the Joint United Nations Programme on HIV/AIDS (UNAIDS)/World Health Organization (WHO) guidelines for second generation HIV surveillance (UNAIDS/WHO, 2000). Available data suggest that vulnerability and risk factors for HIV transmission remain high. Only 17% of the most-at-risk populations can both correctly identify the ways of preventing sexual transmission of HIV and reject major misconceptions about HIV transmission (UNAIDS, 2006).

National sero-surveillance, NSS*[32] *(2004–2005): The sixth round of national HIV sero-surveillance revealed that the highest prevalence of HIV so far among IDUs (from 1.4% in 2000 to 4.9% in 2005) is in the capital city categorized as Central-A. The same survey revealed a pocket in this city where the prevalence was as high as 7.1%. For the first time HIV was detected among IDUs in cities outside Central-A, in the regions referred to as Southeast-D (0.6%) and Northwest-F1 (2%), although the prevalence remains very low. Among heroin smokers, 0.5% tested positive for HIV in Central-A. However, HIV prevalence has remained low (<1%) amongst all groups of female sex workers (FSWs) except the casual FSWs in the city referred to as Northwest-K1, where the rate was found to be 1.7% (95% CI 0.2–5.9). Similarly all other sentinel groups for sexual transmission of HIV had a low prevalence (<1%); for example, only two males who have sex with males (MSM) tested HIV positive out of a sample of 919. Only three transvestites/transsexuals out of a sample of 381 tested positive for HIV, while none of the male sex workers (MSWs) and none of the bridge population (rickshaw pullers, truckers, and dockworkers), who are reported to be major users of sex workers, was HIV positive. The high prevalence of sexual risk behaviors among drug users and sex workers and their clients is alarming. Although a small increase in condom use and a reduction of syphilis have been noted among subsets of high-risk groups in recent years, this is clearly not enough to curb the threat of a possible HIV epidemic (Islam M and Conigrave, 2008).

In 2011 the new cases of AIDS are 251 (231 in 2010) and also new HIV infection are reported 445 persons, which is more (102 persons) than previous year (n=343). Of the newly reported HIV infection, 65% are men, 33% are women and rests of them (2%) are transvestites. According to occupation, 31% (n=138) HIV infected are migrant workers, 22% (n=98) are housewives and 6.74% (n=30) are small businessman. In 2012, 338 and in 2013, 370 new HIV infection was reported. With the latest figure GOB recorded people lived with HIV

[32] (*National HIV serological surveillance, 2004–2005*, Sixth round)

(PLWH) stood at 3241since the first was detected in 1989 and AIDS patients were 1299 (till 2013), and 472 AIDS patients died. However, UN estimated the number to be between 8000 and 16000 which means many were left undetected and untreated[33].

World Bank said certain risk factors of HIV/AIDS bearing by Bangladesh could fuel the spread of HIV/AIDS among high risk groups in spite of a current low prevalence of HIV. These risk factors include large commercial sex industry, low consistent condom use rate by risk groups, high client turnover and high prevalence of STIs among sex workers. However, high STIs also prevails (Shirin et al, 2009, Sabin et al, 2003) even in some pocket of general population. Other factors contributing to Bangladesh's HIV/AIDS vulnerability include large sex industry, multiple partnerships (commercial and noncommercial) with IDUs, CSWs and bridging population, porous cross-border interaction with high-prevalence region in Burma and northeast India, low condom usage among the vulnerable and general population and a general lack of knowledge about HIV/AIDS and other STIs or sexually transmitted infection[34] and also in recent years migrant workers continuously showed HIV positive. A study (Islam M et al, 2010) found poor HIV knowledge among female migrant workers who were flying for overseas jobs

Despite a current low prevalence, Bangladesh has all the potential for a rapid spread. A huge and densely distributed population, a poor economy, geographical and cultural proximity to two more severely affected countries (India and Myanmar), a high prevalence of other sexually transmitted infections (STIs), an enormous underground and mobile population of sex workers, a low rate of condom use, gender inequality, a rising prevalence of HIV among drug users – all these factors, combined with poor access to information on safe-sex and STIs, could fuel a change from the current low prevalence to a sudden epidemic. Even if only 1% of the general population becomes infected with HIV there will be 1.5 million people infected. This massive burden would be almost impossible to cope with should the present socio-economic structure remain unchanged (Islam M and Conigrave, 2008).

Other STIs: A study (Shirin et al, 2009) conducted in outpatient department of Dhaka Medical and Sahid sohrawardy Hospitals showed 86.5% positive for STDs pathogens, of them 42.6% were infected for single pathogens and 43.9% were suffering from multiple infections though no HIV was detected. The prevalence of Chlamydia (4.8%) was the highest among STIs then gonorrhea (39.1%), HSV (38.2%) and syphilis (12.2%) and consistent condom use rate was only 1.7%.

Despite lower prevalence of HIV/AIDS, from the beginning, the prevalence of parasitical, bacterial (*gonorrhea, Chlamidia, trichomoniasis, syphilis*), and viral (*HBsAg, HSV-2*) STIs

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[33] (Bdnews24.com, published 01-12-2013, 17:30:55 BdST)

[34] Health profile: Bangladesh" USAID (March 2008). Retrieved, 2009.

like ulcerative (*syphilis, chancroids, HSV-2)* and non-ulcerative (*gonorrhea, Chlamidia, trichomoniasis*) as well as RTIs (*Bacterial vaginosis (BV), Candiasis*) were reported very high among vulnerable population (Sarker et al, 1998; Rahman et al, 2000; Azim et al, 2000; Bogaerts et al, 2001; Sabin et al 2003; Nessa et al 2004). Considering the risks of these sexually transmitted infections (STIs) among vulnerable population (CSWs, IDUs, Transgender, MSMs, internal & external migrant workers etc.) earlier couples of studies funded by different international agencies were carried out, mainly addressing this population at risk and also others. Previous[35] sentinel surveillance and Azim et al (2000) reported very high syphilis rate among Floating CSWs (56.8%) and Brothel-based CSWs (52.5%) and relatively high HIV prevalence rate in IDUs (2.5%). A study among women attending antenatal clinics (low-risk group) in Dhaka reported the prevalence rates of *N. gonorrhoea, Chlamydia. T, Trichomonas. V* and syphilis were 0.5%, 1.9%, 2.0% and 2.9%, respectively (Bogaerts et al, 2001). A study among slum dwelling men and women (intermediate-risk group) in Dhaka reported prevalence rates of syphilis, *gonorrhoea, chlamydial* infection and hepatitis B virus to be 6.0%, 1.7%, <1% and 3.8%, respectively (Sabin et al 2003). Previous studies among brothel-based (Sarker et al, 1998) street-based (Rahman et al, 2000) and hotel-based (Nessa et al, 2004) female sex workers reported prevalence rates of *bacterial STIs* respectively were [*N. gonorrhea and/ or Chlamydia. T* =28.0%, syphilis=57.1% (Sarker et al, 1998)], [*N. gonorrhea*=35.5%, *Chlamydia. T*= 25.0%, *Trichomonas. V*=45.5% and syphilis=32.6% (Rahman et al, 2000)] and [*N. gonorrhea*=35.8%, *Chlamydia. T*=43.5%, *Trichomonas. V*=4.3%, and syphilis =8.5% (Nessa et al, 2004)].

Role of Government and NGOs on counseling commercial sex workers

Bangladesh was the first country in the region to adopt a comprehensive national policy on HIV/AIDS and STDs (in 1997), then also developed the first national Strategic plan for HIV/AIDS, 1997-2002. This was reviewed in 2005 and the second national Strategic plan for HIV/AIDS (2004-2010) and third national Strategic plan for HIV/AIDS (2005-2015) was adopted. During the period of 2005-2010, there was expansion of the HIV/AIDS program in terms of coverage and involvement of different stakeholders. The major programmes implemented or initiated during this period were

1. HIV/AIDS prevention Project (HAPP) 2004-2007 was the first major project under NASP which was supported by World Bank (WB) and DFID. HAPP had four components: implementing targeted intervention among MARPs (PWID, FSW, MSM and Hijra), advocacy and communication, blood safety and institutional strengthening and program support. HAPP was implemented through GO-NGOs

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[35] Government of Bangladesh. **HIV in Bangladesh: the present scenario. A summary of key findings from the fifth round of serological and behaviour surveillance for HIV in Bangladesh (2003–2004).** Dhaka, Bangladesh: National AIDS/STD Programme, Directorate General of Health Services, Ministry of Health and Family Welfare; 2004;

Government of Bangladesh. *National HIV serological surveillance, 2004–2005.* Sixth round technical report. Dhaka, Bangladesh: National AIDS/STD Programme, Directorate General of Health Services, Ministry of Health and Family Welfare; 2005.

collaboration with assistance from UNICEF, UNFPA and WHO. More than 100 NGOs were involved in implementation of HAPP. The total funding was US $ 26.33 million.

2. The HIV/AIDS Targeted Intervention (HATI) 2008-2009 supported by World Bank financed Health, Nutrition and Population Sector Program (HNPSP). HATI focuses on intervention packages for six high risk groups: PWIDs, brothel based sex workers, street based sex workers, and hotel and residence based sex workers, MSMs, MSWs and hijras and clients of sex workers.
3. The Bangladesh AIDS program (BAP) 2005-2009: the activities included support to interventions for MARPs, support to NGOs for addressing PLHIV, condom promotion, training of health providers, VCT centers staff. In 2009 'Modumita' was launched as the follow on to BAP and implanted through FHI, SMC and BCCP and was continued to 2013.

In addition, based on the satisfactory level of completion of the Round 2 project, Global Fund awarded Bangladesh with the 6 years fund termed as "Rolling Continuation Channel (RCC)" from 2009 to 2015, which is consolidated with Round 6 grant. For high level of performance the project was appreciated as a 'best practice' example in Asia and was rated "A" by the 'Global Fund'. The program was being implemented through 13 technical packages by 13 consortiums comprising 61 organizations nationwide. 'Save the children' was the principle recipient of the grant and others were NASP and ICDDR, B.

In recent years there has been a shift in social development approach due to the steady rejection of top-down methods. Over the past two decades for instance, the United Nations and other international organizations have emphasized the mainstreaming human rights into activities and programs. This shift from a charity or needs approach to a right-based approach highlight the empowerment of socially excluded people by involving them in the development process as active agents rather than beneficiaries. This approach focuses on people's ability to achieve the minimum conditions for living with dignity by recognizing marginalized people as having inherent rights. Sex workers have become increasingly involved in the process of empowerment. For instance, NGOs that were implementing the HIV/AIDS prevention program (HAPP, 2000-2007) trained and employed many sex workers as peer educators and outreach workers.

As mentioned earlier, in collaboration with Government different local and international NGOs such as CARE Bangladesh, Family Health International (FHI) Nari Maitree etc. motivate sex workers to get organized, provide legal aid, medical care and training on human and legal rights and skills training. NGOs have had a pivotal role in the formation of 'self-help' groups. 'Self-help' groups can mobilize the CSW's community and focus on overall health, socio-economic well being and human rights of their members. With the help of NGOs, 'self-help' groups organize savings schemes, information and dissemination sessions, and self-defense classes and nurseries for children of sex workers. They also refer sex workers to relevant organizations when they are in need of assistance. The first organization run by absolutely CSWs in Bangladesh was 'Nari Mukti Sangha' formed in 1997 by sex workers of Tangail brothel. A year later the street-based sex worker's organization 'Durjoy Nari Sangha' was established. Both these organizations were registered with the Ministry of Women and Child Affairs and both have recorded many achievements in improving working conditions. Like sex workers of Tangail did not have the right to burial and their bodies were thrown into the river, now 'Nari Mukti Sangha', Tangail brothel has its own graveyard. However, sometimes these NGO-led activities are limited by funding, resources, program longevity, capacity and scale. Without the support of donar agency, NGOs are unable to continue programs and without NGO-backing self-help groups do not have sufficient funds to implement organizational activities[36].

[36] Exploitation of CSWs in Bangladesh, 2015 http://www.ukessays.com/essays/sociology/exploitation-of-commercial-sex-workers retrieved December, 2015

b. Rationale of the Study

Although most of the researches have been focusing on the global HIV/AIDS prevalence, there has been very little address on the health and immunonutritional indicators of high HIV-risk group and no information is available on the immuno-nutritional indicators of high risk group like ‘sex workers’. There are some studies in developing countries on the lifestyle of high HIV-risk sex workers, sex users, drug addicts but no conclusive systematic study on the high HIV-risk group of population in Bangladesh has so far been performed. Therefore this study was attempted to carry out among the high HIV-risk sex workers, screening and STIs comparisons were made among two categories of sex workers,

GOB and different NGOs are sporadically working only for sex workers, truckers and rickshaw pullers. In the absence of any systematic study on this high HIV-risk group of population, any effective program planning is being hampered. The findings of this study may help the GOB and NGOs to come up with effective program planning in the management and prevention program of deadly infectious disease HIV/AIDS.

Therefore, considering the fatal consequences of the HIV/AIDS epidemic in the world, particularly in the neighboring countries, this research has designed to address risk determinant factors among the vulnerable HIV susceptible group of people and to investigate the nutritional status including anthropometry, biochemical and dietary, infectious disease prevalence and factors associated with STIs, life styles, causes of inconsistent condom usage and immune profile of sex workers. This could help to have an effective intervention program to prevent STIs and HIV/STIs epidemic. Thus our health planner will be able to come up with an effective program design to prevent the spreading of killer infection HIV and as well as other STIs and to control their prevalence at safe level. It will also reduce the cost of healthcare system, as well the national productivity loss through reduction and control of HIV and other infections. As a whole, this could protect the community from spread of STIs and HIV/AIDS, the deadly infectious disease.

Although, HIV prevalence is not yet in threshold level to be epidemic in Bangladesh but the HIV epidemic is expanding very fast in this region. It has been reported that once HIV prevalence reaches to threshold point, it can flare up as an epidemic within 1 year. The overall HIV/AIDS cases have increased more than 25% over a decade till 2012 which is also alarming (World AIDS Report, 2011). All of the risk factors that frightening the devastating explosion of HIV epidemics, are present in Bangladesh today which may shot up of high HIV prevalence within a short period. Without identifying full spectrum of risk indices among the vulnerable groups, an efficient high coverage intervention program could be difficult to design and launching. In addition to the horror of epidemic flare up of HIV infection, behavioral HIV-risk factors among the sex workers, sex users and drug addicts rank them at the highest risk of HIV/STIs infections. Furthermore, study (Shirin et al, 2000) in Bangladesh showed that 61.54% of IDUs has history of sexual promiscuity and multiple partnerships with different categories of CSWs.

Although various studies addressing STIs of different types of CSWs (street-based, hotel-based, brothel-based) but no study in Bangladesh was carried out focusing on the risky as well as unusual sexual practices like “Group sex”, “Anal sex” and non paying sexual partnership (NPSP) other than normal clients etc. Even the existence of these types of sexual practices among higher risk population has not yet fore fronted.

Previous report suggests that Bangladesh has an intermediate prevalence of hepatitis B infection, with the highest recorded rates of HBsAg being in professional blood donors (Mustafa et al 1989a) and in CSWs (Sattar and Islam, 1996; Mustafa et al, 1989b). Besides, little data are available on hepatitis B prevalence in Bangladesh (Gibney et al, 2001). Furthermore, studies focusing on Hepatitis B infections among vulnerable groups like CSWs were also scanty in Bangladesh. No viral screening of STIs (HIV, HBsAg) among hotel-based and floating CSWs was carried out. The few studies (Mustafa et al, 1989; Sattar and Islam, 1996; Gibney et al, 2001) those had been published addressing high risk groups are more than a decade earlier. For hepatitis B surface antigen (HBsAg) which indicates both current and chronic (lifetime carrier) infectious state, previously only few studies reported 8% prevalence in intravenous drug users (Mustafa et al, 1989), 5.9% in truck drivers (Ginbney et al, 2001) 9.7% (Sattar and Islam,1996) and 11% (Mustafa et al, 1989b) in CSWs. Thus, study focus on HIV, hepatitis B and syphilis prevalence among both hotel-based and floating commercial sex workers simultaneously, has never been studied before in Bangladesh. Besides, the study was carried out in combination with the screening of STIs (HIV, hepatitis B and syphilis) along with the information about the socio-demographic, lifestyle factors, risky sexual acts of CSWs which could help future planning and further research of this field. Moreover, in this study an attempt has been made to find out risk variables associated with ‘syphilis’, ‘Hepatitis B’ and ‘inconsistent condom use’ by CSWs as well as tried to explain a clear idea about condom use practices by FCSWs and HBCSWs. In addition, this study tried to investigate the inner thinking as well as insight of these behaviors from both CSW’s and researcher’s point of view. Certainly, this study could help the policy makers of the government and non-government organizations as well as helpful to the researchers.

Moreover, immune functions of garments and apparel workers in Bangladesh were assessed (sultana et al, 2012) but no single study was carried out to assess immune function of CSWs in Bangladesh. Researchers have not given due attention to the immune function of CSWs worldwide rather “STIs and CSWs” especially “HIV” has drawn overwhelming attention to the researchers as a vulnerable infection and “CSWs” as a vulnerable group. Therefore, this study was conducted to assess the immuno-nutritional status of CSWs and its relation to sexually transmitted infection was addressed as well.

c. Research Objectives

The primary aim of this study is to investigate nutritional status, infectious disease prevalence, life style and immune profile of female commercial sex workers (FCSWs). To this end, this study has designed to

- Identify and compare socio-economic pictures and lifestyle factors among floating and hotel-based commercial sex workers.
- Investigate the professional risky behaviors (PRB) of CSWs which make these groups more Susceptible to get infected and to identify factors associated with low 'condom usage' by CSWs.
- Assess nutritional status (anthropometric, dietary and biochemical) of the sex workers.
- Investigate the prevalence of sexually transmitted infections (STIs) including HIV/AIDS, Hepatitis B virus infection (HbsAg) and syphilis infection among the high risk population.
- Analyze immune profile (serum immunoglobulins i.e. IgG, IgA & IgM) of CSWs and variables associated with serum immunoglobulins.
- Estimate immuno-active micronutrients like serum vitamin A, vitamin E, copper, zinc and iron.
- Finally to provide policy recommendations on the management, and prevention of deadly virus HIV and thus improvement of lifestyle of these vulnerable groups of population.

Considering the above aim and objectives the whole dissertation is presented by dividing its contents into several chapters and sub-chapters namely

Chapter-1: Socio-demographic profile of female commercial sex workers (CSWs)

Chapter-2: Nutritional status of CSWs

2.1: Anthropometric Assessment

2.2: Dietary Assessment

2.3: Biochemical Assessment

Chapter-3: Prevalence of Sexually transmitted Infections (STIs) among CSWs

Chapter-4: Micronutrients status of CSWs

4.1: Antioxidant vitamin A and E

4.2: Immuno-active minerals (Cu, Zn, and Fe)

Chapter-5: Serum Immunoglobulins (IgG, IgA and IgM) level of CSWs

d. Study design

The study was conducted among female commercial sex workers (FCSWs) of Bangladesh. The aim of the study was to analyze the lifestyle factors as well as heterosexual risky behaviors (HRB) along with socio-economic status (SES), immuno-nutritional status, screening for seropositivity of sexually transmitted infections (STIs/or STDs) namely HIV, hepatitis B (HBV) and syphilis. Focusing on the aim of the study, the protocol was designed to investigate-

Part I: Socio-demographic profile of FCSWs (n=635)

(i) Socio-economic status (SES): age, education, marital status, income-expenditure pattern etc.

(ii) Life style (LS) related to sex trade: working days in a week, introductory age of sex industry, duration, places (floating/ hotel-based), abortion cases, and use of contraceptives, smoking habit, substance abuse, and knowledge about STIs etc.

(iii) Heterosexual risky behaviors (HRB): Use of condom, weekly client turnover, usual number of client per day, interval between 2 coitus, usual coital frequency per client, weekly/fortnightly/monthly coitus with permanent clients (PC), hygienic measure after coitus, having non paying sexual partners (NPSPs), and history of group sex, Anal sex etc.

Part II: Nutritional status of FCSWs which was evaluated in respect of

(i) Anthropometric assessment (n=635): Height, weight, body mass index (BMI) and chronic energy deficiency (CED)

(ii) Dietary assessment (n=635): Calorie, protein, fat, carbohydrate, protein, calcium, copper, zinc, iron, vitamin A, vitamin C etc.

(iii) Biochemical assessment (n=495): Total protein, albumin

Part III: STIs prevalence of CSWs (n=495)

(i) HIV serology

(ii) Hepatitis B serology

(iii) Syphilis serology

Part IV: Micro-nutrients (n=107)

(i) Antioxidant vitamins: vitamin A and vitamin E

(ii) Micro-minerals: copper, zinc and iron

Part V: Immune profile of FCSWs (n=107)

(i) Serum Immunoglobulins: IgG, IgA, IgM

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CHAPTER-1

Socio-demographic Profile of Commercial Sex Workers of Bangladesh

1.1 Introduction

The existence of selling female sex for the gratification of males is one of the oldest vice in society. Despite commercial sex business is illegal in Bangladesh like other Asia pacific countries; this profession could not be sustained unless there was any demand from the males of different classes. Among the reasons behind the beginning and sustaining of the profession, the subordination and weaker position of women in the socio-economic structure of society can be assumed as the most significant (Hossain et al, 2004).

Sex business considered as a social problem which is also the result of other social problems i.e. poverty, dowry, exploitation, human trafficking etc. Different circumstances under which destitute people are forced to transform their bodies into sex objects and to peddle sexual services as an item of commercial transaction certainly seem to points towards a social existence devoid of basic human rights (Khan, CPD).

Bangladesh is a low-prevalence country for HIV/AIDS as it is less than 0.1% among general population over the years (World Bank, 2009; UNIAIDS, 2010) and <1% both among risk groups and bridging population[37] (with one exception among casual sex workers in Hili). While Bangladesh continues to be a low-prevalence country, its segment of key and bridging population is highly vulnerable for risk behaviours – including CSWs with high numbers of multiple clients but very low condom usage and poor knowledge about STIs and HIV/AIDS, high prevalence of STIs among CSWs, unsafe injecting practices of IDUs and unprotected sex with commercial partners with both IDUs and movable populations like truckers internal and external migrant workers etc. Condom use in Bangladesh is reportedly the lowest in Asia; the figures have been rising although interventions by non-governmental organizations (Sadhana and Sanghamitra, UNAIDS, 2008).

Though Bangladesh has upgraded from low income ($ ≤1045/year) country to lower-middle income country, with annual incomes of $1046 to $ 4125 according to World Bank's (2015)[38] latest estimates of Gross National Income (GNI) per capita and country has also made significant progress in human development in the areas of literacy, gender parity in schooling and reduction of population growth[39] there are still pockets of poverty stricken underprivileged population. Furthermore, a range of structural factors are heightened the vulnerability of the general population to an HIV epidemic. Structural factors include: a low adult literacy rate; low social status of women and the trafficking of women into the commercial sex industry; high population mobility within the country, including interstate and rural-urban as well as international labour migration, particularly across its porous borders with India and Myanmar, both of which are experiencing concentrated epidemics (Sadhana and Sanghamitra, UNAIDS, 2008).

[37] Global AIDS Response Progress Report (GARPR), Annual Progress Report Bangladesh, 2015; 3RD National Strategic Plan for HIV and AIDS Response , 2011-15, National AIDS/STD PROGRAM (NASP), MOHFW, GOB
The Round 9 surveillance, 2011 and Round 8 surveillance, 2007. http://www.aidsdatahub.org/nation

[38] WB update says 10 countries move up in income Bracket (1st July 2015) (www.worldbank.org/en/news/press-release)

[39] United Nations Country profile: Bangladesh.(http://www.un-bd.org/countryprofile/html)

Bangladesh is vulnerable to an extended HIV epidemic due to the prevalence of behavior patterns and risk factors that facilitate the rapid spread of HIV. As mentioned above Bangladesh has to support a large scale commercial sex industry. The internal migration from the country as well as overseas migration from other countries creates demands for the services of the prostitution in Bangladesh. There are only 15 registered brothels out of hundreds hidden sex industry with quite drastic status and inhuman life styles. All other sex-working venues e.g. Hotel-based Sex Work (HSW), Street Sex Work (SSW) or Residential-based Sex Work (RSW) are clearly illegal. Higher rates of Sexually Transmitted Infections (STI) and HIV infection are found among SW and their clients in comparison to other population groups (Mondal et al, 2010). In the 7th round sero-surveillance, active syphilis rates were highest in female intravenous drug users (9.9%) followed by SSW (8.6%) (Azim et al, 2000).

In spite of lower prevalence among CSWs, the overall HIV/AIDS cases are increasing in our country which is also alarming. In 2011 the new cases of AIDS are 251 (231 in 2010) and also new HIV infection are reported 445 persons, which is more (102 persons) than previous year (n=343). Of the newly reported HIV infection, 65% are men, 33% are women and rests of them (2%) are transvestites. According to occupation, 31% (n=138) HIV infected are migrant workers, 22% (n=98) are housewives and 6.74% (n=30) are small businessman. In 2012, 338 and in 2013, 370 new HIV infection was reported. With the latest figure GOB recorded people lived with HIV (PLWH) stood at 3241since the first was detected in 1989 and AIDS patients were 1299 (till 2013), and 472 AIDS patients died. However, UN estimated the number to be between 8000 and 16000 which means many were left undetected and untreated[40].

Economic and political changes over the past decade have apparently created financial hardship in Bangladesh, particularly for women, resulted in a dramatic increase in the number of women and girls in the sex work industry, either voluntary or against their will. Some have moved from village fleeing hardship and poverty in search for better life; others are the victim of sexual exploitation (UNFPA 2002).

All over the world including Bangladesh, young women especially of the age of 13-35 years have high demand in this profession. The profession of CSW is more evident in urban areas than in the rural areas. Approximately one million men buy sexual services from 200,000 prostitute[41] women and children in Bangladesh. They live in brothel, rented houses in affluent areas, hotels, guest houses and mess under urban, semi urban and lesser extent in rural areas. Many live independently and serve as floating CSWs. The floating CSWs do have a

[40] BDnews24.com, 2013.

[41] janmoellerhansen,smugmug.com/Bangladesh/sex-workers/2012
Exploitation of CSWs in Bangladesh, 2015 http://www.ukessays.com/essays/sociology/exploitation-of-commercial-sex-workers retrieved December, 2015

chance to return to their home, on the contrary, brothel-based CSWs cannot return back home once they get registered as professional one[42].

The sexual services were broadly categorized under four main groups-brothel based, hotel based, lodge/home based (sexual business carried on rented houses by small groups of girls hiding identity) and floating/street/ slum based. Some sex workers evicted from brothels were reported to serve as floating sex workers. In Bangladesh, there is a general perception that eviction of brothels might have reduced the bad practice in the country and new recruitment will become difficult. But different reports highlighted the opposite picture, indeed (Hossain et al, 2004). When brothels are evicted, number of floating or hotel or residence based sex worker will be increased obviously. Eviction of brothels never terminates prostitution rather concomitantly increased the number of street/ floating sex workers. Reports of different studies conducted by NGOs in Bangladesh revealed that many evicted eligible sex workers did not quit their professions which prove they can never give up. Interviews with evicted sex workers reveal the fact that eviction cannot be the way to solve this social problem and without proper initiatives to support them financially, otherwise this will increase the number of floating sex workers or unprotected work outside brothel areas (Taposh, 2006).

There exists difference among sex workers, like rich and poor, upper and lower class. In fact, there are more divisions among the sex workers than the class divisions in the society. The lowest rung starts with the homeless variety that totally depends on the street. Having home but extremely poor women also carry on their sex business on street[43].

Gradually, the practice of prostitution took various forms and arrangements which include places of availability, rate, glamour, and associated persons, pimps, shelter, and connection with law enforcing agency (Hossain et al, 2004). In recent years, there has been remarkable change in the nature of the sex industry, possibly due to eviction of brothels from major cities increased demand for SW in non stigmatized and demand for more freedom and opportunity of the income by CSWs (Mondal et al, 2010). Among the four types of CSWs street or FCSWs are found physically most "visible", most harassed but socially "hidden" (Hossain et al, 2004) and often regarded as occupying the lowest point in a status hierarchy of CSWs (Mckeganey and Barnard, 1996).

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[42] Alam, Rainbow Foundation; Karim and Aziz, Banglapedia

[43] FIRM, Maitreya.com (www.thrani.com)(www.firm-kerala.org)

1.2 Materials and Methods

The study was conducted among female commercial sex workers (FCSWs) of Bangladesh.

Study population: a total of 635 female commercial sex workers (FCSWs) comprising both floating CSWs (n=327) and hotel-based CSWs (n=308) were interviewed. The sample size was calculated on the basis standard method.

Sample size determination: The sample size of CSWs was determined by considering the prevalence of HIV infection. The sample size was determined by using the following formula:

$$n = 1.96 \times P\,(100-P)/\,W^2$$

Where, n= minimum sample size for a specified population.

P= prevalence of HIV infection is reported 1.7% (95% CI 0.2–5.9) among FCSWs (National HIV serological surveillance, 2004–2005. Sixth round; Islam and Conigrave, 2008)

W= width of the confidence interval (CI) where the true prevalence will be some known, obviously high probability.

As HIV infection among FCSWs was 2%, so the minimum sample size will become

$$C.I = \frac{1.96 \times \sqrt{[P \times (100-P)]}}{N}$$

$$\text{And}\quad W = \frac{2 \times 1.96\sqrt{P \times (100-P)}}{N}$$

$$N = \frac{2 \times 1.96\sqrt{P \times (100-P)}}{W}$$

$$N = \frac{4 \times 3.84\,[\sqrt{P \times (100-2)}\,]^2}{W^2}$$

$$\text{Now,}\quad n = \frac{4 \times 3.84\,[2 \times (100-2)]}{w^2}$$

$$= \frac{15.36\,(2 \times 98)}{w^2}$$

$$= \frac{(15.36 \times 196)}{w^2}$$

$$= 3010.56 / (3)^2$$

$$= 334.51 = 335 \text{ CSWs.}$$

Here, estimated P value=5%, required precision for the estimate of change ± 5% width of the class interval w= 3%

Study period: The study was conducted during the period of 2011 to 2014.

Questionnaire development: a questionnaire was developed and pretested among 25 FCSWs, who were excluded from the study population. It was the primary research instrument.

Sample survey: The study was conducted among female commercial sex workers (FCSWs) of Bangladesh. With the help of CSW's welfare organizations (Durjoy nari sangha, Ulka nari sangha etc.) which work for all types of CSWs, this study was carried out among randomly selected six hundred and thirty five (n=635) CSWs aged 14-55 years comprising hotel-based (n=308) and floating CSWs (n=327) respectively working either in 'hotel' or on road/park or in cinema hall/cow haat or shrine or shop or elsewhere in Dhaka city. To carry out the study the first approach was contacted the subjects through these welfare organizations (Durjoy nari sangha, Ulka nari sangha etc.) of CSWs and explained to them the objectives of the study in an understandable way and asked for their co-operation and the written consent of the subjects.

The study was approved by ethical review committee of 'Bangladesh medical research council' (BMRC) and 'University of Dhaka'. All CSWs were informed about the study before selection and after briefing the subjects about the perspective of the study 'written consent' was obtained from randomly selected CSWs on the very day of interview by participant's signs or printed 'thumb' on the consent form. The questionnaire was both open-ended and structured questions and off course, was pre-tested. In fact, background information, SES, lifestyle factors, SRB, anthropometry and blood samples were collected sequentially. The participants were interviewed (n=635) and 5 ml venous blood were collected (n=495) by trained social interviewer and paramedics respectively. The most (86.1%) of the subjects of the study were women migrated to the Dhaka city from all over the country from low-income families and only a few (13.9%) were originally from Dhaka city but also poor economic background. Most of them resided either in road/perk/shrine/ cow market or in low-cost accommodation (most cases only one room for all) with no indoor supply of water, no sanitary latrines and densely packed sleeping arrangements.

Socio-economic, life style and hetero sexual risky behaviors (HRB) of FCSWs

Interviewer administered questionnaire were used to assess the Socio-economic status (SES), lifestyle factors of the FCSWs and detailed information on their sexual life, partners, various factors related to sex business, and HRB, as designed in the study, were collected by researchers during interview sessions. The information thus collected were compiled and analyzed statistically using SPSS software program (Version 17). Socio-economic, lifestyle and risk determinant factors were assessed by analyzing questionnaire data.

Statistical analysis

Various statistical tools including ‘descriptive statistics’ and ‘compare means’, were used employing a software package of SPSS (version 17.0: SPSS Inc, Chicago, IL, USA) for the analysis of the data. “Univariate analysis” comprised of simple frequency distribution of selected variables and mean, standard deviation and range for all parameters were determined. “Bivariate analysis” was used to determine the differences between groups (or categories) for various independent variables (viz. background, lifestyle, risky behaves, anthropometric and dietary variables). Categorical socio-demographic characteristics between two types of female CSWs namely hotel-based CSWs (HBCSWs) and floating CSWs (FCSWs) were first statistically compared by using contingency tables ($\aleph^2$ test) and if the variable was continuous then student’s t-test was employed to examine the mean differences between 2 groups of CSWs. Then both univariate and multivariate logistic regression of socio-demographic, lifestyle and HRB variables was done to detect the risk factors (predictors/independent variables) for STIs. $P<0.05$ was considered significant.

1.3 Results

Socio-economic variables: Result showed that all socio-economic (table-1) variables were significantly ($P<0.05$) different between Hotel-based Commercial Sex Workers (HBCSWs) and Female Floating Commercial Sex Workers (FFCSWs) except treatment costs on STIs (t=.748, df=633, P=.455). Result revealed that, generally, young women aged 19-29 were predominant (48.66%=309/635) in sex industry and very young women aged <18 were significantly ($P<0.05$) higher in hotel sex trade (37%) than that of floating (4.59%) where as ≥30 aged CSWs were noticeably higher (between groups & within categories $P<0.05$) in floating community (52.3%) than hotel (8.4%).

Overall, vast majority (65.83%) of CSWs had no formal education but in compare to floating CSWs hotel-based CSWs had higher literacy rate (~52%) and lower illiteracy rate (48.05%), while 82.57% floating CSWs had no formal education.

In generic appearance, proportion of married CSWs was the highest (43.46%) among other categories (unmarried and W/D/AB) and widowed/divorced/abandoned women were the second highest (38.27%) among total CSWs. Although overall unmarried CSWs were only 18.27% but significantly enormous proportion of abandoned (58.71%) and tiny groups of unmarried (1.53%) were present in floating group. In contrast to this, HBCSW's group possessed highest married category of CSWs (47.40%) but smallest portion of abandoned women (16.56%).

Generally, most CSWs (61.6%) did not have children and 38.4% CSWs had children and most (59%) of floating CSWs had children in contrast to HBCSWs who had only 16.6% children ($\aleph^2$=120.87, P=.000). Mean ages (t=-17.211, df=633, P=.000), monthly incomes (t=12.607, df=633, P=.000), last month's expenditure (t=12.704, df=633, P=.000), and expenditure on foods (t= 2.767, df=633, P=.006) of these two groups were also significantly differ each other except 'treatment costs on STIs' (t=.748, P=.179). Mean treatment cost on STIs (last ailment) for all CSWs (n=635) was only 162.63 BDT, surprisingly, it was higher for (175.88 taka) floating CSWs and lower for hotel-based CSWs (140.68 taka), though the difference could not reach at significance level **(table-1).**

Lifestyle factors: In case of lifestyle factors, significant differences (P=.000) were observed between these two groups of CSWs except 'factors behind profession' (P=.235), 'abortion cases' (P=.139), 'smoking habit' (P=.167) and 'NGO counseling on STIs' (P=.054).

Average entering age of floating CSWs into commercial sex trade was higher (21±7 vs. 18±4) than hotel groups (t =- 5.736, P=.000*) but both types had same lower age limit of introducing prostitution (i.e.10y). Although, both under-eighteen and over–eighteen girls were first introduced themselves as CSWs in hotel sex trading (within categories of hotel $P>0.05$), but <18 girls were more likely (between 2 groups $P<0.05$) to join in hotel sex business (50.33%) than floating trade (35.47%). In contrast, ≥18 girls were found abundance in floating sex trade (between 2 groups no difference but within categories $P<0.05$). HBCSWs possessed a tiny portion of women (n=14) who entered into this profession at >25 years of age which was the smallest group among all age categories of CSWs.

Significant difference was reported between two CSW's groups in the length of profession. Generically, more than half (343/635) of CSWs had 1 year to 5 years experience in sex trade but mean professional length of FFCSWs groups was almost triple (FFCSWs: HBCSWs vs. 8.60: 2.79 y) than HBCSWs (t=11.533,p=.000*). Majority (63.31%) of HBCSWs were professionally less experienced (1-5 y) than floating group experienced in ≥6 y (51.1%).

In generic appearance, more than half (52.60%) CSWs did not access to mass media items but in case of floating CSWs it was overwhelmingly high (77%) and opposite (26.6%) for HBCSWs. This appearance indicates comparatively lower economic level of floating CSWs as compare to HBCSWs.

Average working days of floating CSWs (3.65 days) were higher (t=-6.150, P=.000*) than hotel-based sex workers (2.87 days). It is a common phenomenon among all types of CSWs to absent for 1-3 days from work within a week. In our study 5.67% (36/635) did not work for the last week which indicates their inability to work due to tremendous mental and physical stress and also poor physical condition.

Generally, most (54.17%) of the HBCSWs lived in Dhaka city on their own, a remarkable portion (30.24%) of the CSWs were usually lived with their husbands (between 2 groups category P<0.05). Vast majority of floating CSWs (60.86%) lived alone (between and within category P<0.05).

Majority of CSWs (72.9%) of both groups (P=.139) had no abortion history and significant amount of HBCSWs preferred pill (51.62%) to condom (27.60%) as a birth control measure (P=.000) in Contrast to floating CSWs (pill=19.57%, condom=51.07%). In general, most (55.69%) CSWs of both groups were non smokers (P=.167) and vast majority of CSWs did not abused substance (68.03%).

It was also noticeable that most (FFCSWS 238/327 vs. HBCSWs 194/308) of CSWs did not take drug. In general, cannabis (ganja 85/203) and alcohol (44/203) were used most frequently and others (afim, phensedyl, injection 44/203) were used less by them. Proportion of substance abusers were higher in HBCSWs groups (37%) than floating (χ^2=6.99, P=.008) and HBCSWs abused more (χ^2=36.02, P=.000) cannabis (15.3%) and alcohol (18.8%) than floating group [cannabis (8.3%) and alcohol (8.3%)]

Generically, most of the CSW's clients were small businessmen (46.30%, 294/635) but HBCSWs had more than half (60.1%) of businessmen customers in contrast to FFCSWs who had only one third (33.33%) businessmen clients the differences were significant (χ^2=149.6, P=.000*). On the other hand, floating CSWs had significantly (P=.000*) more rickshaw puller (35.17%) and less service-holder clients (9.5%) than HBCSWs (rickshaw puller=.97%, service holder=20.46%) which indicated low wages of FCSWS as compared to hotel-based CSWs.

NGO activity on STIs was not significantly different (P=0.054) between HBCSWs and FFCSWs ***(table-2).***

Risky sexual behaviors of CSWs: Usually, in this study, CSWs (both types) frequented by 6 (mean) customers daily. Hotel-based (HBCSWs) and floating (FCSWs) respectively gave commercial sex services to 8 ±4 (range: 1−30 clients) and 4±2 (1−20) clients per day, that means, HBCSWs handled almost double clients per day in compare to FFCSWs (t=15.01, P=.000*) which was previously reflected in their monthly income (table-1). Although, average working days (3.65 days) of floating CSWs (table-2) were higher (t=-6.150, P=.000*) than hotel-based sex workers (2.87 days) but weekly client turnover were significantly lower (P=.000*) than HBCSWs. Last week 'client turnover history' showed that HBCSWs and FFCSWs met an average of 22 and 12 weekly customers respectively.

In fact, Occasional (inconsistent/irregular) condom use rate was significantly (between group P=.023) higher in FFCSWs (197/327) than HBCSWs (158/3088). In contrast, higher proportions of HBCSWs (150/308) were habituated to use condom consistently than their floating colleagues (130/327). Overall, inconsistent use of condom was higher (55.9%) among all CSWs.

Vast majority (62.34%) of HBCSWs used savlon/dettol as a hygienic measure after coitus with clients as compare to (P=.000**) their counterpart (29.7%). On the other hand, all types of hygienic practices mentioned here equally distributed among FFCSWS (within category P>0.05).

Significant difference (P<0.05) was observed between two CSW's groups (table-3) in case of interval between 2 coitus and coital frequency with usual and permanent clients (PC). In general, majority (67.40%) of CSWs did not take a break between 2 sexual services given to customers; if customers were available, but 32.60% of them asserted that they used to take a break between 2 customer services. Mostly HBCSWs (79.22%) took a break while FFCSWs used to practice both (56.27% took a break and 43.73% didn't). Same type of activity was also observed in case of 'coital frequency' with normal clients, HBCSWs preferred 1-time coitus with usual clients in compare to floating and this difference was statistically significant ($\aleph^2$=19.693, P=.000). On the contrary, FFCSWs Performed both types of coitus (1/ 2 times) with usual clients (within category P>0.05). Similar result was also found in case of weekly, fortnightly and monthly "coital frequency with PC". HBCSWs had higher proportion of permanent clients than FFCSWs (all P=.000). And higher rate of floating CSWs performed "group sex" in a larger group (>6 groups) than HBCSWs (between group P=0.005) ***(table-3)***.

Table-1: Socio-demographic profile of CSWs by type (n=635).

Socio-economic factors	Total CSWs n =635 % n	Hotel-based CSWs, n=308 % n	Floating CSWs % n	Statistical tests	P Value
Present Age (year)					
<18	20.32 (129)	37.01 (114)	4.59 (15)	$\varkappa^2$=184.659	.000*
19-29	48.66 (309)	54.55 (168)	43.12 (141)		
≥30	31.02 (197)	8.44 (26)	52.29 (171)		
Mean P. age ± SD	***25.55 ± 7.61***	***21.13 ± 4.8***	***29.7 ± 7.42***	***t= 17.21,df=633***	***.000****
Range	***(14-55 y)***	***(14-45 y)***	***(15-55 y)***	***(95% CI difference)***	
Education					
[44]No literacy/can sign	65.83 (418)	48.05 (148)	82.57 (270)	$\varkappa^2$=86.909	.000*
1-5 class (primary)	28.35 (180)	41.56 (128)	15.90 (52)		
6-12 class (secondary)	5.82 (37)	10.39 (32)	1.53 (05)		
Marital status					
Married	43.46 (276)	47.40 (146)	39.76 (130)		
Unmarried	18.27 (116)	36.04 (111)	1.53 (05)	$\varkappa^2$=179.196	.000*
Widow/divorced /abandoned	38.27 (243)	16.56 (51)	58.71 (192)		
Having children					
No	61.6 (391)	83.4 (257)	41.0 (134)	$\varkappa^2$=120.872	.000
Yes	38.4 (244)	16.6 (51)	59.0 (193)		
Monthly income					
Did not work	5.67 (36)	5.52 (17)	5.80 (19)	$\varkappa^2$=152.448	.000*
[45]1000-5000 (<12.5 -62.5 **$**)	55.43 (352)	31.82 (98)	77.68 (254)		
5000.1-10000 (62.6-125.0 **$**)	25.5 1(162)	37.66 (116)	14.07 (46)		
>10000 (>125.1 **$**)	13.39 (85)	25.00 (77)	2.45 (08)		
Mean income ±SD	***4658 ± 4438***	***7240± 5043***	***3156.66 ±2892***	***t=12.607, df=633***	***.000****
Range	***(0–31040)***	***(0–31040)***	***(0–21760)***	***(95% CI difference)***	
Last month's Total expenditure					
980-2500 (12.25–31.25 **$**)	14.80 (94)	4.87 (15)	24.16 (79)		
2500.1–5000 (31.26-62.5 **$**)	47.87 (304)	38.96 (120)	56.27 (184)	$\varkappa^2$=106.706	.000*
>5000 (>62.5 **$**)	37.33 (237)	56.17 (173)	19.57 (64)		
Mean exp.± SD	***4432 ± 2172***	***5795±2290***	***3730±1788***	***t=12.704, df=633***	***.000****
Range (min-max)	***(1000–15000)***	***(1400–14500)***	***(1000-15000)***	***(1745–2384)***	
Last month's expenditure on food					
500–2000 (6.25–25.0 **$**)	67.40 (428)	72.40 (223)	62.69 (205)		
2001–3500 (25.1–43.75 **$**)	26.14 (166)	16.88 (52)	34.86 (114)	$\varkappa^2$=38.624	.000*
>3501 (>43.75 **$**)	6.46 (41)	10.72 (33)	2.45 (08)		
Mean ±SD	***2112 ±958***	***2220 ±1060***	***2010±841***	***t=2.767, df=633***	***.006****
Range (min-max)	***(500-7000)***	***(1000-7000)***	***(500-4000)***		
Treatment cost of STIs					
No symptoms	53.9 (342)	58.12 (179)	49.85 (163)		
Self treatment	9.6 (61)	16.88 (52)	24.47 (80)		
5-250 taka	20.8 (132)	18.83 (58)	12.84 (42)		
251-5000 taka	15.7 (100)	6.17 (19)	12.84 (42)	$\varkappa^2$=3.44	.179
Mean Treatment cost± SD	***162.63 ±478***	***140.68±326***	***175.88 ±550***	***t=.748, df=633***	***.455***
Range	***(10-5000)***	***(10-2000)***	***(05-5000)***	***(-127.24–57.24)***	

Legend: Descriptive, cross tab, $\varkappa^2$ test and student's t-test. P<0.05*

[44] Among illiterate CSWs (n=418) 36.36 %(n=152) can sign their own names only.

[45] About 8.0% (51/635) CSWs earned <12.5 $ last month.

Table-2: lifestyle factors of CSWs related to sex business

lifestyle factors	Total CSWs	Hotel-based CSWs	Floating CSWs	Statistical tests	P-value
	n=635 % n	n=308 % n	n =327 % n		
Factors behind profession					
Lower Economy	56.2 (357)	58.12 (179)	54.43 (178)	$\varkappa^2$=.905	.235
Exploited by pimps/others	23.2 (147)	25.97 (80)	20.49 (67)		
Other reasons[46]	20.6 (131)	15.91 (49)	25.08 (82)		
ering into sex trade					
10-17 y	42.68 (271)	50.33 (155)	35.47 (116)	$\varkappa^2$=42.789	.000*
18-24	37.80 (240)	40.58 (125)	35.17 (115)		
≥25 years	20.32 (129	9.09 (28)	29.36 (96)		
Mean age± SD	***19. 79 ± 6.04***	***18. 41 ± 4.10***	***21.09 ± 7.18***	**t= -5.735, df=633**	**.000***
Range	***(10–48 y)***	***(10–37 y)***	***(10–48 y)***	**(-3.601– -1.764)**	
Experience in sex business					
Less than one year	13.54 (86)	24.03 (74)	3.67 (12)	$\varkappa^2$=130.220	.000*
1-5 y	54.02 (343)	63.31 (195)	45.26 (148)		
≥6 y	32.44 (206)	12.66 (39)	51.07 (167)		
Mean year of experience± SD	***5.72 ± 6.50***	***2.71 ± 3.47***	***8.56 ±7.36***	**t= -12.669, df=633**	**.000***
Range	***(<1–38 y)***	***(<1–22 y)***	***(<1 –38 y)***	**(-6.7478–-4.9367)**	
Mass media exposure					
No items	52.60 (334)	26.6 (82)	77.1 (252)	$\varkappa^2$=177.04	.000*
Radio/TV/VCD	31.50 (200)	43.8 (135)	20.2 (66)		
Had all	15.90(100)	29.5 (91)	2.8 (09)		
Working days (last week history)					.002*
Did not work	5.67 (36)	5.52 (17)	5.81 (19)	$\varkappa^2$=24.160	
1–3 days	53.23 (338)	62.99 (194)	44.04 (144)		
4–7 days	41.10 (261)	31.49 (97)	50.15 (164)		
Mean working days ± SD	**3. 72±1.65**	**2.87±1.46**	***3. 65±1.71***	***t= -6.150, d.f=633***	***.000****
Range	**(0–7)**	**(0–7)**	**(0–7)**	***(-1.035–-.534)***	
Monthly absent in work					
1–7 days	83.15 (528)	66.88 (206)	98.47 (322)	$\varkappa^2$=113.536	.000*
8–14 days	9.92 (63)	20.13 (62)	0.31 (01)		
>15 days	6.93 (44)	12.99 (40)	1.22 (04)		
living with					
Own	54.17 (344)	47.08 (145)	60.86 (199)	$\varkappa^2$=40.583	.000*
Husband	30.24 (192)	27.92 (86)	32.42 (106)		
Others[47]	15.59 (99)	25.00 (77)	6.72 (22)		

Legend: Descriptive, cross tab, $\varkappa^2$ test and student's t-test. P<0.01*

[46] Other reasons were -angry with family members (41/635), sold by step mom/others (28/635), raped/tortured (21/635), husband's torture (24/635), self motivation (12/635) and drug addiction (05/635) etc.

[47] Parents (34/635), siblings (30/635), sexual partners (22/635), friends and relatives (13/635) etc.

Table-2 continued: lifestyle factors of CSWs related to sex business

lifestyle factors of CSWs	Total CSWs	Hotel-based CSWs	Floating CSWs	Statistical tests	P-value
	n=635 % n	n=308 % n	n =327 % n		
Cases of abortion					
No abortion cases	72.9 (463)	72.73 (224)	73.09 (239)		
1-2 cases	21.4 (139)	23.70 (73)	20.18 (66)	χ^2=3.94	.139
>3 cases	5.7 (33)	3.57 (11)	6.73 (22)		
Use of contraceptives					
No measure	16.22 (103)	14.94 (46)	17.43 (57)		
Pill	35.11 (223)	51.62 (159)	19.57 (64)	χ^2=75.564	.000
Condom	39.69 (252)	27.60 (85)	51.07 (167)		
Others[48]	8.98 (57)	5.84 (18)	11.93 (39)		
Habit of smoking					
No	55.69 (360)	53.90 (166)	59.33 (194)	χ^2=1.906	.167
Yes	43.31 (275)	46.10 (142)	40.67 (133)		
Substance abuse					
No	68.03 (432)	62.99 (194)	72.78 (238)	χ^2=6.998	.008
Yes	31.97 (203)	37.01 (114)	27.22 (89)		
Substance Types (n=203)					
No drug	68.0 (432)	63.0 (194)	72.7 (238)		
Ganja (cannabis)	11.7 (74)	15.3 (47)	8.3 (27)	χ^2=36.020	.000
Alcohol (local moad)	13.4 (85)	18.8 (58)	8.3 (27)		
Others	6.9 (44)	2.9 (09)	10.7 (35)		
Drug use for fairness					
Yes	4.3 (27)	4.9 (15)	3.7 (12)		
No	95.7(608)	95.1(293)	96.3 (315)	χ^2=1.94	.149
Client profile					
Businessmen (small)	46.30 (294)	60.07 (185)	33.33 (109)		
Rickshaw puller	18.58 (118)	0.97 (03)	35.17 (115)		
Service holder	14.80 (94)	20.46 (63)	9.48 (31)	χ^2=149.552	.000*
Student	7.41 (47)	9.42 (29)	5.51 (18)		
Bus driver	2.99 (19)	0.97 (03)	4.89 (16)		
Don't know	9.92 (63)	8.11 (25)	11.62 (38)		
NGO counseling on STIs					
Yes	48.66 (309)	52.60 (162)	44.95 (147)	χ^2=3.709	.054
No	51.18 (326)	47.40 (146)	55.05 (180)		
Meal skipping (in a day)					
Yes	(62)	1.9 (06)	17.1 (56)	χ^2 =41.46	.000
No	90.2 (573)	98.1 (302)	82.9 (271)		

48 Injection, ligation/tubectomy, menopause

Table-3: Heterosexual Risky Behaviors (HRB) of CSWs related to sex business (n=635)

Risky sexual behaviors of CSWs	Commercial Sex Workers (% n) Total	Hotel-based	Floating	Statistical tests	P-Valu
	100% n=635	48.50%	51.50% n		
Usual no. of clients per day					
1-7	70.24 (446)	46.10 (142)	92.97 (304)	χ^2=166.619	.000*
≥8 clients	29.76 (189)	53.90 (166)	7.03 (23)		
Mean client per day ± SD	***6.18±4.31***	***8.46± 4.53***	***4.04±2.70***	***t=15.010, d.f=633***	***.000****
Range (clients/day)	***(1-30)***	***(1-30)***	***(1–20)***	***(3.834–4.988)***	
Client turnover (last week)					
Did not work	567 (36)	5.53 (17)	5.81 (19)		
1– 14 C	48.03 (305)	30.84 (95)	64.22 (210)	χ^2=75.638	000*
≥15 C	46.30 (294)	63.63 (196)	29.97 (98)		
Mean client last week ± SD	***17.24 ± 14.35***	***2.12±15.63***	***12.65 ± 11.25***	***t=8.80, df 633***	***.000****
Range	***(0-97 clients)***	***(0-97***	***(0-91 clients)***	***(7.355–11.581)***	
Condom use					
No/ irregular use	55.9 (355)	51.30 (158)	60.25 (197)	χ^2=5.149	.023
Consistent use	44.1 (280)	48.70 (150)	39.75 (130)		
Hygienic measure after coitus					
No measure	33.39 (212)	33.11 (102)	33.64 (110)		
Used savlon /soap	45.51(289)	62.34 (192)	29.66 (97)	χ^2=114.915	
Others[@]	21.10 (134)	4.55 (14)	36.70 (120)		.000*
Interval between 2 coitus					
No	67.40 (428)	20.78 (64)	43.73 (143)	χ^2=38.027	.000*
yes	32.60 (207)	79.22 (244)	56.27 (184)		*
Usual coital frequency per client					
One time	62.99 (400)	71.75 (221)	54.74 (179)	χ^2=19.693	.000*
≥2 times	37.01 (235)	28.25 (87)	45.26 (148)		
Weekly coitus with PC					
No weekly PC	59.84 (380)	55.84 (172)	63.61 (208)		
Yes, 1 time/week	25.20 (160)	30.84 (95)	19.88 (65)	χ^2=10.25	.006*
2 times/week	14.96 (95)	13.32 (41)	16.51 (54)		
Fortnightly coitus with PC					
No fortnightly PC	70.39 (447)	58.77 (181)	81.35 (266)		
Yes, 1-2 times	22.52 (143)	27.92 (86)	17.43 (57)	χ^2=51.945	.000*
>3 times	7.09 (45)	13.31 (41)	1.22 (04)		
Monthly coitus with PC					
No monthly PC	60.00 (381)	41.88 (129)	77.06 (252)	χ^2=102.309	.000*
Yes, 1-2 times	21.89 (139)	25.98 (80)	18.05 (59)		
>3 times	18.11 (115)	32.14 (99)	4.89 (16)		
Having NPSP					
No	90.87 (577)	88.31 (272)	93.27 (305)	χ^2=4.702	030*
Yes	9.13 (58)	11.69 (36)	6.73 (22)		
Ever experienced in Gr. sex					
No	74.33 (472)	75.0 (231)	73.70 (241)		
Yes	25.67 (163)	25.0 (77)	26.30 (86)	χ^2=1.40	.708
Gr. Sex history (last month)					
No	78.58 (499)	83.12 (256)	74.31 (243)		
Yes	21.42 (136)	16.88 (52)	25.69 (84)	χ^2=.306	.707
Persons in the last gr. Sex					
No Gr.sex history	78.58 (499)	83.12 (256)	74.31 (243)		
2-5 clients	85.29 (116)	15.58 (48)	20.80 (68)	χ^2=10.428	.005
>6 clients	14.71 (20)	1.30 (04)	4.89 (16)		
Condom used in Gr.sex					
No Gr.sex history	78.58 (499)	83.12 (256)	74.31 (243)	χ^2=8.305	.016*
Yes, used condom	17.17 (109)	14.28 (44)	19.88 (65)		
No , didn't	7.40 (27)	2.60 (08)	5.81 (19)		
Ever experienced in Anal sex					
No	95.59 (607)	93.83 (289)	97.25 (318)	χ^2=4.392	.036
Yes	4.41 (28)	6.17 (19)	2.75 (09)		
Genital discharge (symptom)					
Yes	32.3 (205)	27.6 (85)	36.7 (120)	χ^2=6.008	.014*
No (r)	67.7 (430)	72.4 (223)	63.3 (207)		

Legend: Descriptive, cross tab, χ^2 test, and student's t-test. P<0.05*. Gr.sex=Group sex

NPSP=Non paying sexual partner, Gr.sex=group sex. PC=permanent clients

1.4 Discussion

Socio-economic status of CSWs: Mean age of CSWs (n=635) was 25.55±7.61 y which was close the mean age of CSWs of neighboring country (Altaf et al, 2012; Bibi et al, 2010; Rakhi et al, 2005). And compared to HBCSWs (21.13±4.8), FCSWs had higher mean age (29.7±7.42 y) which was also higher (27.8 y) for street-based CSWs (compared to home-based & brothel) as reported in an Indian study (Rakhi et al, 2006). Studies home (Mondal et al, 2010, 2008; Ullah 2005) and abroad (Rakhi et al, 2006) always pointed at older aged CSWs belonged to street-based or floating group as compared to other categories of CSWs (home-based & brothel-based).

Vast majority (65.83%) of CSWs had no formal education but surprisingly, hotel sex business had higher literate CSWs (51.95%) than illiterate workers (48.05%) but most (82.57%) of the floating CSWs were illiterate and only a few (17.43%) had formal education. Like most studies about sex workers either in home (Mondal et al, 2010; Hossain KJ et al 2010; Mondal et al, 2008; Alam MK; Rahman et al, 2000) or in abroad (Altaf et al, 2012; Rakhi et al, 2006; Rakhi et al, 2005), this study, also elucidated that majority (60-85%) CSWs were illiterate. In contrast, a study in Bangladesh (Nessa et al, 2004) about hotel-based CSWs reported lower (42.0%) illiteracy rate and higher (58.0%) formal education (1-5y=26.0%, 6-12y=30.0%) among CSWs. Furthermore, illiteracy (48.0%) and formal education rate (1-5 y=41.6%, 6-12y=10.4%) of HBCSWs in our study is close to a previous African study (illiteracy=47.2%, 1-5y=36.6%, 6-12y=16.1%) (Wang, 2007) but different from a Tunisian study (Abir Znazen, 2010) about CSWs where no illiteracy was found and >6 years schooling was only 36.7%.

In generic appearance, proportion of married CSWs was the highest (43.46%) category among total CSWs. Highest proportion of married women frequented in sex industry of neighboring countries was also reported (Altaf et al, 2012, Rakhi et al, 2006). Economy is the strong cause for this situation because with increasing poverty and decreasing economic opportunities married women increasingly seeking work outside their households to generate income. And, it is likely that earning potential in sex work for the poor and illiterate women is broader to what they could earn through other types of work (Rakhi et al, 2006).

Although overall percentage of unmarried CSWs was smaller (18.27%) but significantly tiny groups of unmarried (1.53%) and enormous proportion of abandoned (58.71%) were present in floating group. In contrast to this, HBCSW's group possessed highest married category of CSWs (47.40%) but smallest portion of abandoned women (16.56%). Only 1.53% unmarried FCSWs in this study which is very likely to Ullah's comparative study among all types of CSWs namely brothel, hotel and floating (Ullah, 2005) and more unlikely to other studies (Hossain KJ et al, 2010; Rahman et al, 2000; Nessa et al, 2004) as adolescents, and unmarried girls are now booming all over the sex market in Bangladesh.

In fact, proportion of married and unmarried women within HBCSWs group was indifferent (P>0.05) but it was significantly different within female floating CSWs. Moreover, a study about street-based CSWs (Mondal et al, 2008) in Bangladesh showed zero percent unmarried CSWs, 1.3% married (living with husband) and 98.7% W/D/AB (of them 90.7% separated) CSWs. Most of the street-based CSW's studies either in Bangladesh[49] (Mondal et al, 2010 and 2008; Ullah, 2005) or abroad (Rakhi et al, 2006) reported the remarkable presence (more than one third) of W/D/AB women and most of cases being W/D/AB were the main cause of being CSWs like this study. In this study, about 38.27% abandoned women in sex industry which is just 6.3% in ever married women in general population (BDHS, 2011), tells how vulnerable this segment of population is at scale of economy and food security, similar observation was also found in an Indian study (Rakhi et al, 2006). Plausible explanations of this may be, women who are separated/divorced from their husband or are widowed have limited rights, opportunities and economic independence. In addition, if they are illiterate, they are likely to have even fewer labour markets and opportunities other than sex work. Most (276/635) of CWs of this research study were married (43.46%) followed by widow/divorced/abandoned (243/635) and both category had children (43.6%) which indicates illiterate young women (married or abandoned but having children) getting no other ways transform their bodies into commodity of sex industry for livelihoods of them and their children. Marital deprivation backed by poverty was a remarkable contributory factor behind sex trading especially floating sex business in our country. Ullah's comparative study (2005) and other studies (Mondal et al, 2010, 2008; Rakhi et al, 2006)[13] pointed at that significantly (P<0.000) higher percentage of either separated or abandoned women become floating sex workers compared with hotel and brothel sex workers. Sudden break in family bondage put them into economic hardship and initiates into floating sex work as it is easier than choosing other two options.

Monthly average income for both group of CSWs (n=635) was only 4658 taka (58.23 US $) in this study which was close to an Indian study (Rakhi et al, 2005) but far away from a Japanese study (53.8% earned <450 US $ per day, Kinuko Kimoto). Mean 'income' as well as 'expenditure of the last month' was significantly different between two groups of CSWs (income t=12.607, p=.000** & expenditure t=12.704, p=.000**). About 37.66% of the HBCSWs earned 5000-10,000 taka per month (within categories P<0.05) whereas majority (77.68%) of the floating CSWs had monthly income (as well as 56.27% of CSWs' expenditure) within range of 1000-5000 taka which was well agreement with a study conducted by Laboratory Science and Health division of ICDDR,B (2006)[50]. Average monthly income of hotel-based CSWs (HBCSWs) was more than doubled (7240 Taka) than floating CSWs (3156 Taka) (P=.000), this finding is in well agreement with an Indian study (Rakhi et al, 2005) where street-based FSWs had lower income from sex work as compared with the

[49] karim and aziz, Banglapedia; Alam MK, Rainbow Nari O shishu Kallayan Foundation

[50] 'Field evaluation of simple rapid tests in the diagnosis of syphilis'. ICDDR, B. Health and Science Bulletin. Vol. 4 No. 4. December 2006

home and brothel-based FSWs. On the other hand, unlikely to other variables, floating CSWs apparently spent average higher amount (t=.748, P=.455) of money (176 taka) than HBCSWs (141 taka) on treatment of STIs (last ailment).

Mean monthly income (3156 taka) of floating CSWs was very close to another street-based study (Mondal et al, 2010) in Rajshahi where monthly average income of CSWs was only 3000 Tk. Different reports focused on lower income/wages and status of floating CSWs (Mondal et al, 2010; Ullah, 2005) in compare to other types (Nessa et al, 2004; Sarker et al, 1998). A brothel-based CSW's (BBCSWs) socioeconomic and lifestyle study (Hossain KJ et al, 2010) reported that average income and weekly clientele of BBCSWs were 14,460 taka and >40 clients respectively.

Mean treatment cost on STIs (last ailment) for all CSWs (n=635) was only 162.63 BDT which indicates their economic vulnerability and why STIs deep rooted in CSW's community as proper treatment of STIs did not take place ever either for 'low economy' or for 'lack of awareness'. Health condition of CSWs is a major factor for controlling infections or diseases as they have both commercial and non commercial multiple partnerships (including both heterosexual and homosexual). Study (Mondal et al, 2008) observed that floating CSWs who spent money on health purposes had 2.66% syphilis as compared to FCSWs who did not spent on STI treatment (14.66% syphilis) and money spent on health purpose was less likely to infect with STDs (AOR .71, p=.000).

Lifestyle factors: The main 'causal factor' for being CSWs was poverty; in this regard**,** no significant difference (P=.235) was observed between these two groups of CSWs. The universal cause of being a "CSW" was "poverty" was echoed in almost all researches about CSWs in Bangladesh (Mondal et al, 2010; Ullah, 2005)[51] exception is a brothel-based study (Hossain KJ et al,2010) where close to half (47.8%) of CSWs claimed broker/pimps to entice them coming into this profession.

Generally, mean age at starting sex work was 19.79±6 which was slightly lower (18.41±4.10) in case of hotel-based CSWs and higher (21.1±7.18) for floating CSWs (FCSWs). Mean age at starting sex work for FCSWs was also close to an Indian study (Rakhi et al, 2006). It was observed that <18 girls were more likely to join in hotel sex business (50.33%) than floating trade (35.47%). In contrast, ≥18 girls were found abundance in floating sex trade (between 2 groups no difference but within categories P<0.05). HBCSWs possessed a tiny portion of women (n=28) who entered into this profession at >25 years of age which was the smallest group among all age categories of CSWs.

Significant difference was reported between two CSW's groups in the length of profession. In this study mean years of experience was ~6 y for both group (range <1y to 38 y) and only ~3 y for hotel-based CSWs but 8.56 y for floating CSWs. General frequency distribution showed

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[51] karim and aziz, Banglapedia; Alam MK, Rainbow Nari O shishu Kallayan Foundation

that more than half (54%) of CSWs were in this profession for one year to 5 years but in case of HBCSWs this rate was higher (63.31%) and lower (45.26%) in floating. In contrast, length of profession was only <6 months in previous studies about FCSWs in Bangladesh (Rahman et al, 2000; Nessa et al 2004)[16] but consistent with Ulllah's study (2005) which indicated that higher percentage of female floating CSWs have been running this profession for longer period in compare to hotel groups. Free movement and liberty of Floating CSWs compare to other two types predispose them to continue their profession for longer period. However, a study in India (Rakhi et al, 2006) revealed that, the female sex workers (FSWs) engaged in sex work for >5 years were more likely to be non-street-based FSWs –just opposite of this study.

In generic appearance, more than half (52.60%) CSWs did not access to mass media items but in case of floating CSWs it was overwhelmingly high (77%) and opposite (26.6%) for HBCSWs. This appearance indicates comparatively lower economic level of floating CSWs as compare to HBCSWs which also reflected in their monthly income (half of hotel-based CSWs). Also, average working days of floating CSWs (3.65 days) were higher (t=-6.150, P=.000*) than hotel-based sex workers (2.87 days). It is a common phenomenon among all types of CSWs to absent for 1-3 days from work within a week. In our study 5.67% (36/635) did not work for the last week which indicates their inability to work due to tremendous mental and physical stress and also poor physical condition. Thus, floating CSWs worked more days in a week but their income/wages were lower than other group of CSWs[52] (Ullah 2005).

Most of CSWs (72.9%) of both groups (P=.139) had no abortion history and overall condom use rate among both groups was slightly higher (39.69%) than taking pill (35.11%) as a measure of contraception. Significant amount of HBCSWs preferred pill (51.62%) to condom (27.60%) as a birth control measure (P=.000) in Contrast to floating CSWs (pill=19.57%, condom=51.07%). However, prevalence of contraceptive use rate was increased from 56% in 2007 to 61% in 20011 reported among general population (BIDH, 2011).

In general, most (432/635) of CSWs did not take drug. Local alcohol (85/203) and then ganja (74/203) were used most frequently used substances and others (afim, phensedyl, injection 44/203) were used less frequently by them. It was also noticeable that proportion of substance abusers were higher in HBCSWs groups (FFCSWS 238/327 vs. HBCSWs 194/308) than floating ($\aleph^2$=6.99, P=.008) and HBCSWs abused more ($\aleph^2$=36.02, P=.000) ganja (15.3%) and alcohol (18.8%) than floating group [both ganja and alcohol 8.3%]. In this study, overall use of illicit drug through injection was low which was in agreement with a study of neighboring country (Altaf et al, 2012) about CSWs. It is notable that majority (95.7%) of CSWs did not use any drug/medicine for being healthier or fair which is most unlikely for brothel-based sex workers as very young girls in brothel are pushed to take 'Oradexon'

[52] Alam MK, rainbow foundation; Aziz and karim, Banglapedia

(steroid used to be healthier for cow) regularly by sardarni (madam)[53]. Generically, most of the CSW's clients were small businessmen (46.30%, 294/635) but HBCSWs had more than half (60.1%) of businessmen customers in contrast to FFCSWs who had only one third (33.33%) businessmen clients the differences were significant ($\aleph^2$=6.99, P=.000*). On the other hand,

floating CSWs had significantly (P=.000*) more rickshaw puller (35.17%) and less service-holder clients (9.5%) than HBCSWs (rickshaw puller=.97%, service holder=20.46%) which indicates low wages of FFCSWS as compare to hotel-based CSWs. Different report is in agreement of this finding that most of customers of floating CSWs were low income people like small businessmen or rickshaw puller, or factory workers because of their cheapest rate among all categories of sex workers and which ranged from taka 5.00 to 50.00 [54] (Ullah, 2005). NGO activity on STIs was not significantly different (P=0.054) between HBCSWs and FFCSWs.

Risky sexual behaviors: generally, CSWs frequented by 6 (mean) paying customers daily which was 3-fold higher than the mean number of paid clients (2.1±1.2) of our neighboring countries (Altaf et al, 2012; Rakhi et al, 2005). HBCSWs handled almost double clients per day in compare to FFCSWs (t=15.01, P=.000*) which was previously reflected in their monthly income (table-1). Although, average working days (3.65 days) of floating CSWs (table-2) were higher (t=-6.150, P=.000*) than hotel-based sex workers but weekly client turnover were significantly lower (P=.000*) than HBCSWs. Last week 'client turnover history' showed that HBCSWs and FFCSws met an average of 22 ±16 and 12±11 weekly customers respectively. Thus FFCSWs were within the range (12-16/week) reported by Rainbow Nari O Shishu Kallyan Foundation (Alam MK) and HBCSWs were in agreement with a street-based study (21 clients/week) (Rahman et al, 2000) but very inconsistent with an empirical study (mean client 12/day) about all categories of CSWs (Ullah, 2005) and brothel-based study (>40 clients/week) (Hossain KJ et al, 2010). Possible explanation may be, comparatively older age as well as higher length of profession may predispose FFCSWs to physical and mental stress or asymptomatic STIs which ultimately made them slower than other types in catching customers.

In our study, overall 'occasional' or 'never use of condom" rate were 55.9%. Occasional condom use rate was significantly (between group P=.023) higher in FFCSWs (197/327) than HBCSWs (158/3088). This finding was dissimilar with study on street-based CSWs in Dhaka where 63.07% of street-based CSWs used condom regularly (Rahman et al, 2000; Neesa et al, 2004). In contrast, studies held in Rajshahi (Mondal et al 2010) and neighboring country Pakistan (Altaf et al, 2012) reported very low (only 1.3%) consistent condom use (CCU)

[53] www.gurdian.co.uk/society/2010/apr/05/sexworkers-bangladesh-steroid ; janmollerhansen.smugmug.com/Bangladesh/sexworkers

[54] Alam MK, rainbow foundation; Aziz and karim, Banglapedia

among street-based CSWs than brothel-based (68%) and in India (Rakhi et al, 2005) street-based CSWs were 3.5 times less likely to use condom with clients as compared to brothel-based CSWs.

The activities of different NGOs which expand their counseling on safer sex practices as well as training on HIV/AIDS at grass root level in all over the capital city. But the focal point is on the "use of condom appropriately and most consistently" ignoring all problems/odds. In fact, FCSWs did not realize the difference between "occasional (irregular) use of condom" and "regular or consistent use of condom". If an occasional/ irregular condom user used condom with 99 customers but a single sexual course without condom with a STI- positive customer could carry the nasty organism inside the body. The latter case is always riskier than former and should need sustainable counseling. Now focus on former case where CSW answered 'yes' very confidently while encountering the question 'whether she use condom regularly'; from her 'point of view' she made 99 cases successfully and one does not matter but whenever one consider her education, situation, poverty and lots of hatred, harassment around her it was very likely to her. And this is 'the hidden point' where these illiterate as well as vulnerable people based on. In this connection, it is worthy to say for all knowledgeable people that whether CSWs use condom occasionally or totally do not use condom are absolutely indifferent. Everybody, NGOs working with this population at risk should counsel them considering this hidden point/ focal point.

All hetero sexual risky behaviors (HRB) were significantly (all P=.000) different among 2 groups of CSWs except 'group sex', most of the CSWs (74.33%) of both groups were not used to practice this sexual risk behaviors (SRB). However, higher rate of floating CSWs performed "group sex" in a larger group (>6 groups) than HBCSWs (between group P=0.005). Use of condom remarkably hampered during group sex. The risky thing about group sex is CSWs lose control over this in spite of the willingness of using condom as well as it may decrease the possibilities of the use of condom among customers during sexual contacts. Studies of street beggars conducted by Rainbow Nari O Shishu Kallyan Foundation & L.R.B Foundation in mid-2006s at Kamrangir Char, Lalbagh and Polashi in Dhaka city in Bangladesh which showed that 40-45% of homeless beggars (adult male) indulge in multi-partner sex with less than 10% of them reporting condom use. Street Sex Workers are the main sexual partners of them[55].

Significant difference (P<0.05) was observed between two CSW's groups (table-3) in case of interval between 2 coitus and coital frequency with usual and permanent clients (PC). In general, majority (67.40%) of CSWs did not take a break between 2 sexual services given to customers; if customers were available, but 32.60% of them asserted that they used to take a break between 2 customer services. Mostly HBCSWs (79.22%) took a break while FFCSWs used to practice both (56.27% took a break and 43.73% didn't). Same type of activity was

[55] Alam MK, rainbow foundation

also observed in case of 'coital frequency' with normal clients, HBCSWs preferred 1-time coitus with usual clients in compare to floating and this difference was statistically significant ($\varkappa^2$=19.693, P=.000). On the contrary, FFCSWs Performed both types of coitus (1/ 2 times) with usual clients (within category P>0.05). Similar result was also found in case of weekly, fortnightly and monthly "coital frequency with PC". HBCSWs had higher proportion of permanent clients than FFCSWs (all P=.000).

It is well documented from different national and international studies (Gitali et al; Rakhi et al, 2006; Deering et al, 2011; Kimuno kimuto) that CSWs have both commercial (permanent clients or steady clients/same clients) and non-commercial partners (non-paying sexual partners or NPSP with whom did not live and cohabiting partners or husbands or boyfriends or pimps). In this study, 276/635 (43.46%) of CSWs were married but 192/635 (30.24%) lived with husband and only 3.47% (22/635) lived with cohabiting partners. in addition, 58/635 (9.13%) CSWs had NPSP, 31.34% (199/635) had weekly permanent clients (PC), 21.58% (137/635) had fortnightly PCs and 26.61% (169/635) had monthly PCs (table-2, 3). Higher proportion of hotel-based sex workers were engaged in weekly, fortnightly and monthly coitus (P<0.05) with permanent/ regular/same customers than floating CSWs (table-3). Study (Haseen et al, 2012) about youth clients of Hotel-based (HBFSWs) reported that approximately half of them visited female sex workers (FSWs) at least once a month and 25% visited FSWs at least once a week.

In fact, "having permanent client (PC)" "NPSP", "group sex" and "Anal sex" was not a usual phenomenon practiced by all types CSWs. These 'less practiced' habits of CSWs were not uncommon at all. Besides, non paying sexual partner (NPSP) indulged unprotected sex than regular customers as different studies (Gitali et al; Rakhi et al, 2006; Deering et al, 2011; Kimuno kimuto) reported that regular customers were more likely to use condom consistently than NPSP.

It is notable to mention that although the frequency of these risky and unsafe sexual acts (group sex, sex with PC and NPSP and anal sex etc.) performed by FCSWs was not so high, but the main point is that data of these types of sexual acts (e.g. Group sex, NPSP etc.) of CSWs were presented in such a way showed only the performance frequency of CSWs under different categorized variable but whenever we think about the no. of customers met by each CSW under different categorized variables (e.g. 1-5 customers, 2-10 customers etc.) it would be multiplied. For example (table-3), if we consider anal sex performed by floating CSWs, it was only 2.8% (n=9) but when we multiply no. of customers (1-2 customers) with performance frequency of FCSWs (say n=3) it would be actually 6 customers. So, whatever the prevalence (%) of risky sexual acts, it ultimately affects by multiplying the no. of the customers. Moreover, habit of frequent condom use could be hampered more often while performing these risky sexual practices.

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CHAPTER-2

Assessment of Nutritional Status of Commercial Sex Workers

2. Assessment of Nutritional Status of Commercial Sex Workers (CSWs)

Assessment of adult nutritional status is a complex procedure. No single method is available to determine the nutritional status. To have a complete profile nutritional status, it needs to analyze multiple nutrition parameters such as anthropometry, biochemical and dietary indices (Baron, 1999).

2.1 Anthropometric assessment of commercial sex workers (n=635)

2.1.1 Introduction

Compared with western populations, the BMI distribution in East Asian countries is shifted towards lower values, although the prevalence of obesity and overweight has increased in recent years[56]. In South Asian countries, like Bangladesh, the BMI distribution is shifted even further towards low values, especially in rural areas (Rahman et al, 2015; Brandon et al, 2010; Islam MZ et al, 2002, 2003 and 2004).

BMI is a marker or indicator of nutritional status (Doak et al, 2005; Bailey and Ferro-Luzzi, 1995) and low BMI (CED or underweight) may increase susceptibility to a wide range of diseases, both chronic and infectious, thereby increasing mortality risk. Both underweight or chronic energy deficient (BMI<18.5 kg/m^2) and overweight (BMI>25.1-29.9 kg/m^2) or obese (≥30 kg/m^2) tend to die earlier than individuals with intermediate BMI values (Brandon et al, 2010).

Underweight is a term describing a human whose body weight is considered too low to be healthy. Poor nutrition as well as deficit energy consumption in adults can result in underweight. Underweight might be secondary and symptomatic of an underlying disease. Severely underweight individual may have poor physical stamina and a weak immune system, leaving them to open to infection. People who are malnourished raise special concerns, as not only inadequate gross calorie intake, but also intake and absorption of other vital nutrients intake hampered. Being underweight causes increased mortality at rates comparable to that seen in morbidly obese people. The conditions being underweight or overweight can exist in close proximity, such as in the same household (Doak et al, 2005).

Food insecurity is common among the poor households in many developing countries which contributed to nutritional disorder and poor health among the household members. It is found associated with lower macro and micro nutrient intakes. Lower intake of fruits and vegetables and lack of diet diversity which contributes to higher prevalence of underweight and can affect health either directly or indirectly through under nutrition (Rahman et al, 2015; Mohamadpour et al, 2012)

The BMI depends on many socio-economic, demographic and environmental factors like age, education, occupation, household economic status, food habit and sedentary lifestyles (Pryer and Rogers, 2006; Shafique et al, 2007).

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2.1.2 Methods and materials

Of the anthropometric indices, body mass index (BMI) and chronic energy deficiency (CED) is usually used to assess the nutritional status. In other words, underweight (<18.5 kg/m^2) also referred to as 'chronic energy deficiency' or CED (WHO, 2004; James et al, 1988) which is most widely used. BMI or CED is relatively insensitive to 5% weight loss within 30 days or 10% weight loss over 6 months (Baron, 1999). According to WHO[♦], cut off points of International BMI categories (kg/m^2) are 18.5-22.9 kg/m^2 (normal-lower, acceptable), 23.0-24.9 (normal-higher or increasing risk), 25-29.9 (Pre-obese) and ≥30 (obese). In respect to BMI[♦], underweight or CED is categorized into mild underweight or CED-I (17.0-18.4 kg/m^2), moderate underweight or CED-II (16.0-16.9 kg/m^2) and severe underweight or CED-III (<16.0 kg/m^2).

The BMI is a simple index of weight-for-height and an indicator of body composition and BMI of the CSWs were measured according to following formula (WHO, 1995; FAO, 1995; Golden and Golden, 2000)

$$\text{BMI}= \frac{\text{Weight in Kg}}{(\text{Height in meter})^2} \quad (\text{Kg/m}^2)$$

Measurement of body weight and height: Body weight was measured to the nearest 0.5 kg, wearing no shoes with light clothing, on a portable weighing scale. Standing height was measured[57] with a wall-mounted scale to the nearest 0.5 cm, with the head in the Frankfurt horizontal plane, while standing straight on a horizontal surface with heels together, the shoulders relaxed, arms at the sides and without shoes. Height and weight were used to calculate Quetelet index or Body Mass Index (BMI=weight (kg)/height (m^2). The classifications of BMI applied in this study were recommended by the World Health Organization[1] (WHO, 2004). BMI values of <18.5 kg/m^2 and >25 kg/m^2 represented thinness and overweight, respectively.

2.1.2.1 Data Analysis

Normality test: various statistical tools were used employing SPSS for the analysis of BMI data. Before performing analysis "Normality" test of the BMI data set was done by Kolmogorov-Smirnov (K-S) goodness of fit test (statistics=.096, df=635, P=.000) as well as by descriptive statistics. It was observed from the descriptive statistics that, BMI data were positively skewed (skewness=1.611, SE=.097) and both 'logarithmic'/Log_{10} (skewness=.676, SE=.097) and 'Square root transformation' (skewness=1.09, SE=.097) was performed to see which process could eliminate the skewness in a better way. And thus, approximation to a normal distribution

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♦ WHO, Lancet, 2004; James et al, 1988

[57] Tanner J, Hiemaux J, Jarman S. Growth and Physique studies. In: Weiner J, Lourie J, eds. Human biology: a guide to field methods. IBP Handbook No 9. Oxford.

was greatly improved by Log_{10} transformation. After Log_{10} transformation, mean as well as other parametric tests on the transformed scale data was performed and then back transformed by taking the "antilog" and at the same time antilog values were backed in the usual BMI unit (kg/m^2). However, the log transformed 'geometric mean' was always less than the mean of raw data (Bland and Altman, 1996). In fact, extreme observations of a "highly skewed data set" have a large influence on the arithmetic mean, making it more prone to sampling error. Thus, lessening this influence is one advantage of using transformed data (Bland and Altman, 1996).

Outcome variables: the unit of analysis of the study is both 'obesity' and 'underweight'. Both 'obesity' and 'underweight' was measured from BMI. Anthropometric measurement weight and height was used to calculate BMI.

Explanatory variables: those IVs or Independent variables (regarding socio-economic, lifestyle, risky sexual behaviors) had significant influence on BMI of CSWs (both groups) from ANOVA (table-2,3,4) as well as from unadjusted multiple logistic regression model (table-5) all were included in the final multiple logistic regression (ML_oR) model. Nine explanatory variables with categories (shown in parenthesis) were 'category of CSWs' (hotel, 0; floating, 1), 'present age in year' (≤18, 1; 9-29, 2; ≥30, 3), 'marital status' (unmarried,1; married,2; widowed/divorced/abandoned, 3), 'having children' (no, 0; yes,1; unmarried, 2), 'mass media exposure' (none, 0; TV/radio/VCD,1; having all, 2), 'drug habit' (no, 0; regularly, 1), 'monthly absent from sex work' (1-7 days, 1; ≥8 days,2), 'last month's total expenditure' (100-2500 BDT, 1; 2501-5000, 2; >5000; 3), and 'last month's income' (00-2500 BDT,1; 2501-5000, 2; >5000,3).

2.1.2.1a Statistical analysis

All statistical analyses of Anthropometric data (height, weight and BMI) were assessed by using Statistical Package for Social Science version 17.0 (SPSS Inc, Chicago, IL, USA). Two-tailed P values <0.05 were considered statistically significant.

Univariate analysis of the samples**:** Descriptive statistics [Proportions, means (95% CI) and ranges] were used to calculate baseline information. Descriptive data were presented as the mean (SD) for continuous variables and as percentages for categorical variables. Comparisons between continuous variables were performed by Student's t-test.

Bivariate analysis: For participant characteristics analysis, mean differences in BMI levels (dependent variables) were examined in respect to socio-demographic, lifestyle and sexual variables (independent categorical variables) using ANOVA. Also, in **bivariate analysis,** simple logistic regression (SLoR) was firstly used to find the association between independent variables (background characteristics, lifestyle factors and risky behaviors of CSWs) and dependent variables BMI (either CED or Overweight).

Multivariate analysis*:* unadjusted odds ratios erroneously suggested a moderate association between exposure and risk of disease, control for confounding variables is therefore essential (Hermann and Maria, 1997). If the adjusted odds ratio differed from the crude odds ratio by >10%, there was confounding by one or both of the two independent variables[58]. The complementary proportion of bias not removed by imperfect control for confounding is commonly called "residual confounding" (Hermann and Maria, 1997). Most commonly used strategy, inclusion of confounders as categorical variables in multiple regression models. In theory, control for confounding with categorized variables should be increasingly effective if an increasing number of categories are employed. However, inclusions of too many categories decrease precision, particularly if several covariates have to be considered simultaneously, which is the rule rather than the exception in epidemiologic research (Hermann and Maria, 1997). MLoR analysis can be used to account for confounding. The model can be extended to account for several confounding variables simultaneously. Multiple logistic regressions (MLoR) analysis can also be used to assess confounding and effect modification, and the approaches are identical to those used in multiple linear regression analysis. MLoR analysis can also be used to examine the impact of multiple risk factors (as opposed to focusing on a single risk factor) on a dichotomous outcome (Hosmer and Lemeshow, 1989).[59]

In this study, "**multivariate analysis**" was performed, two models of BMI addressing both CED/under nutrition and overweight/obesity were prepared separately to identify the associated factors of overweight/obesity as well as of CED/underweight among CSWs. For this reason, BMI was made a binary response (0=normal BMI; 1=either CED or overweight) in both MLoR models (Rahman et al, 2015). In the MLoR model, dummy variables were created for non-binary categorical variables and the effect of each category of a multi-categorical variable was assessed by keeping the first or last category as reference category. All Covariates (socio-demographic, lifestyle and sexual variables as well nutritional indices) which were found statistically significant ($P \leq 0.05$) or borderline significant ($P<0.09$) in Simple logistic regression (SLoR) analysis were introduced simultaneously in the **multiple logistic regression analysis.** The model was developed by 'backward stepwise elimination', removing the covariate with largest P value at each step until the remaining variables were significant at the 0.05 level in the final adjusted model. Also, 'forward stepwise technique' was applied to confirm the model. Thus relative odds (ORs) and 95% CI were calculated to identify potential risk factors associated with 'CED or under nutrition' and 'overweight/obesity' [0=normal and 1=lower/higher]. Hosmer-Lemeshow goodness of fit and Nagelkarke-pseudo R^2 of the models were also observed. The Statistical Package for Social Science version 17.0 (SPSS Inc, Chicago, IL, USA) was used for statistical analysis.

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[58] Wayne, Boston University. http://sphweb.bumc.bu.edu/otlt/MPH-Modules/EP/EP713_Regression....accessed 7/9/2015

[59] (http://sphweb.bumc.bu.edu/otlt/MPH-Modules/BS/BS704).

"Multiple Logistic Regressions" analysis

Equation of the multivariate regression is: $Yi= b_0+b_1x_1+ b_2 x_2$ - - ----bixi

Here, y=dependent variable, b_0 =intercept, x_1= 1st independent variable (IV), x_2 =2nd IV, b_1=slope with y axis.

The 'Multiple Logistic regression' is a bit advance from 'Multivariate regression equation'. The **logistic regression** function can be written as:

$$E\ (Y/Z=z) = \frac{ez}{1+ez} \text{ where, } z=\beta0+\beta1x1+\beta2x2+\beta3x3+\beta4x4+\beta5x5$$

These are regression parameters, and β_0, β_1, β2, β_3, β4, β_5 are unknown constants to be determined from the data.

2.1.2.1b BMI model focusing on overweight/ Obesity of CSWs

In this study, 'Multiple logistic regressions' analysis was performed in order to observe the effects of different independent variables (X) on the dependent variable (Y). Here, in both SLoR and MLoR analysis, overweight/obesity [Y_i = (0, normal BMI; 1, ≥25 BMI)] was treated as the dependable variable. In this model, **outcome variable** is classified in the following way:

$$Y_i = \begin{cases} 1: \text{if the respondent was overweight/obese } (BMI \geq 25.0\ kg/m^2) \\ 0: \text{normal BMI } (18.5\text{-}24.9\ kg/m^2) \end{cases}$$

And for the explanatory variables

$$X_1 = age = \begin{cases} 1: \text{if the age of respondent} < 18 \\ 0: \text{otherwise} \end{cases}$$

$$X_2 = expenditure = \begin{cases} 1: \text{if monthly expenditure} > 5000 \text{ taka} \\ 0: \text{otherwise} \end{cases}$$

$$X_2 = drug\ habit = \begin{cases} 1: \text{if yes} \\ 0: \text{No} \end{cases}$$

Thus *X_3, X_4 and X_5 explanatory variables were incorporated into the model.*

2.1.2.1c BMI model focusing on chronic energy deficiency (CED)/underweight of CSWs

Secondly, in both SLoR and MLoR model, CED/underweight [Y_i = (0, normal BMI; 1, ≤18.5 BMI)] was treated as the dependable variable. In this model, the dependent variable is classified in the following way:

$$Y_i = \begin{cases} 1: \text{if the respondent was CED/underweight } (BMI \leq 18.5\ kg/m^2) \\ 0: \text{normal BMI } (18.5\text{-}24.9\ kg/m^2) \end{cases}$$

And for the explanatory variables

$$X_1 = \text{Category of CSWs} = \begin{cases} 1: \text{if floating CSWs} \\ 0: \text{Hotel-based CSWs} \end{cases}$$

$$X_2 = \text{Mass media exposure} = \begin{cases} 1: \text{if had Tv/radio/VCD} \\ 0: \text{otherwise} \end{cases}$$

In adjusted MLoR model, 9 explanatory variables (*X, i=1, 2, 3……., 9), viz., category of CSWs* (x_1) *CSW's present age in year* (x_2)*, Marital status* (x_3)*, having children* (x_4)*, mass media exposure* (x_5)*, drug habit* (x_6)*, monthly absent from sex work* (x_7)*, last month's total expenditure* (x_8)*, last month's income* (x_9) *etc. were* entered into the model and "backward stepwise MLoR" procedure was employed. Also, for clarification "forward stepwise MLoR" was applied to the all explanatory variables in the models separately for 2 models (obese and CED). The results of the MLoR models were represented by odds ratio (OR) with 95% confidence interval (CI) for easy understanding of the effects of the associated factors.

2.1.3 Results

Mean height and weight of the CSWs (both groups) was 150 cm (95% CI 149.93-152.07) and 48.3 kg (95% CI 47.38-48.88) respectively. Mean height and weight of these 2 groups of CSWs were significantly different so as BMI (all P<0.000). It was also postulated (table-1) that among 635 CSWs more than half of CSWs (n=387) had normal BMI but about 22.7% (n=144) and 16.3% (n=104) were undernourished (CED) and overweight respectively. Furthermore, HBCSWs had less undernourished and more overweight/obese participants while female FCSWs had more undernourished and less overweight/obese participants. In addition, hotel-based (n=308) CSWs had significantly [F (1,633) =21.01, P=.000**] higher BMI (21.88±1.20) than floating (n=327) CSWs (20.42 ±1.18) (table-1).

Influence of 'socio-economic variables' on BMI of CSWs (ANOVA, table-2) depicted that 'present age' [HBCSWs: F (2,305) =15.9494] and 'having children' [HBCSWs: F (2,305) =8.734] had respectively significant positive and negative influence on the BMI of both types of CSWs (both P=.000). However, greater influence was observed on hotel groups than floating. Marital status (P=.001) had influenced only on hotel-based CSWs and 'mass media exposure' (P=.000), 'monthly income' (P=.012), 'expenditure' (P=.000) and 'STI status' (P=.002) influenced only on floating group.

Influence of 'lifestyle variables' on BMI of CSWs (ANOVA, table-3) showed that 'entering age' of CSWs influenced both types of CSWs and 'monthly absent' from work influenced only hotel groups. However, 'causes behind profession' (P=.026), 'working days in a week' (P=.012), 'experiences in sex business' (P=.000), 'use of contraceptives' (P=.013), 'habit of smoking' (P=.036), 'substance abuse' (P=.002) profoundly influenced BMI of floating group only. No influence of HRB of CSWs on BMI observed (table-4).

2.1.3a BMI model focusing on overweight/ Obesity of CSWs

Critical analysis of BMI (overweight/obese) model-1: before adjustment all 9 unadjusted variables (IVs) were entered together into MLoR model-1 by" backward stepwise" MLoR method and at the 6th step it was "stabled" and adjusted with 5 variables (namely 'present age', UOR=4.17 & 12.67, P=.001; 'monthly expenditure', UOR=.418, P=.038; 'category of CSWs', UOR=1.793, P=.075; 'drug habit' UOR=1.887, P=.019 and 'monthly absent from sex works', UOR=1.99, P=.031) by leaving variable 'marital status' at 2nd step, 'mass media exposure' at 3rd step, 'having children' at 4th step and 'monthly income' at the 5th step. Here "present age (3 categories)" and "category of CSWs (hotel or floating)" were potential mediators which was proved whenever "category of CSWs (question 10)" was removed from the model, odds ratio of all other variables (monthly expenditure, UOR=.359, P=.013; monthly absent from sex works, UOR=2.507, P=.002; Present age, UOR=3.72 & 9.027, P=.002) except "drug habit" (difference 3.4%) differed from the main model by 10.79%-28.60% (data not shown). Furthermore, Hosmer-Lemeshaw goodness of fit (χ^2 =7.99, df=8, P=.435) of BMI (overweight/obese) model, -2LL (449.99), Nagelkerke pseudo R-square (.153), model coefficient (51.07, df=6. P=.000) and overall predicted percentage of DV (78.8%) was also decreased from the main model (table-6). In fact, these 2 variables mediated mainly present age (19-29 y groups by 10.79% and ≥30 by 28.60%) and 'monthly absent from sex works'(≥8 days by 25.0%).

In addition, when both "category of CSWs (question 10)" and "present age" was removed from the model variables loosed consistency and previous 2 variables (significant at table-5) took place in the model ('monthly income' (OR=2.099, P=.010) and 'having children' (OR=2.674, P=.007)) and surprisingly 'category of CSWs' was removed from the model and odds ratio of other 'existing variables' (monthly expenditure, OR=.373, P=.018; monthly absent from sex works, OR=2.241, P=.005) except "drug habit" (OR=1.794, P=.029 difference by 4.92%) differed from the main model by 10.79% to 28.60% (all data not shown). Furthermore, Hosmer-Lemeshaw goodness of fit ($\aleph^2$ =9.59, df=8, P=.295) of BMI (overweight/obese) model, -2LL (464.8), Nagelkerke pseudo R-square (.114), model coefficient (37.57, df=8. P=.000) and overall predicted percentage of DV (79%) was also decreased from the main model (table-6). In fact, these 2 variables mediated mainly monthly expenditure (category 1000-2500 BDT by 10.77%) and 'monthly absent from sex works' (≥8 days by 12.6%).

Among 9 explanatory variables (from "unadjusted model"), only 5 variables namely 'present age', 'last month's expenditure', 'category of CSWs', 'drug habit' and 'monthly absent from sex work' etc. were significantly remained in the "adjusted model" of BMI (focused on overweight). Variables like 'marital status', 'having children' and 'last month's income' which were significant earlier in "unadjusted model" (table-5) were no longer remained significant and ultimately removed from the model by "backward stepwise MLoR" analysis (table-6). Also, variable named "mass media exposure" which was not significant for "obese model" was removed from the model at the first time. On the other hand, "category of CSWs" which was not significant earlier in both "unadjusted models" (of obesity and CED), was remained significant in both of the "adjusted models" was also a "strong confounder" (which influences 'exposures' in 'both directions' and influences the 'outcome' in "one way").

With regard to BMI model-1 (obese model), there were statistically significant differences (P=.001) within age categories of CSWs and older CSWs more likely to develop obesity. CSWs who were within 19-29 age range and ≥30 of age had respectively 4.17 and 12.67 times higher odds ratio of developing obesity as compared to <18 of age category (both P=.001), other socio-economic, lifestyle and risky behaviors remained constant. In short, as age increases BMI also increases positively (3-fold). Thus, being highly significant in unadjusted model (table-5), odds ratio of "present age of CSWs" (UOR=2.98 & 5.186, P=.007) increased and still remained significant (AOR=1.793, P=.055) in the adjusted model (table-6).

The odds of being 'obese' among CSWs were 54%-58% less likely who spent <5000 BDT last month as compared to CSWs who spent >5000 BDT last month. Hotel-based CSWs were nearly 2 times more likely to develop obesity than floating CSWs adjusted for other variables those could influence commercial sex works.

The odds of being obese among CSWs were 1.9 times higher in 'non-drug' abuser as compared to 'drug addicts', adjusted for other variables including age. CSWs who did not take drug regularly became obese as compare to drug addict CSWs. Here "illegal drug habit" acted as a barrier to obtain higher BMI level (>18.5) than normal.

'Monthly absent from work' was also an important predictor of overweight. It was noticed that CSWs who were ≥8 days absent from sex work in a month had almost double risk of being obese [AOR 1.99 (2.064-3.717), P=.031] (table-6).

2.1.3b BMI model focusing on chronic energy deficiency of CSWs

Critical analysis of BMI model-2: before adjustment all 9 unadjusted variables (IVs) were entered together into MLoR model-2 by" backward stepwise" MLoR method and at the 8th step it was "stabled" and adjusted with only 2 variables (namely ''category of CSWs', OR=.587, P=.026 and 'mass media exposure' OR=2.047, P=.055) by leaving variable 'monthly income' at the 2nd step 'marital status' at 3rd step, 'monthly expenditure' at the 4th step, 'present age' at 5th step, drug habit at 6th step, and 'having children' at 7th step. Here being highly significant in unadjusted model (table-5: OR=.439, P=.000) odds ratio of the "category of CSWs" increased and remained significant (OR=.587, P=.026) in the main model (difference from previous by 33.7%) adjusted for mess media exposure. In addition "category of CSWs (hotel or floating)" was a strong mediator as when it removed from the model only one 'mass media exposure' (OR=2.565, P=.006) remained in the model and odds ratio increased by 25.03% from main model.

With regard to CED (model-2) of CSWs, there remained 2 variables one was "category of CSWs" and other was having "mass media exposure". HBCSWs were 56% less likely [AOR=.439 95% CI (.294-.656), p=.000] to develop CED than FCSWs and this is the main influential factor to develop CED among CSWs (Just opposite result of overweight/obese model-1). It was observed that those CSWs did not have any mass media exposure were 2 times more likely to be chronic energy deficient or undernourished as compared to CSWs who had access to radio/TV/VCD [AOR 95% CI (.984-4.257), p=.055].

Table-1: Anthropometric indices of two types of commercial sex workers (CSWs)

	Anthropometric Parameters#	Total CSWs N=635	Hotel-based CSWs n=308	Floating CSWs n=327	Mean difference (95% CI)	p-value
Categories of Height (cm)	105.50-145.0	22.52 (143)	18.2 (56)	26.6 (87)		
	145.1-155.0	57.95 (368)	57.8 (178)	58.1 (190)		
	155.1-165	18.43 (117)	21.8 (67)	15.3 (50)		
	>165.1	1.10 (07)	2.3 (7)	0.0 (0.0)		
Mean height	**G. Mean ± SD**	150.0±1.07	151.02 ± 1.61	149.15 ± 1.82	Mean Difference=.02 95% CI (.01-.02)	.000*
	G. Mean (95% CI)	(149.93–152.07)	(150.0.–152.0)	(148.51–149.78)		
	Range (min-max)	(106-178 cm)	(106-178 cm)	(132-163 cm)	t=3.506, df=633	
Categories of Weight (Kg)	29-45	46.30 (294)	37.7 (116)	54.4 (178)		
	45.1-55	34.96 (222)	39.0 (120)	31.2 (102)		
	55.1-65	14.02 (89)	16.6 (51)	11.6 (38)		
	>65.1	4.72 (30)	6.8 (21)	2.8 (9)		
Mean weight	**G. Mean ± SD**	48.13±1.65	50.31 ± 1.93	46.08 ± 1.91	Mean Difference=4.2 95% CI (2.76–5.7)	.000*
	G. Mean (95% CI)	(47.38–48.88)	(49.20–51.42)	(45.11–47.05)		
	Range (min-max)	(29–94 kg)	(33-94 kg)	(29-90 Kg)	t=5.652, df=633	
Categories of BMI (Kg/m^2)	13.5–16.0 CED III	4.1 (26)	2.3 (7)	5.8 (19)		
	16.1–16.9 CED II	4.6 (29)	2.9 (9)	6.1 (20)		
	17.0–18.4 CED I	14.0 (89)	10.1 (31)	17.7 (58)		
13.5 to 18.4	**Total Under-nutrition**	**22.7 (144)**	**15.3 (47)**	**29.6 (97)**		
18.5–25.0	***#Normal status***	***60.9 (387)***	***65.9 (203)***	***56.3 (184)***		
25.1–29.9	Overweight	13.2 (84)	14.3 (44)	12.2 (40)		
≥30	Obese	3.1 (20)	4.5 (14)	1.8 (06)		
25.1 to onward	**Total Imbalance-nutrition**	**16.3 (104)**	**18.8 (58)**	**14.0 (46)**		
Mean BMI (Kg/m^2)	**G. Mean ± SD**	20.89±1.20	21.88 ± 1.20	20.42 ± 1.19	Mean Difference=1.07 95% CI (1.04-1.1)	.000*
	G. Mean (95% CI)	(20.89–21.4)	(21.38–22.39)	(19.95–20.89)		
	Range (min-max)	(13.45 –51.3)	(4.13-51.2)	(13.51-37.2)	t=4.585 , df=633	

*P<0.000=highly significant. #Anthropometric data were log-normally distributed. 'Normal' BMI was not categorized as 18.5-23.00 because most of CSWs were in low socio-economic status; moreover it mentioned "increasing risk" and also the reason for not to increase no. of category of variable (BMI) as it could decrease cell value in statistical analysis. However, overweight (higher risk) and obese categories were maintained (WHO, Lancet, 2004).

Table-2: Influence of socio-economic variables on BMI of two types CSWs

Socio-economic	All CSWs	Mean BMI of Female Hotel based CSWs (HBCSWs)			Mean BMI of Female Floating CSWs (FFCSWs)		
	n=635	n=308	Mean BMI	ANOVA	n=327	Mean BMI	ANOVA
Present age (y)							
≤18	20.32 (129)	47.40 (114)	**20.41±1.18[t]**	**F (2,305)=15.49**	47.40 (15)	**18.62±1.10[t]**	**F (2,324)=5.436**
19–29	48.66 (309)	36.04 (168)	**21.88±1.18[t]**	**P=.000****	36.04 (141)	**19.95±1.15**	**P=.000****
≥30	31.03 (197)	16.56 (26)	**25.12±1.29[t]**		16.56 (171)	**20.89±1.02[t]**	
Education							
No literacy/can sign[60]	65.83 (418)	48.05 (148)	22.25±1.71	F (2,305)=.372 P=.690	82.57 (270)	20.68±1.77	F (2,324)=.162 P=.851
1-5 class	28.35 (180)	41.56 (128)	22.04±1.96		15.90 (52)	20.82±1.42	
6-12 class	5.83 (37)	10.39 (32)	21.53±1.05		1.53 (05)	19.84±1.78	
Marital status							
Married	43.46 (276)	47.40 (146)	**23.00±1.13[t]**	**F (2,305)=7.222**	39.76 (130)	21.06± 1.17	F (2,324)=1.464
Unmarried	18.27 (116)	36.04 (111)	**20.98±1.17[t]**	**P=.001***	1.53 (05)	18.97±1.33	P=.233
Widow/divorced /abandoned	38.27 (243)	16.56 (51)	**21.87±1.22**		58.71 (192)	20.49±1.36	
Having children							
No	43.30 (275)	47.40 (257)	**23.44±1.23**	**F (2,305)=8.73**	39.45 (129)	**20.89±1.20[t]**	**F (2,324)=3.536**
Yes	38.43 (244)	16.56 (51)	**20.89±1.18[t]**	**P=.000***	59.02 (193)	**19.95±1.18[t]**	**P=.030**
Unmarried	18.27 (116)	36.04 (111)	**21.88±1.20**		1.53 (05)	**19.06±1.15**	
Mass media exposure							
None	52.60 (334)	26.62 (82)	21.38±1.20	F (2,305)=.222	77.06 (252)	**19.95±1.18[t]**	**F (2,324)=9.286**
TV/radio/VCD	31.65 (201)	43.83 (135)	21.88±1.20	P=.802	20.18 (66)	**21.88±1.20**	**P=.000**
Having all	15.75 (100)	29.55 (91)	21.88±1.20		2.76 (09)	**21.38±1.20**	
Monthly income							
[61]<12.5 -62.5 **$**	55.43 (388)	31.82 (98)	22.05±1.15	F (2,305)=.217	77.68 (254)	**19.66±1.00**	**F (2,324)=3.730**
62.6-125.0 **$**	25.5 1(162)	37.66 (116)	22.25±1.04	P=.884	14.07 (46)	**19.78±1.59**	**P=.012**
>125.1 **$**	13.39 (85)	25.00 (77)	21.79±1.59		2.45 (08)	**20.74±1.75**	
Last month's Total expenditure							
1000–2500	14.80 (94)	14.80 (15)	20.57±1.95	F (2,305)=1.175	14.80 (79)	**20.10± 1.51**	**F (2,324)=7.947**
2501–5000	47.87 (304)	47.87 (120)	21.86±1.54	P=.310	47.87 (184)	**20.39±1.33**	**P=.000****
>5001	37.33 (237)	37.33 (173)	22.26±1.31		37.33 (64)	**22.30±1.50[t]**	
Last month's expenditure On food							
500–2000	67.40 (428)	72.40 (223)	21.74±1.22	F (2,305)=2.664	62.69 (205)	20.47±1.71	F (2,324)=1.001
2001–3500	26.14 (166)	16.88 (52)	22.72±1.76	P=.071	34.86 (114)	21.07±1.76	P=.369
>3501	6.46 (41)	10.72 (33)	23.15±1.00		2.45 (08)	21.02±1.54	
#STI status (n=495)		**(n=178)**			**(n=317)**		
STI (HBV/syphilis/both)	43.6 (216)	(40)	21.70±1.01	F (1,176)=.029 P=.864	(176)	**19.95±1.18**	**F (1,315)=9.524**
No STI	56.4 (279)	(138)	21.58±1.29		(141)	**20.89±1.20**	**P=.002**
Treatment cost of STIs							
No symptoms	53.86 (342)	58.12 (179)	22.23±1.26	F (3,304)=1.263	49.85 (163)	20.76±1.62	F (3,323)=.160
Self treatment	9.61 (61)	16.88 (52)	21.35±1.67	P=.287	24.47 (80)	20.71±1.00	P=.923
5-250 taka	20.78 (132)	18.83 (58)	21.84±1.38	P (THV)=.029	12.84 (42)	20.76±1.72	
251-5000 taka	15.75 (100)	6.17 (19)	23.46±1.54		12.84 (42)	20.33±1.51	

Having #STI means either syphilis or hepatitis B or both

[60] Among illiterate CSWs (418) 36.36 %(152) can sign their own names only.

[61] About 8.0% (51/635) CSWs earned <12.5 $ last month.

Table-3: Influence of lifestyle variables on BMI of CSWs

	All CSWs =635	Mean BMI n=308	Female Hotel based CSWs Mean BMI	ANOVA	Mean BMI (FFCSWs) n=327	Female Floating CSWs Mean BMI	ANOVA
Causes behind profession							
Lower Economy	56.2 (357)	58.12 (179)	22.47±1.43	F (2,305)=.372 P=.690	54.43 (178)	**20.31±1.44**	**F (2,324)=3.697 P=.026**
Exploited by pimps/other	23.2 (147)	25.97 (80)	21.24±1.19		20.49 (67)	**20.57±1.38**	
Other reasons*	20.6 (131)	15.91 (49)	22.04±1.14		25.08 (82)	**21.63±1.33**	
Working days (last week)							
Did not work	5.67 (36)	5.52 (17)	22.47±1.65	F (2,305)=.372	5.81 (19)	**22.90±1.18[t]**	**F (2,324)=4.526**
1–3 days	53.23 (338)	62.99 (194)	22.04±1.86	P=.690	44.04 (144)	**20.84±1.75**	**P=.012**
4–7 days	41.10 (261)	31.49 (97)	22.11±1.16		50.15 (164)	**21.30±1.52**	
Monthly absent							
1–7 days	83.15 (528)	66.88 (206)	**21.38±1.20**	**F (1,306)=4.045**	98.47 (322)	20.42±1.18	F (2,324)=.006
8–14 days	9.92 (107)	20.13 (102)	**22.39±1.23**	**P=.045**	0.31 (05)	20.42±1.17	P=.938
Age entering into sex business							
10-17 y	42.68 (271)	50.33 (155)	**21.28±1.10**	**F(2,305)=12.676**	35.47 (116)	**21.43±1.17[t]**	**F (2,324)=3.618**
18-24	37.80 (240)	40.58 (125)	**22.31±1.19**	**P=.000***	35.17 (115)	**20.24±1.17**	**P=.028**
≥25 years	20.32 (129	9.09 (28)	**25.55±1.14[t]**		29.36 (96)	**20.34±1.20**	
Experience in sex business (years)							
<1 y	13.54 (86)	24.03 (74)	21.54±1.38	F (2,305)=2.202	3.67 (12)	**20.53±1.37**	**F(2,324)=10.030**
1-5 y	54.02 (343)	63.31 (195)	22.04±1.18	P=.112	45.26 (148)	**19.73±1.09**	**P=.000***
≥6 y	32.44 (206)	12.66 (39)	23.33±1.91		51.07 (167)	**21.55±1.07[t]**	
living with							
Own	54.17 (344)	47.08 (145)	22.30±1.86	F (2,305)=.710	60.86 (199)	**20.44±1.61**	**F (2,324)=1.159**
Husband	30.24 (192)	27.92 (86)	22.18±1.49	P=.492	32.42 (106)	**21.07±1.06**	**P=.315**
Others♣	15.59 (99)	25.00 (77)	21.58±1.00		6.72 (22)	**21.13±1.48**	
Contraceptives use							
No measure	16.22 (103)	14.94 (46)	22.04±1.80	F (2,632)=.118	17.43 (57)	**20.39±1.11**	**F (2,324)=3.643**
OCP	35.11 (223)	51.62 (159)	22.06±1.61	P=.949	19.57 (64)	**21.69±1.85**	**P=.013**
Condom	39.69 (252)	27.60 (85)	22.26±1.17		51.07 (167)	**21.38±1.40**	
Others♦	8.98 (57)	5.84 (18)	21.63±1.17		11.93 (39)	**22.48±1.70[t]**	
Cases of abortion							
No abortion cases	72.9 (463)	72.73 (224)	21.86±1.22	F (2,305)=1.582	73.09 (239)	**20.48±1.17**	**F (2,324)=7.383**
1-2 cases	21.4 (139)	23.70 (73)	22.49±1.78	P=.267	20.18 (66)	**20.49±1.11**	**P=.001**
>3 cases	5.7 (33)	3.57 (11)	23.91±1.36		6.73 (22)	**23.57±1.12**	
Habit of smoking				F (1,306)=.711			
No	55.69 (360)	53.90 (166)	22.28±1.89	P=.400	59.33 (194)	**21.05±1.19**	**F (1,325)=4.436**
Yes	43.31 (275)	46.10 (142)	21.86±1.60		40.67 (133)	**20.17±1.16**	**P=.036**
Substance abuse							
No	68.03 (432)	62.99 (194)	22.27±1.69	F (1,306)=2.225	72.78 (238)	**21.07±1.16**	**F (1,325)=9.505**
Yes	31.97 (203)	37.01 (114)	21.77±1.66	P=.269	27.22 (89)	**19.67±1.17**	**P=.002**
Types of substance (n=203)							
Ganja (cannabis)	36.46 (74)	41.22 (47)	21.37±1.12	F (2,305)=.810	30.34 (27)	**20.16±1.11**	**F (2,324)=4.125**
Alcohol (local)	41.87 (85)	50.89 (58)	22.24±1.13	P=.489	30.34 (27)	**18.86±1.13**	**P=.007**
Others	21.67(44)	7.89 (09)	20.86±1.16		39.32 (35)	**20.24±1.05**	
Client profile							
Small Business	46.30 (294)	60.07 (185)	22.76±1.57	F (5,302)=2.928	33.33 (109)	20.81±1.22	F (2,324)=.576
Rickshaw puller	18.58 (118)	0.97 (03)	27.46±1.86	P=.021	35.17 (115)	20.29±1.30	P=.718
Service holder	14.80 (94)	20.46 (63)	21.61±1.73		9.48 (31)	20.81±1.06	
Student	7.41 (47)	9.42 (29)	22.76±1.57		5.51 (18)	21.63±1.34	
Bus driver	2.99 (19)	0.97 (03)	27.46±1.44		4.89 (16)	20.87±1.23	
Don't know	9.92 (63)	8.11 (25)	21.25±1.47		11.62 (38)	20.96±1.69	
NGO counseling on STIs							
Yes	48.66 (309)	52.60 (162)	21.95±1.79	F (1,306)=.316	44.95 (147)	20.74±1.69	F (1,325)=.040
No	51.18 (326)	47.40 (146)	22.23±1.89	P=.574	55.05 (180)	20.65±1.72	P=.842

OCP=Oral contraceptive pills. *Other reasons were -angry with family members (41/635), sold by step mom/others (28/635), raped/tortured (21/635), husband's torture (24/635), self motivation (12/635) and drug addiction (05/635) etc. ♣Siblings (30/635), sexual partners (22/635), friends and relatives (13/635) etc. ♦Injection, ligation/tubectomy, menopause.

Table-4: Influence of risky sexual behaviors on BMI of CSWs

Risky sexual behaviors of CSWs	All CSWs n=635	BMI of Female Hotel based CSWs n=308	Mean BMI	ANOVA	BMI of Female Floating CSWs n=327	Mean BMI	ANOVA
Client turnover							
Did not work ,last week	5.67 (36)	5.53 (17)	22.30±1.00	F (2,305)=.112	5.81 (19)	**22.90±1.18** [1]	**F (2,324)=4.029**
1– 14 C	48.03 (305)	30.84 (95)	22.17±1.04	P=.894	64.22 (210)	**20.68±1.13**	**P=.010**
≥15 C	46.30 (294)	63.63 (196)	22.00±1.95		29.97 (98)	**20.30±1.12**	
Usual day clients							
1-7	70.24 (446)	46.10 (142)	22.30±1.00	F (1,306)=.646	92.97 (304)	20.68±1.76	F (1,325)=.026
≥8 clients	29.76 (189)	53.90 (166)	21.90±1.69	P=.422	7.03 (23)	20.81±1.88	P=.872
Condom use							
No/ irregular use	55.7 (354)	51.30 (158)	21.82±1.06	F (1,306)=1.254	60.25 (197)	20.51±1.57	F (1,325)=1.143
Consistent use	44.3 (281)	48.70 (150)	22.37±1.61	P=.264	39.75 (130)	20.96±1.89	P=.286
Hyg. M after coitus							
No measure	33.39 (212)	33.11 (102)	22.18±1.24	F (2,305)=.826	33.64 (110)	20.60±1.38	F (2,324)=.163
Used savlon /soap	45.51 (289)	62.34 (192)	21.94±1.35	P=.439	29.66 (97)	20.87±1.95	P=.850
Others	21.10 (134)	4.55 (14)	23.45±1.96		36.70 (120)	20.63±1.81	
Interval of 2 coitus							
No	67.40 (428)	20.78 (64)	22.80±1.23	F (1,306)=2.224	43.73 (143)	21.02±1.89	F (1,325)=1.981
yes	32.60 (207)	79.22 (244)	21.90±1.07	P=.137	56.27 (184)	20.44±1.54	P=.160
Coital freq. per client							
One time	62.99 (400)	71.75 (221)	22.26±1.44	F (1,306)=1.216	54.74 (179)	20.42±1.70	F (1,325)=2.074
≥2 times	37.01 (235)	28.25 (87)	21.65±1.07	P=.271	45.26 (148)	21.01±1.67	P=.151
Weekly coitus with PC							
No weekly PC	59.84 (380)	55.84 (172)	22.12±1.65	F (2,305)=.896	63.61 (208)	20.60±1.76	F (2,324)=.243
Yes, 1 time/week	25.20 (160)	30.84 (95)	21.72±1.50	P=.409	19.88 (65)	20.96±1.59	P=.784
2 times/week	14.96 (95)	13.32 (41)	22.80±1.77		16.51 (54)	20.74±1.65	
F.nightly coitus with PC							
No fortnightly PC	70.39 (447)	58.77 (181)	22.09±1.56	F (2,305)=.121	81.35 (266)	20.67±1.70	F (2,324)=1.018
Yes, 1-2 times	22.52 (143)	27.92 (86)	21.95±1.57	P=.886	17.43 (57)	20.61±1.62	P=.363
>3 times	7.09 (45)	13.31 (41)	22.36±1.90		1.22 (04)	23.31±1.26	
Monthly coitus with PC							
No monthly PC	60.00 (381)	41.88 (129)	22.42±1.88	F (2,305)=1.018	77.06 (252)	20.54±1.70	F (2,324)=1.130
Yes, 1-2 times	21.89 (139)	25.98 (80)	21.54±1.70	P=.362	18.05 (59)	21.34±1.50	P=.324
>3 times	18.11 (115)	32.14 (99)	22.09±1.06		4.89 (16)	20.74±1.35	
Having NPSP							
No	90.87 (577)	88.31 (272)	22.05±1.34	F (1,306)=.131	93.27 (305)	20.72±1.78	F (1,325)=.368
Yes	9.13 (58)	11.69 (36)	22.33±1.37	P=.717	6.73 (22)	20.23±1.28	P=.545
Ever experienced in group sex (gr. sex)							
No	74.33 (472)	75.0 (231)	22.23±1.58	F (1,306)=1.091	73.70 (241)	20.65±1.77	F (1,325)=.137
Yes	25.67 (163)	25.0 (77)	21.64±1.53	P=.297	26.30 (86)	20.82±1.53	P=.712
Gr.Sex history (last.mo)							
No	78.58 (499)	83.12 (256)	22.21±1.46	F (1,306)=1.361	74.31 (243)	20.74±1.73	F (1,325)=.196
Yes	21.42 (136)	16.88 (52)	21.45±1.67	P=.244	25.69 (84)	20.54±1.63	P=.658
No. of persons in the last gr. Sex							
No Gr.sex history	78.58 (499)	83.12 (256)	22.21±1.46	F (2,305)=.973	74.31 (243)	20.74±1.73	F (2,324)=.572
2-5 clients	85.29 (116)	15.58 (48)	21.31±1.71	P=.379	20.80 (68)	20.73±1.75	P=.565
>6 clients	14.71 (20)	1.30 (04)	23.04±3.10		4.89 (16)	19.73±3.05	
Condom used in Gr.sex							
No Gr.sex history	78.58 (499)	83.12 (256)	22.21±1.46	F (2,305)=.703	74.31 (243)	20.74±1.73	F (2,324)=.286
Yes, used condom	17.17 (109)	14.28 (44)	21.50±1.86	P=.496	19.88 (65)	20.67±1.78	P=.752
No , didn't	7.40 (47)	2.60 (08)	21.13±2.51		5.81 (19)	20.08±3.11	
Ever experienced in Anal sex							
No	95.59 (607)	93.83 (289)	22.01±1.34	F (1,306)=2.225	97.25 (318)	20.72±1.75	F (1,325)=.499
Yes	4.41 (28)	6.17 (19)	23.15±1.29	P=.269	2.75 (09)	19.83±1.10	P=.480

[1] Washed by water, cotton, urine etc. NPSP='Non paying sexual partner', PC= permanent client, Gr.sex=group sex

Table-5: Factors associated with imbalance and under nutrition (crude model of BMI)

Explanatory variables	Unadjusted model of BMI focusing on imbalance nutrition (overweight/obesity) of CSWs. Here n=491 (n_1 having normal BMI=387, n2 BMI				Unadjusted model of BMI focusing on under nutrition (CED) of CSWs. Here n=531 (n_1 normal BMI= 387, n_2 BMI <18.5=144)			
factors associated with higher/lower BMI	**All CS Ws n=**	**0=normal BMI (n=387)**	**1=>25.1 BMI (n=104)**	**unadjusted Odds ratio (95% CI: lower-upper)**	**All CS Ws n=**	**0=normal BMI (n=387)**	**1=<18.5 BMI (n=144)**	**unadjusted Odds Ratio (95% CI: lower-upper)**
Category ofCSWs								
Hotel	261	52.5 (203)	55.8 (58)	1.143 (.739-1.77) P=.548	250	52.5 (203)	32.6 (47)	.439 (.294-.656) **P=.000**
Floating (r)	230	47.5 (184)	44.2 (46)	1	281	47.5 (184)	67.4 (97)	1
Present age (Y)								
<18 (r)	100	23.8 (92)	7.7 (08)	1	121	23.8 (92)	20.1 (29)	1
19-29	243	49.9 (193)	48.1 (50)	2.98 (1.36-6.54) **P=.007***	259	49.9 (193)	45.8 (66)	1.09 (.66-1.79) P=.751
≥30	148	26.3 (102)	44.2 (46)	5.186 (2.33-11.57) **P=.000**	151	26.3 (102)	34.1 (49)	1.524 (.89-2.61) P=.125
Marital status								
Unmarried	223	42.3 (164)	56.7 (59)	1.56 (.96-2.52) **P=.071**	217	42.3 (164)	36.8 (53)	.69 (.451-1.05) **P=.086**
Married	92	20.7 (80)	11.6 (12)	.650 (.318-1.33) P=.238	104	20.7 (80)	16.7 (24)	.64 (.37-1.09) P=.106
#Wi/Di/Ab (r)	176	37.0 (143)	31.7 (33)	1	210	37.0 (143)	46.5 (67)	1
Having children								
No	226	43.9 (170)	53.8 (56)	2.20 (1.12–4.33), **P=.023***	219	43.9 (170)	34.0 (49)	.96 (.55-1.67) P=.888
Yes	173	35.4 (137)	34.7 (36)	1.75 (.86-3.56) P=.121	208	35.4 (137)	49.3 (71)	1.73 (1.0-2.96) **P=.047***
Unmarried (r)	92	20.7 (80)	11.5 (12)	1	104	20.7 (80)	16.7 (24)	1
Mass media exposure								
Did not have	235	49.6 (192)	41.3 (43)	.761 (.42-1.39) P=.372	291	49.6 (192)	68.8 (99)	2.92 (1.51-5.65) **P=.001***
adio/TV/VCD	168	32.8 (127)	39.4 (41)	1.10 (.596-2.02) P=.765	160	32.8 (127)	22.9 (33)	1.47 (.71-3.04) P=.294
Having all (r)	88	17.6 (68)	19.3 (20)	1	80	17.6 (68)	8.3 (12)	1
Drug habit								
NO	329	64.3 (249)	76.9 (80)	1.85 (1.12-3.05) **P=.016***	352	64.3 (249)	71.5 (103)	1.40 (.917-2.114) P=.120
Regularly (r)	162	35.7 (138)	23.1 (24)	1	179	35.7 (138)	28.5 (41)	1
Monthly absent from sex work								
1-7 days (r)	403	84.5 (327)	73.1 (76)	1	452	84.5 (327)	86.8 (125)	1
≥ 8 days	88	15.5 (60)	26.9 (28)	2.0 (1,20-3.36) **P=.008***	79	15.5 (60)	13.2 (19)	.828 (.475-1.44) P=.507
Last month's Total expenditure (BDT)								
1000–2500	64	14.0 (54)	9.6 (10)	.504 (.240- 1.06) **P=.071**	84	14.0 (54)	20.8 (30)	2.27 (1.28-4.04) **P=.005***
2501–5000	226	48.0 (186)	38.5 (40)	.585 (.369-.930) **P=.023***	264	48.0 (186)	54.2 (78)	1.71 (1.092-2.69) **P=.019***
>5000 (r)	201	38.0 (147)	51.9 (54)	1	183	38.0 (147)	25.0 (36)	1
Last month's Income (BDT)								
0.0–2500	159	29.5 (114)	43.3 (45)	.650 (1.01- 2.70) **P=.046**	170	29.5 (114)	38.9 (56)	1.78 (1.12-2.82) **P=.014***
2501–5000	130	28.4 (110)	19.2 (20)	.760 (.421-1.372) P=.362	153	28.4 (110)	29.9 (43)	1.42 (.874-2.30) P=.158
>5000 (r)	202	42.1 (163)	37,5 (39)	1	208	42.1 (163)	31.2 (45)	1

#Wi/Di/Ab=Widow/divorced/Abandoned. Many others explanatory variables including education (P=.567), birth control measure (P=.320) and genital discharge (P=.671) had no significant (P>0.05) influence on both "Obese" and "CED model" of BMI (unadjusted logistic regression). **Legend:** Descriptive, cross tabulation, Simple logistic Regression (SLoR) analysis.

Table-6: BMI model-1 (overweight/obese) focusing on imbalance nutrition

Model-1	**Adjusted model of BMI (0=normal BMI, 1=>25.1) focusing on imbalance nutrition (overweight/obesity) of CSWs. Here n=491 (n_1 having normal BMI=387, n2 BMI >25.1=104)**						
factors associated with higher BMI (outcome)	**All CSWs (n=491) % n**	**0=normal BMI (n=387) % n**	**1=>25.1 BMI (n=104) % n**	**Beta coefficient (B)**	**standard error**	**Adjusted Odds Ratio (95% CI) (lower-upper)**	**P-value**
Present age (Y)							
<18 (r)	20.37 (100)	23.8 (92)	7.7 (08)	-	-	1	-
19-29	49.49 (243)	49.9 (193)	48.1 (50)	1.428	.417	4.17 (1.841-9.441)	.001**
≥30	30.14 (148)	26.3 (102)	44.2 (46)	2.539	.485	12.67 (4.895-32.789)	.000**
Last month's Total expenditure (BDT)							
1000–2500	13.1 (64)	14.0 (54)	9.6 (10)	-.873	.421	.418 (.183-.954)	.038*
2501–5000	46.0 (226)	48.1 (186)	38.5 (40)	-.780	.263	.459 (.274-.768)	.003**
>5000 (r)	40.9 (201)	38.0 (147)	51.9 (54)	-	-	1	-
Category of CSWs							
Hotel	53.2 (261)	52.5 (203)	55.8 (58)	.584	.328	1.793 (.942-3.413)	.075
Floating (r)	46.8 (230)	47.5 (184)	44.2 (46)	-	-	1	-
Drug habit							
No	67.0 (329)	64.3 (249)	76.9 (80)	.635	.271	1.887 (1.109-3.210)	.019*
Regularly (r)	33.0 (162)	35.7 (138)	23.1 (24)	-	-	1	-
Monthly absent from sex work							
1-7 days (r)	82.1 (403)	84.5 (327)	73.1 (76)	-	-	1	-
≥ 8 days	1 7.9 (88)	15.5 (60)	26.9 (28)	.687	.319	1.99 (1.064-3.717)	.031*

Legend: Backward stepwise (5[th] step) Multiple Logistic Regression (MLoR)
Hosmer-Lemeshaw goodness of fit for BMI (obese) model: $\aleph^2$ =9.866, df=8, P=.275
Model summary: -2 Log likelihood=452.781, Nagelkerke pseudo R-square=.163
Model coefficient :Chi-square ($\aleph^2$)=54.273, df=9, P=.000
Overall predicted percentage from classification table of DV (BMI) was 79% (cut value is 50%).
(r)= reference category. *P<0.05, **P<0.01

Table- BMI model-2 (CED/underweight) focusing under nutrition

Model-2	**Adjusted model of BMI (0=normal BMI, 1=<18.5) focusing on under nutrition (CED) of CSWs.**						
factors associated with lower BMI (outcome)	**All CSWs (n=531) % n**	**0=normal BMI (n=387) % n**	**1=<18.5 BMI (n=144) % n**	**Beta coefficient (B)**	**standard error**	**Adjusted Odds Ratio (95% CI) (lower-upper)**	**P-value**
Category of CSWs							
Hotel	47.1 (250)	52.5 (203)	32.6 (47)	-.823	.205	.439 (.294-.656)	.000**
Floating (r)	52.9 (281)	47.5 (184)	67.4 (97)	-	-	1	-
Mass media exposure							
Did not have	54.80 (291)	49.6 (192)	68.8 (99)	.716	.374	2.047 (.984-4.257)	.055
Radio/TV/VCD	30.13 (160)	32.8 (127)	22.9 (33)	.269	.375	1.308 (.628-2.726)	.473
Having all (r)	15.07 (80)	17.6 (68)	8.3 (12)	-	-	1	-

Legend: Backward stepwise (8[th] step) Multiple Logistic Regression (MLoR)
Hosmer-Lemeshaw goodness of fit for BMI (CED) model: $\aleph^2$ =4.237, df=3, P=.237
Model summary: -2 Log likelihood=598.640, Nagelkerke pseudo R-square =.059
Model coefficient : Chi-square ($\aleph^2$) =22.031, P=.000, df=
Overall predicted percentage from classification table of DV (BMI) was 72.9% (cut value is 50%).
(r)= reference category. **P<0.01

2.1.4 Discussion

Data regarding CSW's nutritional status was scanty in Bangladesh as well as in global. National data did not address this segment of population except HIV/AIDS or STI related study or reports. Nevertheless, 2 recent studies mentioning (Huq et al, 2013) nutritional status of floating CSWs and many studies in Bangladesh (previous or recent) other than CSWs, focused on the nutritional status of garments workers (Kaniz et al, 2014; Islam MZ et al, 2008; Ahmed et al, 1997), rural women (Rahman et al, 2015; Brandon et al, 2010; Milton et al, 2010; Rahman and Nasreen, 2009; Shafique et al, 2007; Islam MZ et al, 2002, 2003 and 2004) and destitute women (Islam MZ et al, 2002, 2003 and 2004), slum's women (Pryer and Rogers, 2006) and urban women (Rahman et al, 2015; Khan and Kramer, 2009; Shafique et al, 2007; Islam MZ et al, 2002, 2003 & 2004).

It was observed from this research study that (Table-1) mean height and weight for all (n=635) CSWs (both groups) was 150.0 cm (95% C (149.93−152.07) and 48.13 kg (95% CI 47.38−48.88) respectively. Mean height of this study respondents was much lower than a recent study (158.56 cm) in Bangladesh about floating CSWs (Huq et al, 2013) but mean weight was almost the same like this study (48.60 cm), though both mean height and weight of Huq et al (2013) was higher than the Floating category (46.08 kg and 149.15 kg/m^2) of CSWS of this study. Mean height of floating CSWs was 149.15 cm which was slightly higher than destitute NPNL women of previous studies of Bangladesh (Islam et al, 2003). However, mean weight (50.31 kg) of hotel-based CSWs was higher than that study (Huq et al, 2013). Furthermore, overall, mean BMI of CSWs (n=635) of this study was 20.89 kg/m^2 which was higher (18.54 kg/m^2) than street-based CSWs (Huq et al, 2015) but lower than Indian tribal 'Tharu' women (Mukherjee et al, 2015).

Although, mean BMI of CSW's (n=635) was 20.89 kg/m^2 but hotel-based CSWs (21.88 kg/m^2) had higher BMI than floating (20.42 kg/m^2) group (t=4.585, P=.000). Around 60.9% (n=387) CSWs were in normal status in this study which was close to a female floating CSW's study (Mondal et al, 2010) and an Indian study (Mukherjee et al, 2015) but higher than a recent Floating CSW's study (Huq et al, 2015) which could be, due to the effect of the smaller sample size of the latter study (n=50). Mean BMI (20.89+1.20 kg/m^2**)** was also much lower than a recent study (23.4 kg/m^2) of female garment worker (Kaniz et al, 2014). Though most (60.9%) of CSWs in this study were in normal status, but there existed pockets of both under nutrition (22.7%) and over nutrition (16.3%) simultaneously which recalls the 'double burden of malnutrition' in a previous study (Shafique et al, 2007) and a recent study (Kaniz et al, 2014) (normal BMI=33.9%, underweight=10.3%, obese=21.7%).

In general, 22.7% of CSWs were undernourished and a study about street-based CSWs of Rajshahi city (Mondal et al, 2010) was consistent with the rate of under nutrition of this study. However, under nutrition rate among CSWs was higher than an Indian study (Mukherjee et al, 2015) but slightly lower than national rate (24% in 2011) in general population (BDHS, 2011) which was higher (30%) in BDHS, 2007. Interestingly, prevalence of both adult overweight

(7.8%) and obesity (1.1%) in general population (WHO, 2014)[62] and in rural (Brandon et al, 2010) population (overweight=6.3%, obesity=0.7%) were lower than this study group (overweight=13.2%, obesity=3.1%). Rate of obesity (3.4%) was also consistent with a recent Indian study (Mukherjee et al, 2015) but overweight rate of CSWs was the highest amongst all studies (Brandon et al, 2010; WHO, 2014; Mukherjee et al, 2015). However, Mondal et al (2010) and this study [overweight (13.2%) + obesity (3.1%) = imbalance nutrition (16.48%)] had same rate of over nutrition/obesity or imbalance nutrition reminds that over nutrition is not a problem in general population but also a problem of CSWs community.

Overall CED (or under nutrition) of this study was 22.7%. (Total n=635, HBCSWs-CED=15.3%, n=308; FCSWs-CED=29.6% n=327), but compared to hotel-based CSWs (HBCSWs) female floating CSWs (FFCSWs) were more likely to be underweight (15.3% versus 29.6%), and less likely to be overweight (18.8% versus 14.0%) and normal status (65.9% versus 56.3%). In this study, CSWs suffered from various degree of malnutrition (CED I=4.1%, CED II=4.6% & CED III=14.0%), while it was more than double (52.0%) in a recent study (Huq et al, 2015) about FFCSWs (CED I =6.0%, CED II=16.0% & CED III=30.0%) and was slightly higher than another study about street-based CSWs (15.91%) of Rajshahi city in Bangladesh (Mondal et al, 2010), the variation in malnutrition for both cases, may be due to small participants especially former (only 50) and then latter (n=176).

Influence of ‘socio-economic variables’ on BMI of CSWs (ANOVA, table-2) depicted that ‘having children’ [HBCSWs: $F(2,305) = 8.734$] had significant negative influence on the BMI of both types of CSWs (both $P=.000$). However, greater influence was observed on hotel groups than floating and those CSWs had children had the lowest mean BMI (20.89 kg/m^2) than who didn’t have children (23.44 kg/m^2) in post hoc test (Tukey). This finding was supported by other recent studies conducted in Bangladesh (Rahman et al, 2015; Corsi et al, 2011; Rahman and Nasrin, 2009). Marital status ($P=.001$) had influenced only on hotel-based CSWs and observed that married HBCSWs had higher BMI than widowed/divorced/abandoned CSWs which also in agreement with the findings of other studies (Corsi et al, 2011; Kamal and Islam, 2010; Khan and Kremer, 2009).

Lifestyle factors (ANOVA, table-3) like ‘causes behind profession’ ($P=.026$), ‘working days in a week’ ($P=.012$), ‘experiences in sex business’ ($P=.000$), ‘use of contraceptives’ ($P=.013$), ‘habit of smoking’ ($P=.036$), and ‘substance abuse’ ($P=.002$) profoundly influenced BMI of floating group only. It was observed that contraceptive users [$F(2,324) = 3.643$, $P=.013$] had higher BMI (21.38-22.48 kg/m^2) than non users. Similar conceptual finding was observed in other studies in Bangladesh (Corsi et al, 2011; Milton et al, 2010) where showed that OCP use reduced the vulnerability of women to underweight.

[62] Global Nutrition Report. 2014 Nutrition Country Profile Bangladesh. (www.globalnutritionreport.org)

BMI model focusing on overweight: in a multivariate **overweight/ Obesity** model, the beta coefficient is the independent effect of variable age (Independent variable) on BMI (DV) after adjusting for last month's expenditure, category of CSWs (hotel or floating), drug habit and monthly absent from sex work. In fact, 'Present age' is very important indicator of BMI, as age increases BMI is also increases. Older age CSWs like 19-29 years and ≥30 years were respectively almost 4 times [AOR 4.17 95% CI (1.841-9.441), P=.001] and 13 times [AOR 12.67 CI (4.895-32.789), P=.000] more likely to be overweight/obese as compare to <18 years age of CSWs. ANOVA also postulated that 'present age' [HBCSWs: F (2,305) =15.9494] had greater positive influence on the BMI of both types of CSWs (both P=.000) though greater influence was observed on hotel groups than floating. This observation is in agreement with a very recent population based study (Rahman et al, 2015) which showed that 25-49 aged of both urban and rural women were (P<0.001) less likely to be underweight than younger (15-24 years) women and also consistent with other studies (Kamal and Islam, 2010; Khan and Kramer, 2009) conducted in Bangladesh and India (Subramanian and Smith, 2006). This is due to the metabolism rate, which slows as age increases, so, body requirements decreases as age increases. Thus by taking same calorie in older age (as taken in younger age) one can easily gain weight as well as BMI. Also, PAL is an important influential factor for BMI. However, the 95% confidence interval for the odds ratio comparing ≥30 y CSWs versus <18 y CSWs who develop obesity was very wide (4.895 to 32.789). While the odds ratio is statistically significant, the confidence interval suggests that the magnitude of effect could be anywhere from 4.90-fold increase to a 32.8-fold increase. This could be, due to the fact that there were small number of outcome events (>BMI 25.1=104 in 491 CSWs) and small number of women had >25.1 BMI in reference category (n=08). Thus the association should have interpreted with caution. A large study is needed to generate a more precise estimate of effect. However, as point prevalence was high (AOR 12.67) so as its logical magnitude of effect.

The odds of being 'obese' among CSWs were 54%-58% less likely who spent <5000 BDT last month as compared to CSWs who spent >5000 BDT last month [AOR .459(.274-.768), P=.003; AOR .418 (.183-.954), P=.038]. In other word, CSWs who spent monthly >5000 BDT (by inverting odds ratio) had 2 times higher risk of being obese than who spent <5000 BDT that is CSWs who spent <5000 taka were more likely to be underweight. Similar conceptual finding was observed in a previous study about slums people of Dhaka (Pryer and Rogers, 2006) and also in another recent study in Bangladesh (Rahman et al, 2015) where both urban and rural (middle class and rich) women had less risk to be underweight than their poor counterparts or food unsecured households. Same result was also reported by another study (Ramdath et al, 2011) in Trinidad where authors found significant association of food insecurity with underweight. In fact, economic condition showed an inverse relation with underweight and wealth is positively associated with obesity. Different recent studies in Bangladesh (Corsi et al, 2011; Kamal and Islam, 2010; Milton et al, 2010; Khan and Kramer, 2009; Rahman and Nasrin, 2009; Pryer and Rogers, 2006) were consistent with this finding.

It was observed from MLoR model of overweight/obese that hotel-based CSWs were nearly 2 times more likely to develop obesity than floating CSWs adjusted for other variables those could influence commercial sex works. Plausible explanation for this is HBCSWs had better economic condition than floating CSWs. Socio-economic data of this study was also consistent with this finding that HBCSWs earned almost double than FCSWs and younger age women were more likely to engage in hotel sex trading which is the key point for earning money in sex industries especially in capital city where lower to middle income people migrated frequently and harbored from all over the countries throughout the year. Thus, in sex industry, being a HBCSWs was more obvious to become obese than being a FCSWs as economy is the underlying factor. Different researches[63] also in agreement with that floating or street based CSWs had less social and economic status among all types of CSWs (Mondal et al, 2010, 2008; Rakhi et al, 2005; Ullah, 2005 ;Rahman et al, 2000; McKeganey and Barnard, 1996).

MLoR model of BMI (overweight/obese) revealed that non drug addict CSWs were 1.9 times higher odds [AOR 1.9, 95% CI (1.109-3.210), P=.019) of having ≥18.5 BMI. In other words (by inverting the odds ratio) drug addicts CSWs were 53% less likely to have ≥18.5 BMI. This was due to the poor condition of the 'health, hygiene and nutrition' of the drug dependent FCSWs. In fact, FCSWs are deprived of all well being including personal, social, economical, mental, physical, and sound health and nutrition etc. and their habit of "drug addiction" is like "add coal to fire" and made them worsen than anything else. Different studies supported (Islam et al, 2002) the below normal BMI as well as lower biochemical values, poor health and nutritional status, chronic Energy deficiency of drug addicts that contributed to increase susceptibility to infection and nutritional deficiencies (Chandra, 1997; Varela et al, 1997). This deficiency is because of using immunosuppressive illicit drugs that impair or alter food habit (Himmelgreen et al, 1998; Varela et al, 1997). However, the finding of this study was disagreement with a recent study result in USA (Richard et al, 2014) about US high school students where obese sexually active high school students were more likely than normal weight sexually active students to inject illegal drugs especially obese female students were 1.98 times more likely to inject illegal drugs. In other words overweight/obesity significantly associated with increased likelihood of injecting illegal drugs among US high school students. The discrepancy between this study and US study (Richard et al, 2014) might be due to, this is a study absolutely about female 'commercial sex workers', who were basically destitute, exploited by pimps or by others reason they migrated from throughout the country to "Dhaka city" and entered into the 'sex industry' because of 'extreme lower economical status' (from the answers why they came this profession) to save them or their children (as 38.43% or n=244/635 had children) or their families simply from "extreme hunger". Moreover, different studies including this reported that before entering into sex trade these women were not

[63] The floating sex workers are the most disadvantaged among all commercial sex workers Bangladesh, 2008 (www.insticeage.studies.com)

addicts (but destitute or abandoned or divorced housewives), instead they became addicts after tolerant of lots of pressures, stresses abuses to cope with.

'Monthly absent from work' was also an important predictor of overweight. It was noticed that CSWs who were ≥8 days absent from sex work in a month had almost double risk of being obese [AOR 1.99 (2.064-3.717), P=.031]. ANOVA (table-3) also revealed the fact; those CSWs absent 8-14 days in a month had significantly higher BMI (22.39 kg/m^2) as compared to CSWs (21.38 kg/m^2) who took leave for 1-7 days in a month. This might be due to extra 'physical and psychological activity and stress' faced by CSWS who did not take enough time to take rest as compared to others who took ≥8 days resting time in a month. This observational concept was consistent with different studies conducted in Bangladesh (Rahman et al, 2015; Kamal and Islam, 2010; Rahman and Nasrin, 2009) where currently working women were found more underweight.

BMI model focusing on underweight: hotel-based CSWs (HBCSWs) were 56% less likely [AOR=.439 95% CI (.294-.656), P=.000] to develop CED than floating CSWs and this is the main influential factor to develop CED among CSWs (Just opposite result of overweight/obese model-1). It was also observed that, those CSWs did not have any mass media exposure were 2 times more likely to be chronic energy deficient or undernourished as compared to CSWs who had access to radio/TV/VCD [AOR 2.047 95% CI (.984-4.257), P=.055]. ANOVA (table-2) also showed this finding in case of floating group [F (2, 324) =9.286, P=.000] and in post hoc test it was also postulated that those floating CSWs did not have mass media exposure owned the lowest BMI (19.95 kg/m^2) than who were exposed (21.88 kg/m^2) to mass media items (like TV, VCD,radio). This finding is reinforced by a very recent study (Rahman et al, 2015) where 'exposure to mass media' was a significant predictor of 'under nutrition' for both rural and urban women and women having 'exposure to mass media' had lesser risk of being underweight as compared to women did not have access to mass media. Another urban women study (Khan and Kramer, 2009) in Bangladesh reported lower prevalence of underweight among women who watch television and read newspaper daily. However, having small floor areas, no electricity and tube well water as drinking sources were associated with under nutrition reported by a study (Pryer and Rogers, 2006)

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CHAPTER-2.2

Dietary Assessment of the Nutritional Status of Commercial Sex Workers

2.2.1 Introduction

Food intake reflects what either individuals or group consume in terms of solid foods, beverage including drinking water, and supplements. Food consumption can be estimated through food consumption surveys at individual (individual dietary survey) or household level (household budget survey) or approximated through food supply Data derived from food balanced sheets[64]. Several dietary assessment tools directed at the individual level are of two basic categories: prospective methods-those that record data at the time of eating (i.e. so called weighted and estimated record methods) and retrospective /interview methods-those that collect data about the diet eaten in the recent past and or over a longer period of time (Van Staveren and Ockè, 2006).

Food insecurity is the converse state of food security, is often associated poverty and low income, and has important implications for the health and nutrition of individuals. Given women their contribution to food production and preparation, their role in society as child bearers and caregivers, the increasing number of female-headed households worldwide, and their disproportionately poor economic status, women need special consideration in discussions of food insecurity and its effect on health, nutrition and behavior (Louise and Kimberly, 2011).

Food insecurity is associated with obesity, anxiety, and depressive symptoms: risky sexual behaviors; poor coping strategies; and negative pregnancy outcomes in women, although evidence about the direction and causality of association is unclear. There is lack of evidence and understanding the effects of food insecurity in resource-poor settings, including its effect on weight, nutritional outcome, and pregnancy outcomes, as well as its effect on progression on diseases such as HIV infection (Louise and Kimberly, 2011).

Nutritional assessment by diet analysis is a two stepped process. The first step is the evaluation of food consumption, and the second the conversion of food into nutrient intake. To do this a 'food composition' data base is urgently required which lists the mean nutritional values for a given food portion (especially per 100 g). Then multiplying food intake by the mean nutrient content of that amount of food (obtained from the food composition database) (Willett, 1998).

The major sources of error in dietary studies have been reviewed in details by Bingham (1987), Four possible sources of error occur to some degree with all dietary methods, but can be minimized by careful study design and execution: sampling bias, response bias, inappropriate coding and use of food composition tables in place of chemical analysis (MacIntyre, 2009).

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[64] EFSA, European Food Safety Authority, 2009

Dietary assessment is usually affected by an underreporting bias (Islam et al, 2013) and also over reporting or 'stockpiled quantities' are also observed (Bermudez et al, 2012). Coding errors are also likely to arise when more than one person is involved in coding and there is no agreed procedure and/ or comprehensive coding manual. Coding errors arising exclusively from inadequate description of the foods have resulted in coefficient of variation ranging from 3% to 17% for different nutrient (Maclntyre, 2009). Results of a pilot study (Tamara et al, 2012) showed that variability of calculated data induced by food coding lonesome is acceptable but putting other errors into account, results can be misleading.

Energy to power the body's metabolic processes is derived from the food that we eat. The process by which energy is transformed into ATP is known as cellular respiration. Glucose is the body's preferred source of energy for the production of ATP but, if necessary, other carbohydrates, fats and proteins can also be metabolized to acetyl coenzyme A (CoA), enter the citric acid (Krebs) cycle and be oxidized to carbon dioxide and water (Huskisson et al, 2007).

Even in developed countries, many sections of the population do not receive the essential vitamins and minerals needed from their diet. Several groups in the population are at increased risk for inadequate micronutrient status, usually due to insufficient intake caused by weight-reducing diets, insufficient and/or imbalanced nutrition, eating disorders, or demanding periods such as extensive exercise or emotional and/or physiological stress. Increased requirements may also cause an inadequate vitamin and mineral status; for example, as may occur in pregnancy and lactation, during growth, in the elderly, smokers and chronic alcohol abusers, and in patients with certain underlying diseases.

The concept of marginal micronutrient deficiency first proposed by Pietrzik (1985) showed that, long before the clinical symptoms of deficiency appear micronutrient deficiencies develop progressively through several sub-clinical stages. Marginal deficiencies may occur as a (Pietrzik K, 1985) concept of borderline vitamin deficiencies.

Data regarding CSW's dietary intake are scanty. Nothing is known about dietary habits and different nutrients intake (calorie, protein, fat, calcium, zinc, iron) of CSWs in Bangladesh. No research focused on or carried out CSW's health nutrition, food habit etc. We have our previous 4 health and Nutrition surveys references. Very recently 3 surveys namely "Bangladesh Integrated Household Survey" (BIHS, Akhter et al, 2013) "Nutrition, Health, Demographic Survey, Bangladesh, 2011 (Akhtaruzzaman et al, NHDS, BD, 2011) and National Micronutrients status survey, Bangladesh, 2011-12 (NMSS, 2011-12) were carried out but both surveys focused on general population (male and female) under 4 strata (national, rural, urban and slum).

2.2.2 Materials and Methods

2.2.2a Study setting and subjects

The dietary survey was conducted among female commercial sex workers (FCSWs) of Bangladesh (n=635). With the help of CSW's welfare organizations (Durjoy nari sangha, Ulka nari sangha etc.) which work for all types of CSWs, this study was carried out among randomly selected six hundred and thirty five (n=635) CSWs aged 14-55 years comprising hotel-based (n=308) and floating CSWs (n=327) respectively working either in 'hotel' or on road/park or in cinema hall/cow haat or shrine or shop or elsewhere in Dhaka city. The study was approved by both ethical review committee of 'Bangladesh medical research council' (BMRC) and 'University of Dhaka'. All CSWs were informed about the study before selection and after briefing the subjects about the perspective of the study 'written consent' was obtained from randomly selected CSWs on the very day of interview by participant's signs or printed 'thumb' on the consent form. The questionnaire had both open-ended and structured questions and was pre-tested.

2.2.2b Dietary Survey

Dietary information of each CSW and their children (if any) was collected by interviewing them. Seven days foods frequency data were collected (7 day recall) and one-day "24 hours recall" method was applied to obtain habitual dietary intake of each respondent (n=635). Food data consisted of individual or multiple foods including beverages and foods eaten away from home, some of which were mixed meals (e.g. fish and vegetable dishes). The amount of food eaten was assessed by showing them 'portion size display' such as measuring spoons, cups, glasses and plates of different sizes (used as standards in different earlier nutritional surveys by INFS, DU). The subjects were asked which food/beverages were eaten either in home or outside during the 'last 24-hours' and amounts were recorded after confirmed by displaying 'portion size'. Individual food acquisition data were transformed into standard units of weight (grams) or volumes (milliliters) after coding the food items by 'food code list'. The consumed quantities of foods (in grams) by CSWs were multiplied by the food values of each food items (using a food composition data base) (Islam SN et al, 2012) and they added together to estimate the average daily intake of calorie, protein, carbohydrate, calcium, copper, zinc, Iron, retinol (vitamin A) and vitamin C by using SPSS software. To obtain this, especial syntax (of SPPS version 17) was prepared endorsing all nutrients values of per 100 gm (Islam SN et al, 2012) according to food code list (Helen Keller Worldwide) starts from serially. Fortunately, we have our own "food composition database" (Islam SN et al, 2012) and in this study this database was used for the nutrient conversion during dietary analysis (by applying SPSS version 17) of the foods consumed by the CSWs. Moreover, dietary intake of phytic acid, interaction of phytic acid with zinc and its effect upon bioavailability of zinc has been theoretically calculated. "Phytate to zinc molar ratio", an indicator of zinc bioavailability, calculated to find out whether zinc intake of CSWs' diet biologically available or absorption of zinc hampered by anti-nutrient (Phytic Acid).

2.2.2c Data analysis

Normality test: various statistical tools were used employing SPSS for the analysis of dietary data. Before performing analysis "Normality" test of the food data set was done by Kolmogorov-Smirnov (K-S) goodness of fit test as well as by descriptive statistics. It was observed from the descriptive statistics that, dietary data including all nutrients were positively skewed, calcium data was mostly skewed skewness=9.713, SE=.097, then fat and vitamin C, both of which skewness was 5.20 and carbohydrate had the lowest skewness i.e. .288, SE=.097 and skewness of the rest of the nutrients were from .597 to 3.5. Both 'logarithmic'/Log_{10} and 'Square root transformation' was performed to see which process could eliminate the skewness of all nutrients in a better way. And thus, approximation to a normal distribution was greatly improved by 'Square root transformation'. After 'Square root transformation', mean as well as other parametric tests on the transformed scale data was performed and then back transformed by taking the "square" and at the same time the values were backed in the correspondent nutrient unit (g/mg/µg). However, the Square root transformed 'geometric mean' was always less than the mean of raw data (Bland and Altman, 1996). In fact, extreme observations of a "highly skewed data set" have a large influence on the arithmetic mean, making it more prone to sampling error. Thus, lessening this influence is one advantage of using transformed data (Bland and Altman, 1996).

2.2.2d Statistical Analysis

All statistical analyses of dietary data were assessed by using Statistical Package for Social Science version 17.0 (SPSS Inc, Chicago, IL, USA). Two-tailed P values <0.05 were considered statistically significant. Comparisons between continuous variables were performed by Student's t-test.

Univariate analysis of the samples: Descriptive Statistics including mean (with 95% CI), Standard deviation, range, percentage and frequency distribution of Data was done to calculate baseline information. Descriptive data were presented as the mean (with 95% CI) for continuous variables and as percentages for categorical variables. A comparison of the mean intake of all nutrients with the Reference RDA (ICMR, India and IOM, USA) i.e. 'adequacy of the nutrients' and 'national level comparison' (NMSS, 2011-12; NHDS, 2011) was also addressed.

Bivariate analysis: **in** bivriate analysis Pearson's correlation was applied to assess extent of linear correlation between two continuous variables, hence correlation (2-tailed) between different dietary nutrients were performed. Influences of dietary nutrients on serum nutrients were observed by 'ANOVA.

Multivariate analysis: multivariate analysis was done to understand the association of different independent variables with dichotomous (0, 1) dependent variable (different dietary nutrients). To explore which factors/variables influence >50% or <50% different dietary nutrients intake (dichotomous dependent variables), in this study multiple logistic regression analysis was performed (MLoR) for all nutrients except fat, as no CSWs took >50% RDA of fat and fat intake of all (n=635) CSWs was well below <50% of RDA. Thus, multiple logistic regression analysis (MLoR models) of different dietary nutrients was accomplished to observe which factors positively associated (positive deviance) with ≥50% of RDA (of calorie, protein, copper and iron: table-9 to 12) and factors negatively associated with <50% of RDA (of zinc, calcium, retinol, and vitamin C: table-13 to 16). All Covariates (socio-demographic, lifestyle and sexual variables as well nutritional indices) which were found statistically significant (P≤0.05) or borderline significant (P<0.09) in Simple logistic regression (SLoR) analysis were introduced simultaneously in the **multiple logistic** regression analysis. The model was developed by 'backward stepwise elimination', removing the covariate with largest P value at each step until the remaining variables were significant at the 0.05 level in the final adjusted model. Also, forward stepwise technique applied in same way to confirm the model. Thus relative odds (ORs) and 95% CI were calculated to observe factors associated with >50% or <50% different dietary 'nutrient intake'. Hosmer-Lemeshaw goodness of fit and Nagelkarke-pseudo R^2 of the model was also observed.

The plausibility of apparent food and nutrients intake was assessed with exploratory analysis, including simple counts of food items by CSWs, frequency tables, simple identification of extreme values, plot charts and others. Obvious 'stockpiled quantities' were also detected and labeled as 'outliers'. To further correct for stockpiling or over reporting, CSWs identified with mean individual intake above 3500 kcal. After applying these steps, 4 CSWs were identified only with implausible data, which were excluded from the analysis.

2.2.3 Results

2.2.3.1 Amount of foods consumed by CSWs

Table-1 revealed that mean weight of the foods consumed by CSWs (n=635) was only 545.39 gm, comprised of total 365.17 g cereal (mainly from rice, 294.28 g and others cereal were one fourth of rice i.e. 70.28 g) and 308.59 g non-cereal plants (like pulses, leafy or non-leafy vegetables and fruits etc.) and 295.25 gm animal foods (like fish, meat, egg and milk). Mean amount (g) of other cereals, pulses, green leafy vegetables (GLVs), meat, egg and even milk consumption by CSWs were noticed higher than national averages (NHDS, 2011), while rice, non-leafy vegetables (NLVs) oils and fish consumptions by CSWs were much lower than national average. Interestingly, mean amount (295.25 g) of animal food intake by CSWs was 120 gm higher than national intake and 104 g than that urban intake (table-1).

2.2.3.2 Nutrients consumption and RDA met by CSWs

Dietary survey of CSWs (n=635) indicates that average energy intake (table-2 and 3) was only 1362.79 Kcal/day (95% CI 1321.13−1405.10) with a range of 58.76−3277.49 kcal/day which was, of course, inadequate for energy intake and met 61% of RDA (ICMR, 2010) of energy by CSWs. This may be due to low fat intake (met only one third of RDA, 32.2%, ICMR) as found in this study. Most of calorie came from carbohydrate, mainly 1635.11 kcal from plant sources (cereals i.e. rice, Ata etc. 1289.92 kcal +other plants 345.19 kcal) and around one fourth from animal sources (430.96 kcal) (table-2). Mean fat intake was 8.05 g, most of which came mainly from animal foods (23.05 gm) and a very small segment (5.39 g) came from cooking oils. Mean carbohydrate intake was 275.22 g and met 89.76% of RDA (ICMR) and most of which came from mainly from rice (mean 232.51 g), secondly from other cereals (mean 49.59 g) collectively total carbohydrate from cereal was 282 g, and other plant origin mean carbohydrate was 69.86 g and a tiny portion of carbohydrate (mean 8.18 g) came from animal origin (table-2)

In fact, dietary intake of CSWs lacked severely in fat (8.05 g met only 32.2% RDA adequacy of ICMR) which directly affects calorie intake and vitamin A (only met 27.14% RDA, IOM), and calcium (only met 21.60%, IOM). In addition, CSW's diet lacked in zinc (met 43.48% RDA, IOM), vitamin C (met 43.70% RDA, IOM) and also mean protein intake was only 40 g (fulfilled 72.86% RDA, ICMR), which was not up to the mark (though animal intake was slightly higher than plant source; 39.69 g plant and 47.74 g animal). In contrast to this, iron consumption (met 110.2% RDA, IOM) exceeded RDA limit and level of apparent copper intake (met 320.62% RDA, IOM) was abnormally high as RDA is set only 900 mcg by IOM, USA (table-3).

Mean vitamin C intake by CSWs (32.82 mg) was not up to the mark but pretty good amount than vitamin A consumption and fulfilled 43.76% RDA (IOM) of vitamin C. Vitamin A/retinol intake of CSWs was remarkably very poor (183.96 µg), most of which came from plant origin

(423.77 µg=1.03 cereal+ 422.74 green vegetables/pulses/fruits) and rest of RDA cleaved from animal sources (228.72 µg) and attained only 27.14% of RDA (IOM) of the study subjects.

All the participants were found severely deficient in their macro mineral intake i.e. mean calcium intake was 237.63 mg/day/person (95% CI 218.88−257.15), fulfilled only 21.60% of RDA (according to IOM 1100 mg/day). Micro mineral zinc intake was extremely low, consumed only 3913.24 µg/day (95% CI 3736.52−4094.04), which was less than half of their requirements (8000-9000 µg, IOM). However, iron intake (19.83 mg) was surprisingly exceeded RDA (110.17%) and national average (intake only 7.83 mg in slums, NMSS, 2012) and copper intake was overwhelmingly higher (met 320.62% RDA) than other nutrients (table-2, 3).

2.2.3.3 Adequacy of the intake of nutrients

In short, CSWs' diet was mainly rice-based carbohydrate-rich (met exactly 89.76% RDA, ICMR) which was deficient of all nutrients except copper and iron. Diet of CSWs (comparing among nutrients) was severely deficient in calcium, vitamin A, fat, zinc and vitamin C and met only 21.60%, 27.14%, 32.2%, 43.48% and 43.78% RDA respectively (IOM). The mean apparent intake levels of energy by CSWS (1362.79 kcal) were not up to the mark and were equivalent to 61% adequacy when compared with (ICMR, India, 2010) the requirements for the nutrients. The lowest proportion of adequacy of nutrients was observed in vitamin A (31.66% adequacy of RDA) then in fat (met only 32.2% of RDA), zinc (fulfilled 39.13% RDA), calcium (39.69% adequacy), while dietary copper (met 213.75% of RDA) intake was higher than requirement. And apparent intake level of iron was equivalent to 94.43% adequacy when compared with the requirement of National Institute of India (ICMR, 2010) and was equivalent to 110.17% when compared with the requirement of National Institute of Medicine (IOM, USA). Similar findings were observed also for vitamin C (ICMR-40 mg vs. IOM-75 mg) and calcium (ICMR-600 mg vs. IOM-800 to 1100 mg) as RDA amounts were remarkably different between these two institutes (ICMR vs. IOM). However apparent intakes of other macronutrients like protein (met 72.86% of RDA) and carbohydrate (fulfilled 89.76% of RDA) were not reached in full adequacy of requirement levels (table-3).

2.2.3.4 Comparison of nutrient intake of CSWS with national data

Comparison of nutrient intake of CSWS with national data (table-4, 5 & 6) showed that all nutrients intake by CSWs were lower than national average intake (NMSS, 2012) except iron intake which was higher not only in quantity (CSWs: 19.83 mg vs. national: 7.42) but also by quality (table-5 & 6). It is important to mention that as mean phytic acid (492.86 mg), calcium intake (237.63 mg) and mean phytate to zinc molar ratio (7.70) of the diet of CSWs was pretty low, so most probably it could not affect adversely on the iron and zinc absorption of CSWs. Mean phytate to zinc molar ratio (7.70) of the diet of CSWs was lower than national ratio, 12 (NMSS, 2012). Moreover, 41.4% of CSWs had diet containing <5 phytate to zinc molar ratio while nationally only 2.1% slum people had diet containing <5 phytate to zinc molar ratio. Also

42.2% CSWs had diet containing 5-15 phytate to zinc molar ratio but it was nationally 64.1% for slum people (table-6).

2.2.3.5 Frequency distribution, RDA attainment (<50% and ≥ 50%) of different nutrient by CSWs

The apparent intake of fat (8.0 g), calcium (237.63 mg), vitamin A (189.96 µg), zinc (3.91 mg) and vitamin C (32.82 mg) were insufficient to satisfy the recommended intakes for ≥50% of RDA. All CSWS could not even fulfilled >50% of RDA of fat, rather absolutely 100% CSWs met only <50% of RDA of fat, while apparent intake of calorie (1362.79 kcal), protein (40.07 g), carbohydrate (275.22 g), copper (2.89 mg) and iron (19.83 mg) were satisfied the requirements of ≥50% of RDA of most (70.2-92%) of the CSWs (table-7).

Dietary 'calcium' and 'vitamin A' intake by CSWs was the highest proportion of nutrient (IOM, USA) with insufficient apparent intake, secondly 'fat', RDA adequacy of which met only 32.2 % (ICMR, 2010) and almost all (100%) CSWs fulfilled their fat RDA by <50% and none remain left to have ≥50% of fat RDA. Over 80.9 % CSWs had attained <50% of RDA of vitamin A while rest i.e. 19.1% had apparently ≥50% of RDA attainment. The probability of inadequate intake of zinc was also very high according to the estimates based on our dietary data of CSWs. As observed in table-7, 71.3% of the population was identified as having a high probability of <50% of RDA of zinc intake. Even though apparent intakes of vitamin C attained 82% (accordingly ICMR, 2010) of RDA, almost half (47.9%) of CSWs was at risk for <50% RDA attainment of this antioxidant vitamin.

In addition, frequency distribution of 'dietary intake of different nutrients' by CSWs (table-7) also shows that vast majority (65.8%) of CSWs consumed within range of 1000-4000 µg zinc (or less than half of RDA) and a very small segment (5.3%) fulfilled their RDA (8000-9000 µg). Nevertheless, CSWs consumed slightly lower total mean zinc (CSWs 3913.24 µg/day vs. slums 4230 mcg/day) and remarkably more zinc intake from animal sources than the participants of National level Survey (NMSS, 2011-12) (table-7)

2.2.3.6 Influence of dietary nutrients on serum nutrients level

It was observed from table-8 that dietary copper and serum copper (r=.225, P=.020) and dietary zinc and serum zinc (r=.781, P=.000) were significantly, positively correlated. In addition, higher copper intake (>4-fold than RDA/ >3600 µg) was associated with higher serum copper level [F (2.104) =3.321, P=.040]. Similar positive effect was also observed for dietary zinc [F (2.104) =30.11, P=.000] and dietary protein. It was observed that >50 g dietary protein intake was associated with higher serum IgG. Dietary protein and serum IgG correlated positively.

2.2.3.7 Correlation (2-tailed) among different dietary nutrients

Dietary intake of "calorie (kcal)" had highly significant (for all P<0.01) strong positive correlation with all macro (fat, protein and carbohydrate) and micro nutrients (calcium, zinc, copper, iron, vitamin c and retinol.). Similar findings were observed for dietary "carbohydrate" and "fat" except fat insignificantly correlated with vitamin C (P=.072). Dietary protein had highly significant (for all P<0.01) strong positive correlations with calcium (r=.658) and all micro minerals (Cu=.558, Zn=.281 & Fe=.458) and with vitamin C (r=.226) and vitamin A (r=.246). In addition, dietary calcium had highly significant (for all P<0.01) positive correlations with iron (r=.232), zinc (r=.093), Copper (r=.166), vitamin C (r=.229) and vitamin A (r=.192). Also, dietary zinc had highly significant (P<0.01) positive correlations with Copper (r=.319 and iron (r=.162) and was significant vitamin A (P=.027, r=.088) except for vitamin C (P=.75.). With the exception for vitamin C (P=.537), there were strong positive correlations of copper with iron (r=.213, P=.000) and vitamin A (r=.132, P=.001). Similarly, iron with vitamin c (r=.621, P=.000)), iron with vitamin A (r=.490, P=.000) and vitamin C and vitamin A (r=.646, P=.000) were strongly correlated together (not shown in table form).

2.2.3.8 Regression analysis (MLoR models) of different dietary nutrients (≥50% of RDA)

Calorie: It was observed that (table-9) CSWs who did not skip meal were 2.25 times more likely to attain ≥50% adequacy of calorie as compared to CSWs who skipped meal in a day [AOR 2.254 (1.304-3.893), P=.004] (table-9).

Protein: In MLoR model of protein, marital status, income>2001 taka and no meal skipping in a day were associated with ≥50% RDA of protein. Married CSWs had 2 times higher odds of meeting ≥50% RDA of protein whenever compared with widowed/divorced/abandoned CSWs [AOR 1.79 (1.074-2.966), P=.025].

Copper: In MLoR model of copper (table-11), 'no abortion cases' and ≥6 year's length of profession were associated with ≥50% RDA of copper. Commercial sex workers (CSWs) having no history of abortion were 3.56 times more likely to attain ≥50% RDA of copper as compared to CSWs had history of abortion [AOR 3.564 (1.080-11.76), P=.037].

Iron: 'Introductory age (10-17 y) at sex trade' and 'marital status' both were associated with higher or ≥50% RDA of iron (table-12) and were strong covariates in MLoR model of **iron** as two were border line significant in simple logistic models but significantly remained in MLoR models.

2.2.3.9 Regression analysis (MLoR models) of different dietary nutrients (<50% of RDA)

Zinc: In MLoR model (table-13), having children and STIs were associated with <50% RDA of zinc. 'Having children' of CSWs was associated with 1.57 times higher odds of attaining <50%

RDA of dietary zinc as compared to CSWs who did not have children [AOR 2.065 (1.07-2.32), P=.022]. Moreover, CSWs infected with STIs also associated with <50% RDA attainment of dietary zinc, though the effect could not reach at significant level (P=.095).

Calcium: adjusted model of calcium postulated that extreme income (1000-2500 BDT) earner and lower income earner (2501-5000 BDT) CSWs were respectively 1.9 and 2.6 times more likely to attain <50% of adequacy of calcium than CSWs who earned >5000 BDT per month (table-14).

Retinol: In MLoR model (table-15) of retinol, only one variable i.e. 'category of CSWs' remain associated with <50% RDA of retinol. Interestingly, hotel-based CSWs (HBCSWs) were almost 2 times more likely to attain <50% RDA of retinol as compared to floating or street-based CSWs [AOR 1.695 (1.130-2.543), P=.011]. As only one variable remained in the model of vitamin A, so 'Homer-Lemeshaw goodness of fit' for model showed 'zero'.

Vitamin C: In MLoR model (table-16) of vitamin C, two variables namely nutrition risk index (NRI) and 'category of CSWs' remain associated with <50% RDA of attainment of dietary vitamin C.

Table-1: Mean food consumption of CSWs (gm/person/day) by food groups (n=635).

Food item (gm)	No. of CSWs consumed each foods (multiple responses) (n %)	Mean amount of food items consumed by CSWs (gm/person/day)	National[2] level consumption (gm/person/day)	
			Only Urban	National
Rice	640 (20.0)	294.28	406.3	435.9
Other cereals	237 (7.54)	70.89	30.0	24.6
Total cereal plant	**n=877 (27.54%)**	**365.17 (37.69%)**	**436.9 g**	**461.1 g**
Pulses	307 (9.64)	25.72	18.5	15.9
Green leafy vegetables	218 (6.85)	113.23	33.5	36.4
Non-leafy vegetables	603 (18.93)	75.12	234.4	232.1
Fruits	47 (1.48)	65.56	22.5	20.4
Oils	**47 (1.48)**	**4.0**	**25.3**	**21.7**
Miscellaneous	273 (8.57)	66.75	10.6	10.8
Total non-cereal plant	**n=1495 (46.94%)**	**308.59 (31.85%)**	**344.8 g**	**337.3 g**
Fish	434 (13.63)	64.74	137.0	126.7
Meat	88 (2.76)	81.18	28.6	24.9
Egg	125 (3.93)	90.35	8.6	7.1
Milk	166 (5.21)	58.91	17.1	16.9
Total animal food	**n=813 (25.53%)**	**295.25 (30.47%)**	**191.3 g**	**175.6**
Total (sum)	n =3185 (100%)	969.01 gm (100%)	-	-
Total (Mean)	**n=635**	**545.39±165.34**	**907.8 g**	**914.9 g**

Nutrition, Health, Demographic Survey, Bangladesh, 2011 (Akhtaruzzaman et al, NHDS, BD, 2011)[2]

Table-2: Per day mean foods and nutrients consumption of CSWs (n=635) by food groups.

Mean intake of Food groups	Mean weight of food consumed by CSWs (gm)	Mean consumption of different nutrients (gm) by CSWs										Anti-nutrien
		Calorie (Kcal)	Protein (gm)	Fat (gm)	CHO (gm)	Ca (mg)	Copper (µg)	Zinc (µg)	Iron (mg)	Vita A (µg)	Vit C (mg)	Phytic acid (mg)
Rice	294.28 n=640	1047.6	18.86	1.17	232.5	26.72	1492.2	907.04	11.82	0.00	0,0	347.58
Other Cereals	70.89 n=237	242.33	8.48	1.14	9.59	31.89	337.16	1429.4	7.39	1.03	0.0	220.14
Total cereal intake	**365.17 n=877**	**1289.9**	**27.34**	**2.31**	**282.1**	**58.61**	**1829.4**	**2336.5**	**19.2**	**1.03**	**0.00**	**567.72**
Pulse	25.72 n=307	89.04	6.31	0.32	15.20	17.57	405.96	999.46	1.36	3.26	0.03	135.10
Leafy vegetable	113.23 n=218	40.86	3.69	0.34	17.90	136.68	312.67	741.65	7.47	411.71	77.44	6.31
Other vegetable	75.12 n=603	53.18	1.16	0.42	11.47	12.67	182.97	341.45	0.66	4.46	13.43	10.55
Fruits	65.56 n=47	63.61	0.46	0.46	14.56	11.28	41.49	411.05	0.56	3.26	15.62	13.59
Oils	5.39 n=47	48.50	0.00	5.39	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.0
Miscellaneous	23.57 n=273	50.00	0.73	0.0	10.73	3.24	0.44	0.66	0.15	0.08	0.08	-
Total non-cereal plant intake	**308.59** n=1495	**345.19**	**12.35**	**6.93**	**69.86**	**181.44**	**943.53**	**2494.3**	**10.2**	**422.74**	**106.6**	**165.55**
Fish	64.74 n=434	99.73	11.83	4.82	1.80	197.95	246.69	307.15	1.0	1.27	9.58	-
Meat	81.18 n=88	90.46	19.63	1.32	0.00	14.24	2057.5	1346.4	0.31	90.35	0.78	-
Egg	90.35 n=125	158.07	12.12	12.22	0.40	59.17	2662.5	1526.7	2.34	123.33	0.0	-
Milk	58.91 n=166	82.70	4.16	4.69	5.98	154.34	15.90	577.28	0.16	13.78	1.38	-
Total animal intake	**295.25** n=813	**430.96**	**47.74**	**23.05**	**8.18**	**425.7**	**4982.6**	**3757.5**	**3.81**	**228.72**	**11.74**	**-**
Mean Total	**545.39 gm**	1362.79±53.3	40.07 ±2.42	8.05 ±2.73	275.2 ±9.90	237.63 ±63.41	2885.62 ±288.43	3913.2 ±336.2	19.83 ±1.14	189.96 ±90.71	32.82 ±16.89	492.86 ±19.06

Table-3: Comparison of mean nutrients intake of studied population (CSWs) with reference

Name of the nutrients consumed by CSWs (n=635)	Ranges of the dietary nutrients	Mean nutrient Intake of CSWs	Comparison of the mean nutrient requirements per day per person (moderately active, age 19-50) of the studied population with the standard RDA references.			
			[1]ICMR, 2010	RDA met (%) by CSWs	[2]IOM, 2004	RDA met (%) by
Calorie (K cal)	58.76–3277.49	1362.79±53.26	2230	61.11	-	-
Protein (gm)	3.79–198.33	40.07±2.42	55.0	72.86	46	87.11%
Fat (gm)	0.19–175.01	8.05±2.73	25	32.2	-	-
Carbohydrate (gm)	16.26–744.10	275.22±9.90	306.63	89.76	-	-
Calcium (mg)	6.31–6423.78	237.63±63.41	600	39.61	800-1100#	21.60%
Copper (µg)	115.03–94515.0	2885.62±288.4	1350	213.75	890-900#	320.62%
Zinc (µgm)	1013.0–21000.0	3913.24±336.2	10,000	39.13	8000-9000#	43.48 %
Iron (mg)	0.03–113.84	19.83±1.14	21	94.43	15-18#	110.17%
Vitamin A (µg)	0.00–7501.6	189.96±90.71	600	31.66	700	27.14%
Vitamin C (mg)	0.00–842.14	32.82±16.89	40	82.05	65-75#	43.76%

[1]ICMR, NIN, India. [2]IOM, USA

#About 20.3% (n=129/635) CSWs were <18 years and lower limit of copper, vitamin C and iron for <18 y and upper RDA[1, 2]

Table-4: Comparison of mean nutrients (macro and micronutrients) intake of CSWs with National average

Nutrients n=244	Mean nutrient Intake of CSW	National level consumption (gm/ person/day)		
		[1]NHDS, 2011		[2]NMSS, 2011-12
		Only Dhaka	National Urban	
@Calorie (K cal)	1362.79±53.26	2055.2 kcal	2086.8	-
Protein (gm)	40.07±2.42	56.3 gm	59.4	-
Fat (gm)	8.05±2.73	33.6 gm	33.6	25.9 g oils/person/day -slums
Carbohydrate (gm)	275.22±9.90	375.1 gm	381.6	-
Calcium (mg)	237.63±63.41	499.9 mg	511.5	-
Copper (micro gm)	2885.62±288.43	-	-	-
Zinc (micro gm)	3913.24±336.17	-	-	4057.14 (mean)-slums
Iron (mg)	19.83±1.14	24.0 mg	25.0	7.83 (mean)-slums
Vitamin A (µg)	189.96±90.71	899.9µg	778.8	412.5 (median)-slums
Vitamin C (mg)	32.82±16.89	70.2 mg	69.8	-

Nutrition, Health, Demographic Survey, Bangladesh, 2011 (NHDS, BD, 2011)[1]
National micronutrient status survey (NMSS, 2011-12)[2]
NPNL=non pregnant non lactating women.

Table-5: Dietary micronutrients intake of CSWs and NPNL women

NPNL vs. CSWs	Nutrients /day	Intake patterns	Total intake	Plant sources	Animal sources
NMSS, 2011-12 NPNL women	**Iron (mg) (mean)**	National	7.42	6.25	1.16
		Urban	8.24	7.06	1.18
		Slums	7.83	6.74	1.09
	Zinc (µg) (mean)	National	4242.86	2942.86	1300.0
		Urban	4614.29	3271.43	1342.86
		Slums	4057.14	2900.0	1157.14
	Vitamin A (µg) (Median)	National	372.1	285.8	31.8 µg
		Urban	467.2	392.3	40.2
		Slums	412.5	373.2	30.3
Study women CSWs	**Nutrients /day**	**Mean intake**		**Plant sources**	**Animal sources**
	Iron (mg)	19.83±1.14		(19.2+10.2=29.2 mg) (Cereal+ non-cereal plant)	3.81 mg
	Zinc (µg)	3913.24±336.2		(2336.47+2494.27= 4830.74) (Cereal+ non-cereal plant)	3757.51 µg
	Vitamin A (µg)	189.96±90.71		(1.03+422.74=423.77) (Cereal+ non-cereal plant)	228.72 µg

National micronutrient status survey (NMSS), 2011-12.
NPNL=non pregnant non lactating women

Table-6: Comparison of nutrient intake of CSWs and other dietary parameters with National Data

Daily Mean foods and Nutrients intake gm/person/day N=635	Mean Plant sources Total cereals	pulses/ vegetables/fruits/others	Plant Total (sum)	Animal sources	Total dietary intake (Mean)	% of RDA[1] met	National surveys[2,3] 2011-12
Mean Weight of the foods (gm) consumed by CSW/day	365.17	308.59	673.76	295.25	545.39 gm	-	[2]Urban: 907.8 gm
Calorie (K cal/day/person)	1289.92	345.19	1635.11	430.96	1362.79	*61.1%	[2]Dhaka-female: 2055.2 kcal
Protein (gm/day/person)	27.34	12.35	39.69	47.74	40.07	87.11%	[2]Dhaka-female: 56.3 g
Fat (gm/day/person)	2.31	6.93	9.24	23.05	8.05	*32.2%	[2]Dhaka-female: 33.6 g
Carbohydrate (gm/day/person)	282.1	69.86	351.96	8.18	275.22	*89.76%	[2]Dhaka-female: 375.1 g
Calcium (mg/day/person)	58.61	181.44	240.05	425.7	237.63	23.76%	[2]Dhaka-female: 499.9 g
Copper (mcg/day/person)	1829.39	943.53	2772.92	4982.6	2885.62	320.62%	-
Zinc (mcg/day/person)	2336.47	2494.3	4830.7	3757.5	3913.24	43.48%	[3]Slums: 4230 mcg/day (2900 mcg for plant, 1330 animal, RDA met 47.0%)
Iron (mg/day/person)	19.2	10.2	29.4	3.81	19.83	110.17%	[3]Slums: 7.83 mg/day (6.74 mg plant, 1.09 animal sources) [2]Dhaka-female: 24.0 g
Vitamin A (µg/day/person)	1.03	422.74	423.8	228.72	189.96	27.14%	[2]Dhaka-female: 899.9 µg [3]Slums median-412.5 µg, slum-animal-30.3 µg Slum-Plants-373.2 µg National-372 µg
Vitamin C (mg/day/person)	0.0	106.6	106.6	11.74	32.82	43.76%	[2]Dhaka-female: 70.2 mg
Other parameters related to micro-minerals							
Anti-nutrients Phytic acid (mg/day/person)	567.72	165.55	733.27 (sum)	NA	492.86 (mean)		-
Mean Phytate to zinc molar Ratio 95% CI	7.53±7.11 (6.22–8.78)	1.18±1.4 (.92–1.4)	7.70±8.35 (7.05–8.4)	NA	7.70		[3]Slums:13.45 (CI 12.7–14.1) National 12.0 (10.6-13.5)
Grades[1] of phytate-Zinc molar ratio in CSWs <5 ratio 5–15 >15.0	**(% n)** 41.4 (263) 42.2 (268) 16.4 (104)	-	-	-			**[3]Grades[1] of phytate-Zinc molar ratio in slums** <5 ratio 2.1% 5-10 64.1% >15.0 33.7%
Dietary Zinc to Copper ratio	1.277	2.64	1.74	1.08	1.71±0.9		-

[1]IOM
[2]Nutrition, Health, Demographic Survey, Bangladesh, 2011 (NHDS, BD, 2011)
[3]National Micronutrients status survey, Bangladesh, 2011-12 (NMSS, 2011-12)
*ICMR, India

Table-7-: Frequency distribution and RDA attainment of different dietary nutrients by CSWs

Dietary intake of different nutrients	n=635	Mean and Mean ±SD	95% CI of Dietary nutrients	RDA attainment for dietary nutrients by CSWs		
				@Net RDA met by CSWs	Met <50% RDA % (n)	Met ≥50% of RDA
Calorie (Kcal)						
58.76 to1000.0	19.2 (122)	1362.79±53.3	1321.13–1405.	61.11%	29.8 (189)	70.2 (446)
1000.1 to 1500.0	37.3 (237)					
1500.1 to 1804.0	22.7 (144)					
1805.1 to 2122.0	14.0 (89)					
2122.1 to 4277.5	6.8 (43)					
Protein (gm)						
3.79 to 45.0	59.5 (378)	40.07±2.42	38.55–41.62	87.11%	16.9 (107)	83.1 (528)
45.1 to 50.0	14.6 (93)					
50.1 to 198.33	25.8 (164)					
Fat (gm)						
0.9 to 7.5	58.0 (368)	8.05±2.73	7.34–8.80	32.2%	100% (635)	0.0 (00)
7.6 to 10.0	7.6 (48)					
10.1 to 20.0	24.4 (155)					
20.1 to 175.0	10.1 (64)					
Carbohydrate						
16.26 to 250.0	38.7 (246)	275.22±9.90	267.15–283.42	89.96%	8.0 (51)	92.0 (584)
250.1 to 280.0	9.8 (62)					
280.1 to 744.0	51.5 (327)					
Calcium (mg)						
6.231 to 250.0	62.5 (397)	237.63±63.4	218.88–257.15	23.76%	86.6 (550)	13.4 (85)
250.1 to 500.0	24.1 (153)					
500.1 to 1000.0	12.0 (76)					
>1000.1	1.4 (09)					
Copper (mcg)						
00 to 900	4.1 (26)	2885.62±288	2686.67–2968.	320.62%	1.9 (12)	98.1 (623)
900.1 mcg to 1800	19.4 (123)					
1800.1 to 3600	50.8 (322)					
>3600.1 to 2221.59	25.8 (164)					
Zinc (mcg)						
1000.0 to 4000.0	65.8 (411)	3913.24±336	3736.52–4094.0	43.48%	71.3 (453)	28.7 (182)
4000.1 to 7999.0	29.0 (181)					
8000.1 to 9000.0	1.8 (11)					
9000.1 to 21000.0	3.5 (22)					
Iron (mg)						
.03 to 7.5	4.9 (31)	19.83±1.14	19.1–20.58	110.17%	8.2 (52)	91.8 (583)
7.6 to 18.0	43.9 (279)					
18.1 to 113.84	51.2 (325)					
Vitamin C (mg)						
0.0 to 25.0	52.9 (336)	32.82±16.89	29.25–36.59	43.76%	65.0 (413)	35.0 (222)
25.1 to 50	21.3 (135)					
50.1 to 75	6.8 (43)					
>75.1 to	19.1 (121)					
Retinol (µg)						
0.0 to 180.18	72.0 (457)	189.96±90.71	163.92–216.38	27.14%	80.9 (514)	19.1 (121)
180.2 to 450.45	13.2 (84)					
450.5 to 700.0	7.6 (48)					
700.1 to 2252.55	7.2 (46)					

@IOM, USA except calorie, fat and carbohydrate (ICMR, 2010)

Table-8: Influence of the dietary intake of nutrients on their respective serum micronutrients

Dietary Nutrients /Nutrients consumed by CSWs	CSWs % n	Serum nutrients Mean ±SD	95% CI	Statistical analysis	
Dietary Protein (gm) n=495		**Serum TP (g/L)**	**lower–upper**	**ANOVA**	**Correlation**
3.79 to 45.0	60.0 (297)	52.24±5.90	48.31–56.33	F (2,492)=.574	r= –.032
45.1 to 50.0	15.3 (76)	48.70±7.02	40.60–57.52	P=.563	P=.480
50.1 to 198.33	24.7 (122)	48.82±6.25	42.76–55.29		
Dietary Copper (µg) n=107		**Serum cu µmol/L**			
115.0 to 1800	22.43 (24)	16.57±1.34[t]	14.62–18.73	F(2.104)=3.321	r=.225
1800.1 to 3600.0	55.14 (59)	18.54±1.32	17.26–19.90	P=0.040	P=.020
3600.1 to 9451.59	22.43 (24)	20.83±1.49[t]	17.60–24.67		
Dietary Zinc (mcg) n=107		**Serum zinc µmol/L**			
1000.0 to 4000.0	42.99 (46)	10.49±1.29	9.74–11.30	F(3.103)=	r=.781
4000.1 to 7999.0	27.10 (29)	13.92±1.43	12.15–15.95	30.110	P=.000
8000.1 to 9000.0	15.89 (16)	19.98±1.49	16.16–24.71	P=.000	
9000.1 to 21000.0	14.02 (16)	27.60 ±1.90[t]	19.60–38.86		
Dietary Iron (mg) n=107		**Serum Iron µmol/L**			
.03 to 18.0	53.27 (57)	19.99±1.59	17.08–23.13	F(1.105)=2.123 P=.148	r= –.110 P=.258
18.1 to 113.84	46.73 (50)	16.88±1.30	13.99–20.04		
Dietary Retinol (µg) n=107		**Serum retinol µmol/L**			
0.0 to 180.18	76.64 (82)	0.53±0.10	0.43–0.63	F(2.104)=2.029	r=.019
180.2 to 450.45	10.28 (11)	0.85±0.03	0.66–1.06	P=.137	P=.815
450.5 to 2252.55	13.08 (14)	1.58±0,09	0.35–0.87		
Dietary Protein (gm) n=107		**Serum IgG µmol/L**			
3.79 to 45.0	64.49 (69)	2.13±0.04	0.97–2.29	F(2.104)=3.130	r= .078
45.1 to 50.0	14.95 (16)	1.32±0.09	0.80–2.45	P=.048	P=.427
50.1 to 198.33	20.56 (22)	5.61±0.09	1.14–8.08		
Dietary Protein (gm) n=107		**Serum IgA µmol/L**			
3.79 to 45.0	64.49 (69)	0.49±0.01	0.27–0.71	F(2.104)=1.189	r= .051
45.1 to 50.0	14.95 (16)	0.26±0.07	0.17–0.35	P=.309	P=.604
50.1 to 198.33	20.56 (22)	0.72±1.01	0.18–1.25		
Dietary Protein (gm) n=107		**Serum IgM µmol/L**			
3.79 to 45.0	64.49 (69)	0.40±0.04	0.24–0.55	F(2.104)=.095	r= –.036
45.1 to 50.0	14.95 (16)	0.45±0.03	0.05–0.94	P=.909	P=.712
50.1 to 198.33	20.56 (22)	0.47±0.08	0.12–0.81		

[t]This category (within variable) was significantly (P<0.05) different than other categories in Post Hoc test.

Table-9: Factors associated with ≥50% RDA attainment of dietary calorie by CSWs (MLoR)

Explanatory variables	All CSWs n=635 % n	0=<50% RDA of calorie n=189	1=≥50% RDA of calorie n=446	@Unadjusted Odds Ratio (95% CI) (lower-upper)	Adjusted model of RDA for kilo calorie		
					Beta coefficient (B), SE	Odds Ratio (95% CI) (lower-upper)	P-value
BMI (Kg/m²)							
Underweight (r)	22.7 (144)	24.9 (47)	21.7 (97)	1			
Normal	60.9 (387)	64.0 (121)	59.6 (266)	1.065 (.707-1.604) P=.762	-	No longer remained in the model	
Overweight	16.4 (104)	11.1 (21)	18.7 (83)	1.915 (1.059-3.462) **P=.031***			
Mass media exposure							
No items (r)	52.6 (334)	57.7 (109)	50.4 (225)	1	-	1	-
TV/radio/VCD	31.7 (201)	32.2 (61)	31.4 (140)	1.112 (.762-1.622) P=.582	B= -.006 SE=.198	.994 (.674-1.466)	.975
Had all items	15.7 (100)	10.1 (19)	18.2 (81)	2.055 (.192-3.578) **P=.010**	B=-.592 SE=.285	1.807 (1.033-3.2)	**.038***
Meal skipping							
Yes (r)	9.76 (62)	15.9 (30)	7.2 (32)	1	-	1	-
No	90.24 (573)	84.1 (159)	92.8 (414)	2.441 (1.436-4.150) **P=.000****	B=.813 SE= .279	2.254 (1.304-3.9)	**.004****

Legend: Cross tabulation, Simple Logistic Regression (SLoR) @
Backward stepwise Multiple Logistic Regression Model (MLoR)
Hosmer-Lemeshaw goodness of fit for RDA of calorie model (n=635): $\aleph^2$=.917, df=2, P=.286
Model summary: -2 Log likelihood (-2LL) ratio=757.113, Nagelkerke R-square=.035
Model coefficient: Chi-square ($\aleph^2$)=15.692, df35, P=.001
Overall predicted percentage from classification table of dietary calorie (DV) was 70.0% (cut value is 50%).
(r)= reference category. P<0.05*, P<0.01**

Table-10: Factors associated with ≥50% RDA attainment of dietary protein by CSWs (MLoR model)

Explanatory variables	All CSWs n=635 % n	0=<50% RDA of protein n=107	1=≥50% RDA of protein n=528	@Unadjusted Odds Ratio (95% CI) (lower-upper)	Adjusted model of RDA for protein		P-value
					Beta coefficient (B), SE	**Odds Ratio (95% CI) (lower-upper)**	
Marital status							
Married	43.47 (276)	30.8 (33)	46.0 (243)	1.860 (1.151-3.006) **P=.011***	B=.579 SE=.259	1.785 (1.074-2.9)	**.025***
Unmarried	18.27 (116)	23.4 (25)	17.2 (91)	.919 (.535-1.581) P=.761	B=-.226 SE=.293	.798 (.449-1.42)	.439
Widowed/divorced/abandoned (r)	38.27 (243)	45.8 (49)	36.7 (194)	1	-	1	-
Expenditure on Food (BDT)							
500-2000 (r)	67.40 (428)	66.4 (71)	67.6 (357)	1	-	1	-
2001-3500	26.14 (166)	23.4 (25)	26.7 (141)	1.844 (.883-3.850) P=.103	B=.867 SE=.392	2.381 (1.105-5.13)	**.027**
>3500	6.46 (41)	10.2 (11)	5.7 (30)	2.068 (.919-4.655) **P=.079**	B=.906 SE=.428	2.475 (1.069-5.73)	**.034***
Meal skipping							
Yes	9.76 (62)	17.8 (19)	8.1 (43)	.411 (.229-.738) **P=.003****	B=-.930 SE= .317	.395 (.212-.74)	**.003****
No (r)	90.24 (573)	82.2 (88)	91.9 (485)	1	-	1	-

Legend: Cross tabulation, Simple Logistic Regression (SLoR) @
Backward stepwise Multiple Logistic Regression Model (MLoR)
Hosmer-Lemeshaw goodness of fit for RDA of protein model (n=635): $\aleph^2$=1.617, df=6, P=.996
Model summary: -2 Log likelihood (-2LL) ratio=555.113, Nagelkerke R-square=.054
Model coefficient: Chi-square ($\aleph^2$)=20.840, df=5, P=.001
Overall predicted percentage from classification table of protein (DV) was 83.0% (cut value is 50%).
(r)= reference category. P<0.05*, P<0.01**

Table-11: Factors associated with ≥50% RDA attainment of dietary copper by CSWs (MLoR model)

Explanatory variables	All CSWs n=635 % n	0=<50% RDA of copper n=12	1=≥50% RDA of copper n=623	@Unadjusted Odds Ratio (95% CI) (lower-upper)	Adjusted model of RDA for copper		P-value
					Beta coefficient (B), SE	Odds Ratio (95% CI) (lower-upper)	
Abortion cases							
No cases	72.9 (463)	50.0 (06)	73.4 (457)	2.753 (.876-8.655) **P=.083**	B=1.271 SE=.609	3.564 (1.080-11.8)	**.037***
≥1 cases (r)	27.1 (172)	50.0 (06)	26.6 (166)	1	-	1	-
Length of profession							
<1 year (r)	13.5 (86)	25.0 (03)	13.3 (83)	1	-	1	-
1-5 years	54.0 (343)	66.7 (08)	53.8 (335)	1.514 (.393-5.830) P=.547	B=.711 SE=.714	2.037 (.502-8.26)	.319
≥6	32.5 (206)	8.3 (01)	32.9 (205)	7.410 (.760-72.260) **P=.085**	B=2.396 SE=1.18	10.98 (1.07-112.3)	**.043***

Legend: Cross tabulation, Simple Logistic Regression (SLoR) @
Backward stepwise Multiple Logistic Regression Model (MLoR)
Hosmer-Lemeshaw goodness of fit for RDA of Cu model (n=635): $\aleph^2$=.992, df=3, P=.803
Model summary: -2 Log likelihood (-2LL) ratio=110.42, Nagelkerke R-square=.079
Model coefficient: Chi-square ($\aleph^2$)=8.592, df=3, P=.035
Overall predicted percentage from classification table of dietary Cu (DV) was 98.1% (cut value is 50%).
(r)= reference category. P<0.05*, P<0.01**

Table-12: Factors associated with ≥50% RDA attainment of dietary Iron by CSWs (MLoR model)

Explanatory variables	All CSWs n=635 % n	0=<50% RDA of iron n=52	1=≥50% RDA of iron n=583	@Unadjusted Odds Ratio (95% CI) (lower-upper)	Adjusted model of RDA for Iron		
					Beta coefficient (B), SE	Odds Ratio (95% CI) (lower-upper)	P-value
Present age (y)							
≤18	20.3 (129)	25.0 (13)	19.9 (116)	.471 (.203-1.123) **P=.090**		No longer existed in the model	
19-29	48.7 (309)	55.8 (29)	48.0 (280)	.516 (.246-1.085) **P=.081**			
≥30 (r)	31.0 (197)	19.2 (10)	32.1 (187)	1	-	1	-
Introductory age at sex trade							
10-17 y	42.7 (271)	50.0 (26)	42.0 (245)	.396 (.148-1.057) **P=.064**	B= -1.036 SE=.517	.355 (.129-.98)	**.045***
18-24 y	37.8 (240)	40.4 (21)	37.6 (219)	.438 (.161-1.192) P=.106	B= -.951 SE=.522	.386 (.139-1.1)	.068
≥25 y (r)	19 .5 (124)	9.6 (05)	20.4 (119)	1	-	1	-
Marital status							
Married	43.47 (276)	30.8 (16)	44.6 (260)	1.781 (.923-3.437) **P=.085**	B=.708 SE=.341	2.029 (1.04-3.96)	**.038***
Unmarried	18.27 (116)	23.1 (12)	17.8 (104)	.950 (.457-1.973) P=.890	B=.201 SE=.385	1.22 (.575-2.59)	.602
Widowed/divorced/abandoned (r)	38.27 (243)	46.1 (24)	37.6 (219)	1	-	1	-

Legend: Cross tabulation, Simple Logistic Regression (SLoR) @
Backward stepwise Multiple Logistic Regression Model (MLoR)
Hosmer-Lemeshow goodness of fit for RDA of iron model (n=635): $\aleph^2$ =1.759, df=6, P=.940
Model summary: -2 Log likelihood (-2LL) ratio=351.01, Nagelkerke R-square=.032
Model coefficient: Chi-square ($\aleph^2$)=8.856, df=4, P=.065
Overall predicted percentage from classification table of dietary iron (DV) was 91.8% (cut value is 50%).
(r)= reference category. P<0.05*, P<0.01**

Table-13: Factors associated with <50% RDA attainment of dietary zinc by CSWs (MLoR model)

Explanatory variables	All CSWs n=495 % n	0=≥50% RDA of zinc n=147	1=<50% RDA of zinc n=348	@Unadjusted Odds Ratio (95% CI) (lower-upper)	Adjusted model of RDA for zinc: Beta coefficient (B), SE	Odds Ratio (95% CI) (lower-upper)	P-value
Present age (y)							
≤18	17.8 (88)	16.3 (24)	18.4 (64)	1.408 (.862-2.30) P=.172		No longer existed in the model	
19-29	46.3 (229)	44.2 (65)	47.1 (164)	1.395 (.945-2.058) **P=.094**			
≥30 (r)	35.9 (178)	39.5 (58)	34.5 (120)	1			
Category of CSWs							
Hotel	35.96 (178)	29.3 (43)	38.8 (135)	1.51 (1.067-2.140) **P=.020***		No longer existed in the model	
Road/park (r)	64.04 (317)	70.7 (104)	61.2 (213)	1			
Having children							
No (r)	56.36 (279)	48.30 (71)	59.8 (208)	1	-	1	-
Yes	43.64 (216)	51.70 (76)	40.2 (140)	1.473 (1.038-2.089) **P=.030***	B=.454 SE=.199	1.574 (1.066-2.32)	**.022***
STIs							
No	56.4 (279)	50.3 (74)	58.9 (205)	1	-	1	-
Yes (HBV/SY/both) (r)	43.6 (216)	49.7 (73)	41.1 (143)	1.414 (.960-2.083) P=.080	B=.332 SE=.199	1.394 (.944-2.059)	.095

Legend: Cross tabulation, Simple Logistic Regression (SLoR) @

Backward stepwise Multiple Logistic Regression Model (MLoR)

Hosmer-Lemeshaw goodness of fit for RDA of zinc model (n=495): $\aleph^2$=2.658, df=2, P=.265

Model summary: -2 Log likelihood (-2LL) ratio=593.86, Nagelkerke R-square=.024

Model coefficient: Chi-square ($\aleph^2$)=8.296, df=2, P=.016

Overall predicted percentage from classification table of zinc (DV) was 70.3% (cut value is 50%).

(r)= reference category. P<0.05*

SYP=syphilis

Table-14: Factors associated with <50% RDA attainment of dietary calcium by CSWs (MLoR model)

Explanatory variables	All CSWs n=635 % n	0=≥50% RDA of calcium n=85	1=<50% RDA of calcium n=550	@Unadjusted Odds Ratio (95% CI) (lower-upper)	Adjusted model of RDA for calcium		P-value
					Beta coefficient (B),	Odds Ratio (95% CI) (lower-upper)	
BMI (Kg/m^2)							
Underweight	22.7 (144)	20.7 (25)	23.1 (119)	1.482 (.761-2.884) P=.247	B=.431 SE=.348	1.538 (.777-3.045)	.217
Normal	60.9 (387)	60.3 (73)	61.1 (314)	2.024 (1.140-3.594) **P=.016***	B=.723 SE=.300	2.060 (1.144-3.710)	**.016***
Overweight (r)	16.4 (104)	19.0 (23)	15.8 (81)	1	-	1	-
Having children							
No (r)	61.6 (391)	55.4 (67)	63.0 (324)	1	-	1	-
Yes	38.4 (244)	44.6 (54)	37.0 (190)	1.778 (1.123-2.814) **P=.014***	B=.725 SE=.249	2.065 (1.267-3.366)	**.004****
Monthly income (BDT)							
1000-2500	33.9 (215)	29.8 (36)	34.8 (179)	1.408 (.841-2.358) P=.194	B=.624 SE=.280	1.866 (1.079-3.229)	**.026***
2501-5000	27.2 (173)	33.1 (40)	25.9 (133)	2.394 (1.265-4.530) **P=.007****	B=.970 SE=.332	2.638 (1.376-5.056)	**.003****
>50001 (r)	38.9 (247)	37.1 (45)	39.3 (202)	1	-	1	-

Legend: Cross tabulation, Simple Logistic Regression (SLoR) @
Backward stepwise Multiple Logistic Regression Model (MLoR)
Hosmer-Lemeshow goodness of fit for RDA of calcium model (n=635): $\aleph^2$=7.445, df=6, P=.282
Model summary: -2 Log likelihood (-2LL) ratio=477.775, Nagelkerke R-square=.063
Model coefficient: Chi-square ($\aleph^2$)=22.168, df=5, P=.000
Overall predicted percentage from classification table of calcium (DV) was 86.6% (cut value is 50%).
(r)= reference category. P<0.05*, P<0.01**

Table-15: Factors associated with <50% RDA attainment of dietary retinol by CSWs (MLoR model)

Explanatory variables	All CSWs n=635 % n	0=≥50% RDA of retinol n=121	1=<50% RDA of retinol n=514	@Unadjusted Odds Ratio (95% CI) (lower-upper)	Adjusted model of RDA for retinol: Beta coefficient (B),	Adjusted model of RDA for retinol: Odds Ratio (95% CI) (lower-upper)	P-value
Present age (y)							
≤18	20.3 (129)	14.9 (18)	21.6 (111)	2.098 (1.160-3.793) **P=.014***		No longer existed in the model	
19-29	48.7 (309)	43.8 (53)	49.8 (256)	1.643 (1.065-2.542) **P=.026***			
≥30 (r)	31.0 (197)	41.3 (50)	28.6 (147)	1			
Category of CSWs							
Hotel	48.5 (308)	38.0 (46)	51.0 (262)	1.695 (1.130-2.543) **P=.011***	B=.528 SE=.207	1.695 (1.130-2.543)	**.011***
Road/park (r)	51.5 (327)	62.0 (75)	49.0 (252)	1	-	1	-

Legend: Cross tabulation, Simple Logistic Regression (SLoR) @
Backward stepwise Multiple Logistic Regression Model (MLoR)
Hosmer-Lemeshaw goodness of fit for RDA of retinol model (n=635): $\aleph^2$=00, df=0,
Model summary: -2 Log likelihood (-2LL) ratio=111.872, Nagelkerke R-square=.017
Model coefficient: Chi-square ($\aleph^2$)=6.645, df=1, P=.010
Overall predicted percentage from classification table of retinol (DV) was 80.9% (cut value is 50%).
(r)= reference category. P<0.05*

Table-16: Factors associated with <50% RDA attainment of dietary vitamin C by CSWs (MLoR model)

Explanatory variables	All CSWs % n	0=≥50% RDA of vitamin C	1=<50% RDA of vitamin C	@Unadjusted Odds Ratio (95% CI) (lower-upper)	Adjusted model of RDA for vitamin C: Beta coefficient (B), SE	Adjusted model of RDA for vitamin C: Odds Ratio (95% CI) (lower-upper)	P-value
BMI (Kg/m^2), n=635	**n=635**	**n=222**	**n=413**				
Underweight	22.7 (144)	18.5 (41)	24.9 (103)	1.842 (1.083-3.134) **P=.024***			
Normal	60.9 (387)	61.7 (137)	60.5 (250)	1.338 (.861-2.081) P=.196		No longer remained in the model	
Overweight (r)	16.4 (104)	19.8 (44)	14.6 (60)	1			
NRI, n=495	**n=495**	**n=174**	**n=321**				
Well nourished (r)	36.4 (180)	44.3 (77)	32.1 (103)		-	1	-
Malnourished	63.6 (315)	55.7 (97)	67.9 (218)	1.680 (1.149-2.457) **P=.007**	B=.508 SE=.195	1.661 (1.135-2.433)	**.009***
Category of CSWs, n=635	**n=635**	**n=222**	**n=413**				
Hotel	48.5 (308)	43.7 (97)	51.1 (211)	1.346 (.970-1.869) **P=.076**	B=.359 SE=.202	1.432 (.964-2.128)	**.075**
Road/park (r)	51.5 (327)	56.3 (125)	48.9 (202)	1	-	1	-

Legend: Cross tabulation, Simple Logistic Regression (SLoR) @
Backward stepwise Multiple Logistic Regression Model (MLoR)
Hosmer-Lemeshaw goodness of fit for RDA of vitamin C model (n=495): $\aleph^2$=2.490, df=2, P=.288
Model summary: -2 Log likelihood (-2LL) ratio=631.539, Nagelkerke R-square=.028
Model coefficient: Chi-square ($\aleph^2$)=10.357, df=2, P=.006
Overall predicted percentage from classification table of dietary vitmin C (DV) was 70.3% (cut value is 50%).
(r)= reference category. P<0.01*

NRI (Nutrition Risk Index) =1.519*SERUM ALBUMIN+41.7*PRESENT WT/USUAL WT (Le Banh, 2006)

2.2.4 Discussion

Some research studies addressed different Bangladeshi women of both rural and urban (Islam et al, 2003, 2004; Combs and Hasan, 2005; Kabir et al, 2010) settings. And also some valuable researches (present and past) on 'female garments workers' focusing on bone mineral calcium (Islam et al, 2013, 2008), and vitamin A of garments workers (Ahmed et al, 1997) were carried out who also belonged to general population. In this discussion, this study population (CSWs) was compared with these women (Islam et al, 2003, 2004, 2008, 2013; Combs and Hasan, 2005; Kabir et al, 2010; Ahmed et al, 1997) and also with slum population (NMSS, 2011-12) and found this special segment of population was far from slum dwellers in terms of economy, lifestyle, human rights, ethics, food habit, health status and nutrients intake. Combs and Hasan (2005) studied the local food system in southeastern Bangladesh, and found among a sample of 480 households, the dominated foods produced included rice and starchy vegetables, such as potatoes and gourds. Very few micronutrient-dense foods were consumed on a regular basis. Only one third of households used pulses (bean, peas, lentils), and one quarter used leafy vegetables. Households very rarely used meat, eggs, dairy products, or fruits. However, fish was consumed in 73 households that lived close to water (Combs and Hasan, 2005). If this is the scenario of the food and nutrients intake of normal population of Bangladesh what could be the 'food and nutrients intake level' of CSWs is easily guessable.

Calorie intake by CSWs: as reflected in the table-2, 3 & 4, the apparent daily intake of energy fluctuated from 58.76 kcal to 3277.49 kcal with 1362.79 kcal as mean intake which was, of course, inadequate for RDA of energy. The low intake of energy of CSWs is could be explained by the remarkably low mean fat (8.05 g) intake as well as by extremely low amount of oil consumption (only 5.9 g) by CSWs. Energy insufficiency could be ascertained by examining trends in weight loss over time which also reflected in lower BMI of floating CSWs (chapter-2.1, table-1). As fat intake of CSWs met less than half of RDA (32.2%, ICMR), so calorie intake was met only 61.1% (RDA, ICMR. 2010), most of which came from carbohydrate (cereals i.e. rice, Ata etc. 1289.92 kcal +other plants 345.19 kcal) (table-2) and it is well known that 'cereals' or rice-based diets are the main source of calorie in most of the Bangladeshi diets (Akhter et al, 2013) which also reflected in different studies (Islam et al, 2004, 2003).

Female CSWs of this study was poor, underprivileged group so as their calorie (mean calorie 1362.79 kcal/day/CSW) as well as other nutrient intake pattern being mostly based on staples (rice and wheat), with a big amount of vegetables and a small amount of animal products (meat, fish, eggs) and very tiny amounts of milk and milk products. Most of the mean calorie derived from cereals but calorie from animal intake (430.96 kcal) was higher than plant intake (345.19 kcal) (table-2). Similar poor energy (1438 kcal) and nutrients consumption was also reported in 'low income NPNL group' of a previous study (Islam et al, 2004, 2003) where low income NPNL group (n=101) was rural destitute women mainly from the households of day laborers, beggars, landless farmers and individuals/farmers with small holdings (owned <1 acre of land) but much lower (1547 kcal) than a recent study on garments workers (Islam et al, 2013). Energy consumption by CSWs was far away from a micronutrient (Bermudez et al, 2012) survey report (Dhaka 2195 kcal) as well as a recent surveys [BIHS, Akhter et al, 2013; Akhtaruzzaman et al, NHDS, 2011 (2055.2 kcal for female-Dhaka)] conducted in Bangladesh. However, even the richest income groups in the FTF zone and the country as a whole consumed insufficient amount of calories and adult women were among the worst off in terms of calorie adequacy reported by a recent survey (Akhter et al, 2013, BIHS).

Poverty measurement by calorie: poverty is the major cause of food insecurity. Different measures (income, expenditure and calorie intake) provide different figure regarding its incidence. Among three available approaches (DCI or direct calorie intake, CBN or cost of basic need and a qualitative approach using perception of respondents) to measure poverty none of one is comparable to each other across time and space (Ravallion and Sen, 1996). For Bangladesh, the minimum calorie threshold is 2122 kcal/person/day, which is known as the 'food poverty line'. Bangladesh Bureau of Statistics (BBS, 2007) identifies people or households failing to acquire this level are termed as 'absolute poor' and those who cannot acquire 1805 kcal/person/day are termed as 'hard-core poor'. In this study, only 6.8% (43/635) CSWs took up to the mark calorie (>2123 kcal), about 79.2% (503/635) and 14.0% (89/635) CSWs were in **'Hard core Poverty** (or consumed ≤1805 kcal/person)' and 'Absolute **poverty** (or consumed ≤ 2122 kcal/person)" respectively (table-7).

Multiple logistic regression (MLoR) model of calorie: MLoR model of calorie (table-9) revealed that, having mass media access and no meal skipping in a day were associated with ≥50% RDA of calorie. CSWs had mass media exposure were 2 times more likely to attain ≥50% RDA of calorie as compared to CSWs who did not have TV/radio/VCD [AOR 1.807 (1.03-3.160), P= .038]. No skipping of meal (no starvation) was associated with 2 times higher odds of having ≥50% RDA of calorie as compared to CSWs who skipped one or two times meal in a day [AOR 2.254 (1.30-3.893), P=.004]. Similar finding was observed in USA (Rose and Oliveria, 1997) that women from in-sufficient household were more likely to consume <50% of the recommended energy intake than were those from food sufficient households.

Protein intake by CSWs: animal protein is 1st class protein as well as pretty good source of iron, calcium, copper, zinc and vitamin A. In this study mean protein intake (40.07 g) of CSWs was not up to the mark (met only 72.86%, ICMR) and far away from national survey average (Akhter et al, 2013, BIHS; Akhtaruzzaman et al, NHDS, 2011) as well as lower than female garments workers (Islam et al, 2013). Surprisingly, animal intake was higher than plant origin was noticed (39.69 g plant and 47.74 g animal) (table-2).

Multiple logistic regression (MLoR) model of dietary protein: in MLoR model of protein, marital status, income>2001 taka and no meal skipping in a day were associated with ≥50% RDA of protein. Married CSWs had 2 times higher odds of meeting ≥50% RDA of protein whenever compared with widowed/divorced/abandoned CSWs [AOR 1.79 (1.074-2.966), P=.025]. Higher expenditure on food either 2001-3500 BDT or >3500 BDT both was associated with 2 times higher odds of acquiring ≥50% RDA of protein as compared to CSWs who spent lower (500-2000 BDT) amount of taka on food. Meal skipping or starvation was also associated with having <50% RDA of protein and meal skippers were 60% less likely to have ≥50% RDA of protein whenever compared with CSWs who did not skip meals in a day [AOR .395 (.212-.735), P=.003] (Table-10).

Fat intake by CSWs: mean fat intake of our study participants was only 8.05 g which was lower than RDA (met only 32.2% RDA adequacy) and also far away from national average (Akhtaruzzaman et al, NHDS, 2011). Slightly higher fat intake (9.9 mg) than CSWs in female garments workers (Islam et al, 2013) and also lower fat intake than RDA even in urban adolescent college girls (Kabir et al, 2010) were reported.

Multiple logistic regression (MLoR) model of dietary fat: In this study, multiple logistic regression analysis was performed (MLoR) to explore which factors/variables influence >50% or <50% different dietary nutrients intake (dichotomous dependent variables) for all nutrients except fat, as no CSWs took >50% RDA of fat and fat intake of all (n=635) CSWs was well below than <50% of RDA.

Copper intake by CSWs: data regarding dietary intake of micro minerals especially copper was scanty. Mean intake of copper by CSWs was 2885.62 mcg/day/person which was very higher than an Italian study (median copper intake for both men and women was only 1500 µg/day/person) (Simona Bo et al, 2008). The major sources of dietary copper of both Italian men and women were dairy products (24.6%), fruit (22.0%), bread (13.4%), fish (12.9%), Pasta (11.8%), vegetables (5.5%) and legumes (5.5%).

In contrast to dietary zinc, mean intake of copper (2885.62 µg) apparently seems to be higher than RDA as DRI set by IOM was only 900 µg per day. But, in reality, maximum foods with few exceptions contain higher level of zinc than copper and these two micro-minerals always maintain a natural balanced ratio, for example among all Bangladeshi green leafy vegetables (GLVs/ shak) most have higher zinc contents than copper except Kalmi shak (swamp morning glory) which contain lesser zinc than copper (Islam SN *et al, 2012*). On the other hand, high copper rich foods are mainly oysters, crabs, Lobsters, mushrooms, sesame seeds, cashew nuts, and chick peas etc. and our study population did eat none of this but 9.64% (n=307) of CSWs consumed lentils (not chick peas) and interestingly, lentils contains 1620.91 mcg copper and 4166 mcg zinc (Islam SN *et al, 2012*). Thus, calculative dietary copper did not necessarily exceeds limit, but other sources of copper like drinking water source, cooking pot made of copper could easily take into account. In our study, most of the drinking water sources were Municipal Corporation's public water supply and respondents did not use copper pots as these are not available in our country, so there was no possible reason to exceed dietary copper intake of CSWs. In spite of 'mean dietary copper' intake (2885.62 µg /person/day) exceeded RDA limit (met 320.62%, IOM), frequency distribution of copper showed that (table-7) most (59.0%) of CSWs consumed double (1800.1 mcg/day/person) to fourfold (3600 mcg/person/day) dietary copper and interestingly, 22.43% CSWs ingested more than four times dietary cu than RDA and other 22.43% (24/107) consumed 115.03 to 1800 mcg (double RDA) dietary cu as well. As copper is an essential micro mineral, so tolerable upper limit (TUL) of dietary copper (only intake through foods) is set to 10000 mcg (IOM, USA; Linus polling institute; WHO). Thus, our study subjects' dietary intake was not only within permissible limit of Cu (range 115.03–9451.59 mcg/person/day) but also reflected in serum copper level (18.34 µmol/L) which was within range of normal level (11.0-22.0 µmol/L, Young, 1998). Furthermore, a controlled study (Hunt and Vanderpool, 2001) using females (USDA Nutrition Center in Grand Forks, North Decota), was observed that lacto-ovo vegetarians took in 1.45 mg of coppers daily of which 33% (0.48 mg) was taken into the body. In contrast, the non-vegetarian took 0.94 mg of copper daily of which 42% (0.39 mg) was taken into the body, thus vegetarians have higher levels of copper in the diet than non-vegetarians. In this study, copper intake by every CSW was found 2885.62 µg (or 2.89 mg/per CSW/day) daily.

Multiple logistic regression (MLoR) model of dietary copper: in MLoR model of copper (table-11), 'no abortion cases' and ≥6 year's length of profession were associated with ≥50% RDA of copper. Commercial sex workers (CSWs) having no history of abortion were 3.56 times more likely to attain ≥50% RDA of copper as compared to CSWs had history of abortion [AOR 3.564 (1.080-11.76), P=.037]. Longer length of the profession (≥6 years) was also attributable for higher RDA attainment of dietary copper of CSWs as compared to <1 year professional length [AOR 10.98 (1.07-112.34), P=.043].

Iron intake by CSWs: mean Iron intake of this study subjects was surprisingly more than (19.83 mg/CSW/day) recommendation level of IOM (15-18 mg) which was also overwhelmingly higher than previous (Jahan and Hossain, 1998) and recent national survey reports [Bermudez et al, 2012 (Dhaka-0.78 mg/household/day); NMSS, 2011-12, (slums-7.83 mg/person/day); BIHS, 2013, Akhter et al] but lower than another national survey [Akhtaruzzaman et al, NHDS, 2011 (Dhaka-24.0 mg/person/day)] and from a recent study of female garments workers in Dhaka (Islam et al, 2013). However, mean intake of iron was also reported higher in a study (Kabir et al, 2010) of urban adolescent college girls.

Although, iron intake of CSWs was up to the mark of RDA, but most of the iron came from cereals (19.2 mg) and then non-cereal plant sources (10.2 mg), only 3.81 mg of Iron provided by animal origin, and animal foods are the richest sources of "Heme Iron". Nevertheless, mean iron consumption (19.97 g) as well as both plant (total plant 29.4 g) and animal (3.81 g) intake of our study group was remarkably higher than national average (National micronutrient survey 2011-12). Plausible explanation for up to the mark intake of iron by CSWs could be (i) as mean phytic acid (492.86 mg) content was pretty low, so most probably it could not affect adversely on the iron absorption (Hunt, 2003; Hallberg, 1981) of CSWs, certainly which also reflected in their serum iron (18.49 µmol/L) for maintaining normal range [(11-29 mg) young, 1998] (ii) moreover, mean dietary calcium was also low (237.63 mg) in CSW's diet which might be causal factor for optimum iron absorption (Hunt, 2003; Hallberg, 1981) (iii) mean vitamin C content (32.82), if compare to RDA of ICMR, India (2010) was pretty good which could enhance iron absorption (iv) Evidence indicates that iron absorption is regulated to some extent by physiological demand. Persons who are iron deficient tend to absorb iron more efficiently and in greater quantities than do normal subjects. Healthy subjects normally absorb only 5 to 10% of the iron (0.75 to 1.5 mg from 15 mg of iron contained in diet) in their foods, whereas iron deficient subject may absorb twice (~3mg from 15 mg of iron contained in diet) that amount (Hunt, 2003; Hunt and Roughead, 2000; Cook, 1990).Thus, because of apparent up-regulation of non-heme iron absorption, non-heme iron contributes more than heme iron to the total amount of iron absorbed in CSWs with low body iron stores (v) heme iron of animal origin (meat, fish & poultry) has higher absorption rate (~15-40%) than non-haem iron of plant origin (~1-15%), thus 'the complement' effect of iron absorption is happened when people eat meals (not a single food), if a small amount of heme iron consumed with a meal where most of the iron is non-heme iron will enhance the absorption of all the iron. Thus, the addition of a quite small amount of heme iron from perhaps fish or meat (like CSWs ate only 3.81 mg animal origin iron) to a large helping of rice or maize containing non-heme iron will result in much greater absorption of iron from the cereal staple (Hunt, 2003; Roughead and Hunt, 2000; Cook, 1990).

Multiple logistic regression (MLoR) model of dietary iron: 'Introductory age (10-17 y) at sex trade' and 'marital status' both were associated with higher or ≥50% RDA of iron (table-12). It was found that, '10-17' years of introductory ages (at sex business) were 64% less likely to attain higher dietary iron of RDA (≥50%) as compared to ≥25 years of introductory age's [AOR 0.36 (.129-.979), P=.045]. Married CSWs had 2 times higher odds of meeting ≥50% RDA of iron whenever compared with widowed/divorced/abandoned CSWs [AOR 2.03 (1.04-3.96), P=.038]. The same effect of marital status was also observed in MLoR model of protein**.** Possible explanation could be protein rich foods are also iron rich foods

Zinc intake by CSWs: the mean zinc intake of CSWs was 3.91 mg/day/person which was surprisingly higher than a micronutrient (Bermudez et al, 2012) survey report (Dhaka 2.18 mg/household/day) but a bit lower than a recent (NMSS, 2011-12) national survey (slums-4.06 mg/person/day) and far away (7.4 mg) from 'Bangladesh Integrated Household survey report' (BIHS, Akhter et al, 2013). In this connection it is noted that, the mean phytic acid (anti-nutrient) content (492.86 mg) and "phytate to zinc molar ratio" (7.70), an indicator of 'zinc bioavailability' has been theoretically calculated to find out whether absorption of zinc hampered by anti-nutrient and found "phytic acid" was pretty low and "phytate to zinc molar ratio" (7.70) was within permissible range [at the same time lower than national survey (NMSS, 2011-12)(slums-13.45)] thereby could not seriously hampered 'zinc absorption' that also reflected in 'pearson's correlation' which showed that 'dietary zinc' and 'serum zinc' level of CSWs were significantly correlated (r=.781, P=.000).

In this study, mean zinc intake was only 3913.24 mcg /person/day which was well below the IZiNCG (2007) recommendations as well as IOM for dietary zinc. Although zinc intake of CSWs was slightly lower but very close to zinc intake (National Micronutrient Status Survey, 2011-12) of national average (4230.0 mcg /day/person) but much lower than a previous Korean study [(Hee et al, 1999) (6410 µg/day/person, did not have phy/zn molar] and a recent Indian study [(Herbst et al, 2014) (median zinc intake 5300 µg/person/day)] where median phytate/zinc molar ratio was remarkably higher (26) than mean phytate/zinc molar ratio (7.70) of this study. As a result, by taking comparatively lower amount of zinc (3913.24 µg /person/days vs. 5300 µg/person/day) than Indian women (Herbst et al, 2014) CSWs managed to absorb more bio-available dietary zinc which also reflected in their serum zinc (CSWs: 14.41 µmol/L vs. Indian women: 10.8 µmol/L). Furthermore, Pearson's correlation (r=.781, P=.000) as well as Multiple Logistic Regression (ML_oR) Model of serum zinc (chapter 4.2) supported the relation between 'dietary zinc' and 'serum zinc'. In this study, "dietary intake of zinc" is an important and strong predictor of serum zinc level.

Possible explanations of discrepancy between 'dietary zinc intake' of CSWs and 'national level intake' could be explained by addressing different points (table-6) regarding zinc intake of CSWs and national participants, **such as: (1)** The dietary total mean zinc which was taken by CSWs (3.91 mg) was a bit lower in quantity (than national average 4.23 mg) but higher in quality, CSWs consumed 1.6 times higher amount of zinc of plant sources (CSWs:4830.74 mcg/day plant origin zinc vs. slums: 2900mcg/day) and 2.9 times higher animal zinc than the national average (CSWs: 3757.51 µg/day animal origin zinc vs. slums:1330 µg/day) **(2)** As, animal origin zinc is more bio-available than plant sources, so zinc easily consumed by CSWs, this also reflected in their mean serum zinc level (14.41±1.67 mmol/L) which was within normal range. Thus CSWs consuming less than half of RDA of dietary zinc but with pretty good amount of animal origin Zn (3757.51 µg/day animal origin zinc) helped them to keep normal serum zinc level (14.41 mmol/L 95% CI:14.48–19.33) which was also higher (1.7 times) than national average (9.67 mmol/L, NMSS, 2011-12). Furthermore, low phytic acid content (492.86 mg/day) in CSW's diet could boost up the bio-availability of dietary zinc **(3)** bio-availability of zinc also enhanced by protein (Hunt, 2003; Sandström et al, 1980:) and positively correlated to zinc absorption but plant sources protein are also generally high in phytic acid, fortunately CSWs consumed more animal protein (47.74 g) than plant protein (39.69 g) (table-2) which also could help them to make zinc more bio-available **(4)** in addition**,** the amount of zinc in a meal, will, in itself affect zinc absorption and with increasing amounts of zinc in a meal, fractional zinc absorption (%) decreases (Lönnerdal, 2000; Sandström and

Cederblad, 1980; Sandström et al, 1980), as dietary zinc intake of CSWs was only 3.91 mg, so their fractional absorption rate might be increased for zinc **(5)** also for long-term zinc intake i.e. zinc status can affect absorption of dietary zinc and low zinc status increased absorption (Istfan et al, 1983), as this study subjects were consuming only 3.91 mg dietary zinc/day, so it could also influenced their zinc absorption **(6)** other indicators of dietary zinc bioavailability are contents of phytic acid and calcium in diet and “phytate to zinc molar ratio”. In this study both mean phytic acid content (492.86 mg/person/day) and mean calcium content (237.63 mg/person/day) was remarkably low and might not hamper zinc absorption. As phytic acid content of this study was low, so mean ‘phytate to zinc molar ratio’ was also low i.e. 7.70 which was less than half of ‘phytate to zinc molar ratio’ of slums in national survey (slums-13.45, national-12.0, NMSS 2011-12) **(7)** Moreover, Grades of phytate-Zinc molar ratio in CSWs revealed that (table-6) diets of the most (268/635) CSWs were within 5-15 grades of ‘phytate to zinc molar ratio’ which was in agreement with national level. In contrast, only 16.4% (104/635) CSWs’ diet was less bio-available (15% absorption) i.e. 16.4% CSWs consumed >15.0 Grades of phytate-Zinc molar ratio diet and another 41.4% (263/635) CSWs consumed <5.0 grades of phytate-Zinc molar ratio containing diet (table-6) resulted that a remarkable portion (41.4%) of CSWs consumed high-zinc bio-available diet that has absorption rate 50-55% (Hunt, 2003).

Thus, in conclusion, though the study population consumed slightly lower amount of total dietary zinc (CSWs: 3913.24 mcg/person/day vs. national: 4230 mcg/person/day) than national level but this consumption of zinc was more bio-available than national level in terms of origin and quality (1.6 times more plant origin and 2.9 times more animal origin than national). Moreover, low calcium and phytic acid contents and low ‘phytate to zinc molar ratio’ (CSWs: 7.70 vs. national: slums13.45) also made dietary zinc bioavailable. Feeding low zinc diets increases zinc absorption in all age groups and that homeostatic mechanism up-regulate zinc absorption and retention so previous intake may therefore has an effect on zinc bioavailability (Lönnerdal, 2000; Sandström and Cederblad, 1980; Sandström et al, 1980).

Although mean dietary zinc intake of CSWs was close to national data (NMSS, 2011-12) but Frequency distribution of zinc showed that 65.8% (n=411/635) CSWs consumed lesser than half of DRI of zinc (IOM) and only 5.3% CSWs took dietary zinc according to RDA (9 mg/day). Similar result also found in an UK based extensive survey[65] where data from more than 2250 dietary interviews were gathered, along with more than 1700 7-day dietary records, the intake data for vitamins and minerals were satisfactory, showing an average intake from food sources and supplements combined that met or exceeded the local recommended daily allowance (RDA) for each individual micronutrient. However, when only dietary intake was considered and when looking at the stratified intake data, significant proportions of the population were found to have intakes below the RDA and 45% women and 43% men’s dietary intake was below the RDA.

[65] Food Standards Agency, 2003

Zinc[66] is an element which cannot be stored in the body (Culha and sangun, 2007; Zarebavani et al, 2012), regular dietary intake of this mineral is important in maintaining the integrity of the immune system (Linus Pauling). Discrepancy of mean dietary zinc intake obtained from one 24-h recall might be due to economic variation, inadequate zinc intake due to poor diet quality and low consumption of flesh foods rich in zinc (Herbst et al, 2014

About 52% of the Indian participants (Herbst et al, 2014) had a low serum zinc concentration according to the International Zinc Nutrition Consultative Group (IZiNCG, 2007) while 24.3% of CSWs of this study had serum Zn <10.1 µmol/L. Diets low in energy with poor bioavailability of dietary zinc is likely to be the primary cause of the high proportion of Indian women with zinc deficiency (Herbst et al, 2014).

Multiple logistic regression (MLoR) model of dietary zinc: in MLoR model (table-13), having children and STIs were associated with <50% RDA of zinc. 'Having children' of CSWs was associated with 1.57 times higher odds of attaining <50% RDA of dietary zinc as compared to CSWs who did not have children [AOR 2.065 (1.07-2.32), P=.022]. Similar effect was also observed for dietary calcium model (table-14). Moreover, CSWs infected with STIs also associated with <50% RDA attainment of dietary zinc, though the effect could not reach at significant level (P=.095).

Calcium intake by CSWs: studies on 'dietary calcium intake of women of childbearing age' are scanty, let alone CSWs. Nevertheless, a recent study (Islam et al, 2013) focused on the association of dietary calcium intake with parathyroid hormone (PTH) of young Bangladeshi female garments factory workers, also another study about vitamin D status of Bangladeshi women of reproductive age (Ann Micka, 2014) , both study focused on poor calcium and vitamin D intake by Bangladeshi women. Adequate dietary calcium intake is very important for optimum bone health (Islam *et al*, 2013). Calcium intake has been identified as one of the several factors that affect high peak bone mass which is inversely associated with bone fragility (Islam et al, 2013; Heaney *et al*, 2000; Matkovic, 1992).

The mean apparent intake of dietary calcium by CSWs (237.63 mg/CSW/day) were not up to the mark and were equivalent to 39.61% adequacy when compared with (ICMR, India, 2010) the Indian requirements (600 mg) for the calcium but that was only 21.60% adequacy according to dietary reference intake (DRI: 800-1100 mg) of IOM (USA). However, intake of calcium by CSWs was much lesser than a recent study on Dhaka-based female garments workers [(Islam et al, 2013), (465 mg)] as well as the national survey [(Akhtaruzzaman et al, 2011, NHDS, BD) (Dhaka- 499.9 mg)]. About 86.6% of study participants consumed <50% of DRI for calcium (IOM, USA) and remarkable portion of them (62.5%) took 6.231 to 250.0 mg

[66] Linus Pauling Institute. Oregon State University. http://lpi.oregon state.edu/mic/micronutrients-health/immunity. accessed 9/11/2015

of calcium per day per person and only 12% (n=76/635) consumed 500-1000 mg of calcium (table-7). Dietary calcium intake was correlated with protein intake (r=.658, P=.000) (table-8).

Calcium intake of this study was severely poor (met only 21.60% RDA, IOM) as milk intake by CSWs was only 5.21% (166/3185) (table-1), which was second provider (154.34 mg) of calcium after fish (197.95 mg). About 59.17 mg and 14.24 mg calcium out of 425.7 g total animal calcium provided by Egg and meat respectively (table-2). It is well established that milk is the richest source of calcium (whole milk contains 120 mg/100g calcium, Islam SN *et al,* 2012) and milk product i.e. cheese, curd, yogurt contain more as these are concentrated form of milk but in this study consumption of milk was very scanty and consumption of milk products were almost zero. In this study, most of the calcium came from fish (intake frequency 13.63%, n=434/3185) which was much lower than national amount (table-1) and ingestion of small fish was almost zero, this finding is consistent with another previous study (Comb and Hassan, 2005) of Bangladesh.

Previous studies showed that inadequate dietary intake of calcium (Kabir et al, 2010; Islam et al, 2003; Jahan and Hossain, 1998) and vitamin D (Ann Micka, 2014; Islam et al, 2002) among different groups of Bangladeshi women including women garments workers (Islam et al, 2008, 2013). Moreover, existing data either recent (Ann Micka, 2014; Islam *et al*, 2013, 2008; Kabir *et al,* 2010) or older (Islam *et al*, 2003, 2002; Harinarayan *et al*, 2007; Jahan and Hossain, 1998) both suggested that calcium and vitamin D intake among Bangladeshi women and other South Asian populations is typically well below the present recommendation. This study also supported the 'old claim' (Islam *et al*, 2013, 2003, 2004) that low dietary calcium intake prevails among low income Bangladeshi female population.

In fact, inadequate consumption of milk and milk products is the leading cause of lower calcium intake which also seen in this study subjects (mean milk intake was only 58.91 gm which yielded 154.34 mg calcium). Nevertheless, in this study, mean calcium derived from animal intake (425.7 mg) was surprisingly 2.3 times higher than that from plant origin (181.44). Furthermore, as mean phytic acid content was only 492.86 mg, so dietary calcium absorption might not be hampered by phytic acid which was reported in an earlier Indian study that high content of phytate in Indian diet acted as a barrier for calcium absorption in the gut (Harinarayan et al, 2007).

Multiple logistic regression (MLoR) model of dietary calcium: in MLoR model (table-14) of calcium, even having normal BMI, income<5000 taka and having children were associated with <50% RDA of calcium. Even having normal BMI of CSWs were 2 times more likely to possess <50% RDA of calcium as compared to overweight CSWs [AOR 2.06 (1.144-3.710), P=.016]. 'Having children' of CSWs was associated with 2 times higher odds of attaining <50% RDA of dietary calcium as compared to CSWs who did not have children [AOR 2.065 (1.267-3.366), P=.004]. Monthly income <5000 BDT was associated with <50% RDA of calcium. It was observed from MLoR model of calcium that CSWs having monthly income

1000-2500 and 2501-5000 BDT, were associated with 1.86 times (P=.026) and 2.64 times (P=.003) higher odds of getting <50% RDA of calcium respectively as compared to higher monthly income (>5000 BDT). This finding is reinforced by a study in USA (Rose and Oliveria, 1997) which revealed that mean intakes of women from food-insufficient households were below two-thirds of the recommended daily allowance for calcium, iron, vitamin E, magnesium, and zinc.

Vitamin A intake by CSWs: vitamin A has been considered one of the most limiting micronutrients in diets of the Bangladeshi population (Bermudez et al, 2012) since its first National Nutrition Survey,1977 (National Nutrition Survey of rural Bangladesh,1977.1975/76) and onward surveys (Ahmed and Hassan, 1983; Jahan and Hossain, 1998; NMSS, 2012). According to the national nutrition surveys of 1975/76 and 1981/82 vitamin A intake was highly inadequate with just 36% of the RDA reported in former survey (National Nutrition Survey, 1977) and 39% in latter (Ahmed and Hassan, 1983).

Mean dietary intake of vitamin A by CSWs was only 189.96±90.71 µg of retinol activity equivalents (RAE) which was consistent with older national nutrition surveys (National Nutrition Survey of rural Bangladesh,1977.1975/76; Ahmed and Hassan, 1983; Jahan and Hossain, 1998) but much lower (Dhaka 285 µg/household/day) than a recent Bangladesh Integrated Household Survey [BIHS, 2013, Akhter et al (224 µg)] and a micronutrient survey report (Bermudez et al, 2012) and 4.7 times lower (Dhaka=899.9 µg/person/day) than recent Nutrition, health and demographic survey (Akhtaruzzaman et al, 2011) and far from national micronutrient status survey (slums=412.5 µg/person/day)(NMSS, 2011-12).

Multiple logistic regression (MLoR) model of dietary vitamin A: in MLoR model (table-15) of retinol, only one variable i.e. 'category of CSWs' remain associated with <50% RDA of retinol. While in unadjusted model of vitamin A, '<18' and '19-29' age groups and 'hotel-based CSWs' was associated with lower RDA attainment of retinol but in MLoR model of vitamin A 'present age' was no longer remained in the model and the only one factor remained associated with <50% attainment of retinol was 'category of CSWs'. Interestingly, hotel-based CSWs (HBCSWs) were almost 2 times more likely to attain <50% RDA of retinol as compared to floating or street-based CSWs [AOR 1.695 (1.130-2.543), P=.011]. As only one variable remained in the model of vitamin A, so 'Homer-Lemeshaw goodness of fit' for model showed 'zero'.

Vitamin C intake by CSWs: mean vitamin C intake of CSWs 32.82 mg which was not up to the mark (40 mg) according to ICMR (2010) and far away from national data (Akhtaruzzaman et al, 2011, NHDS, BD) as well as also lower than female garments workers (Islam et al, 2013) and adolescent college girls of Dhaka city (Kabir et al, 2010).

Multiple logistic regression (MLoR) model of dietary vitamin C: in MLoR model (table-16) of vitamin C, two variables namely nutrition risk index (NRI) and 'category of CSWs' remain associated with <50% RDA of attainment of dietary vitamin C. NRI is an index (Le Banh, 2006) based on 'serum albumin level' and >100 recognized as well nourished and <43.5 as malnourished. It was observed that malnourished CSWs were 66% more likely to meet <50% RDA of vitamin C as compared to well nourished CSWs (>100 NRI) [AOR 1.66 (1.135-2.433), P=.009]. Interestingly, hotel-based CSWs (HBCSWs) were 43% more likely to attain <50% RDA of vitamin C as compared to floating or street-based CSWs [AOR 1.43 (.964-2.128), P=.075]. Similar finding was observed in case of vitamin A.

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CHAPTER-2.3

Biochemical Assessment of the Nutritional Status

2.3 Biochemical Assessment of the Nutritional Status (n=495)

In biochemical assessment of nutritional status of CSWs, serum total protein (TP) and albumin (ALB) level were measured using standard methods.

2.3.1 Introduction

Proteins are the most abundant compounds in serum (the rest of blood after removing all cells). Amino acids are the building blocks of all proteins and in turns proteins are the building blocks of all cells and body tissues. They are the basic components of enzymes, many hormones, compliments, antibodies and clotting agents. Proteins act as transport substances for hormones, vitamins, minerals, lipids and other materials. In addition, protein helps balance the osmotic pressure of the blood and tissues. Osmotic pressure is part of what keeps water inside a particular compartment of one's body. Protein plays a major role in maintaining the delicate acid-alkaline balance of human body. Serum proteins serve as a reserve source of energy for tissues and muscle when an adequate amount was not ingested. The major measured serum proteins are divided into two groups, albumins and globulins[67].

Serum total proteins represent non-visceral protein status in the body (Anderson et al, 1982). Hundreds of proteins are dissolved in the plasma. By measuring the concentration of these proteins, the clinician can obtain information regarding disease states in different organ systems. The measurement of protein is done on serum, which is the fluid that remains after plasma has clotted, thus removing fibrinogen and most of the clotting factors. Serum total protein (TP) in the plasma is made up of 'albumin' and 'globulin'. Total protein content provides some information regarding a patient's general status; more clinically useful data are obtained from fractionating the total protein. The normal serum 'total protein' level is 6 to 8 g/dl, 'albumin' makes up 3.5 to 5.0 g/dl, and the remainder is the 'total globulins'. The 'total globulin' fraction is generally determined by subtracting the 'albumin' from the 'total protein'. The globulin fraction includes hundreds of serum proteins including carrier proteins, enzymes, complement, and immunoglobulin. Most of these are synthesized in the liver, although the immunoglobulins are synthesized by plasma cells. Serum Protein Electrophoresis (SPEP) is the most common means of further fractionating serum proteins.

During the 1990s, the heart shaped crystalline structure of albumin has been described and a new protein, termed α-albumin (afamin), has been added to the albumin super family (Peter, 1996a). In humans, albumin is the most abundant plasma proteins, accounting for 55-60% of the measured serum protein (Gosling, 1995). It consists of a single polypeptide chain of 585 amino acids with a molecular weight of 66500 Da. The chain characterized by having no carbohydrate moiety, a scarcity of tryptophan and methionine residues, and abundance of charged residues, such as lysine, arginine, glutamic acid and aspartic acid (Peter, 1996b). Albumin attaches to and transports hormones, including thyroid hormone, binds with calcium

[67] http://www.drkaslow.com/html/proteins_albumin_globulin_etc.html

and also helps carry free fatty acids to the liver; it transports bilirubin, some medicines, metals, vitamins and others throughout the blood and is important for tissue growth and healing. It has an important role in fat Metabolism by binding fatty acids and keeping them in a soluble form in the plasma. This is one reason why hyperlipemia occurs in clinical situations of hypoalbuminemia. Albumin also helps maintain the blood acidity in a narrow range. Because of its negative charge, albumin is also able to furnish some of the anions needed to balance the cations of the plasma. It's most important function is that it maintains osmotic pressure, which keeps the plasma inside the blood vessels where it belongs. More importantly, albumin is a pretty good barometer of one's overall health (Busher, 1990).

Albumin is made mainly in the liver with a relatively large body pool size, only 5% of which is synthesized daily and very little of the albumin pool is comprised of newly synthesized ALB. Redistribution between the extra vascular and intravascular space occurs frequently. The majority of the changes in ALB are likely due to this redistribution in response to the many factors. Albumin itself a negative acute phase reactant, levels decrease during the acute phase response (Le Banh, 2006). Albumin will be synthesized only in a suitable nutritional, hormonal and osmotic environment. the colloid osmotic pressure (COP) of the interstitial fluid bathing the hepatocyte is the most important regulator of albumin synthesis and synthesis requires (i) mRNA for translation (ii) an adequate supply of amino acid, activated binding to tRNA (iii) ribosomal machinery for assembly (iv) energy in the form of ATP or GTP (Oratz et al, 1970).

Numerous physiological (anemia, cancer, increasing age, hormonal levels, decreased physical activity, muscle mass and strength) and lifestyle-related factors (cigarette smoking) influence serum concentration of albumin. More broadly, serum albumin is inversely related to morbidity and mortality (Le Banh, 2006; Gersoviz et al, 1980; Hostmark et al, 2005). The primary factors affecting albumin synthesis include protein and amino acid nutrition, colloidal osmotic pressure, the action of certain hormones, and disease status. Fasting or a protein-deficient diet causes a decrease in albumin synthesis as long as the deficiency state is maintained. In the normal individual, the liver increases albumin synthesis in response to the increased availability of amino acids provided by the portal blood following each protein-containing meal (Anna et al, 2007). A decrease in extra vascular colloidal pressure serves as a stimulus for albumin synthesis and is thought to act within the liver. Thyroid hormone, corticosteroids, growth hormone, and insulin all can increase albumin synthesis it helps keep the blood from leaking out of blood vessels. Albumin accounts for 70% of the colloid osmotic pressure. It exerts a greater osmotic force than can be accounted for solely on the basis of the number of molecules dissolved in the plasma, and for this reason it cannot be completely replaced by inert substances such as dextran. The reason is that albumin has a negative charge at normal blood pH and attracts and retains cations, especially Na+ in the vascular compartment. This is called the Gibbs-Donnan effect. Albumin also binds a small number of Cl^- ions that increase its negative charge and ability to retain Na+ ions inside the capillaries.

This enhanced osmotic force causes the colloid osmotic pressure to be 50% greater than it would be by protein concentration alone (Busher, 1990).

Globulins are proteins that include gamma globulins (antibodies) and a variety of enzymes and carrier/transport proteins. Globulins are divided into four fractions by electrophoresis and they are α1 (antitrypsin), α2 (macro globulin and haptoglobin), β (transferrin, complements C3, C4 & C5), and γ globulins (IgG, IgA & IgM), depending on their migratory pattern between the anode and the cathode. Concentrations below the reference range usually reflect low albumin concentration, for instance in liver disease or acute infection. Rarely, low total protein may be a sign of immunodeficiency (Busher, 1990; Wikipedia).

Finally, macronutrient (carbohydrate, fat & protein) deficiency directly reduces BMI and its long-term deficiency may result in Chronic Energy Deficiency.

2.3.2 Materials and Methods

Total protein is measured in serum to give an indication of total immunoglobulin concentration since [(total protein-albumin) =globulins] of which the major component is immunoglobulin[68].

2.3.2.1 Estimation of serum total protein

Serum total protein was determined by “Biuret method” (welchselbaum, 1946) using a commercially available kit (cat. No. TP 245, Randox Laboratories Ltd. UK)

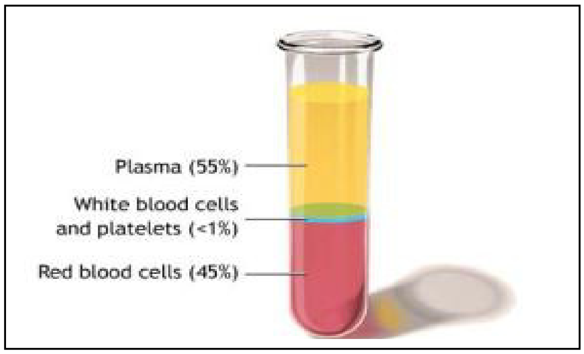

Image-1: Blood serum (yellow part)

Composition of Reagents

	Contents	**Concentration of solutions**
i.	Stock Biuret reagent (R1)	
	Sodium hydroxide	100 mmol/L
	Sodium-potassium-tartrate	16 mmol/L
	Potassium iodide	15 mmol/L
	Cupric sulphate	6 mmol/L
ii.	Blank reagent (R2)	
	Sodium hydroxide	100 mmol/L
	Sodium-potassium-tartrate	16 mmol/L
iii.	Standard protein	
	CAL. Standard (Lot specific)	5.98 g/dl (Lot no. 682TP)

Stability and preparation of reagents

R1 (working): The contents (100 ml) of bottle R1 (biuret reagent) was diluted with 400 ml of double distilled water, rinsing the bottle (R1) thoroughly. This solution was up to the mark for 1 year as sealed and stored at +2 to +25°C.

R2: The contents (100 ml) of bottle R2 (blank reagent) was diluted with 400 ml of double distilled water, rinsing the bottle (R1) thoroughly. This solution stabled for 1 year as sealed and stored at +2 to +25°C.

CAL. Standard: Ready for use and stabled for expiry date as sealed and stored at +2 to +25°C.

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68 Association for Clinical Biochemistry, 2012: www.acb.org.UK>Total Protein

Procedure

1. About 1.0 ml (1000 µl) of biuret working solution (R1) was pipetted into a 'test tube', and then .02 ml (20 µl) "serum" sample was further pipetted into that 'test tube' and well mixed.
2. This mixture was incubated for 30 minutes at room temperature.
3. For standard, 0.02 ml protein solution was taken and treated in the same way as serum sample. (Biuret working R1 (1.0 ml) solution+0.02 ml protein→ incubation 30 minutes)
4. "Reagent blank" was prepared by mixing 0.02 ml double distilled water with biuret working solution (R1).
5. The absorbance of the color complexes i.e. sample and standard were measured by spectrophotometer (UV-1201, UV-VIS, Spectrophotometer, Shimadzu Corporation, Japan) at 546 nm against the reagent blank.

Calculation

$$\text{Total protein concentration in serum (g/L)} = \frac{\text{Absorbance of the sample}}{\text{Absorbance of the standard}} \times \text{Standard concentration}$$

Here, Standard concentration of TP was 59.80 g/L (Lot number 682 TP, Randox Laboratories Ltd. UK)

2.3.2.2 Estimation of serum Albumin

Serum Albumin was estimated by BCG method or "Bromocresol-Green method" (Doumas et al, 1971) using a commercially available kit (cat. No. ALB 362, Randox Laboratories Ltd. UK).

Composition of Reagents

	Contents	Concentration of solutions
i.	BCG concentrate (R1)	
	Succinate buffer	75 mmol/L; P^H 4.2
	Bromocresol-Green	0.15 mmol/L
	Brij 35 Preservative	
ii.	CAL. Standard (Lot specific)	4.68 g/dl (Lot no. 400 AB)

Stability and preparation of reagents

R1 (working): The contents (13.5 ml) of bottle R1 (BCG concentrate) was diluted with 87 ml of double distilled water, rinsing the bottle (R1) thoroughly. This solution was up to the mark for 3 months as sealed and stored at +15 to +25^{o}C.

CAL. Standard: Ready for use and stabled up to expiry date when sealed and stored at +15 to +25^{o}C.

Procedure

6. About 3.0 ml (1000 µl) of BCG working solution (R1) was pipetted into a 'test tube', and then .01 ml (10 µl) "Serum" sample was further pipetted into that 'test tube' and well mixed.
7. This mixture was incubated for 30 minutes at room temperature.
8. For standard, 0.01 ml protein solution was taken and treated in the same way as serum sample. (BCG working R1 (3.0 ml) solution+0.01 ml protein→ incubation 30 minutes)
9. "Reagent blank" was prepared by mixing 0.01 ml double distilled water with BCG working solution (R1).
10. The absorbance of the color complexes (i.e. samples and standards) were measured by spectrophotometer (UV-1201, UV-VIS, Spectrophotometer, Shimadzu corporation, Japan) at 630 nm against the reagent blank.

Calculation

$$\text{Albumin concentration in serum (g/L)} = \frac{\text{Absorbance of the sample}}{\text{Absorbance of the standard}} \times \text{Standard concentration}$$

Here, Standard concentration of Albumin was 46.75 g/L (Lot number 400AB, Randox Laboratories Ltd. UK)

2.3.2.3 Data Analysis

Normality test: various statistical tools were used employing Statistical Package for Social Science for the analysis of biochemical (TP and ALB) variables. Before performing statistical analysis, "Normality" test of the biochemical data set was done by "Shapiro-Wilk (S-W)" goodness of fit test (TP: statistics=.962, df=495, P=.000; ALB: statistics=.923, df=495, P=.000) as well as by doing descriptive statistics. It was observed from 'descriptive statistics' that both TP and ALB data were positively skewed (TP skewness=.421, SE=.110; ALB skewness=.963, SE=.110) and both "logarithmic or Log_{10}" (TP skewness= -1.324, SE=.110; ALB: skewness= -1.693, SE=.110) and "Square root transformation" (TP skewness= -.365, SE=.110; ALB skewness=.193, SE=.110) was performed to see which process could eliminate the skewness in a better way and thus approximation to a normal distribution was greatly improved for both TP and ALB data by "Square root transformation". After Square root transformation, mean as well as other parametric tests were performed on the transformed scale data and then back transformed by taking the "square" and at the same time "Square root transformed" values were backed in the usual TP/ALB units (g/L). However, the Square root transformed 'geometric mean' was always less than the mean of raw data (Bland and Altman, 1996). In fact, extreme observations of a "highly skewed data set" have a large influence on the arithmetic mean, making it more prone to sampling error. Thus, lessening this influence is one advantage of using transformed data (Bland and Altman, 1996).

2.3.2.3.1 Statistical Analysis

All statistical analyses of biochemical data (TP, ALB) were assessed by using Statistical Package for Social Science version 17.0 (SPSS Inc, Chicago, IL, USA). Two-tailed P values <0.05 were considered statistically significant. Comparisons between continuous variables were performed by Student's t-test.

Univariate analysis of the samples: Descriptive statistics (Proportions, means, and SDs) were used to calculate baseline information. Descriptive data were presented as the mean (SD) for continuous variables and as percentages for categorical variables.

Bivariate analysis: **in** bivriate analysis Pearson's correlation (2-tailed) was applied to assess extent of linear correlation between two continuous variables. For participant characteristics analysis, differences in serum TP and ALB levels (dependent continuous variables) were examined in respect to socio-demographic, lifestyle and sexual variables (independent categorical variables) using ANOVA (comparing mean TP and ALB among categories).

Multivariate analysis: multivariate analysis was done to understand the association of different independent variables with dichotomous dependent variable. Multiple logistic regression models (MLoR), with the outcome variables dichotomized (0, 1) as normal to higher [TP: (60 to ≥80 g/L); ALB: (40 to ≥60 g/L) or lower [TP: (1.14-59.9 g/L); ALB: (0.1-39.9 g/L)] was developed to examine the strength of relationship between dependent variables (TP and ALB) with independent categorical variables. In the MLoR model, generally, dummy variables were created for non-binary categorical variables; the effect of each category of a multi-categorical variable was assessed by keeping the first or last category as reference category. All Covariates (socio-demographic, lifestyle and sexual variables as well nutritional indices) which were found statistically significant (P≤0.05) or borderline significant (P<0.09) in Simple logistic regression (SLoR) analysis were introduced simultaneously in the multiple logistic regression analysis**.** The model was developed by 'backward stepwise elimination', removing the covariate with largest P value at each step until the remaining variables were significant at the 0.05 level in the final adjusted model. Also, forward stepwise technique applied in same way to confirm the model. Thus relative odds (ORs) and 95% CI were calculated to identify potential risk factors associated with 'total protein' and 'albumin' deficiency [0=normal to higher and 1=lower]. Hosmer-Lemeshaw goodness of fit and Nagelkarke-pseudo R^2 of the models were also observed.

2.3.3 Results

2.3.3.1 Serum total protein and albumin level of CSWs (n=495)

In general, mean total protein (TP) and Albumin (ALB) values of the study subjects (CSWs) were 50.84 g/L (95% CI 47.75−54.02) and 31.81g/L (95% CI 29.59−34.11) respectively. Although, HBCSWs had higher serum TP (54.18 µmol/L) and FCSWs had higher ALB level (32.39 µmol/L), but no significance differences were observed between mean serum 'total protein' (t=1.553, P=.121) and 'albumin' (t=-.701, P=.484) of these two groups of CSWs. The macronutrient levels of CSWs were found to be remarkably low, at the same time, a remarkable portion of CSWs had elevated level (than normal range, TP: 60-80 and ALB: 40-60 g/L) of serum TP (25.7%) and albumin (19.4%). In fact, mean serum total protein and Albumin were observed vulnerably lower than their normal lower limits (NLL) whenever compare with the standard normal values (total protein= 60−80 g/L and Albumin= 40−60 g/L) (Young, 1998). Moreover, 'Frequency distribution' showed most CSWs (total protein=49.7% and Albumin=63.6%) had below serum total protein and Albumin. Conversely, more than one fourth (127/495) and less than that of CSWs (n=96/495) had elevated serum total protein (>80.1 g/L) and Albumin level (>60.0 g/L) respectively. However, around 24.6% and 17.0% CSWs possessed normal serum total protein and Albumin level respectively. Like total protein and albumin serum total globulin (19.03 g/L) and albumin to globulin ratio (1.67) was found also low among CSWs.

2.3.3.2 Influence of Socio-demographic variables, business location and STIs status of CSWs

Present age was significantly associated with both total protein [TP: F (2,492) =4.075, P=.018*) and Albumin [ALB: F (2,492) =4.099, P=.017*]. And 19 to 29 age groups of both TP and ALB possessed significantly the lowest mean TP (46.47 g/L) and ALB (28.51) values. In contrast, ≥30 age category belonged to the highest mean Albumin (35.56 g/L) and <18 belonged to the highest TP (58.41g/L) values than other age groups in Post Hoc Test of multiple comparisons (Tukey). In addition, last month's total expenditure was significantly associated (F (2,492) =4.225, P=.015*) with serum TP concentration of CSWs and in Post Hoc test (i.e. Tukey for multiple comparisons) the lowest TP concentration (41.42 g/L) belonged to (P<0.05) the lowest expenditure category (00-2500 taka or 31.25 $). Also, highest TP concentration (53.84 g/L) belonged to the highest expenditure category (>5000.1 BDT or >62.6 **$**). However, no influence of education, marital status, monthly income, STI status, STI treatment cost and sex business location of CSWs on serum TP and albumin was noticed (table-2).

2.3.3.3 Influence of lifestyle factors on macronutrients status

Influence of lifestyle factors of CSWs was depicted in table-3. Years of professional experiences positively associated with total protein and albumin, in both cases, highest values belonged to the highest experience category but TP values were not significantly different (P=.409) and albumin had borderline significant (P=0.55). Use of contraceptives significantly associated with only serum albumin concentration (F (3,491) =3.913, P=.009*) and in case of TP it was indifferent (P=.173). Moreover, in Post Hoc Test (Tukey), the lowest albumin concentration (32.95 g/L) belonged to (P<0.01) the condom users whereas "other category" (those used injection/ or had ligation/tubectomy or having menopause) had the highest albumin values (45.27 g/L). Types of substances for abuse significantly associated with both serum TP (F (3,491) =3.519, P=.015*) and ALB (F (3,491) =4.970, P=.002*). CSWs who abused substances (like afim, charush, phensedyl, injection (pethidin) and Heroin etc) other than Alcohol and ganza were more likely to have higher TP (69.56 g/L) and ALB (47.32 g/L) concentration than Alcohol and ganza abuser (significantly different in Post Hoc Tukey test). A weird and Interesting thing was observed about "NGO counseling on STIs", CSWs who counseled on STIs by Non-Government Organization (NGO) had significantly (P=.000) lower TP (49.69 g/L) and Albumin (31.41 g/L) concentration than who did not counseled by NGO workers (TP=63.95 g/L, ALB=41.91 g/L). However, no association of other lifestyle factors of CSWs with serum TP and albumin was observed.

2.3.3.4 Influence of risky sexual behaviors of CSWs on total protein and albumin

Hygienic measures (after coitus with clients) influenced serum TP and albumin level, CSWs who used savlon/soap after coitus had the lowest TP (53.89 g/L) and albumin (33.01 g/L) concentration in the serum. On the other hand, who did nothing or used cotton or simply washed by water had higher TP and albumin values but in case of TP the difference was borderline significant (P=.058). In Post Hoc test (Tukey), both the highest (39.67 g/L) and lowest albumin values (33.01 g/L) belonged to "No users" and 'savlon/soap users' groups respectively (F (2,492) =3.474, P=.032*).

"Group Sex performed last month" and "number of persons involved in last Gr. Sex" was negatively associated with only serum TP and in case of 'serum albumin' both were indifferent. It was observed from the study that, CSWs who used to perform Gr.sex last month (F (1,493) =5.371, P=.021*) had significantly less likely to have higher serum total protein (50.48 g/L) than who did not participate in Gr. Sex (58.88 g/L). Similarly, who used to perform Gr. Sex with >6 clients together had lower TP values (42.12 g/L) than who performed Gr. Sex with lesser (2-5) clients (TP=52.18 g/L, F (1,493) =3.391, P=.034*). Same type of association with TP was also observed in case of 'non paying sexual partner' (NPSP), and 'usual coital frequency per clients' but both association possessed borderline significance (P=.055–.057). Furthermore, 'interval between 2 coitus' and 'usual coital frequency per clients' significantly as well as negatively associated with serum TP (F (1,493) =5.089, P=.025*) and albumin level (F (1,493) =3.391, P=.034*) respectively. Experienced ever in "Anal sex"- this risky sexual behavior of CSWs was positively associated with serum TP and Albumin levels though TP was not significantly different (P=.409) but CSWs who experienced with this risky behavior had significantly higher albumin values (50 g/L) than who (36.28 g/L) did not perform it (F (1,493)=4.578, P=.033*). However, other risky sexual behaviors i.e. condom use, number of clients per day, weekly/fortnightly/monthly coitus with permanent clients (PCs) etc did not show any significant relationship with serum TP and albumin.

2.3.3.5 Influence of dietary factors, immunoglobulins and micronutrients on TP and ALB level

ANOVA (table-5) revealed that serum micro minerals like copper zinc and iron did not have any significant ($P<0.05$) association with serum TP and Albumin. Serum IgA and IgM had great positive influence on only serum total protein (TP) and it is obvious that no association would exist between serum albumin and serum IgG, IgA and IgM, as Immunoglobulins and albumin are part of "total protein". In fact, CSWs who had below normal serum Immunoglobulin A and M had the below normal level of serum TP and who had elevated level (above normal range) of Immunoglobulins had also elevated level of TP [IgM: $F(1, 105) =5.256$, $P=.024$] IgA [$F(1, 105)=8.712$, $P=.004$]. Similar positive influence also noticed in case of serum vitamin E one both TP ($P=.061$) and ALB ($P=.049$). Besides, NRI, an index derived from serum albumin exhibited logical influence on both TP and ALB and also showed strong correlation with them.

Correlation: serum TP ($P=.480$, $r= -.032$) as well as albumin ($P=.664$, $r= -.020$) had insignificant negative correlation with dietary intake of protein. However, serum TP and ALB were strongly correlated ($P=.000$, $r= .462$). Serum TP had insignificant negative correlation with serum Zinc ($r= -.064$, $P=.512$), Copper ($r= -.102$, $P=.295$) and iron ($r= -.137$, $P=.160$) and serum ALB had insignificant negative correlation with serum Zinc ($r= -.022$) and iron ($r= -.016$) and borderline significant with vitamin A ($r= -.182$, **$P=.060$**) (table-5).

2.3.3.6 Crude model of total protein and albumin

In crude model of TP (table-6), present age (19-29 age group), NGO counseling on STI, interval between 2 coitus, use of contraceptive (condom), NGO counseling on STI, meal skipping or starvation, Nutrition Risk Index (NRI) and low serum immunoglobulin A were associated with ($P<0.05$) low serum total protein.

In crude model of ALB (table-7), 19-29 age group, NGO counseling on STI, use of drug, use of contraceptive (condom), <1 to 5 y professional length of CSWs and coital frequency of clients showed significant ($P<0.05$) association with low serum albumin (table-7).

2.3.3.7 Multiple logistic regression (MLoR) models of total protein

Multiple Logistic Regression models (table-8 and 9) were fitted for total protein (dichotomous outcome variables) adjusted for various independent variables (socio-economic, lifestyle, dietary, and nutritional). For the analysis, those variables had scored significance ($P\leq0.05$) or borderline ($P<0.09$) with serum TP from ANOVA (table-2, 3 & 4) as well as from 'Simple logistic regression model', performed separately (table-6) all were included in the final multiple logistic regression (ML_oR). To explore which factors would be associated with<normal serum TP values, backward stepwise and forward stepwise ML_oR analysis was done consecutively

for better precision. In MLoR model of total protein, variables those retained in the model were present age, meal missing habit, NRI and receiving counseling from NGO on STI.

2.3.3.8 Multiple logistic regression (MLoR) model of albumin

Multiple Logistic Regression model (table-10) was fitted for albumin (dichotomous outcome variables) adjusted for various independent variables (socio-economic and lifestyle). For the analysis, those variables had scored significance ($P \leq 0.05$) or borderline ($P < 0.09$) with serum ALB from ANOVA (table-2, 3 & 4) as well as from 'Simple logistic regression model' (table-7), all were included in the final ML_oR. To explore which factors would be associated with <normal serum ALB values, backward stepwise and forward stepwise **ML_oR** analysis was done consecutively for better precision. In MLoR model of albumin, variables those retained in the model were present age, use of contraceptives and receiving counseling from NGO on STI.

Table-1: Serum total protein and albumin level of CSWs (n=495)

Serum Macro nutrients Levels	Amount (g/L)	All CSWs (n=495) % n	All CSWs (% n) Hotel-based n=178	Floating n=317	Statistical analysis Mean differences (MD), t-value, df	p-value
Total Protein/TP						
Low	1.14 to 59.9	49.7 (246)	45.5 (81)	52.1 (165)		
Normal	60.0 to 80	24.6 (122)	37.1 (66)	17.7 (56)		
High	≥80.1	25.7 (127)	17.4 (31)	30.3 (96)		
Range	(Min-max)	1.14–167.19	1.90–167.19	1.14–165.38		
G. Mean ±SD	-	**50.84±5.90**	**54.18±4.45**	**49.08±7.07**	MD=5.1, t=1.553, df=493	.121
95% CI of mean	(Lower-upper)	47.75–54.02	49.68–58.88	44.98–53.20		
Albumin (g/L)						
Low	0.01 to 39.9	63.6 (315)	66.3 (118)	62.1 (197)		
Normal	40 to 60	17.0 (84)	16.3 (29)	17.4 (55)		
High	≥60.1	19.4 (96)	17.4 (31)	20.5 (65)		
Range	(Min-max)	0.01–135.07	1.88–113.42	0.01–135.07		
G. Mean ±SD	-	**31.81±5.02**	**30.74±4.92**	**32.39±5.05**	MD=1.65, t= -.701 df=493	.484
95% CI of mean	(Lower-upper)	29.59–34.11	27.52–34.49	29.63–35.28		
Total Globulin (g/L)= (total protein-albumin)		**19.03 g/L**	-	-		
Albumin to globulin ratio		**1.67**	-	-		

Human normal serum Total protein[69] and albumin[70] are (60-80 g/L) and (40-60 g/L) respectively (Young, 1998). Human normal serum globulin is (2.3-2.8 g/L)[71], proper albumin to globulin ratio is= (2.1)[4]

[69] Association for Clinical Biochemistry, 2012: total protein (adults) 60-80 g/L (www.acb.org.UK>Total Protein)

[70] Association for Clinical Biochemistry, 2012: albumin (adults) 35-50 g/L (www.acb.org.UK>Albumin)

[71] http://www.drkaslow.com/html/proteins_albumin_globulin_etc.html ; Wikipedia: globulin-2.6 to 4.6 g/L

Table-2: Influence of socio-demographic variables, STIs status and business location of CSWs on serum TP and ALB

Sociological factors	All CSWs n=495 % n	Total protein	ANOVA	Albumin	ANOVA
Present age (y)					
<18	17.8 (88)	**58.41±4.93**[t]	**F (2,492)=4.075**	**33.16±4.8**	**F (2.492)=4.099**
19–29	46.2 (229)	**46.47±5.08**[t]	**P=.018***	**28.51±4.9**[t]	**P=.017***
≥30	36.0 (178)	**52.99±4.11**		**35.56±5.1**[t]	
Education					
No literacy/can sign	69.50 (344)	4.77±4.93	F (2,492)=1.123	37.59±4.32	F (2, 492)=.609
1-5	26.06 (129)	53.72±3.08	P=.326	25.29±5.01	P=.545
6-12 class	4.44 (22)	53.70±5.05		32.72±4.94	
Marital status					
Married	41.6 (206)	57.46 ±3.03	F (2,492)=.595	34.65±5.02	F (2, 492)=1.137
Unmarried	13.7 (68)	40.44±5.09	P=.552	37.66±4.70	P=.322
Widow/divorced /abandoned	44.7 (221)	50.47±4.18		28.49±5.07	
Monthly income (BDT/$)					
0.0 – 2500 BDT (00- 31.25 **$**)	38.38 (190)	47.06±6.12	F (2,492)=.109	36.08±5.33	F (2,492)=.670
2500.1- 5000 BDT (31.26 -62.5 **$**)	28.89 (143)	55.96±5.05	P=.897	35.47±4.09	P=.512
>5000.1 BDT (>62.6 **$**)	32.73 (162)	50.78±3.10		28.75±4.18	
Last month's Total expenditure					
0.0 – 2500 BDT (00- 31.25 **$**)	17.58 (87)	**41.42±7.18**[t]	**F (2,492)= 4.225**	32.40±4.8	F (2,492)=1.995
2500.1- 5000 BDT (31.26 -62.5 **$**)	49.90 (247)	**52.58±6.50**	**P=.015***	36.56±5.01	P=.137
>5000.1 BDT (>62.6 **$**)	32.52(161)	**53.58±4.84**		29.48±5.20	
Treatment cost of STIs (last ailment, n=238/495) [72]					
Self treatment	22.7 (54)	56.22 ±3.92	F (2, 235)=.553	37.83±5.02	F (2,235)=.386
5-250 taka	47.9(114)	44.62±4.32	P=.576	34.24±5.01	P=.680
251-5000 taka	29.4 (70)	51.13±5.06		26.75±3.70	
STIs status					
STIs (either syphilis+ or HBV+ or both)	43.64 (216)	57.21±5.41	F (1,493)=.018	37.81±5.11	F (1,493)=.569
No STIs	56.36 (279)	6.80±3.75	P=.892	35.98±4.28	P=.451
Places of sex trade					
Hotel	35.96 (178)	48.61±4.67	F (1,493)=.647	35.64±4.20	F (1,493)=.503
Floating (road/perk/shrine)	64.04 (317)	56.06±3.52	P=.422	37.42±5.17	P=.479

[t] In post Hoc tests (TUKEY/multiple comparisons) this category (within an individual variable) was significantly different than other categories (of that variables) for having the lowest or highest mean value.

[72] No symptoms appeared in 51.9% (257/498) of CSWs.

Table-3: Influence of lifestyle variables on serum macronutrients status

Lifestyle Factors	All CSWs % n	Total protein	ANOVA	Albumin	ANOVA
Factors behind profession					
Lower Economic status	56.6 (280)	54.98 ±5.63	F (2,492)=1.273	38.44±2.84	F (2,492)=1.278
Exploited by pimps/other	21.2 (105)	60.87±4.14	P=.281	34.11±3.98	P=.279
Other reasons[73]	22.2 (110)	58.38±4.27		35.08±2.63	
Working days (last week)					
Did not work	5.1 (25)	60.65±5.60	F (2,492)=.654	39.15 ±2.13	F (2,492)=.110
1–3 days	50.5 (250)	58.25±2.75	P=.520	36.79±5.40	P=.896
4–7 days	44.4 (220)	55.12±5.02		36.49±2.18	
Age entering into sex business					
10-17 y	43.5 (215)	59.41±3.17	F (2,492)=1.008	36.98±2.57	F (2,492)=.016
18-24	34.9 (173)	55.54±3.45	P=.366	36.76±2.61	P=.984
≥25 years	21.6 (107)	54.43±3.80		36.40±4.23	
Experience in sex business (Y)					
Less than one year	10.7 (53)	56.51±2.29	F (2,492)=.895	**32.59±5.00**	**F (2,492)=2.360**
1-5 y	52.1 (258)	55.22±3.46	P=.409	**35.34±5.52**	**P=.055**
≥6 y	37.2 (184)	59.58±6.98		**40.00±3.72**	
living with					
Own	55.2 (273)	56.46±5.33	F (2,492)=.196	37.45±2.13	F (2,492)=.285
Husband	32.1 (159)	56.89±4.29	P=.822	37.18±.2.13	P=.752
Others[74]	12.7 (63)	59.43±6.01		35.46±6.66	
Use of contraceptives					
No measure	16.8 (83)	61.47±5.66	F (3,491)=1.668	**41.32±3.53**	**F (3,491)=3.913**
Pill	30.7 (152)	58.14±4.31	P=.173	**36.95±2.91**	**P=.009****
[75]Condom	42.8 (212)	53.33±5.98		**32.95±2.08[t]**	
[76]Others	9.7 (48)	61.61±3.80		**45.27±6.56[t]**	
Cases of abortion					
No abortion cases	72.9 (361)	55.94±3.50	F(2,492)=.914	35.56±2.69	F(2,492)=2.099
1-2 cases	21.4 (106)	60.92±4.95	P=.401	41.48±3.62	P=.124
>3 cases	5.7 (28)	55.47±5.15		34.72±4.41	
Habit of smoking					
No	57.4 (284)	55.83±5.86	F (1,493)=.767	36.91±6.25	F (1,493)=.016
Yes	42.6 (211)	58.53±4.09	P=.382	36.60±2.61	P=.899
Substance abuse					
No	69.5 (344)	57.40±4.22	F (1,493)=.178	37.94±27.63	F (1,493)=2.123
Yes	30.5 (151)	56.00±3.25	P=.673	34.13±24.72	P=.146
Types of substances (n=151)					
Alcohol (maod)	39.07 (59)	**55.87±3.89**	**F (3,147)=3.519**	**30.65±5.99**	**F (3,147)=4.970**
Gaza	35.76 (54)	**46.61±3.08**	**P=.015***	**28.65±3.95**	**P=.002***
Others[77]	25.17 (38)	**69.56±3.69[t]**		**47.32±4.64[t]**	
Client profile					
Businessmen (small)	43.0 (213)	56.35±3.57	F (5,489)=1.071	36.68±6.78	F (5,489)=1.115
Rickshaw puller	23.6 (117)	60.85±5.50	P=.476	40.21±3.75	P=.352
Service holder	13.1 (65)	50.97±3.48		31.00±2.30	
Student	6.2 (30)	62.76±3.21		35.34±5.99	
Bus driver	4.2 (21)	53.10±4.41		34.60±2.13	
Didn't know	9.9 (49)	56.52±3.36		38.46±3.85	
NGO counseling on STIs					
No	51.1 (253)	**63.95±3.63**	F (2,492)=22.848	**41.91±7.19**	**F (2,492)=19.676**
Yes	48.9 (242)	**49.69±3.90**	P=.000**	**31.41±5.37**	**P=.000****

[t] In post Hoc test (TUKEY/multiple comparisons) who used 'condom' as contraceptive had significantly (P=.020) lower mean albumin than others and who used 'other' methods (like injection, ligation or having menopause) had significantly (0.02*) higher mean albumin than others.

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[73] Angry with family members, sold by step mom/others, raped/tortured, pushed by husband, self motivation (2.2%) and drug addiction.

[74] Parents, siblings, sexual partners etc.

[75] Injection, ligation/tubectomy, menopause etc.

[77] Cannabis (afim, charush), phensedyl, injection (pethidin) and Heroin etc.

Table-4: Influence of Risky sexual variables on serum macronutrients status

Risky sexual behaviors of CSWs	Total CSWs	Total protein	ANOVA	Albumin g/L	ANOVA
Client turnover (last week)					
Did not work	5.1 (25)	60.65±5.66	F (2,492)=.419	39.15±5.13	F (2, 492) = .198
1– 14 C	52.7 (261)	55.76±6.23	P=.658	36.17±5.06	P=.820
≥15 C	42.2 (209)	58.06±0.61		37.26±6.32	
Usual no. of clients per day					
1-7	76.2 (377)	56.02±6.17	F (1,493)=1.272	36.35±6.0	F (1.493)=.395
≥8 clients	23.8 (118)	60.05±5.20	P=.260	38.13±5.36	P=.530
Condom use					
No/ irregular use	54.1 (268)	55.40±4.23	F(1,493)=1.269	35.36±6.13	F (1, 493)=1.626
Consistent use	45.9 (227)	58.84±3.49	P=.260	38.44±5.00	P=.203
Hygienic measure after coitus					
No measure	33.3 (165)	61.45±4.18	**F (2,492)=2.339**	39.67±6.23[t]	**F (2,492)=3.474**
Used savlonl/soap	41.2 (204)	53.89±3.74	**P=.058**	33.01±5.36[t]	**P=.032***
Others[78]	25.5 (126)	56.12±5.47		39.08±4.23	
Interval between 2 coitus					
No	63.6 (315)	59.57±4.70	**F(1,493)=5.089**	36.99±4.63	**F(1,493)=.053**
yes	36.4 (180)	52.45±5.09	**P=.025***	36.41±4.84	**P=.818**
Usual coital frequency/client					
One time	59.2 (293)	59.26±5.23	**F (1,493)=3.259**	**39.02±4.94**	**F (1,493)=5.054**
≥2 times	40.8 (202)	53.67±3.67	**P=.057**	**33.53±4.0**	**P=.025***
Weekly coitus with permanent client (PC)					
No weekly PC	59.8 (296)	57.27±5.53	F (2,492)=1.501	37.06±4.82	F (2,492)=1.501
yes			P=.224		P=.224
1 time/week	23.8 (118)	53.13±3.57		34.90±4.87	
2 times/week	16.4 (81)	61.52±3.11		38.49±5.94	
Fortnightly coitus with PC					
No fortnightly PC	72.3 (358)	57.69±5.62	F (2,492)=1.201	37.72±4.93	F (2,492)=.814
yes			P=.302		P=.444
1-2 times	21.8 (108)	53.09±0.25		34.59±4.16	
>3 times	5.9 (29)	62.70±2.51		33.34±4.50	
Monthly coitus with PC					
No monthly PC	65.9 (326)	56.72±3.06	F (2,492)=.105	36.28±5.93	F (2,492)=.656
yes			P=.900		P=.519
1-2 times	20.2 (100)	58.34±4.76		39.44±4.66	
>3 times	13.9 (69)	56.25±4.64		35.26±4.38	
Having NPSP[79]					
No	91.5 (453)	**57.80±3.77**	**F (1,493)=3.189**	37.32±4.85	F (1,493)=2.161
Yes	8.5 (42)	**48.06±4.47**	**P=.055**	30.97±4.95	P=.142
Ever experienced in group sex (gr. sex)					F (1,493)=1.649
No	74.1 (367)	58.46±3.76	F (1,493)=2.707	37.69±4.44	P=.200
Yes	25.9 (128)	52.74±3.04	P=.101	34.16±4.83	
Gr. Sex history (last month)					**F (1,493)=3.511**
No	77.4 (383)	**58.88±3.99**	**F (1,493)=5.371**	37.99±3.56	**P=.062**
Yes	22.6 (112)	**50.48±3.93**	**P=.021***	32.61±4.71	
No of persons in the last gr. Sex (n=112)					
2-5 clients	83.04 (93)	**52.18±3.54**	**F(1,110)=3.391**	32.81±3.84	F(1,110)=1.766
>6 clients	16.96(19)	**42.12±4.44**	**P=.034***	31.66±3.64	P=.172
Ever experienced in Anal sex					
No	96.4 (477)	56.73±3.95	F (2,492)=.684	**36.28±5.80**	**F (1,493)=4.578**
Yes	3.6 (18)	63.47±5.69	P=.409	**50.00±4.13**	**P=.033***

[78] Washed by water, cotton, urine etc.
[79] NPSP means Non paying sexual partner

Table-5: influence of dietary factor, serum micronutrients and immunoglobulins on TP and ALB

Dietary and micronutrient Variables	Total CSWs	Total protein	Statistics	Albumin	Statistics
Meal skipping (n=495)			ANOVA		ANOVA
One-two meals	9.8 (59)	43.56±6.60	F (1,493)=3.001, P=.084	32.49±5.20	F (1,493)=.049, P=.825
No	90.2 (436)	51.84±6.05	r=.074, P=1.0	31.70±4.97	r= -.011, P=.807
[@]**Nutrition Risk Index (495)**			ANOVA		ANOVA
≥100 (well nourished)	36.4 (180)	70.90±3.61	F (1,493)=91.016, P=.000	65.00±1.35	F (1,493)=1035.32, P=.000
≤ 83.5 (malnourished)	63.6 (315)	40.83±6.1	Pearson's correlation r = .452 , P=.000**	18.06±1.77	Pearson's correlation r = .977 , P=.000**
Copper (µ mol/L) (n=107)			ANOVA		ANOVA
8.18 to 10.9	6.5 (07)	52.71±4.17	F (2,104)=.447, P=.641	38.19±4.17	F (2, 104) = .008 P=.992
10.10 to 22.0	68,3 (73)	55.50±7.99	Pearson's correlation	37.33±5.01	Pearson's correlation
22.1 to 63.27	25.2 (27)	47.20±2.08	r = -.059 , P=.547	36.97±3.87	r = .002 , P=.980
Zinc (µ mol/L) (n=107)			ANOVA		ANOVA
5.97 to 11.5	37.4 (40)	56.25±5.62	F (2,104)=.209, P=.812	39.43±3.45	F (2, 104)=.300, P=.741
11.6 to 18.5	37.4 (40)	52.27±5.23	Pearson's correlation	37.45±4.68	Pearson's correlation
18.6 to 99.30	25.2 (27)	50.11±2.85	r = -.046 , P=.637	33.64±2.47	r = -.030 , P=.757
Iron (µ mol/L)			ANOVA		ANOVA
0 .11 to 10.9	15.0 (16)	63.52±3.25	F (2,104)=1.235, P=.295	37.58±3.06	F (2, 104) = .545 P=.581
11.0 to 29.0	64.5(69)	54.17±4.12	Pearson's correlation	37.18±3.24	Pearson's correlation
29.1 to 70.74	20.5 (22)	43.56±2.76	r = -.151 , P=.120	35.05±1.38	r = .079 , P=.417
Immunoglobulin G (IgG) (n=107)			ANOVA F (1,105)=1.686, P=.197		ANOVA F (1,105)=.268, P=.606
0 .04 to 4.99	85.05 (91)	51.12±5.41	Pearson's correlation	36.48±3.31	Pearson's correlation
5.0 to 33.0	14.95 (16)	65.61±5.66	r = .150 , P=.123	40.12±4.20	r = .039 , P=.339
Immunoglobulin A (IgA) (n=107)			ANOVA		ANOVA
0.00 to 0.49	79.44 (85)	47.89±5.0	**F(1,105)=8.712, P=.004**	36.00±3.31	F(1,105)=.268, P=.606
0.50 to 5.75	20.56 (22)	76.74±3.0	Pearson's correlation r = .146 , P=.135	42.20±5.20	Pearson's correlation r = .053 , P=.547
Immunoglobulin M (IgM) (n=107)			ANOVA		ANOVA
0.02 to 0.29	67.3 (72)	47.33±3.24	**F (1,105)=5.256, P=.024**	32.38±2.33	F (1,105)=6.819, P=.010
0.30 to2.85	32.7 (35)	66.26±4.67	Pearson's correlation r = .065 , P=.508	48.44±2.07	Pearson's correlation r = .019 , P=.470
Vitamin A (µmol/L) (n=107)			ANOVA		ANOVA
0 .02 to o.34 (severe)	19.6 (21)	72.25±3.73	**F (2,104)=2.870, P=.061**	51.12±3.71	**F (2,104)=3.101, P=.049**
0.35 to 1.04 (sub-clinical)	68.2 (73)	49.70±3.62	Pearson's correlation	35.52±2.39	Pearson's correlation
≥1.05 (normal)	12.2 (13)	44.62±4.6	r = -.122 , P=.211	27.14±3.9	r = -.182 , **P=.060**
Vitamin E (µmol/L) (n=107)			ANOVA		ANOVA
0.00 to 5.99	42.06 (45)	44.89 ±3.92	F (2,104)=2.641, **P=.076**	30.69±3.71	F (2,104)=2.411, **P=.095**
6.0 to 6.66	41.12 (44)	54.76±5.06	Pearson's correlation	40.70±2.49	Pearson's correlation
13.0 to 35.23	16.82 (18)	70.56±6.55	r = .056 , P=.565	46.79±3.0	r = .136 , P=.164

[@]NRI (Nutrition Risk Index) =1.519*SERUM ALBUMIN+41.7*PRESENT WT/USUAL WT (Le Banh, 2006)

Table-6: Independent variables associated with lower serum TP level of CSWs (Crude model)

factors associated with lower serum TP	All CSWs n=495	Unadjusted model of serum TP focusing on **lower serum TP** level of CSWs.			
	% n	0=≥normal % n	1=<normal % n	95% CI (lower-upper)	p-value
Present age (Y)					
<18 (r)	17.78 (88)	23.7 (59)	11.8 (29)	1	-
19-29	46.26 (229)	37.3 (93)	55.3 (136)	2.975 (1.774–4.989)	P=.000**
≥30	35.96 (178)	39.0 (97)	32.9 (81)	2.054 (1.132-3.724)	P=.052
Monthly expenditure (BDT)					
1000-5000	67.5 (334)	65.9 (164)	69.1 (170)	1.662 (.979-2.819)	P=.060
>5001 (r)	32.5 (161)	34.1 (85)	30.9 (76)	1	-
NGO counseling on STIs					
Yes	48.7 (242)	39.8 (99)	58.1 (143)	2.104 (1.470-3.011)	P=.000**
No (r)	51.3 (253)	60.2 (150)	41.9 (103)	1	-
Ever experienced in Gr.sex					
No (r)	74.1 (367)	77.5 (193)	70.7 (174)	1	-
Yes	25.9 (128)	22.5 (56)	29.3 (72)	1.426 (.951-2.138)	P=.086
Interval between 2 coitus					
No (r)	60.75 (65)	69.49 (41)	50.0 (24)	1	-
Yes	39.25 (42)	30.51 (18)	50.0 (24)	1.47 (1.01-2.09)	P=.049*
Use of contraceptive					
No measure (r)	16.77 (83)	18.9 (47)	14.6 (36)	1	-
Pill/others	40.40 (200)	44.6 (111)	36.2 (89)	1.05 (.625-1.754)	P=.862
Condom	42.83 (212)	36.5 (91)	49.2 (121)	1.74 (1.040-2.897)	P=.035*
Meal skipping					
1-2 meals	9.8 (59)	8.0 (20)	15.9 (39)	2.157 (1.219-3.818)	P=.008**
No (r)	90.2 (436)	92.0 (229)	84.1 (207)	1	-
Nutrition Risk Index[#]					
>100 (w nourished) (r)	36.4 (180)	56.6 (141)	15.9 (39)	1	-
<83 (malnourished)	63.6 (315)	43.4 (108)	84.1 (207)	6.929 (4.534-10.59)	P=.000**
BMI					
Low (<18.5)	24.4 (121)	22.1 (55)	26.8 (66)	1.733 (.944-3.182)	P=.076
Normal (18.5-24.9)	62.2 (308)	62.2 (155)	62.2 (153)	1.426 (.832-2.445)	P=.197
High (≥25) (r)	13.4 (66)	15.7 (39)	11.0 (27)	1	-
Immunoglobulin A (IgA) (n=107)					
Low (0.00 to 0.35)	64.5 (69)	52.5 (31)	79.2 (38)	4.168 (1.38-12.57)	P=.011*
Very low (0.36 to 0.49)	15.0 (16)	18.6 (11)	10.4 (05)	1.545 (.361-6.610)	P=.557
≥Niormal (0.50 to 5.75)	20.5 (22)	28.8 (17)	10.4 (05)	1	-

Legend: Simple Logistic Regression (SLoR)
(r)= referent category
P<0.05*, P<0.01**
[#]NRI (Nutrition Risk Index)=1.519*SERUM ALBUMIN+41.7*PRESENT WT/USUAL WT (ref)
♣ Most CSWs (57/635) skipped meal one time and only 0.8% (05/635) skipped meals two times.

Table-7: Variables associated with lower serum albumin level of CSWs (Crude model)

Explanatory variables	All CSWs	Unadjusted model of serum ALB focusing on **lower serum ALB** of CSWs.			
factors associated with lower serum ALB	n= 495	0=≥normal	1=<normal	(95% CI: lower-upper)	p-value
	% n	% n	% n		
Present age (Y) (n=495)					
<18 (r)	17.78 (88)	17.2 (31)	18.1 (57)	1.501 (.805-2.545)	P=.131
19-29	46.26 (229)	38.3 (69)	50.8 (160)	1.893 (1.258-2.848)	P=.002**
≥30 (r)	35.96 (178)	44.4 (80)	31.1 (98)	1	-
NGO counseling on STIs					
Yes	48.7 (242)	36.1 (65)	56.2 (177)	2.289 (1.557-3.308)	P=.000**
No (r)	51.3 (253)	63.9 (115)	43.8 (138)	1	-
Drug used					
No drug (r)	69.5 (344)	73.3 (132)	67.3 (212)	1	
Ganja (cannabis)	10.9 (54)	6.7 (12)	13.3 (42)	2.179 (1.107-4.290)	P=.024*
Alcohol	11.9 (59)	7.8 (14)	14.3 (45)	2.001 (1.057-3.788)	P=.033*
Others	7.1 (38)	12.2 (22)	5.1 (16)	.453 (229-.894)	P=.022*
Use of contraceptive					
No measure (r)	16.77 (83)	20.6 (37)	14.6 (46)	1	
Pill/others	40.40 (200)	46.6 (84)	36.8 (116)	1.111 (.663-1.861)	P=.690
Condom	42.83 (212)	32.8 (59)	48.6 (153)	2.086 (1.232-3.533)	P=.006**
Length of profession (y)					
<1 y to 5	62.8 (311)	56.7 (102)	66.3 (209)	1.508 (1.035-2.196)	P=.032*
>5.1 y (r)	37.2 (184)	43.3 (78)	33.7 (106)	1	
Coital frequency per client					
One time (r)	59.2 (293)	65.6 (118)	55.6 (175)	1	
≥ 2 times	40.8 (202)	34.4 (62)	44.4 (140)	1.523 (1.042-2.225)	P=.030*

Legend: Simple Logistic Regression (SLoR)
(r)= referent category
P<0.05*, P<0.01**

Table-8: Multiple Logistic Regression (MLoR) model of TP adjusted for independent variables

factors associated with lower than normal TP (n=495)	Adjusted model of TP (without immunoglobulin)						
	All CSWs (n=495) % n	0=≥normal n=249 % n	1= <normal TP n=246 % n	Beta coefficient (B)	standard error	Adjusted Odds Ratio (95% CI) (lower-upper)	P-value
Present age (Y)							
<18 (r)	17.78 (88)	23.7 (59)	11.8 (29)	-	-	1	-
19-29	46.26 (229)	37.3 (93)	55.3 (136)	1.077	.289	2.937 (1.668–5.172)	.000*
≥30	35.96 (178)	39.0 (97)	32.9 (81)	.720	.304	2.054 (1.132-3.724)	.018*
Meal skipping							
♠One-two meals	9.8 (59)	8.0 (20)	15.9 (39)	1.080	.340	2.944 (1.511–5.739)	.002*
No meal skipping (r)	90.2 (436)	92.0 (229)	84.1 (207)	-	-	1	-
Nutrition Risk Index (NRI)							
>100 (well nourished) (r)	36.4 (180)	56.6 (141)	15.9 (39)	-	-	1	-
<83 (malnourished)	63.6 (315)	43.4 (108)	84.1 (207)	1.964	.230	7.128 (4.542–11.188)	.000*
NGO counseling on STIs							
Yes	48.7 (242)	39.8 (99)	58.1 (143)	.510	.209	1.665 (1.106–2.506)	.014*
No (r)	51.3 (253)	60.2 (150)	41.9 (103)	-	-	1	-

Legend: Backward stepwise Multiple Logistic Regression (MLoR)
Hosmer-Lemeshaw goodness of fit for TP model (n=495)l: $\aleph^2$=4.402, df=8, P=.869
Model summary: -2 Log likelihood (-2LL)=559.447, Nagelkerke R-square=.302
Model coefficient :Chi-square ($\aleph^2$)=126.751, P=.000, df=5
Overall predicted percentage from classification table of DV (TP) was 70.5% (cut value is 50%).
(r)= referent category,
NRI (Nutrition Risk Index)=1.519*SERUM ALBUMIN+41.7*PRESENT WT/USUAL WT (Le Banh, 2006)

Table-9: Multiple Logistic Regression (MLoR) model of TP adjusted for independent variables including immunoglobulin A.

factors associated with lower than normal TP (n=107)	Adjusted model of TP (with serum immunoglobulin A)						
	All CSWs (n=107) % n	0=≥normal TP n=59 % n	1= <normal TP n=48 % n	Beta coefficient (B)	standard error	Adjusted Odds Ratio (95% CI) (lower-upper)	P-value
Meal skipping							
♠One-two meals	26.17 (28)	20.34 (12)	33.33 (16)	1.277	.561	3.584 (1.194–10.756)	.023*
No meal skipping (r)	73.83 (79)	79.66 (47)	66.67 (32)	-	-	1	-
Nutrition Risk Index (NRI)							
>100 (well nourished) (r)	51.40 (55)	71.19 (42)	27.08 (13)	-	-	1	-
<83 (malnourished)	48.60 (52)	28.81 (17)	72.92 (35)	2.115	.498	8.286 (3.124–21.974)	.000**
Interval between 2 coitus							
No (r)	60.75 (65)	69.49 (41)	50.0 (24)	-	-	1	-
Yes	39.25 (42)	30.51 (18)	50.0 (24)	.984	.490	2.68 (1.025–6.991)	.044*
Serum Immunoglobulin A (IgA)(g/L)							
Low (0.00 to 0.35)	64.5 (69)	52.5 (31)	79.2 (38)	1.647	.641	5.193 (1.479–18.239)	.010*
Very low (0.36 to 0.49)	15.0 (16)	18.6 (11)	10.4 (05)	.806	.862	2.426 (.497–13.155)	.304
≥Normal (0.50 to 5.75)	20.5 (22)	28.8 (17)	10.4 (05)	-	-	1	-

Legend: Backward stepwise Multiple Logistic Regression Model (MLoR)
Hosmer-Lemeshaw goodness of fit for TP model (n=107): $\aleph^2$=7.090, df=8, P=.527
Model summary: -2 Log likelihood (-2LL)=108.927, Nagelkerke R-square=.402
Model coefficient: Chi-square ($\aleph^2$)=38.274, P=.000, df=5
Overall predicted percentage from classification table of DV (TP) was 73.8% (cut value is 50%).
(r)= referent category
P<0.05*, P<0.01**
NRI (Nutrition Risk Index)=1.519*SERUM ALBUMIN+41.7*PRESENT WT/USUAL WT (Le Banh, 2006)

♠ Most CSWs (57/635) skipped/missed meal one time and only 0.8% (05/635) skipped meals two times.

Table-10: Multiple Logistic Regression (MLoR) model of ALB adjusted for independent variables

Factors associated with lower than normal ALB level (n=495)	Adjusted model of serum ALB						
	All CSWs (n=495) % n	0= ≥normal ALB 36.4% (n=180) % n	1= <normal ALB 63.6% (n=315) % n	Beta coefficient (B)	standard error	Adjusted Odds Ratio (95% CI) (lower-upper)	P-value
Present age (Y)							
<18	17.78 (88)	17.2 (31)	18.1 (57)	.485	.278	.624 (.942–2.800)	.081
19-29	46.26 (229)	38.3 (69)	50.8 (160)	.542	.216	.720 (.391-.919)	.012*
≥30 (r)	35.96 (178)	44.4 (80)	31.1 (98)	-	-	1	-
Use of contraceptive							
No measure (r)	16.77 (83)	20.6 (37)	14.6 (46)	-	-	1	-
Pill/others	40.40 (200)	46.6 (84)	36.8 (116)	.097	.271	1.102 (.648-1.875)	.720
Condom	42.83 (212)	32.8 (59)	48.6 (153)	.669	.276	1.953 (1.136-3.357)	.015*
NGO counseling on							
Yes	48.7 (242)	36.1 (65)	56.2 (177)	.707	.197	2.028 (1.378-2.985)	.000**
No (r)	51.3 (253)	63.9 (115)	43.8 (138)	-	-	1	-

Legend: Backward stepwise Multiple Logistic Regression Model (MLoR)
Hosmer-Lemeshaw goodness of fit for ALB model (n=107): $\aleph^2$=7.319, df=7, P=.396
Model summary: -2 Log likelihood (-2LL)=614.275, Nagelkerke R-square=.093
Model coefficient: Chi-square ($\aleph^2$)=34.652, df=5, P=.000
Overall predicted percentage from classification table of DV (ALB) was 64.8% (cut value is 50%).
(r)= referent category. P<0.05*, P<0.01**

2.3.4 Discussion

Mean serum total protein (50.84±5.90 g/L) and Albumin (31.81g/L) were observed vulnerably lower than their normal lower limits (NLL) whenever compared with the standard normal values [TP: 60-80 g/L; ALB: 40-60 g/L (Young, 1998)]. As total globulin was derived from serum total protein, so it was also below normal (19.03 g/L), so as 'albumin to globulin ratio' (1.67).

Although, HBCSWs had higher serum TP (54.18 µmol/L) and floating CSWs had higher ALB level (32.39 µmol/L), but no significance differences were observed between mean serum 'total protein' (t=1.553, P=.121) and 'albumin' (t=-.701, P=.484) of these two groups of CSWs (table-1). Both mean serum TP and ALB of were far away from garments workers (TP: 84.0±6.0 g/L) in a previous study (Ahmed, 1997) and Chinese healthy control (ALB: 50 g/L) of a recent study respectively (WeiLin Mao et al, 2015). In fact, serum albumin is an indicator of protein malnutrition and its depletion reflects a severe protein imbalance. Low total protein only occurs as a result of conditions causing low values of the major components i.e. albumin and immunoglobulins particularly serum IgG. However, serum total globulins may be low in patients with humoral immunodeficiency (primary or secondary) but because IgG is the major component, deficiencies of IgA or IgM may not significantly affect total globulins[80]. Study (Coin et al, 2008) reported <40 g/L serum albumin in women also emerged as an independent risk factor (OR=2.6).

'Frequency distribution' (table-1) showed most CSWs had below serum total protein (49.7%) and Albumin (63.6%) than standard values. However, only **24.6%** and **17.0%** CSWs possessed normal serum total protein and Albumin level respectively. Study (Visser, 2005) showed that lower albumin concentrations even above the clinical cut off 38 g/L, were associated with future loss of appendicular skeletal muscle mass (ASMM) in older persons. Plausible explanation of lower serum protein and albumin level of CSWs might be as follows (i) malnutrition (inadequate protein intake) and malabsorption could lower serum protein (Rigaud et al, 2000; Kaslow[81]); from dietary analysis (Chapter-2.2: table 2 & 3) it was observed that mean protein (40.07g) intake by CSWs was not up to mark, fulfilled only 72.86% RDA of protein (ICMR, India, 2010). In spite of slightly higher animal intake (47.74 g) of protein than plant protein (39.69 g) consumed by CSWs did not ultimately get adequate and normal serum level of protein, rather which was much below than standard normal values. Anna et al (2007) measured albumin synthesis as a function of protein intake and most important findings of the study were that as dietary protein increased, so did the synthesis of albumin and these changes held across the ages of the subjects. The protein-dependent differential albumin fractional synthesis rate (FSR) response to nutrient ingestion suggests that decreasing the availability of amino acid substrate by consuming meals with inadequate protein reduces the rate of albumin synthesis. In addition, other proteins

[80] Association for Clinical Biochemistry, 2012: www.acb.org.UK>Total Protein

[81] http://www.drkaslow.com/html/proteins_albumin_globulin_etc.html

(globulins) like serum IgA and IgM positively correlated with serum TP level resulted from ANOVA (table-5). CSWs possessed below normal IgA [F (1, 105) =8.712, P=.004] and IgM [F (1, 105) =5.256, P=.024] had also below normal TP and vice versa which also indicative of possible malnutrition as well as low dietary intake of dietary protein, calorie, fat, zinc and vitamin A etc. that was reflected in dietary assessment (Chapter-2.2: table1, 2, and 3). (ii) different level of stressors (Le Banh, 2006) like physical, mental, social, psychological and emotional endured by CSWs could possibly the reason of lower serum protein level (TP,ALB, globulin- immunoglobulins) of CSWs as it has been previously reported that in the case of serious physical stress, the level of zinc and total protein decreased in serum (Tietz, 1986). However, a Turkish study (Demir, 1996) also showed insignificant (P>0.05) change of serum total protein (but not in albumin) in stressor group than control. In biology, most biochemical processes strive to maintain equilibrium (homeostasis), a steady state that exists more as an ideal and less as an achievable condition. Environmental factors, internal or external stimuli, continuously disrupt homeostasis; an organism's present condition is a state of constant flux moving about a homeostatic point that is that organism's optimal condition for living (Dattatreya and Shruthi, 2014). The age at which the stress is experienced can dictate its effect on health. Research suggests chronic stress at a young age can have lifelong impacts on the biological, psychological and behavioral responses to stress later in life (Miller et al, 2009). Stress can have many profound effects on the human biological systems. The central nervous system (brain and spinal cord) plays a crucial role in the body's stress-related mechanisms and works closely with the body's endocrine system to regulate these mechanisms. Experiments show that when healthy human individuals are exposed to acute laboratory stressors they show an adaptive enhancement of some markers of natural immunity but a general suppression of functions of specific immunity. By comparison, when healthy human individuals are exposed to real-life chronic stress, this stress is associated with a biphasic immune response where partial suppression of cellular and humoral function coincides with low-grade, nonspecific inflammation[82] (iii) study (Doweiko and Nompleggi, 1991) showed low intake of calories inhibits the synthesis of albumin. In this study, dietary calorie intake was severely low (1362.79 Kcal met 61.11% RDA) which might indicative of low serum TP and albumin level of CSWs (iv) Infection/inflammation (Le Banh, 2006) and liver disease could reduce serum albumin; in this study, 52.4% floating CSWs and 18% hotel-based CSWs inflicted with 'syphilis' (P<0.05), and 'hepatitis B' infection rate was 5% and 6.2% respectively for floating CSWs and hotel-based CSWs (P>0.05) (chapter-3: table-1) (v) Zinc deficiency is indicative of low serum albumin level (Le Banh, 2006); in this study, more than one third (37.4%) CSWs had below normal serum zinc, of them 24.3% had <10.1 µmol/L (NMSS, 2012; IZiNCG, 2007) serum zinc or zinc deficiency, though mean serum zinc level was within normal level (14.41 µmol/L) (Chapter -4.2: table-1).

[82] http;//en.Wikipedia.org/wiki/stress_(biology)

Multiple logistic regression model of total protein

In MLoR model of total protein, variables those retained their independent explanatory role on lowering serum total protein of CSWs were their present age, meal missing habit, NRI and receiving counseling from NGO on STI.

Present age: age groups 19-29 [AOR 2.937 (1.668-5.172), P=.000] and ≥30 [AOR 2.05 (1.132-3.724), P=.018] had respectively 3- folds and 2-folds risks to have <normal serum total protein as compared to <18 age group. This effect of increasing age on diminishing serum protein both conflicted (Anna et al, 2007; Fu and Nair, 1998) and reinforced (Hostmark et al, 2005; Gersoviz et al, 1980) by many previous studies. However, study revealed that an increase in dietary protein intake promotes the synthesis of more albumins and raises albumin levels in the blood, even in older people (Anna et al, 2007).

Meal skipping: the predicted odds for CSWs having <normal serum TP were almost 3 times higher in meal skippers than the odds for non meal skippers [AOR 2.944 95% CI (1.511-5.739), P=.002]. In other words, those CSWs did not take meals 3 times in a day had 3 times lower serum total protein than who took meals 3 times in a day. Similar result was also found in TP model with immunoglobulin (table-9). Notably, serum 'total protein' is the addition of two serum proteins namely 'albumin' and 'globulins' and low total protein only occurs as a result of conditions causing low values of the major components i.e. albumin and immunoglobulins particularly serum IgG[83]. In fact, serum albumin is an indicator of protein malnutrition and its depletion reflects a severe protein imbalance. Study showed that hepatic albumin synthesis is suppressed during an extended fasting period and stimulated with nutrient ingestion (Anna et al, 2007). Fasting or a protein-deficient diet causes a decrease in albumin synthesis as long as the deficiency state is maintained. In the normal individual, the liver increases albumin synthesis in response to the increased availability of amino acids provided by the portal blood following each protein-containing meal (Busher, 1990; Wikipedia[84]). Fasting reduces albumin production, but specifically omitting protein from the diet causes a greater reduction in synthesis (De Feo, 1992). When dietary intake is interrupted, the body turns to endogenous 'stored' calorie to provide energy for essential functions. By using different metabolic process like 'Cori cycle', 'oxidation of free fatty acids' to produce ketone bodies, 'gluconeogenesis', body tries to meet energy need. Early in starvation, approximately 75 g of body protein and 160 g of adipose tissue are metabolized each day for every 1800 Kcal utilized. All endogenous proteins are utilized, including those that play important metabolic roles, such as plasma and organ proteins and digestive enzymes. Serum albumin is used in the ratio of 1 g albumin to 30 g tissue protein lost. The most clinically evident protein loss is from skeletal muscles[85].

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[83] Association for Clinical Biochemistry, 2012: www.acb.org.UK>Total Protein

[84] http;//Wikipedia.org/wiki/serum albumin

[85] http://www.tpnteam.com/secure/starvation_metabolism.html

Nutrition risk index (NRI)***:*** low nutrition risk index (<83.5) was significantly associated with higher odds of low TP level, CSWs who were malnourished (had <83.5 NRI) were 7 times more likely to have low serum TP value as compared to CSWs who were not malnourished (had >100 NRI) [AOR 7.13 (4.542-11.188), p=.000] (table-8). Similar result was also found in TP model with immunoglobulin (table-9). NRI[86] is an index derived from serum albumin, thus, it was in agreement with different studies about low protein intake leads to malnutrion which also reflected in low serum albumin level of the subjects (Anna et al, 2007; De Feo et al, 1992; Busher, 1990). Also, Peter (1996c) reported that the rate of albumin synthesis depend on nutritional intake, more so than other hepatic protein. In addition, study (Doweiko and Nompleggi, 1991) showed low intake of calories inhibits the synthesis of albumin. In this study, dietary calorie intake was severely low (1362.79 Kcal met 61.11% RDA) which might indicative of low serum TP and albumin level of CSWs as well as malnutrition.

NGO counseling on STI: NGO counseling on STIs was significantly negatively associated with higher odds of low TP level. CSWs those were counseled by NGO on STIs were 1.67 times more likely to have low serum TP value as compared to CSWs who were not counseled by NGO on STIs [AOR 1.665 (1.106-2.506), p=.014]. In this study, NGO mostly (30.7%) counseled CSWs on 'how to use condom', 26.7% CSWs reported they were asked to 'go to NGO clinic', 25.3% reported free condom given by NGO and 17.5% said they were given training on HIV/AIDS (STIs/Chapter-3: table-4). However, most (326/635) of participants (CSWs) of this study were not counseled by NGOs and no difference was observed between 2 groups of CSWs in this regard ($\varkappa^2$=3.709, P=.054) but studies (Rakhi et al, 2006; Deering et al. 2011) in India observed that, compared to brothel-based CSWs, floating or street-based CSWs were remained inaccessible to HIV prevention programmes, thereby undermining the efforts of HIV prevention by NGO. Also, to organize floating CSWs as a group that could be empowered to protect themselves and participate in HIV prevention effort as compared to non-brothel-based CSWs was difficult. The turnover of CSWs is relatively more among street-based CSWs but they are unorganized as compared with non street-based CSWs (Rakhi et al, 2006).

Interval between two coitus: MLoR model of 'total protein' (n=107) detailed that CSWs who gave interval between 2 coitus with clients were 2.68 times more likely to have low serum TP as compared to CSWs who did not give interval between 2 coitus with clients [AOR 2.68 (1.025–6.991), P=.044] (table-10). This effect is not clear, as majority (60%-67%)(Chapter-1: table-3) of the CSWs in this study did not take interval and rest of them took a break between 2 coitus. This may be due to economic condition of the CSWs. Interestingly, most of hotel-based workers (79.22%) as compared to floating group took a break between coitus (P=.000) (socio-economic table-3). It is noteworthy that HBCSWs had slightly higher TP value (54.18

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[86] [NRI (Nutrition Risk Index) = (1.519*SERUM ALBUMIN+41.7*PRESENT WT/USUAL WT)] (Le Banh, 2006)]

g/L) than floating CSWs (P=.121). Moreover, the usual norm of CSWs about customers dealing which was informed from CSWs (from unstructured questionnaire) that, if a customer was already available just after an 'episode with client' they did not refuse. It could happen to CSWs that, they took a break as because they didn't have customers, and no or low customers means livelihoods of CSWs and their children were at great risks. Thus giving interval between 2 coitus with clients was negatively influenced their daily income, livelihood and low dietary intake which ultimately lower serum protein days after days.

Serum immunoglobulin A: Serum immunoglobulins are indicator of proper immune function. It was observed from the MLoR model of 'total protein' by CSWs (n=107) that having very low serum IgA (0.00-.35 g/L) was associated with higher odds [AOR 5.193 (1.479-18.239), P=.010] of low serum TP. Pearson's correlation (r=.261, P=.007) and ANOVA (F (2,104) =5.712, P=.004) also revealed the fact that (table-5) serum immunoglobulins are positively associated with serum total protein.

Multiple logistic regression model of albumin

Present age: age group 19-29 years were 28% less likely to have low serum albumin as compared to ≥ 30 age group [AOR .720 (.391-.919), P=.012]. Contrary to the effect of increasing age on diminishing serum albumin Anna et al (2007) described that older people who consumed as much protein as younger people made the same amount of albumin as younger people. However, this finding was in agreement with many previous studies (Hostmark et al, 2005; Gersoviz et al, 1980).

Use of contraceptive: condoms are known as a mean of contraception and are not widely used in Bangladesh. In this study, as method of contraception, condom use rate was slightly higher (42.8%) than oral pill/other contraceptive users (40.4%) and 'MLoR model of ALB' (table-10) revealed that condom users were 95% more likely to have low serum albumin value [AOR 1.953 (1.136-3.357), P=.015] as compared to CSWs who did not take any measure for contraception (reference category). However, oral contraceptive pill (OCP) had no significant effect on low serum albumin (P=.720) of this study subjects (CSWs). Study (Obisesan et al, 2002) reported significant increased of TP, globulin and cholesterol in both oral contraceptive users and their control counterparts but albumin/globulin ratio in subjects on OCP users was significantly (P<0.001) decreased compared with controls due to increased serum globulin level; while serum albumin level showed no significance difference (P=.20) in OCP users compared with controls. Also, an old study (Liappis, 1973) documented that serum 'albumin' and 'gamma globulin' was higher (P<0.05) among German women using OCP than men and women who did not use OCP than men respectively. Another study supported such higher albumin level among OCP users (Le Banh, 2006). The association of 'condom use with low serum albumin' (table-10) was not available in literature, however, no effect (P>0.05) of intra-uterine device (UniplantR, 19-nor-progesterone derivative) during one year of use on serum immunoglobulin A and M, albumin and total protein except IgG ($P<0.05$) were reported in Nigerian women (Adekunle et al, 2001).

The effect of 'condom use' as contraception on low serum albumin was not clear but CSWs might not use condom consistently (with each and every commercial and non commercial partners) and correctly (from the beginning and not just before insertion or ejaculation) either as a method of contraception or a barrier against STIs which leads to infection and morbidity and this study showed 'inconsistent habit of condom use' was associated with 'lower expenditure on food' and 'meal skipping' especially among floating CSWs (chapter-3: table-14 & 15). In fact, low income is the leading cause of low dietary intake which ultimately affect health and nutritional status of CSWs. Notably, in this study mean serum albumin level of CSWs was found much lower (32.39 g/L) than standard normal lower limit (NLL) of albumin and only 17% CSWs had normal mean albumin level.

***NGO counseling on STI*:** it was observed that NGO counseling on STIs was significantly associated with higher odds of low albumin level. CSWs those were counseled by NGO on STIs were 2 times more likely to have low serum albumin value as compared to CSWs who were not counseled by NGO on STIs [AOR 2.028 (1.378-2.985), P=.000]. Similar effect of '*NGO counseling on STI'* on serum TP was observed previously in MLoR model of TP.

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CHAPTER-3

Prevalence of sexually transmitted infections (STIs) among commercial sex workers of Bangladesh

Screening of STIs

HIV Serology

Hepatitis B Serology

Syphilis Serology

3.1 Introduction

Sexually transmitted sickness also called, venereal diseases or Sexually transmitted diseases (STDs) or Sexually transmitted infections (STIs), are passed from person to person through sexual acts (UNAIDS, 2010). There are 26 STDs including HIV carry the tag “sexually transmitted” because they spread that way (i.e. by sexual contact such as vaginal, anal and oral sex). In addition, women suffer from more frequent and more serious complications from STDs than men. If left untreated, STDs can cause serious health problems including cervical cancer, liver disease, pelvic inflammatory disease (PID), Infertility and pregnancy problem etc. HIV is a STD and attacks immunological system which is used to fight off diseases. The final stage of the infection has been named AIDS. Having STD of some kind (i.e. chancroid, herpes, syphilis and trichomoniasis) can increase the risk of getting HIV, though he is HIV-negative. One can greatly reduce the risk of getting many STDs by practicing safer sex but should aware of some STDs like genital warts, herpes and syphilis which can be spread through contact with infected skin and here condom cannot prevent transmission[1].

STI and Reproductive Tract Infections (RTI) represent a major public health problem in Bangladesh. Female sex workers (FSW) have been identified as one of the highest risk groups for the acquisition and transmission of STI and HIV because they are characterized by a high number of partners and poor healthcare-seeking behavior (Thomas and Tucker, 1996) and act as a bridging group to the general population. The floating SWs are most disadvantaged among all the commercial SW in Bangladesh. They face a tremendous discrimination and violation of human rights. The floating prostitutes are present in large number but their precise distribution and prevalence and life styles are quite mysterious (Deering et al, 2011; Rakhi et al, 2006; Ullah et al, 2005; Hossain KT, 2004; Mckeganey and Barnard, 1996; Alam MK). Most of them are insisted to take part in sexual intercourse without condoms. The proportion of condom use is rather unsatisfactory among them, the main reason of which was client’s unwillingness in this regard. To strengthen the negotiation skill of them for using condom is the most important issue. Actually, safe sex is the effective way to prevent the HIV transmission. The CSW frequently lacks the personal or social status to negotiate safe sexual practices being under the threat of violence or loss of clients. But condoms are playing more effective role in preventing the majority of STI including HIV. Hence practices of condom use are very important for them because of their high frequency of sexual contacts with a variety of clients.

Again another important and alarming factor to situation analysis of prostitution is HIV/AIDS. Though socio-economic and cultural barriers are hindering the overall progress of the country in many sectors including the status of health of the people, the heath issues concerned with the commercial SWs are more severe and worst among all. As a matter of fact, the issue of CSWs and high risk of STIs through their risky sexual practices are very poorly addressed in

[1] www.stds.help.org/stds you and me

theory and practice in Bangladesh. During the last decade the previous scenario has changed a bit as HIV/AIDS as well as STIs are global health issues. Although Bangladesh remains a low HIV/AIDS prevalence country with less than 0.1% overall prevalence in general population over the years (World Bank, 2009; UNIAIDS, 2010) and <1% both among risk groups and bridging population[2] (with one exception among casual sex workers in Hili), but from the beginning, the prevalence of parasitical, bacterial (*gonorrhea, Chlamidia, trichomoniasis, syphilis*), and viral (*HBsAg, HSV-2*) STIs [(Ulcerative; *syphilis, chancroids, HSV-2)* and (non-ulcerative; *gonorrhea, Chlamidia, trichomoniasis*)] as well as RTIs (*Bacterial vaginosis (BV), Candiasis*) were reported very high among vulnerable population (Sarker et al, 1998; Rahman et al, 2000; Azim et al, 2000; Bogaerts et al, 2001; Sabin et al 2003; Nessa et al 2004). Considering the risks of these sexually transmitted infections (STIs) among vulnerable population (CSWs, IDUs, Transgender, MSMs, internal & external migrant workers etc.) earlier couples of studies funded by different international agencies were carried out, mainly addressing this population at risk and also others. Previous[3] sentinel surveillance and Azim et al (2000) reported very high syphilis rate among Floating CSWs (56.8%) and Brothel-based CSWs (52.5%) and relatively high HIV prevalence rate in IDUs (2.5%). A study among women attending antenatal clinics (low-risk group) in Dhaka reported the prevalence rates of *N. gonorrhoea, Chlamydia. T, Trichomonas. V* and syphilis were 0.5%, 1.9%, 2.0% and 2.9%, respectively (Bogaerts et al, 2001). A study among slum dwelling men and women (intermediate-risk group) in Dhaka reported prevalence rates of syphilis, *gonorrhoea, chlamydial* infection and hepatitis B virus to be 6.0%, 1.7%, <1% and 3.8%, respectively (Sabin et al 2003). Previous studies among brothel-based (Sarker et al, 1998) street-based (Rahman et al, 2000) and hotel-based (Nessa et al, 2004) female sex workers reported prevalence rates of *bacterial STIs* respectively were [*N. gonorrhea and/ or Chlamydia. T* =28.0%, syphilis=57.1% (Sarker et al, 1998)], [*N. gonorrhea*=35.5%, *Chlamydia. T*= 25.0%, *Trichomonas. V*=45.5% and syphilis=32.6% (Rahman et al, 2000)] and [*N. gonorrhea*=35.8%, *Chlamydia. T*=43.5%, *Trichomonas. V*=4.3%, and syphilis =8.5% (Nessa et al, 2004)].

In fact, to establish effective behavioral changes including "safer sex practices" among this vulnerable group of population as well as their clients, comprehensive study should be carried out for each type of sex workers namely brothel, hotel, street or residence-based and also floating sex workers. Such research may help the Government in funding for CSWs or NGOs

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[2] Global AIDS Response Progress Report (GARPR), Annual Progress Report Bangladesh, 2015; 3[RD] National Strategic Plan for HIV and AIDS Response , 2011-15, National AIDS/STD PROGRAM (NASP), MOHFW, GOB
The Round 9 surveillance, 2011 and Round 8 surveillance, 2007. http://www.aidsdatahub.org/nation

[3] Government of Bangladesh. **HIV in Bangladesh: the present scenario. A summary of key findings from the fifth round of serological and behaviour surveillance for HIV in Bangladesh (2003–2004).** Dhaka, Bangladesh: National AIDS/STD Programme, Directorate General of Health Services, Ministry of Health and Family Welfare; 2004;

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to contribute in the improvement of the quality of lifestyle of CSWs and their children. Outcome of the study may help in preventing the further deterioration of the existing situation.

Although various studies addressing STIs of different types of CSWs (street-based, hotel-based, brothel-based) but no study in Bangladesh was carried out focusing on the risky as well as unusual sexual practices like “Group sex”, “Anal sex”, “oral sex” and non paying sexual partner (NPSP) other than normal clients etc. Even the existence of these types of sexual practices among higher risk population has not yet fore fronted.

Viral infections including Human immunodeficiency virus (HIV) and hepatitis B infections with shared percutaneous, mucosal and perinatal routes of transmission are responsible for serious morbidity and mortality globally. Previous report suggests that Bangladesh has an intermediate prevalence of hepatitis B infection, with the highest recorded rates of HBsAg being in professional blood donors (Mustafa et al 1989) and CSWs (Sattar and Islam, 1996; Mustafa et al, 1989). Besides, little data are available on hepatitis B prevalence in Bangladesh. Furthermore, studies focusing on Hepatitis B infections among vulnerable groups like CSWs were also scanty in Bangladesh. No viral screening of STIs (HIV, HBsAg) among hotel-based and floating CSWs was carried out. The few studies (Mustafa et al, 1989; Sattar and Islam, 1996; Gibney et al, 2001) those have been published addressing high risk groups are nearly a decade earlier. For hepatitis B surface antigen (HBsAg) which indicates both current and chronic (lifetime carrier) infectious state, previously only few studies reported 8% prevalence in intravenous drug users (Mustafa M et al, 1989), 5.9% in truck drivers (Ginbney et al, 2001) 9.7% (Sattar and Islam,1996) and 11% (Mustafa et al, 1989) in CSWs. Thus, study focus on HIV, hepatitis B and syphilis prevalence among both hotel-based and floating commercial sex workers simultaneously, has never been studied before in Bangladesh. Besides, the study was carried out in combination with the screening of STIs (HIV, hepatitis B and syphilis) along with the information about the socio-demographic, lifestyle factors, risky sexual acts of CSWs which could help future planning and further research of this field. Moreover, in this study an attempt has been made to find out variables associated with ‘syphilis’, ‘Hepatitis B’ and ‘inconsistent condom use’ by CSWs as well as tried to explain a clear idea about condom use practices by FCSWs and HBCSWs. In addition, tried to investigate the inner thinking as well as insight of these behaviors from both CSW’s and researcher’s point of view. Certainly, this study could help the policy makers of the government and non-government organizations as well as to the researchers.

3.2 Materials and Methods

3.2.1 Collection of the information and blood specimen

The study was conducted between December 2011 and June 2012 among female commercial sex workers (FCSWs) of Bangladesh. With the help of CSW's welfare organizations (Durjoy nari sangha, Ulka nari sangha etc.) which work for all types of CSWs, this study was carried out among randomly selected six hundred and thirty five (n=635) CSWs aged 14-55 years comprising hotel-based (n=308) and floating CSWs (n=327) respectively working either in 'hotel' or on road/park or in cinema hall/cow haat or shrine or shop or elsewhere in Dhaka city. The first approach was to contact welfare organizations (Durjoy nari sangha, Ulka nari sangha etc.) of CSWs to explain the purpose of the study and to ask for their co-operation, then contacted the subjects through these organizations and explained to them the objectives of the study in an understandable way and asked for the written consent of the subjects.

The study was approved by ethical review committee of 'Bangladesh medical research council' (BMRC) and 'University of Dhaka'. All CSWs were informed about the study before selection and after briefing the subjects about the perspective of the study 'written consent' was obtained from randomly selected CSWs on the very day of interview by participant's signs or printed 'thumb' on the consent form. The questionnaire had both open-ended and structured questions and was pre-tested. In fact, background information, SES, lifestyle factors, SRB, anthropometry and blood samples were collected sequentially. The participants were interviewed (n=635) and 5 ml venous blood were collected (n=495) by trained social interviewer and paramedics respectively. However, about 130 HBCSWs and 10 FFCSWs (total 140) refused to give blood thus interviewed participants (CSWs) were 635 but blood specimens were 495. The height-weight was measured with a physician adult metric scale.

3.2.2 Processing and Preservation of the blood samples

The blood specimens were kept undisturbed for 60 minutes and then centrifuged at 3000 rpm for 20 minutes to extract serum, after that aliquoted into 1.5 ml eppendorf tubes (denoted as main and duplicate for each sample). Duplicate samples (for estimation of biochemical indices) stored at -80°c for long storage but working samples (for screening of STIs) were kept at -20°c until the time for assay.

3.2.3 Serological screening of STIs (HIV, hepatitis and syphilis) among female CSWs

About 495 blood samples of CSWs were screened for HIV serology (anti-HIV), hepatitis B serology [Hepatitis B surface antigen (HBsAg)] and syphilis serology (both non-treponemal and treponemal test). Screening of both HIV and Hepatitis B surface Antigen (HBsAg) was carried out by Enzyme-Linked Immunosorbent Assay (ELISA) using standard immunoassay kits. Syphilis was tested by agglutination method using standard kit (Omega Diagnostics, UK).

3.2.3a HIV Serology (ELISA)

Sera (495) were tested for antibodies against HIV by ELISA (Bio-Rad laboratories, USA) and conformation of the test results were done with immuno chromatographic strip (Truong et al, 2000) (ICS/cassette, Atlas, china) as no HIV positive was found among sera.

3.2.3b Hepatitis "B" serology (HBsAg-ELISA)

CSW's Sera were first screened by ELISA (Pathozyme, omega diagonostisc, UK) and conformation was made by both Line Immunoassay (LIA) and rapid ICS test (Excel, china) (Truong et al, 2000).

3.2.3c Syphilis Serology (VDRL, TPHA, ICS)

CSW's Sera were first screened by non-treponemal antibody test (immutrep, omega diagonostic, UK) and reactive sera were then confirmed by treponemal antibody test (TPHA, omega diagnostic, UK) to avoid Biological False Positive [BFP=VDRL(+) and or TPHA (-)]. Besides, immuno chromatographic strip (ICS, Be sure, china) was used for all 495 sera to ascertain firm conformation (Larsen et al, 1995; Truong et al, 2000, Pinar et al, 2002).

3.2.4 Statistical Analysis

All statistical analyses of STI data were assessed by using Statistical Package for Social Science version 17.0 (SPSS Inc, Chicago, IL, USA). Two-tailed P values <0.05 were considered statistically significant. Comparisons between continuous variables were performed by Student's t-test.

Univariate analysis of the samples: Descriptive statistics (Proportions, means, and SDs) were used to calculate baseline information. Descriptive data were presented as the mean (SD) for continuous variables and as percentages for categorical variables.

Bivariate analysis: For participant characteristics analysis, differences of STIs status (dichotomized outcome variables) were examined in respect to socio-demographic, lifestyle and sexual variables (independent categorical variables) using ANOVA.

Multivariate analysis: multivariate analysis was done to understand the 'association' and 'risk factors' of different socio-demographic, lifestyle, HRB and Nutritional variables with STIs (syphilis and hepatitis B) and with 'inconsistent use of condom' by FCSWs respectively. In multiple logistic regression model (MLoR) of STIs (0, 1), outcome variable dichotomized as 'no STIs (0)' and 'having STI (1)' was developed to examine the strength of relationship between dependent variable with independent categorical variables (socio-demographic, lifestyle and sexual variables). Also, in multivariate analysis of 'inconsistent condom use model', the association of 'no' or 'inconsistent condom use' by CSWs for penetrative vaginal sex with clients associated with others characteristics to identify those that may play a significant role in determining 'inconsistent use of condom' by FCSWs. In the MLoR model, generally, dummy variables were created for non-binary categorical variables; the effect of each category of a multi-categorical variable was assessed by keeping the first or last category as reference category. All Covariates which were found statistically significant ($P \leq 0.05$) or borderline significant ($P < 0.09$) in Simple logistic regression (SLoR) analysis were introduced simultaneously in the **multiple logistic regression analysis.** The models were developed by 'backward stepwise elimination', removing the covariate with largest P value at each step until the remaining variables were significant at the 0.05 level in the final adjusted model. Also, forward stepwise technique applied to confirm the model. Thus relative odds (ORs) and 95% CI were calculated to identify potential risk factors associated with 'STIs' and 'inconsistent condom use' [0=no, 1=yes]. Hosmer-Lemeshaw goodness of fit and Nagelkarke-pseudo R^2 of the models were also observed.

3.3.1 HIV screening among Female CSWs

HIV-1 and HIV-2 are responsible for acquired immunodeficiency syndrome (AIDS). Both the viruses show substantial antigenic cross reactivity in their core protein, but the envelope glycoproteins are less reactive (Denies et al 1988). To ensure detection of antibodies against both of the viruses following infection, it is therefore, necessary to use epitops on the envelop region and major cross reacting core proteins of the viruses (Gurtler et al, 1994). Following HIV infection, the earliest specific antibody response may be of IgM followed by IgG (Loussert-Ajaka, 1994). Immunoassay has been developed to detect IgM and IgG to the envelop glycoproteins and the cross reacting core proteins of HIV-1 and HIV-2 viruses. As HIV-1 is strongly virulent in comparison to HIV-2 and infection with HIV-1 is only prevalent in Asia, screening was carried out for HIV-1 seropositivity among the female commercial sex workers (FCSWs) of Bangladesh.

ELISA Ag-Ab (Antigen-Antibody) sandwich method was used for screening of HIV seropositivity among the sera of FCSWs. A commercial HIV kit (HIV Ag-Ab ELISA, cat no 1975055, Bio-Rad Laboratories, USA) was used. HIV Ag-Ab ELISA is a test for the quantitative determination of the antibodies (Abs) to HIV-1 and HIV-2 and Antigen of HIV-1 in human serum or plasma.

Description of the HIV Ag-Ab ELISA kit (reagents)

1. Micro plate (96 wells): colorless polystyrene plate, each well contained HIV antigen and antibody (100 µl) and phosphate buffer (q.s).
2. Negative control: liquid phase of light yellow, 1 vial contained normal human serum and preservative (proclin 300)
3. Antibody positive control: liquid phase of light yellow, 1 vial contained inactivated anti-HIV-1serum with normal human serum (diluents) and preservative (proclin 300).
4. Antigen positive control: liquid phase of light yellow, 1 vial contained recombinant HIV-1 p^{24} antigen with normal human serum (diluents) and preservative (proclin 300).
5. Washing solution (20X concentrated): liquid phase of colorless or light yellow (crystal precipitation may be formed), 1 vial contained 3M phosphate buffer saline with Tween 20 (surfactant) and preservative (proclin 300).
6. Conjugate (51X concentrated): liquid phase of colorless or light yellow, 1 vial contained HIV Antigen and Antibody/peroxidase conjugate with bovine serum albumin (stabilizer) and kathon CG (preservative).
7. Conjugate diluent: liquid phase of blue, 1 vial contained phosphate saline casein buffer with preservative (proclin 300).
8. Substrate (10X concentrated): liquid phase of colorless or light yellow, 1 vial contained Tetramethyle benzidine (TMB) with Dimethyle sulfoxide (solvent).
9. Substrate buffer: liquid phase of light pink, 1 vial contained 30% hydrogen peroxide (H_2o_2) with citrate phosphate buffer (solvent).
10. Stop solution: liquid phase of colorless or transparent, 1 vial contained 1.6 N Sulfuric acid.

3.3.1a Preparation of reagents

1. All reagents were brought to room temperature for 15 to 30 minutes before use.
2. Micro plate has 96 wells (8 rows χ 12 columns) coated with antigen and antibody. According to the requirement each row strip (total 8 strips in a plate) can be removed from the plate but remained strips must be kept at 2-8° C with a silica gel in an aluminum pouch.
3. *Preparation of washing solution:* A 1:20 dilution of washing solution was made with distilled or ionized water to the extent of required amount (e.g. 10 ml washing solution + 190 distilled/ionized H_2o)
4. *Preparation of conjugate:* a 1.51 dilution of conjugate with conjugate diluent to the extent of required amount 10 minutes before use (e.g. per strip: 20 µl of conjugate + 1 ml (1000 µl) of conjugate diluent). Before use it was shaken.
5. *Preparation of substrate:* after completely thawing at 37°c in the water bath, a 1:101 dilution of substrate with substrate buffer 5 to 10 minutes before use (e.g. per strip: 20 µl of substrate+ 2ml (2000 µ) of substrate buffer). Exposure to light was avoided.

3.3.1b Assay procedure

1. Required numbers of strips were taken and fixed them to frame plate.
2. The following steps were done consecutively
   i. 100 µl of "Negative control" into each well of 1A to 1D (out of A-H of column 1)
   ii. 100 µl of "Antibody positive control" into each well of 1E to 1F (out of A-H of column 1)
   iii. 100 µl of "Antigen positive control" into each well of 1G to 1H (out of A-H of column 1)
   iv. And then, 100 µl of each 'serum sample' pipetted into the remained wells (rest of 2-12 columns)
3. Incubated for 60 minutes at 37°c ±1° c after sealing the plate with cover sealer.
4. Before the last 5 to 10 minutes first incubation, 1:51 dilution of conjugate with conjugate diluent was made.
5. The contents from all the wells were aspirated and washed each one 5 times with diluted washing solution (300-400 µl/well/times).
6. The plate was inverted and tapped it on absorbent paper to remove the remaining washing solution, and then pipetted 100 µl of prepared diluted 'conjugate' into each well.
7. Incubated for 30 minutes at 37°c ±1° c after sealing the plate with cover sealer.
8. Before the last 5 to 10 minutes second incubation, 1:101 dilution of substrate with substrate buffer was made.
9. The contents from all the wells were aspirated and washed each one 5 times with diluted washing solution (300-400 µl/well/times).

10. The plate was inverted and tapped it on absorbent paper to remove the remaining washing solution, and then pipetted 100 µl of prepared 'substrate' into each well and was incubated at room temperature for 30 minutes. Exposure of sunlight was avoided.
11. 100 µl of 'stop solution' pipetted into each well and tapped the plate gently to homogenize the coloring materials.
12. Absorbance of the plate was observed against air in an ELISA plate reader (**iMark, BIORAD, Japan. Serial no.103222, manufacture date 2008/06/17)** at 450 nm within 30 minutes after pipetting of stop solution.

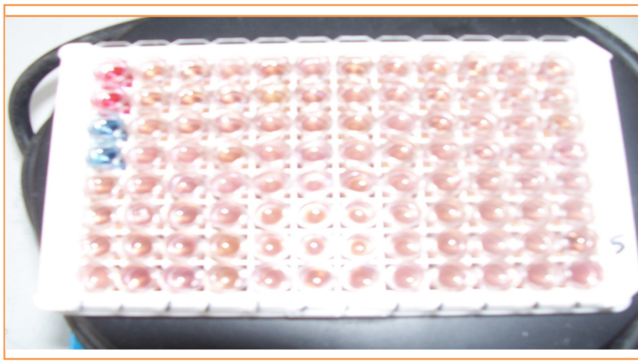


Image-1: ELISA plate before performing test (positive control red, negative control, blue)

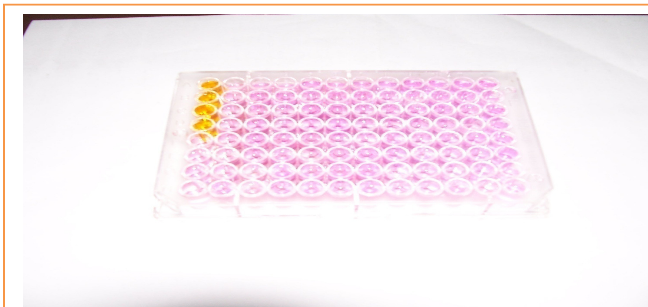


Image-2: ELISA plate showing the HIV sero-negativity.

3.3.1c Conformation test of HIV

Although ELISA indicated that, none of the FCSW found positive for HIV among two groups of CSWS, but some randomly selected sera among 495 sera were re-examined by rapid test (Truong et al, 2000) using ICC or immunochromatographic cassette (Atlas, china).

3.3.2 Screening of Hepatitis B among FCSWs

3.3.2a Principal of the test HBsAg

Monoclonal antibodies, specific for the eight known HBsAg subtypes, recognized by the WHO, are bound to the surface of microtitration wells. Undiluted test sera are added followed by anti-HBsAg antibody conjugated to Horseridish Perioxidase (HRP). If HBsAg is present in the sample it binds to the antibody in the wells and conjugate binds to the captured viral antigens. If HBsAg is not present binding does not take place and an unbound material is washed away. On addition of the substrate, stabilized 3, 3´, 5, 5´ Tetramethyl Benzidrine (TMB), a color will develop only in those wells in which HRP is present, indicating the presence of HBsAg. The reaction is stopped by the addition of dilute sulfuric acid and the absorbance is then measured at 450 nm. Any result with an optical density greater than the cut-off should be considered positive

3.3.2b Description of the Pathozyme HbsAg EIA kit (reagents)

Acute Viral Hepatitis, caused by the Hepatitis B virus, is a common and serious infection. It is characterized by inflammation and necrosis of the liver and with the exception of chronic liver disease; it is the single most significant cause of mortality by liver cancer. Transmission of Hepatitis B virus occurs by direct human transmission via parental routes such as infective serum, blood, blood transfusion and contaminated needles or by non parental transmission through body fluids such as saliva, urine and semen.

Pathozyme HbsAg (Ref OD207, Omega Diagnostic LTD, Scotland, UK) is an Enzyme-immunoassay for the detection of Hepatitis B Surface Antigen (HBsAg) in human serum. Pathozyme HbsAg is classified as a third generation test according to the specification of the US, FDA. The kit contained were

1. **Microtitre plate:** breakable wells coated with monoclonal antibody contained in a resealable bag with a desiccant.
2. **Negative control:** clear solution of human serum negative for $HBsAg^-$
3. Positive control: clear solution of human serum containing $HBsAg^+$
4. Wash buffer: tris based buffer containing detergents.
5. Conjugate: anti-HBsAg conjugated to Horseridish Perioxidase (HRP)
6. Substrate solution: 3, 3´, 5, 5´ Tetramethyl Benzidine (TMB) in a citrate buffer.
7. Stop solution: sulfuric acid diluted in purified water.

3.3.2c Combined Assay procedure/protocol

Total time (90 minutes) = [sample + conjugate (60 minutes @37°C) + Substrate (30 minutes @37°C)]

1. All the kit components and the test sera were brought to the room temperature (20°C to 25°C) prior to the start of the assay.
2. One set of control sera (4 positive and 4 negative controls) were run with each batch of test serum. The position of the standards and the test sera were recorded on the provided EIA Data recording sheet.

3. 100 µl of test serum (samples), positive and negative controls were dispensed into the previously marked appropriate wells.
4. Then, 100 µl of anti-HBsAg HRP "conjugate" was dispensed into each of the well. After that, plate was gently shaken for 60 seconds and subsequently placed into the humidity chamber at 37°C for 60 minutes.
5. Wash buffer was diluted by distilled water by 1 part concentrated wash buffer +9 part distilled H_2o (total volume for one plate (96 wells) =30 ml wash buffer +270 ml dist.H_2o).
6. At the end of the 1st incubation period (60 minutes), the plate was removed from the water bath
   i. The plate was washed by wash buffer with 300 µl/ per well
   ii. Plate was washed by 5 times with diluted wash buffer with a 60 seconds soak during each cycle.
   iii. Excessive fluid was removed by tapping the inverted plate on absorbent paper
7. 100 µl "substrate solution" was then dispensed into each well, then plate was shaken gently for 5 seconds.
8. The plate was then kept in the dark at 37°C for 30 minutes to allow the reaction developed
9. The reaction was stopped by adding 100 µl of stop solution into each well. This will produce a color change from **blue** to yellow in wells containing enzyme which indicate the presence of HBsAg
10. The absorbance of each well was measured at 450 nm filter by EIA reader immediately after stopping the reaction.

3.3.2d Calculation and interpretation of results

For each test, optical density (OD) of the control sera (both positive & negative) and sample sera in the wells were determined.

3.3.2e Assay validation

OD of the Negative controls must be lower than 0.15

OD of the positive controls must exceed 0.300

Cut off level= Average OD of (4) Negative controls + 0.10

Negative result

A negative result should have an OD less than the cut off level (Negative HBsAg = OD<cut of level).

Positive result

A positive result should have an OD equal or higher than the cut off level (positive HBsAg = OD ≥ cut of level).

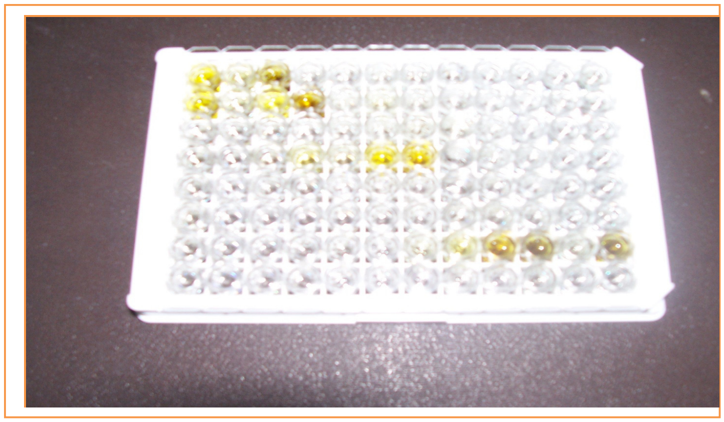


Image-3: Plate showing positive results of Hepatitis B (yellow colored wells)

3.3.2f Conformation of the positive results of Hepatitis B

Positive result was assayed further (2 consecutive test) if the samples were positive in first time. Conformation was carried out by 2 ways-

1. **By using same HBsAg EIA kit (further):** Positive and doubtful results of "first time screening" were assayed further with same Pathozyme HBsAg EIA kit (omega diagnostic, USA) by using 2/ or 4 wells per column of ELISA plate for every positive result (line immune assay/LIA).
2. **By using rapid strip/ or Immuno chromatographic strip (ICS):** All blood samples including sera, which showed positive result by both of the above test were tested by ICS (Excel, china) for firm conformation.

Image-4: Immuno chromatographic strip for HBsAg

3.3.2f-2 Rapid test or Immuno chromatographic strip (ICS) for Hepatitis B

Immuno chromatographic strip (ICS) is, in fact, a rapid chromatographic immunoassay, one step test for the qualitative detection of Hepatitis B surface Antigen (HBsAg) in serum and plasma.

3.3.2f-2a Principle of ICS: one step Hepatitis B surface Antigen (HBsAg) test strip is a qualitative, lateral flow immunoassay for the detection of HBsAg in serum and plasma. The membrane is pre-coated with anti- HBsAg antibodies on the test line region of the strip. During testing, the serum or plasma specimen reacts with the particle coated with anti-HBsAg antibody. The mixture migrates upward on the membrane chromatographically by capillary action to react with anti- HBsAg antibodies on the membrane and generate a colored line. The presence of this colored line in the test region indicates a positive result, while its absence indicates a negative result. To serve as a procedural control, a colored line will always appear in the control line region indicating that proper volume of specimen has been added and membrane wicking has occurred. The test utilizes a combination of monoclonal and polyclonal antibodies to selectively detect elevated levels of HBsAg in serum and plasma.

3.3.2f-2b Reagents

The test strip contains anti-HBsAg particles and anti-HBsAg coated on the membrane

3.3.2f-2c Assay procedure

1. Test (ICS) strip, sera specimens were equilibrated to room temperature (15°C-30°C) prior to testing.
2. The test strip was removed from sealed pouch and was used within one hour.
3. The test strip with arrows pointing toward the serum or plasma specimen was immersed vertically in the serum or plasma for at least 10-15 seconds. Care was taken for not to cross maximum (MAX) line on the test strip while immersing the strip.
4. The test strip was placed on a non-absorbent flat surface, then timer was started and waited for whether the red line (s) would be appeared. The result was read/noticed at 15 minutes.

3.3.2f-2d Interpretation of ICS result for hepatitis B

ICS positive: two distinct red lines (red) were appeared. One line was in the control (C) region and another was in the test (T) region. The intensity of the red color in the test line region (T) will vary depending on the concentration of HBsAg present in the specimen. Therefore, any shade of red in the test region (T) was considered positive.

ICS negative: one red line appeared in the control region (C). No apparent red or pink line was appeared in the test region (T).

Invalid ICS: if control line failed to appear, insufficient specimen volume or incorrect procedural techniques were the most likely reasons for control line failure.

Quality control: a procedural control, mentioned before, was included in the test. A red line appearing in the control region (C) is the **internal** procedural control. It confirmed sufficient specimen volume and correct procedural technique.

Cautions taken were

1. As Humidity and temperature can adversely affect results, so test was carried out in a fully air-conditioned environment.
2. Inadequate serum/or a low HBsAg concentration might result in no line/ or a weak line appearing in the test (T) region respectively after an extended period of time. Therefore, no result was interpreted after 30 minutes.
3. In case of **invalid ICS,** the procedure was repeated with a new strip.

3.3.3 Screening of Syphilis

Syphilis is a complex disease which is normally sexually transmitted. This venereal disease caused by Spirochete bacterium called *Treponema pallidum* (TP). If untreated, the organisms move throughout the body and can cause damage to many organs. As the organism cannot be cultured on artificial media the diagnosis of syphilis depends on the **correlation** of clinical data with the detection of specific antibody by serological test. The serological response to syphilis involves production of antibodies to a wide range of antigens, including non-specific antibodies (non-TP Antibodies) and specific anti-TP antibodies. Antibody to non-treponemal antigen is found (normally) in active disease and the levels subside after successful treatment. In contrast, specific antibody persists long after the infection has been successfully treated (WHO, 1982; Larson et al, 1995).

In this study, Venereal Disease Research Laboratory (VDRL) test was used (IMMUTREP CARBON ANTIGEN, CAT NO.OD 031, omega diagnostics, Scotland, UK) primarily for the serological screening of syphilis. IMMUTREP CARBON ANTIGEN (Serodiagnosis of syphilis, by slide or automated method) was used for non-treponemal flocculation test for the qualitative determination of regain antibodies in serum or plasma.

3.3.3a Principle: IMMUTREP CARBON ANTIGEN was a modified form of IMMUTREP VDRL ANTIGEN which contained carbon particles to improve the visual reading of the result. When binding occurs between cholesterol/cardiolipin/ lecithin (suspension of carbon appx. 0.2 g/L, 0.003% Cardiolipin, 0.02% Lecithin & 0.09% cholesterol) in the reagent and the regain antibodies in the sample, the results can be seen microscopically in the form of black clumps. No visual flocculation indicates negative result. The test can be performed in the **manual slide test** and on single and multi-channel auto analyzer instrument. This test has been calibrated to WHO Reference serum for sero diagnosis tests for treponemal infection (WHO, 1982; Larson et al, 1995).

3.3.3b Assay procedure

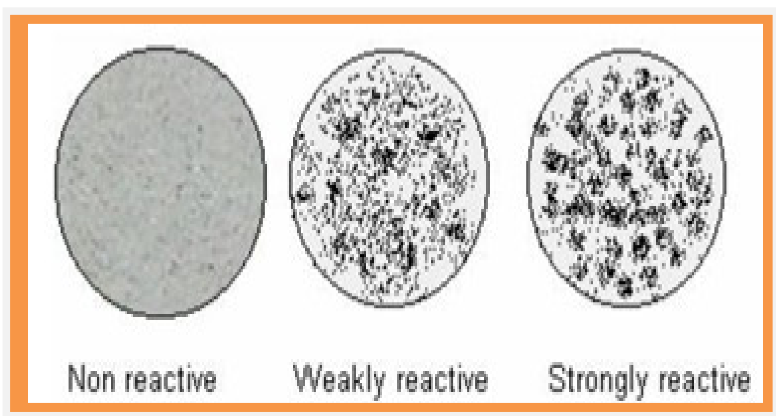
1. 50 µl of test sample was dispensed onto IMMUTREP RPR cards (with 18 mm diameter circles) or a glass slide (white backed ringed slide) and was spread to cover the entire circle.
2. 16 µl of shaken antigen was then added to the sample (there was no need to mix these two compounds).
3. The slide / RPR card was rotated for 8 minutes at 100 r.p.m
4. Immediately after the eight minutes, the result was inspected visually in good light.

3.3.3c Results and interpretation

Medium and large aggregates = Reactive

No aggregates visible, smooth grey appearance = Non Reactive

Finely Dispersed Aggregates = Weak Reactive



Imagee-5: results of VDRL test for syphilis (non-treponemal test)

Cautions taken were-

1. Known level value samples were tested with each test run. If known samples did not give expected results, test results were considered 'invalid'.
2. Primary screening for syphilis (In VDRL) used 'cardiolipin' and 'lecithin' as "antigens" are simple to perform but biological false positive (BFP) reactions occurs frequently if the patient has infections other than syphilis as because the tests use non-treponemal antigens.
3. A low or suspected positive result was reassessed. Diagnosis of syphilis should not be made solely on the findings of only one assay like VDRL (clinical assay).

3.3.3d Syphilis conformation tests

Active syphilis was diagnosed if the serum of CSW became positive on the three consecutive tests. Moreover, if VDRL (-) but last 2 tests were positive they were also positive. Conversely, VDRL (+) but both of the last 2 tests was negative was absolutely "negative" or BFP.

3.3.3e Conformation of the positive results of VDRL test

It should be necessary to test for both groups of antibody since the non-treponemal antibody may arise for reasons other than syphilis reaction. Thus, Positive result was assayed further for conformation. Conformation was carried out by 2 consecutive tests-

a. **By using (specific anti-TP antibodies) *Treponema Pallidum* Haemaagglutinatin (TPHA) test kit** (same brand, omega diagnostics, UK): doubtful results of "VDRL screening" as well as "Positives" were also assayed further with IMMUTREP, TPHA (ref OD 071, omega diagnostic, Scotland, UK) for conformation.
b. **By using rapid strip/ or Immuno chromatographic strip (ICS):** All blood samples which showed either "negative" or "positive" result by both of the above test were tested by ICS (Be sure, syphilis ultra rapid test strip, china) for firm conformation.

3.3.3f TPHA test (or treponemal antibodies kit)

IMMUTREP TPHA is a specific, sensitive passive haemagglutination test for the detection of antibodies to *Treponema pallidum* in serum or CSF. Here, syphilis was confirmed by detecting antibodies specific for T. pallidum for CSW's serum. The first detectable response to infection is the production of specific anti-treponemal IgM, which can be detected within 4 to 7 days after the chancre appears and until the end of the second week of infection; anti-treponemal IgG appears at about 3-4 weeks later following exposure and may remain at detectable levels for long periods after treatment. By the time syphilis disease symptoms develop; most patients have both detectable IgG and IgM.

3.3.3f-1 Principle of TPHA: IMMUTREP TPHA comprises T.pallidum sensitized formolized tanned fowl erythrocytes; un-sensitized formolised tanned fowl erythrocytes, diluent and control sera (both positive and negative). When diluted positive samples are mixed with sensitized erythrocytes, antibody to the sensitizing antigen causes agglutination of the cells. The cells form a characteristic pattern of the cells in the bottom of a micro titration plate well. In the absence of antibody, they form a compact button in the well.

3.3.3f-2 Reagents

1. Test cells: T. Pallidum antigen coated preserved fowl erythrocytes in buffer (sensitised).
2. Control cells: preserved fowl erythrocytes in buffer (un-sensitised).
3. Diluent: Selected rabbit serum (approximately 0.4%) in buffer.

4. Positive Control: Serum pre-diluted (1/20) in buffer containing antibodies to T. pallidum.
5. Negative Control: Serum pre-diluted (1/20) in buffer free of antibodies to T. pallidum.

3.3.3f-3 one well dilution protocol for TPHA

Each test (per sample) as well as 'positive' and 'negative control' requires 2 wells of a micro titration plate

1. About 190 µl of diluent was dispensed into row 1 of the U-well (u-shaped well) microtitration plate.
2. After that, 10 µl of serum sample was dispensed to row 1 and thoroughly mixed by taping.
3. Then, 150 µl (mixture) was discarded from row 1 (remained only 50 µl)
4. 25 µl of mixture was added from row 1 to row 2 (now both row contained 25 µl)
5. 75µl of well mixed (Re-suspended) 'Test Cells' (sensitised with TP antigen) was added to row 1

   75µl of well mixed (re-suspended) 'Control Cells' (unsensitised, no antigen) was added to row 2

   The plate was tapped gently to mix. The final dilutions in wells in rows 1 and 2 were 1/80.
6. Covered and was let stand at room temperature for 45 to 60 minutes (alternatively the plates can be left overnight).
7. Besides, 4 wells (2 positives and 2 negatives) were remarked as "positive control" and "negative control" on the 3rd and 4th row of the plate respectively and 25 µl pre-diluted 'positive control' (TP Ab) and 25 µl pre-diluted 'negative control' (no Ab) serum was poured in wells. After that, 75µl of well mixed (Re-suspended) test Cells (sensitised with TP antigen) was added to "positive control" (having TP Ab) wells and 75µl of well mixed (Re-suspended) control Cells (un sensitised) to "negative control" wells –and was placed at room temperature for 45 to 60 minutes.
8. After 45 to 60 minutes 'agglutination patterns' were carefully examined with comparing 'positive' and 'negative' control.

3.3.3f-4 Results and interpretation

1. Agglutination of cells occurred in the bottom of the U-shaped micro titration plate well (after 45-50 minutes)
2. In contrast, the absence of antibody, they form a compact button in the well (after 45-50 minutes)

Caution taken was

1. U-well (u-shaped well) microtitration plate was used in TPHA and it is mandatory for TPHA test, plate other than U-shaped (flat bottom) would not show any negative (red button) or positive result (agglutination).

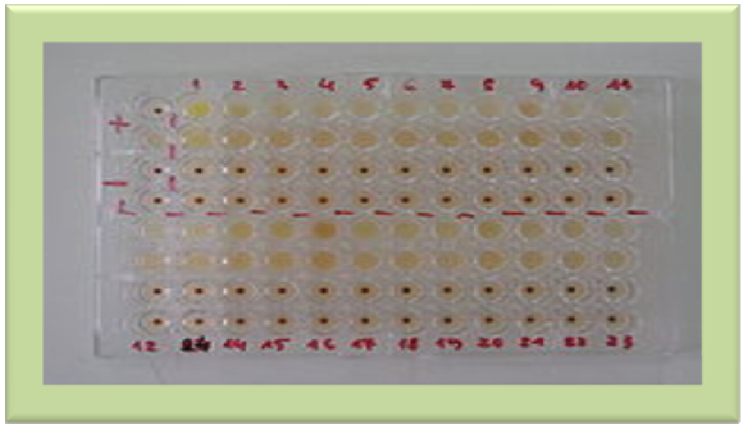


Image-6: TPHA U-well microtiter-plate showing positive (mate like) and negative result (button like)

Image-7: Examples of positive results

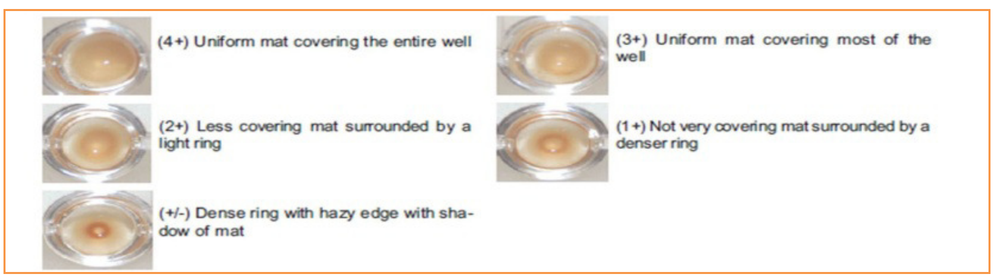


Image-8: Examples of negative results:

The images above correspond to reactions carried out in U-well microtiter-plates. Results are expressed given the agglutination intensity (- to 4+). All samples showing a positive or weakly positive result (+/- to 4) must be tested with a quantitative procedure.

3.3.3g **Rapid test or Immuno chromatographic strip (ICS) for syphilis**

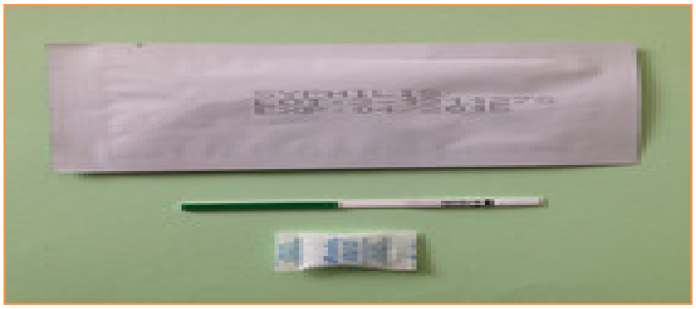


Image-9: ICS for syphilis

The Syphilis ultra rapid test strip♦ (Truong et al, 2000; Pinar et al, 2002) is a rapid chromatographic immunoassay for the qualitative detection of antibodies (IgG and IgM). It is also known as one step syphilis tests, are a rapid direct binding test for the visual detection of syphilis antibodies in human in-vitro serum specimens (whole blood/ serum/plasma), as an aid in the diagnosis of syphilis infection. IVD syphilis rapid test is based on the principle of "double antigen sandwich" immunoassay for detection of syphilis antibodies in human serum. The Syphilis test strip contains 'syphilis antigen coated particles' and 'syphilis antigen coated' on the membrane. Thus, it utilizes a 'double antigen' combination of a syphilis antigen coated particles and syphilis antigen (together) immobilized on the membrane to detect TP antibodies (IgG and IgM) qualitatively and selectively in whole blood, serum or plasma.

With TP rapid test, purified recombinant syphilis antigen is immobilized in the test line region of the strip. After a serum specimen is added to the specimen pad it reacts with syphilis antigen coated particles that have been applied to the specimen pad. This mixture migrates chromatographically along the length of the test strip and interacts with the immobilized syphilis antigen. The 'double antigen' test format can detect both IgG and IgM in blood specimens. If the serum contains TP antibodies, a red line will appear in the test line region, indicating a positive result. If the serum does not have TP antibodies, a red line will not appear in this region, indicating a negative result. To serve as a procedural control, a pink line will always appear in the control line region indicating that proper volume of specimen (serum) has been added and membrane wicking has occurred.

This rapid TP test is very sensitive, less costly and only takes about 10 to 20 minutes to know a screening result. Syphilis Rapid test kits are highly sensitive and specific;

- With one-step TP test, results are read visually without any instrument;
- Syphilis immunoassay test kit is easy to operate

♦ 'Field evaluation of simple rapid tests in the diagnosis of syphilis'. ICDDR, B. Health and Science Bulletin. Vol. 4 No. 4. December 2006.

- No other reagent is needed to perform the rapid TP test kits.
- Less costly

3.3.3g-1 Necessary Materials

1. Test strip
2. Test cards (on which test strip would be placed/glued)
3. Buffer (one drop of buffer would be poured after 50 µl serum was poured on the specimen pad of the test strip pointing downward)
4. Disposable specimen droppers.

3.3.3g-2 Procedure

1. The 'Test strip' was removed from the sealed foil pouch.
2. The tape was peeled off from the 'Test card' and the 'Test strip' was stuck in the middle of the 'Test card' with the arrows pointing downwards as illustrated.
3. The dropper was hold vertically and 2 drops of serum (app. 50 µl) were transferred onto the specimen pad of the test strip, then 1 drop of buffer (40 µl) was added and at the same time the timer was started.
4. Should wait until red line (s) to be appeared. The result should read at 10 minutes later.

3.3.3g-3 Interpretation of the result

Positive: two distinct red lines appeared. One line should have been in the control line region (C) and another line should have been in the test line region (T). The intensity of the red color in the test line region (T) could vary depending on the concentration of TP antibodies present in the specimen serum. Therefore, any shade of red in the test line region (T) should be considered positive.

Negative: One red line appeared in the control line region (C). No apparent red or pink line appeared in the test line region (T).

Invalid result: if control line failed to appear, the result was considered as "Invalid". Insufficient specimen volume or incorrect procedural techniques are the most likely reasons for control line failure.

Cautions taken were -

1. After removing the rapid 'Test strip' from foil pouch it was used as soon as possible. Best result would be obtained if the assay would perform within one hour.
2. The result was not interpreted after 30 minutes.
3. In case of "control line fails to appear", the procedure was reviewed and repeated the test with a new test strip.

3.4 Results

3.4.1 Screening of STIs

Screening of STIs resulted in the **table-1**. **ELISA** indicated that none of the CSW among the two groups of CSWs (n=495) was found HIV-positive. Prevalence of syphilis and hepatitis B (HBsAg) for Hotel-based (HBCSWs) was 18.0% and 6.2% respectively and for floating CSWs (FCSWs) syphilis was 52.4% and hepatitis B was 5.0%. It was revealed from the study that higher rate of floating CSWs significantly infected with syphilis (HBCSWs 18.0% vs. FFCSWs 52.4%) than their counter group (P<0.05), on the contrary, slightly higher proportion of hotel-based CSWs afflicted with hepatitis B than floating group (P>0.05).

3.4.2 Symptom-based knowledge on STIs

Presence of symptoms and treatment patterns of two CSW's group are depicted in table-2. Almost half (51.6%) of hotel-based CSWs claimed they knew all symptoms than floating group (P=.000), although both groups had nearly equal amount of CSWs (HBCSWs 17.9% vs. FFCSWs 17.2%) who did not know any symptoms. It was observed from the study that most (58%) of the hotel-based CSWs and nearly half (49.8%) of floating CSWs did not claim (P=.037) about symptoms but rest of the CSWs asserted that all symptoms of STIs e.g. smelly vaginal discharge, vaginal scar and lower abdominal pain without menstruation were present in both CSW's group. No significance difference (P>0.05) was noticed in the presence of different symptoms between the two CSW's groups except smelly v. discharge which was higher in floating group (P=.014). In contrast, significant difference was observed in 'treatment pattern' (P=.006) and 'delay time before treatment' (P=.000) between 2 CSW groups. Self treatment, treatment in NGO clinic, and 1 to 15 days delay in taking treatment was significantly (P=.006) higher among FCSWs than HBCSWs **(table-2)**.

3.4.3 Knowledge on HIV and HIV risk assessment among FCSWs

"hearing about HIV", "mosquito bite spread HIV" and "proper use of condom during anal sex reduce HIV" these pattern of HIV knowledge among 2 groups of CSWs was indifferent (P>0.05) but whenever they were asked question about 'sharing food causes HIV' (P=.001*) and "sharing needles cause HIV" (P=.005*) in each cases answers were different among 2 groups **(table-3)**.

In this study, most of CSWS (97.6% both groups) heard about the name of HIV, which was surprisingly, opposite to the national finding in general population about "have heard about HIV" (Bangladesh Demographic and Health Survey, 2011). According to BDHS (2011) only 40.3% of non-educated ever married women and 57.0% of widow/divorced/abandoned women have heard about HIV and older and widow/divorced/abandoned women were less likely to know about HIV. In contrast to this, as overwhelming majority (69.5%) of this study had no formal education, moreover, vast majority of them (221/495) were widow/divorced/abandoned and 36% (178/495) were in >30 age, so it is very unlikely that poor, destitute, illiterate study subject had had knowledge about HIV. Professional awareness and NGO counseling on STIs would be the possible reason as different report revealed about strong GOB-NGO collaboration focus on STIs by giving free counseling, condom and STI screening (Naimul Haq). This could be plausible explanation for the 'zero' prevalence of HIV among CSWs in this study. In addition, more than half (55.3%) of CSWs (both type) claimed that they had no risk of HIV and more FFCSWs strongly supported that than HBCSWs (P=.000**)**(table-3)**.

3.4.4 Multiple responses of STIs answered by CSWs

It was observed from the table that majority of CSWs knew all answers of STIs symptoms and Smelly V. discharge and V. scar were most predominant responses answered by most of CSWs especially FFCSWs which was also in agreement of higher prevalence (%) of syphilis among FFCSWs. Vast majority of CSWs denoted their job 'as risky" while responding the question 'the reason of HIV risk' but only 23.8% mentioned that as they did not use condom, so this job could bring HIV risky for them. In this study, most of CSWs were trained how to use condom in drop in center or in NGO clinic which also consistent with this finding that 44.1% (table-3) of CSWs used condom consistently. NGO mostly (30.7%) counseled on 'how to use condom', 26.7% CSWs reported they were asked to 'go to NGO clinic', 25.3% reported free condom given by NGO and 17.5% said they were given training on HIV/AIDS (table-4).

Table-1: Prevalence of STIs among hotel-based and floating commercial sex workers (n=495)

STIs	Tests including conformation	Hotel-based sex workers (HBCSWs) n=178			Floating sex workers (FFCSWs) n =317			Total no. of CSWs for STI screening n=495	
		+Ve % n	–Ve % n	total % n	+Ve % n	–Ve % n	total % n	+Ve % N	–Ve % N
HIV	ELISA Immunochromatographic strip (ICS)	Nil (00)	100 (178)	100 (178)	Nil (00)	100 (317)	100 (317)	Nil (00)	100 (495)
Syphilis	VDRL, TPHA & Immunochromatographic strip (ICS)	18.0* (32)	82.0 (146)	100 (178)	52.4* (166)	47.6 (151)	100 (317)	40.0 (198)	60.0 (297)
Hepatitis B	ELISA, LIA & ICS	6.2# (11)	93.8 (167)	100 (178)	5.0# (16)	95.0 (301)	100 (317)	5.5 (27)	94.5 (468)

Legend: Descriptive, cross tab, proportion test: *P<0.05, P>0.05#

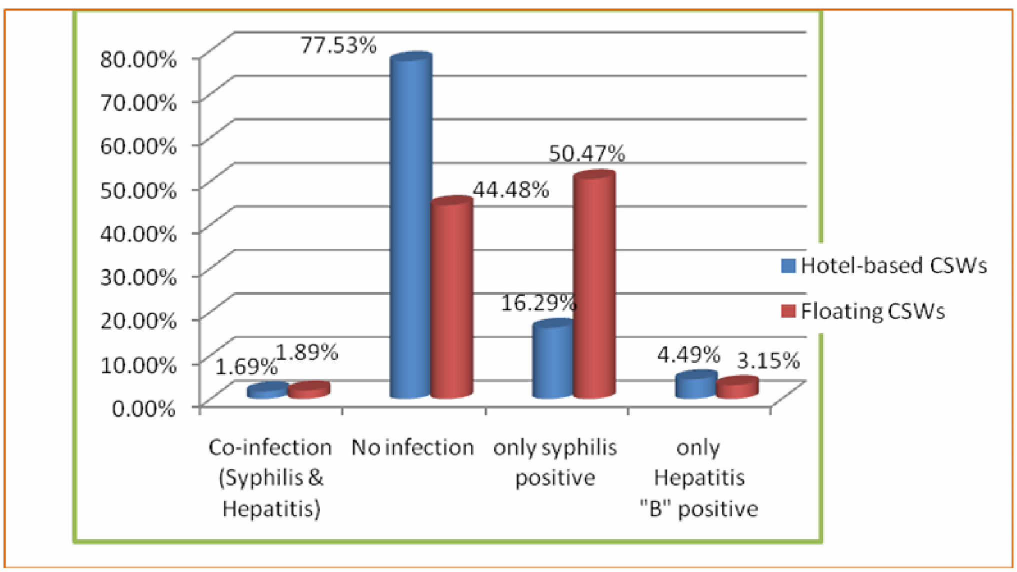


Figure-1.1: Prevalence of STIs between two groups of CSWs

Table-2: Knowledge on symptoms of STIs, presence of symptoms and treatment pattern of STIs among all CSWs (n=635)

Knowledge on STI Symptoms, presence of symptoms	Total CSWs (n=635)	Hotel-based CSWs 48.50% 308	Floating CSWs 51.50% 327	Statistical test	P Value
	% n	% n	% n		
Knowledge on different symptoms of STIs					
known all symptoms of STIs	43.94 (279)	51.6 (159)	36.7 (120)		
Known any one/two Symptoms	38.58 (245)	30.5 (94)	46.1 (151)	$\varkappa^2$=49.359	.000**
Did not know any	17.48 (111)	17.9 (55)	17.2 (56)		
Total	100 (635)	100 (308)	100 (327)		
Presence of symptoms					
Yes	46.1 (293)	41.9 (129)	50.2 (164)	$\varkappa^2$=4.365	.037
No	53.9 (342)	58.1 (179)	49.8 (163)		
Symptomatic presence of STIs					
Smelly V. discharge					
Yes	33.9 (205)	27.6 (85)	36.7 (120)	$\varkappa^2$=6.008	.014*
No	66.1 (430)	72.4 (223)	63.3 (207)		
Lower abdominal pain without menstruation					
Yes	32.3 (215)	31.2 (96)	36.4(119)	$\varkappa^2$=1.932	.165
No	67.7 (420)	68.8 (212)	63.6 (208)		
V. scar /infection					
Yes	15.4 (98)	14.0 (51)	14.8 (47)	$\varkappa^2$=.580	.446
No	84.6 (537)	(257)	(280)		
Treatment after symptoms appeared					
No symptoms appeared	**53.9 (342)**	**58.1 (179)**	**49.8 (163)**		
Sought treatment n=293	**46.1 (293)**	**41.9 (129)**	**50.2 (164)**		
Self treatment[4] (n=61)	9.6 (61)	6.2 (19)	12.8 (42)		
Others (n=232): In hospital	8.3 (53)	9.7 (30)	7.0 (23)	$\varkappa^2$=16.418	.006*
From Dispensary	5.8 (37)	7.1 (22)	4.6 (15)		
Private Doctor	6.9 (44)	6.5 (20)	7.3 (24)		
In NGO clinic	15.4 (98)	12.3 (38)	18.3 (60)		
Delaying time before taking formal treatment (n=232)					
1-15 days	89.22 (207)	79.1 (87)	98.4 (120)		
>16 days	10.78 (25)	20.9 (23)	1.6 (02)	$\varkappa^2$=22.340	.000*

Legend: Descriptive, cross tab, $\varkappa^2$ test, proportion test. P<0.05*

4 Used hot water, dettol or savlon etc.

Table-3: knowledge on HIV, HIV risk assessment among CSWs (n=635)

HIV related knowledge and risk	Total CSWs (n=635)	Hotel-based CSWs 48.50% 308	Floating CSWs 51.50% 327	Statistical test	P Value
	% n	% n	% n		
Heard about HIV/AIDS					
Yes	97.8 (621)	98.7 (304)	96.9 (317)	$\varkappa^2$=2.277	.131
No	2.2 (14)	1.3 (04)	3.1 (10)		
Can "mosquito bite" spread HIV?					
Yes	32.9 (209)	30.5 (94)	35.2 (115)	$\varkappa^2$=1.552	.213
No	67.1 (426)	69.5 (214)	64.8 (212)		
"Share food with HIV infected person" causes HIV?					
Yes	26.0 (165)	19.8 (61)	31.8 (104)	$\varkappa^2$=11.874	.001*
No	74.0 (470)	80.2 (247)	68.2 (223)		
"Sharing needles" with HIV infected person could infect with HIV					
Yes	88.2 (560)	91.9 (283)	84.7 (277)	$\varkappa^2$=7.836	.005**
No	11.8 (75)	8.1 (25)	15.3 (50)		
Abstinence from "Anal sex" could lower the risk of HIV in general people					
Yes	41.0 (253)	33.8 (104)	45.6 (249)	$\varkappa^2$=9.213	.002*
No	59.0 (382)	66.2 (204)	54.4 (178)		
"Proper use of condom" during "Anal sex" could reduce the HIV risk					
Yes	49.4 (314)	45.8 (141)	52.9 (173)	$\varkappa^2$=3.222	.073
No	50.6 (321)	54.2(167)	47.1 (154)		
"Preventive measure" for not infecting with HIV/STD					
Consistent use of condom	44.1 (280)	48.7 (150)	39.75 (130)		
Irregular condom use	24.3 (154)	6.5 (20)	40.98 (134)	$\varkappa^2$=74.281	.000**
No measure	29.4 (187)	41.2 (127)	18.35 (60)		
Taking medicine/washed by dettol	2.2 (14)	3.6 (11)	0.92(03)		
Having the risk of HIV?					
Yes	51.0 (324)	62.7 (193)	40.1 (131)	$\varkappa^2$=32.421	.000*
No	49.0 (311)	37.3 (115)	59.9 (196)		
Risk intensity of HIV					
No risk	49.0 (311)	45.5 (113)	56.2 (198)		
More risk	32.3 (205)	43.3 (153)	19.2 (52)	$\varkappa^2$=82.792	.000**
Medium risk	18.7 (119)	11.2 (42)	24.6 (77)		
Why risk (n=324)					
Risky work	65.1 (211)	60.1 (116)	72.5 (95)	$\varkappa^2$=5.296	.021**
Did not use condom	34.9 (113)	39.9 (77)	27.5 (36)		

Legend: Descriptive, cross tab and $\varkappa^2$ test. Significant* P<0.05*, P<0.01**

Table-4: Multiple responses related to the symptoms of STIs (n=495)

Multiple responses	All CSWs (N=495) Responses % n	All CSWs % of cases	HB CSWs (n=178) Responses % n	HB CSWs % of cases	FFCSWs (n=317) Responses % n	FFCSWs % of cases
Multiple responses on the different STI's symptoms (general)						
• Vaginal discharge	12.05 (80)	16.2	14.6 (31)	17.4	10.8 (49)	15.5
• Smelly V. discharge	18.37 (122)	24.6	11.8 (25)	14.0	21.5 (97)	30.6
• Vaginal scar/ infection	18.82 (125)	25.3	9.0 (19)	10.7	23.5 (106)	33.4
• Lower abdominal pain (without menstruation)	3.77 (25)	5.1	6.1 (13)	7.3	2.7 (12)	3.8
• Known all symptoms	31.18 (207)	41.8	42.9 (91)	51.1	25,7 (116)	36.6
• Did not know any	15.8 (105)	21.2	15.6 (33)	18.5	15.9 (72)	22.7
Total	100.0 (664)	134.1	100 (212)	119.1	100 (452)	142.6
Multiple responses on the 'reasons of HIV risk'						
• Risky job	67.7 (176)	76.2	66.3 (63)	70.1	71.1 (113)	83.7
• No use of condom	23.8 (62)	26.8	29.5 (28)	31.1	21.4 (34)	25.2
• Others [5]	8.5 (22)	09.5	4.3 (04)	4.4	7.6 (12)	8.1
Total	100 (260)	112.6	100 (95)	76.0	100 (165)	120.6
Multiple responses of the NGO counseling on STIs						
• Advice to go NGO	26.5 (121)	49.8	34.0 (54)	54.0	22.6 (67)	46.9
• Condom given	25.3 (115)	47.3	22.0 (35)	35.0	26.9 (80)	55.9
• Trained on how to use condom	30.7 (140)	57.6	23.9 (38)	38.0	34.3 (102)	71.3
• Trained on HIV/AIDS	17.5 (80)	32.9	20.1 (32)	32.0	16.2 (48)	33.6
Total	100 (456)	187.1	100 (159)	159.0	100 (297)	207.7

Legend: Descriptive, chi- square test, multiple responses.

[5] Did not know, frequent 'Anal sex', sharing needles etc.

3.4.5 Bivariate and multivariate analysis for the models of Syphilis, Hepatitis B and 'Condom use' by CSWs

3.4.5a Crude logistic regression models of 'syphilis'

Crude logistic regression models of 'Syphilis' for different independent variables of CSWs are postulated in table-5 (syphilis and socio-demographic variables), table-6 (syphilis and lifestyle factors) and table-7 (syphilis and sexually risk behaviors or SRB). From these tables it clearly revealed that most of these variables significantly (P=.000) influenced 'syphilis positivity' except 'having children' (P=.505) and 'treatment cost of STIs' (P=.434) (table-5); 'introductory age in sex trade' (P=.149), 'working days' (P=.457), 'substance abuse' (P=.750) and 'NGO counseling on STIs' (P=.971) (table-6); 'use of condom' (P=.484), 'hygienic measure after coitus' (P=.119), 'usual coital frequency per client' (P=.179), 'having NPSP' (p=.118) and 'ever experienced in anal sex' (P=.182)(table-7).

3.4.5b Multiple logistic regression model of 'syphilis'

MLoR model (table-8) of syphilis (dichotomous dependent or outcome variable) adjusted for different socio-economic, lifestyle and sexually risk variables of CSWs. At first, simple logistic regression (SL_oR) of each and every socio-demographic variables (total 6 variables), lifestyle variables (n=11), and HRB or heterosexual risky behaviors (n=15) of CSWs were performed separately (table-5, 6 and 7 respectively). After that, variables scored significance level $P \leq 0.05$ or borderline level ($P<0.09$) were included together by block into MLoR analysis. Thus, around 19 categorical variables from socio-economic, lifestyle and sexually risk groups participated in MLoR analysis through both backward and forward stepwise process and finally 'factors behind profession (2 categories or cat)', 'present age (3 categories)', 'education (2 cat)', 'monthly income (2cat)', 'length of profession (3cat)', 'cases of abortion (3cat)', 'types of contraceptives (3 cat)' and 'weekly permanent clients (PC)' significantly ($P<0.05$) remained in the final model (table-8) like previous simple logistic regression (SL_oR) models (table 5, 6, 7). On the other hand, some variables like marital status', 'monthly expenditure', 'places of sex trade (hotel/street)', 'number of clients per day', 'group sex experienced' etc. which were individually significant in simple logistic model (table-5,6,7), were no longer remained significant in MLoR model (table-8) of syphilis. Smaller categories (≤3) of variables were preferred so that range of 95% CI might not be wider thus shorter magnitude of effect could be achieved. "Hosmer-Lemeshaw goodness of fit" test was done and thus **"adjusted model of syphilis"** is presented in table-8 adjusted for all types of variables including socio-demographic variables, lifestyle factors and heterosexual risk behaviors (HRB) of CSWs.

3.4.5c Multiple Logistic Regression model of hepatitis B

Multiple Logistic Regression models (table-9) of hepatitis B (dichotomous outcome variable) adjusted for mainly heterosexual risky behaviors (HRB). At first, simple logistic regression (SL_oR) of each and every socio-demographic variables (total n=6), lifestyle variables (n=11), and HRB or heterosexual risky behaviors (n=15) of CSWs were performed separately (full tables shown in appendix as only 3 variables were found statistically significant). After that, variables scored significance level $P \leq 0.05$ or borderline level ($P<0.09$) were included together into MLoR analysis. Only 3 categorical variables namely 'genital discharge', 'ever experienced in group sex' and 'last month's history of group sex' participated in MLoR analysis through backward stepwise process and finally 'genital discharge' and 'ever experienced in group sex' (both were 2 categories) remained in the adjusted model of hepatitis B.

3.4.5d Crude logistic regression models of 'STIs' for all separate nutritional parameters

In the "Unadjusted model of STIs", all nutritional parameters (categorical variables) were included separately in simple logistic regression analysis (SLoR). Crude logistic regression models of 'STIs' for different independent variables of CSWs are resulted in table-10 (STIs and BMI & macronutrients), table-11 (STIs and micronutrients). In crude model of STIs for BMI and macronutrients (table-10) most of the nutritional variables did not significantly associated with 'STI positivity' except 60-80 g/L category of total protein (P=.022) and >25.1 BMI category (P=.057). Similarly, in crude model of STIs for micronutrients (table-11) majority of biochemical variables did not significantly (p<0.05) associated with 'STI positivity' except serum iron (P=.032), serum vitamin A (P=.025) and serum IgA (P=.051) which also remained significant in adjusted model (table-12).

3.4.5e Multiple logistic regression model of 'STIs' adjusted for nutritional variables

To examine the association of different nutritional parameters such as BMI, serum macronutrients (total protein and albumin) as well as serum micronutrients (immune active vitamins and minerals) with "STI positivity" (both syphilis and hepatitis) simple logistic regression analysis (SLoR) was done first. In the "adjusted model of STIs", all above mentioned nutritional categorical variables which were found statistically significant (P≤ 0.05) or borderline significant (P<0.09) in SLoR (table-10 & 11) were included together by using both "forward" and "backward stepwise" multiple regression method. Furthermore, "Hosmer-Lemeshaw goodness of fit" test was done to see how the model fitted best (table-12).

Six independent nutritional variables namely 'BMI', 'serum vitamin A', 'serum zinc', 'serum iron', 'serum IgA' and 'serum IgM' were remained in the 'adjusted model of STIs' (table-12). About half of predictors of the model namely 'serum vitamin A', 'serum iron' and 'serum IgA' significantly (P<0.05) associated with "STIs" and half of them (BMI, serum zinc & serum IgM), however, could not reach in significance level, instead, they remained marginally significant in the adjusted model. It is notable that serum zinc (P=.341 & P=.850) & serum IgM (P=.407) was insignificant in crude model of STIs (table-11) and BMI had (P=.057) borderline significance (table-10).

CSWs who found to have normal BMI (18.5-25 kg/m^2) were less likely [AOR .498, 95% CI (0.154-1.616), P=.093) to inflict with STIs as compared to CSWs who possessed <18.5 kg/m^2 BMI (reference category. From the fitted model of STIs, it was also observed that CSWs who had severe vitamin A deficiency (serum level 0.0 to o.34 µ mol/L) had 4.461 times higher odds (P=.114) for infecting STIs as compare with CSWs having normal serum vitamin A (>1.05 µ mol/L). Moreover, CSWs suffered from sub-clinical vitamin A deficiency ((<0.7 µ mol/L serum retinol) had 10.792 folds more risks to have STIs [AOR 10.792 (1.93-60.29), p=.007] as compared to CSWs having normal serum vitamin A (>1.05 µ mol/L). Higher serum IgA (.50-5.75 g/L) holders (CSWs) were 7.49 times more likely to [AOR 7.489: 95% CI 1.370-

40.94, P=.020) inflict with STIs than CSWs who had lower level of serum IgA levels (0.00-.49 g/L) was also noticed. Conversely, lower serum IgM holder CSWs (0.02-.29 g/L) were 3 times more likely to [AOR 3.03 95% CI (.880-10.458), P=.079] inflict with STIs than who had higher level of serum IgM levels (0.30-2.85 g/L).

3.4.5f Crude logistic regression models of "condom use" by CSWs

Simple logistic regression analysis of different indicator variables with **"condom use" by CSWs** is tabulated in table-13. It was observed from the unadjusted model of 'condom use' by CSWs (table-12) that most of the variables associated with **inconsistent** 'use of condom' by CSWs except 'NGO counseling on STIs' (P=.139) and 'interval between 2 coitus' (P=.577).

3.4.5g Multiple logistic regression model of "condom use" by CSWs

To observe the association of different variables with inconsistent **"condom use" by CSWs** multiple logistic regressions (MLoR) analysis was done by applying 'backward stepwise' MLoR analysis. For clarification 'Forward stepwise' MLoR analysis was also performed. All categorical variables which were found statistically significant (P≤ 0.05) or borderline significant (P<0.09) in 'simple logistic regression' model (table-13) were included together in the **multiple logistic regression analysis.** "Hosmer-Lemeshaw goodness of fit" test was done and thus **"adjusted model of condom use" by CSWs** is presented in table-14 adjusted for all types of variables including socio-demographic variables, lifestyle factors and HRB of CSWs and table-15 adjusted for all types of variables (socio-demographic variables, lifestyle factors and SRB) including serum immunoglobulin A of CSWs.

3.4.5g-1 "Condom use" model (without immunoglobulin A, n=635)

Five independent variables namely 'category of CSWs', 'uses of contraceptive', 'having NPSP', 'monthly expenditure on food' and 'NGO counseling on STIs' were remained both in the 'unadjusted model' (table-13) as well as in the 'adjusted model' of "Condom use" by CSWs (table-14) except 'NGO counseling on STIs' which is a strong covariate and was insignificant (P=.139) in the 'unadjusted model' (table-12) but remained significantly in 'adjusted model' of "Condom use" by CSWs (table-13). All (P<0.05) these variables remarkably associated with inconsistent "Condom use" by CSWs.

3.4.5g-2 "Condom use" model (including immunoglobulin A , n=107)

Five independent variables namely 'category of CSWs', 'monthly expenditure on food', 'meal skipping', 'interval between 2 coitus' and 'serum IgA' were remained both in the 'unadjusted model' (table-13) as well as in the 'adjusted model' of "Condom use" by CSWs (table-15) except 'meal skipping' and 'interval between 2 coitus' both of which were strong covariates and former was borderline significant (P=.090) and latter was insignificant (P=.577) in the 'unadjusted model' (table-13) but both remained significantly in 'adjusted model' of "Condom use" by CSWs adjusted for all types of variables including IgA. All (P<0.05) these variables significantly associated with inconsistent "Condom use" by CSWs including IgA.

Table-5: 'Socio-demographic' variables associated with "syphilis" (Crude model)

Socio-economic Variables	Total (N=495)	Syphilis n=297 (60%) negative (0)	Syphilis n=198 (40%) positive (1)	Unadjusted Odds ratio (UOR) with 95% Confidence interval (lower–upper)	P-value
Present age (y)					
<18 (r)	17.8 (88)	26.6 (79)	4.5 (09)	1	
19-29	46.3 (229)	49.2 (146)	41.9 (83)	4.990 (2.380 –10.462)	.000**
>30	36.0 (178)	24.2 (72)	53.5 (106)	12.923 (6.094 –27.404)	.000**
Education					
No literacy (r)	69.5 (344)	60.3 (179)	83.3 (165)	1	
1-12 class	30.5 (151)	39.7 (118)	16.7 (33)	.303 (.195 –.471)	.000**
Marital status					
Married (r)	41.6 (206)	40.1 (119)	43.9 (87)	1	
Unmarried	13.7 (68)	19.9 (59)	4.5 (09)	.209 (.098– .443)	.000**
Widow/divorced /abandoned	44.7 (221)	40.1 (119)	51.5 (102)	1.172 (.800–1.719)	.415
Having children					
No (r)	56.4 (279)	82.0 (146)	41.9 (133)	1	
Yes	43.6 (216)	18.0 (32)	58.1 (184)	1.131 (.787-1.625)	.505
Monthly income					
12.5 -62.5 **$** (r)	67.3 (333)	56.9 (169)	82.8 (164)	1	
>62.6 **$**	32.7 (162)	43.1 (128)	17.2 (34)	.274 (.177–.423)	.000**
Last month's Total expenditure	67.5 (334)	62.0 (184)	75.8 (150)	1	
12.5 -62.5 **$** (r)	32.5 (161)	38.0 (113)	24.2 (48)	.521 (.349–.778)	.001**
>62.6 **$**					
Treatment cost of STIs[6] (last ailment, n=238/495)					
Self treatment	22.7 (54)	18.7 (25)	27.9 (29)	1.963 (.954–4.041)	.067
5-250 taka	47.9(114)	48.5 (49)	47.1 (49)	1.276 (.693–2.349)	.434
251-5000 taka (r)	29.4 (70)	32.8 (44)	25.0 (26)	1	

Legend: Descriptive (cross tab) and Simple Logistic Regression (SLoR)
'r' means reference category. P<0.05*, P ≤0.001**

[6] No symptoms appeared in 51.9% (257/495) of CSWs.

Table-6: "Lifestyle" variables associated with "syphilis" (Crude model)

lifestyle Variables of all CSWs♥	Total (N=495)	Syphilis negative (0)	Syphilis positive (1)	Unadjusted Odds ratio (UOR) with 95% Confidence interval (lower–upper)	P-value
Factors behind profession					
Lower Economic status (r)	56.6 (280)	61.3 (182)	49.5 (98)	1	
Other reasons[7]	43.4 (215)	20.5 (115)	22.4 (100)	1.615 (1.123–2.323)	.010*
Entering age into sex trade					
10-17 (r)	43.5 (215)	42.1 (125)	45.5 (90)	1	
18-24	34.9 (173)	38.0 (113)	30.3 (60)	.737 (.487–1.116)	.149
>25	21.6 (107)	19.9 (59)	24.2 (48)	1.130 (.708–1.803)	.609
Length of profession as CSW					
Less than one year (r)	10.7 (53)	16.2 (48)	2.5 (05)	1	
1-5 year	52.1 (258)	61.6 (183)	37.9 (75)	3.934 (1.507–10.270)	.005**
>6 y	37.2 (184)	22.2 (66)	59.6 (118)	17.164 (6.513– 45.234)	.000**
Places of sex business					
Floating	64.0 (317)	50.8 (151)	83.8 (166)	5.016 (3.225–7.880)	.000**
Hotel (r)	36.0 (178)	49.2 (146)	16.2 (32)	1	
Mass media exposure					
No items	60.4 (299)	55.2 (164)	68.2 (135)	3.442 (1.716-6.905)	.001**
Radio/TV/VCD	28.1 (139)	29.3 (87)	26.2 (52)	2.499 (1.190-5.250)	.016*
Had all (r)	11.5 (57)	15.5 (46)	5.6 (11)	1	
Working days (last week, n=470)[8]					
1–3 days (r)	50.5 (250)	54.3 (155)	51.1 (95)	1	
4–7 days	44.4 (220)	45.4 (129)	48.9 (91)	1.151 (.795–1.667)	.457
Abortion cases					
No abortion cases (r)	72.9 (361)	78.1 (232)	65.2 (129)	1	
≥1 cases	27.1 (134)	17.2 (65)	27.7 (69)	1.909 (1.278–2.852)	.002**
Use of contraceptives					
No measure (r)	16.8 (83)	15.5 (46)	18.7 (37)	1	
Pill/others	40.4 (200)	37.7 (141)	20.2 (59)	.520 (.307–.883)	.015*
Condom	42.8 (212)	37.0 (110)	51.5 (102)	1.153 (.692–1.919)	.585
Habit of Smoking					
No (r)	57.4 (284)	60.3 (179)	53.0 (105)	1	
yes	42.6 (211)	39.7 (118)	47.0 (93)	1.344 (.934–1.932)	.111
Substance abuse					
No (r)	69.5 (344)	70.0 (208)	68.7 (136)	1	
Regularly	30.5 (151)	30.0 (89)	31.3 (62)	1.065 (.722– 1.573)	.750
Client profile					
Businessmen (small) (r)	43.0 (213)	47.5 (141)	36.4 (72)	1	
Rickshaw puller	23.6 (117)	20.9 (62)	27.8 (55)	1.737 (1.096–2.754)	.019*
Service holder	13.1 (65)	14.1 (42)	11.6 (23)	1.072 (.599–1.920)	.814
Student	6.2 (30)	5.7 (17)	6.6 (13)	1.498 (.689–3.254)	.308
Bus driver	4.2 (21)	4.0 (12)	4.5 (09)	1.469 (.591–3.648)	.408
Don't know	9.9 (49)	7.7 (23)	13.1 (26)	2.214 (1.180–4.152)	.013*
NGO counseling on STIs					
No (r)	51.1 (253)	51.2 (152)	51.0 (101)	1	
Yes	48.9 (242)	48.8 (145)	49.0 (97)	1.007 (.703–1.443)	.971
Meal skipping (usually a day)					
Yes	11.9 (59)	11.8 (35)	12.1 (24)	1.033 (.594-1.796)	.910
No (r)	88.1 (436)	88.2 (262)	87.9 (174)	1	

Legend: Descriptive (cross tab) and Simple Logistic Regression (SLoR)
'r' means reference category. P<0.05*, P ≤0.001**

[7] About 21.2% (n=105/495) CSWs exploited by pimps, also Angry with family members (HB 6.9%, FF 7.6%), sold by step mom/others (HB 4.8%, FF 6.3%), raped/tortured (HB 4.0%, FF 6.0%), pushed by husband (HB 3.4%, FF 1.2%), self motivation (both 2.2%) and drug addiction (HB 0.8%, FF 1.3%).

[8] About 5.1% (25/495) did not work during the last week.

Table: 7: 'Risky sexual behaviors' and symptom associated with "syphilis" (Crude model)

Risky sexual behaviors of all CSWs	Total (N=495)	Syphilis negative n=297 (0)	Syphilis positive n=198 (1)	Unadjusted Odds ratio (UOR) with 95% Confidence interval (lower–upper)	P-value
Client turnover (last week, n=470)					
1– 14 C	52.7 (261)	51.4 (146)	61.8 (115)	1.531 (1.051–2.231)	.027*
≥15 C (r)	42.2 (209)	48.6 (138)	38.2 (71)	1	
Usual no. of client per day					
1-7	76.2 (377)	68.7 (204)	87.4 (173)	3.155(1.941 –5.128)	.000**
>8 clients (r)	23.8 (118)	31.3 (93)	12.6 (25)	1	
Interval between 2 coitus					
No (r)	63.6 (315)	68.7 (204)	56.1 (111)	1	
Yes	36.4 (180)	31.3 (93)	43.9 (87)	1.719 (1.184–2.496)	.004**
Use of condom					
No use/occasional	54.1 (268)	52.9 (157)	56.1 (111)	1.138 (.793–1.633)	.484
Consistent use (r)	45.9 (227)	47.1 (140)	43.9 (87)	1	
Hygienic measure after coitus					
No measure (r)	33.3 (165)	32.7 (97)	34.3 (68)	1	
Use savlon/soap	41.2 (204)	45.8 (136)	34.3 (68)	.713 (.466–1.091)	.119
others[@]	25.5 (126)	21.5 (64)	31.4 (62)	1.382 (.866–2.204)	.175
Usual Coital frequency per client					
One time	59.2 (293)	61.6 (183)	55.6 (110)	1	
More than two	40.8 (202)	38.4 (114)	44.4 (88)	1.284 (.891–1.850)	.179
Weekly coitus with PC					
No weekly PC (r)	59.8 (296)	63.0 (187)	55.1 (109)	1	
Yes 1 time	23.8 (118)	22.9 (68)	25.3 (50)	1.261 (.817 – 1.949)	.295*
2 times	16.4 (81)	14.1 (42)	19.7 (39)	1.593 (.970–2.615)	.056*
Fortnightly coitus with PC					
No fortnightly PC (r)	72.3 (358)	66.3 (197)	81.3 (161)	1	
Yes					
1-2 times in fortnight	21.8 (108)	25.9 (77)	15.7 (31)	.493 (.301–.785)	.003**
>3 times	5.9 (29)	7.7 (23)	3.0 (06)	.319 (.127–.813)	.015 *
Monthly coitus with PC					
No monthly PC (r)	65.9 (326)	60.3 (179)	74.2 (147)	1	
Yes					
1-2 times in a month	20.2 (100)	21.5 (64)	18.2 (36)	.685 (.431–1.088)	.109
>3 times	13.9 (69)	18.2 (54)	7.6 (15)	.338 (.183–.624)	.001**
Having NPSP					
No (r)	91.5 (453)	89.9 (267)	93.9 (186)	1	
Yes	8.5 (42)	10.1 (30)	6.1 (12)	.574 (.287–1.151)	.118
Ever experienced in Anal sex					
No	96.4 (477)	94,9 (282)	98.5 (195)	.289 (.083–1.013)	.182*
Yes (r)	3.6 (18)	51.0 (03)	1.5 (15)	1	
Ever experienced in Gr.S					
No (r)	74.1 (367)	77.4 (230)	69.2 (137)	1	
yes	25.9 (128)	22.6 (67)	30.8 (61)	1.528 (1.018–2.295)	.041*
Group sex history (last month)					
No (r)	77.4 (383)	81.8 (243)	70.7 (140)	1	
yes	22.6 (112)	18.2 (54)	29.3 (58)	1.864 (1.219–2.852)	.004**
No. of Persons in Gr.S (last)					
No Gr.S experienced (r)	77.4 (383)	81.8 (243)	70.7 (140)	1	
2-5 clients	18.8 (93)	14.8 (44)	24.7 (49)	1.933 (1.224–3.053)	.004**
>6	3.8 (19)	3.4 (10)	4.5 (09)	1.562 (.620–3.937)	.344
Genital discharge (symptom)					
Yes	34.7 (172)	34.3 (102)	35.4 (70)	1.045 (.715-1.524)	.817
No (r)	65.3 (323)	65.7 (195)	64.6(128)	1	

Legend: Descriptive (cross-tabulation) and Simple logistic regression (SLoR), *P<0.05, **P<0.01
PC ="permanent client"
NPSP ="Non Paying Sexual Partner"
Gr. S= "group sex"
[@]Washed by water (douching), cotton & urine

Table-8: 'Multiple Logistic Regression (MLoR) model of syphilis' adjusted for socio-demographic', 'lifestyle' and 'HRB' variables.

Explanatory variables for syphilis model	All CSWs	Syphilis (outcome variable)		MLoR model of Syphilis adjusted for different background, lifestyle and risky sexual			
	Total =495	No (0) 60.0% N=297	Yes (1) 40.0% n=198	Beta coefficien	Standard	Odds ratio 95% CI (lower–upper)	P-value
Factors behind profession							
Lower Economic status (r)	56.6 (280)	61.3 (182)	49.5(98)			1	
Other reasons[9]	43.4 (215)	20.5 (115)	22.4(100)	.389	.228	1.476 (.944–2.307)	.016*
Present age (y)							
<18 (r)	17.8 (88)	26.6 (79)	4.5 (09)			1	
19-29	46.3 (229)	49.2 (146)	41.9 (83)	.966	.415	2.628 (1.166–5.923)	.020*
>30	36.0 (178)	24.2 (72)	53.5 (106)	1.502	.455	4.492 (1.843–10.948)	.001**
Education							
No literacy	69.5 (344)	56.9 (169)	83.3 (165)	.714	.270	2.042 (1.203–3.466)	.008*
1-12 class (r)	30.5 (151)	39.7 (118)	16.7 (33)			1	
Monthly income (BDT)							
1000-5000 (12.5 -62.5 $)	67.3 (333)	67.3 (333)	82.8 (164)	.761	.266	2.141 (1.272–3.603)	.004**
>5000.1 (>62.6 $) (r)	32.7 (162)	43.1 (128)	17.2 (34)			1	
Length of profession							
<1 y (r)	10.7 (53)	16.2 (48)	2.5 (05)			1	
1-5 y	52.1 (258)	61.6 (183)	37.9 (75)	.960	.522	2.613 (.939-7.271)	.066
>6 y	37.2 (184))	22.2 (66)	59.6 (118)	1.789	.547	5.984 (2.048–17.484)	001*
Abortion cases							
No abortion cases (r)	72.9 (361)	78.1 (232)	65.2 (129)			1	
Having abortion (≥1 c)	27.1 (134)	17.2 (65)	27.7 (69)	.728	.249	2.072 (1.272–3.376)	.003**
Use of contraceptives							
No measure (r)	16.8 (83)	15.5 (46)	18.7 (37)			1	
Pill/other methods	40.4 (200)	37.7 (141)	20.2 (59)	-.756	.322	.470 (.250–.882)	019*
Condom	42.8 (212)	37.0 (110)	51.5 (102)	.145	.304	1.156 (.637–2.095)	.634
Weekly coitus with PC							
No (r)	59.8 (296)	63.0 (187)	55.1 (109)			1	
Yes 1 time	23.8 (118)	22.9 (68)	25.3 (50)	.636	.270	1.890 (1.114-3.206)	.018*
≥2 times	16.4 (81)	14.1 (42)	19.7 (39)	.761	.310	2.141 (1.166-3.932)	.014*

Legend: Backward stepwise Multiple Logistic Regression Model (MLoR)
Hosmer-Lemeshaw goodness of fit for "Syphilis" model (n=495): $\aleph^2$=4.072, df=8, P=.851
Model summary: -2 Log likelihood (-2LL)=513.254, Nagelkerke R-square=.359
Model coefficient: Chi-square ($\aleph^2$)=153.028, df=12, P=.000
Overall predicted percentage from classification table of DV (syphilis) was 74.9% (cut value is 50%).
(r)= reference category. P<0.05*, P<0.01**
PC=permanent client
1 US $=80 BDT (24-10-2013)

[9] About 21.2% CSWs exploited by pimps, also Angry with family members, sold by step mom/others, raped/tortured, pushed by husband, self motivation and drug addiction.

Table-9: Multiple Logistic regression (MLoR) model of Hepatitis B

Explanatory variables for Hepatitis B model	All CSWs n=495 % n	Hepatitis B No (0) 94.5% n=468	Hepatitis B Yes (1) 5.5% n=27	*Unadjusted OR (95% CI) (lower-upper)	Adjusted Odds Ratio (AOR) Beta coefficient (B) SE	Adjusted Odds Ratio (AOR) (95% CI) (lower-upper)
Genital discharge (symptom)						
Yes (r)	34.7 (172)	35.7 (167)	18.5 (05)	1	-	1
No	65.3 (323)	64.3 (301)	81.5 (22)	2.441 (.908-6.565) P=**.077**	.902 SE=.508	2.465 (.910-6.677) P=**.076**
Ever experienced in group sex						
No (r)	74.1 (367)	75.6 (354)	48.1 (13)	1	-	1
1-5 class	25.9 (128)	24.4 (114)	51.9 (14)	3.344(1.527–7.323) **P=.003****	1.213 SE=.402	3.364 (1.531-7.395) P=**.003***
Group sex history (last month)						
No (r)	77.4 (383)	78.4 (367)	59.3 (16)	1		
yes	22.6 (112)	21.6 (101)	40.7 (11)	2.498 (1.124–5.562) **P=.025***	No longer exist in the model	

Legend: Descriptive (cross tab) and *Simple Logistic Regression (SLoR).
Backward stepwise Multiple Logistic Regression Model (MLoR)
Hosmer-Lemeshaw goodness of fit for Hepatitis B model: $\aleph^2$ =.143, df=2, P=.931
-2 Log likelihood (-2LL) ratio=197.083, Nagelkerke R-square=.072
Model coefficient :Chi-square ($\aleph^2$)=12.488, P=.002, df=2
Overall predicted percentage from classification table of Hepatitis B (DV) was 94.5% (cut value is 50%).
(r)= reference category. P<0.05*, P<0.01**

Table-10: Nutritional variables associated with STIs (Crude model)

Nutritional variables (NRI,BMI & serum Macronutrients) n=495	All CSWs (n=495) % n	Sexually Transmitted Infection (STIs)[♣] No 56.4% (n=279) % n	Yes 43.6% (n=216) % n	Unadjusted odd ratio (UOR) 95% CI (lower–upper)	P-value
Nutrition risk Index (NRI)					
>100 (well nourished) (r)	36.4 (180)	34.8 (97)	38.4 (83)	1	
<83 (malnourished)	63.6 (315)	65.2 (182)	61.6 (133)	.854 (.591-1.235)	.401
BMI (kg/m^2)					
<13.51-18.4 (CED) (r)	24.4 (121)	24.7 (69)	24.1 (52)	1	
18.5-25 normal	62.2 (308)	65.2 (182)	56.5 (126)	.919 (.600– 1.406)	.696
>25.1 overweight	13.33 (66)	15.8 (28)	15.7 (38)	1.801 (.982 – 3.303)	.057
Mean (95% CI)	**20.89 (20.89–21.4)**				
Range	13.51–51.3				
Serum Total Protein (g/L) (n=495)					
1.14 to 59.9	49.7 (246)	48.7 (136)	50.9 (110)	.822 (.535–1.262)	.370
60.0 to 80	24.6 (122)	28.4 (79)	19.9 (43)	.553 (.332–.920)	**.022***
≥80.1 (r)	25.7 (127)	22.9 (64)	29.2 (63)	1	
Mean (95% CI)	**50.84 (47.75–54.02)**				
Range	1.14 to 167.19				
Serum Albumin (g/L)					
.01 to 39.9	63.6 (315)	65.2 (182)	61.6 (133)	.940 (.592–1.490)	.791
40 to 60	17.0 (84)	15.4 (43)	19.0 (41)	1.226 (.681–2.207)	.497
≥60.1 (r)	19.4 (96)	19.4 (54)	19.4 (42)	1	
Mean (95% CI)	**31.81 (29.59–34.11)**				
Range	0.01 to 135.07				

Legend: Descriptive (cross-tabulation) and Simple Logistic Regression (SLoR)
r=reference category. P<0.05

[♣] Here STIs "yes" (1) means these CSWs had either syphilis (38.18%) or Hepatitis B positive (3.64%) or having the both infections (1.82%) and "no" infection (0) means none (56.36%). of these CSWs had infected with either syphilis spirochete or hepatitis B virus.

Table-11: Micronutrients and immunological factors associated with STIs (crude model).

Nutritional parameters (Micronutrients and Ig)	All CSWs (n=107) % n	Sexually Transmitted Infection (STIs)		Unadjusted odd ratio (UOR) 95% CI (lower–upper)	p-value
		No 31.78% (n=34) % n	Yes 68.22% (n=73) % n		
Serum Copper (µmol/L)					
8.18 to 10.9 (r)	6.5 (07)	5.9 (02)	6.8 (05)	1	-
10.9 to 22	68.3 (73)	76.5 (26)	64.4 (47)	.723 (.131–3.991)	.710
22.1 to 63.27	25.2 (27)	17.6 (06)	28.8 (21)	1.400 (.215–9.121)	.715
Mean (95% CI)	**18.34 (17.46–19.71)**				
Range	8.13 to 63.10				
Serum Zinc (µmol/L)					
5.97 to 11.5 (r)	37.4 (40)	32.4 (11)	39.7 (29)	1	-
11.6 to 18.5	37.4 (40)	44.1 (15)	34.2 (25)	.632 (.246–1.625)	.341
18.6 to 99.30	25.2 (27)	23.5 (08)	26.1 (19)	.901 (.306–2.650)	.850
Mean (95% CI)	**14.41 (13.15–15.91)**				
Range	5.37 to 99.30				
Serum Iron (µmol/L)					
0.11 to 10.9	15 (16)	8.8 (03)	17.8 (13)	5.200 (1.149–23.540)	032*
11.0 to 29.0	64.5 (69)	55.9 (19)	68.5 (50)	3.158 (1.172–8.512)	.023*
29.1 to 70.74(r)	20.6 (22)	35.3 (12)	13.7 (10)	1	-
Mean (95% CI)	**18.49 (16.43–20.69)**				
Range	0.11 to 70.73				
Serum Vitamin A (µmol/L)					
0.0 to o.34 (Severe)	19.6 (21)	17.6 (06)	20.5 (15)	5.625 (1.241–15.492)	.025
0.35 to 1.04 (sub-clinical)	68.2 (73)	55.9 (19)	74.0 (54)	6.395 (1.763–13.198)	.005
>1.05 (Normal) (r)	12.2 (13)	26.5 (09)	5.5 (04)	1	-
Mean (95% CI)	**0.66 (0.56–0.75)**				
Range	0.0 to 4.18				
Serum Vitamin E (µmol/L)					
0.0 to 5.99	42.06 (45)	50.0 (17)	38.4 (28)	.633 (.192–2.092)	.454
6.0 to 6.66	41.12 (44)	35.3 (12)	43.8 (32)	1.026 (.301–3.496)	.968
13.0 to 35.23 (r)	16.82 (18)	14.7 (05)	17.8 (13)	1	-
Mean (95% CI)	**6.29 (5.36–7.20)**				
Range	0.00 to 35.23				
@Serum IgG (g/L)					
0 .04 to 4.99	85.00 (91)	95.0 (38)	79.1 (53)	1.350 (.447–4.079)	.595
5.0 to 33.0 (r)	15.0 (16)	5.0 (02)	20.9 (14)	1	-
Mean (95% CI)	2.73 (1.52–3.93)				
Range	0.04 to 33.0				
@Serum IgA (g/L)					
0.0 to 0.49 (r)	79.44 (85)	91.2 (31)	74.0 (54)	1	-
0.50 to 5.75	20.56 (22)	8.8 (03)	26.0 (19)	3.636 (.996–13.278)	.050
Mean (95% CI)	**0.50 (0.33–0.68)**				
Range	.00 to 5.75				
@Serum IgM (g/L)					
0.02 to 0.29	67.3 (72)	61.8 (21)	69.9 (51)	1.435 (.611–3.361)	.407
0.30 to 2.85 (r)	32.7 (35)	38.2 (13)	30.1 (22)	1	-
Mean (95% CI)	**0.42 (0.28–0.55)**				
Range	0.02 to 2.85				

Legend: Descriptive (Frequeny, SD and cross tab) and Simple Logistic Regression (SLoR)
(r)= reference category. P<0.05*, P<0.01**
@For obtaining greater cell numbers and small magnitude (or range) of 95% CI, serum immunoglobulin (IgG) categories were rearranged by adding "very low" and "low" frequencies (namely <below normal) and "normal" and "high" frequencies (≥normal) together

Table-12: 'Multiple Logistic Regression' model of STIs adjusted for nutritional variables

Explanatory variables	All CSWs	♣STI (outcome variable)		B	Adjusted Odds ratio	
(n=107)	% (n)	Negative (n=34)	Positive (n=73)	Coeffi	95% CI (lower–upper)	P-value
BMI (kg/m^2)						
<13.51-18.4 (CED) (r)	31.8 (34)	24.7 (12)	24.1 (22)	-	1	-
18.5-25 normal	51.4 (55)	65.2 (21)	56.5 (34)	-.696	.498 (.154– 1.616)	.246
>25.1 overweight	16.8 (18)	15.8 (01)	15.7 (17)	1.970	7.169 (.719 – 71.472)	.093
Vitamin A (µ mol/L)						
0.0 to o.34 (Severe)	19.6 (21)	17.6 (06)	20.5 (15)	1.528	4.611 (.693-30.68)	.114
♦0.35 to 1.04 (S.clinical)	68.2 (73)	55.9 (19)	74.0 (54)	2.379	10.792 (1.93-60.29)	.007**
>1.05 (Normal) (r)	12.2 (13)	26.5 (09)	5.5 (04)	-	1	-
Serum Zinc (µ mol/L)						
5.97 to 11.5 (r)	37.4 (40)	32.4 (11)	39.7 (29)	-	1	-
11.6 to 18.5	37.4 (40)	44.1 (15)	34.2 (25)	-.517	.596 (.181– 1.968)	.396
18.6 to 99.30	25.2 (27)	23.5 (08)	26.1 (19)	1.441	4.227 (.828 – 21.587)	.083
Serum Iron (µ mol/L)						
0.11 to 10.9	15 (16)	8.8 (03)	17.8 (13)	2.145	8.545 (1.197– 61.02)	.032*
11.0 to 29.0	64.5 (69)	55.9 (19)	68.5 (50)	1.437	4.208 (.975 – 18.160)	.054
29.1 to 70.74(r)	20.6 (22)	35.3 (12)	13.7 (10)	-	1	-
@Serum IgA (g/L)						
0.0 to 0.49 (r)	79.44 (85)	91.2 (31)	74.0 (54)	-	1	-
0.50 to 5.75	20.56 (22)	8.8 (3)	26.0 (19)	2.013	7.489 (1.370-40.94)	.020*
@Serum IgM (g/L)						
0.02 to 0.29	67.3 (72)	61.8 (21)	69.9 (51)	1.110	3.034 (.880-10.458)	.079
0.30 to 2.85 (r)	32.7 (35)	38.2 (13)	30.1 (22)	-	1	-

Legend: Backward stepwise Multiple Logistic Regression Model (MLoR)
Hosmer-Lemeshaw goodness of fit for "STI" model (n=107): $\aleph^2$ =5.986, df=7, P=.541
Model summary: -2 Log likelihood (-2LL)=98.266, Nagelkerke R-square=.396
Model coefficient: Chi-square ($\aleph^2$)=35.520, df=10, P=.000
Overall predicted percentage from classification table of DV (STI) was 73.8% (cut value is 50%).
(r)= reference category. P<0.05*, P<0.01**
@For obtaining greater cell numbers and small magnitude (or range) of 95% CI, serum immunoglobulin (IgA, IgM) categories were rearranged by adding "very low" and "low" frequencies together and named <below normal and made ≥normal by adding "normal" and "high" frequencies

♣ Here STIs "yes" (1) means these CSWs had either syphilis (38.18%) or Hepatitis B positive (3.64%) or having the both infections (1.82%) and "no" infection (0) means none (56.36%). of these CSWs had infected with either syphilis spirochete or hepatitis B virus.

♦ Only 5 CSWs had 0.7 t0 1.04 µ mol/L (Mild deficiency) serum vitamin A and rest (n=68) of all had subclinical vitamin A deficiency (<0.7 µ mol/L serum retinol).

Table-13: Factors associated with inconsistent 'condom use' by CSWs (crude model)

Factors associated with inconsistent condom use by CSWs	All CSWs n=635 % n	Unadjusted model "condom use" by CSWs focusing on factors associated with inconsistent condom use			
		0=consistent % n	1=inconsistent % n	95% CI (lower-upper)	p-value
Category of CSWs					
Hotel-based (r)	48.5 (308)	53.6 (150)	44.5 (158)	1	-
Road/perk/shrine	51.5 (327)	46.4(130)	55.5 (197)	1.439 (1.050-1.971)	P=.023*
Monthly expenditure on food (BDT)					
500-2000	67.4 (428)	66.8 (187)	67.9 (241)	2.014 (1.045-3.880)	P=.036*
2001-3500	26.1 (166)	24.3 (68)	27.6 (98)	2.252 (1.119-4.533)	P=.023*
>3500 (r)	6.5 (41)	8.9 (25)	4.5 (16)	1	-
NGO counseling on STIs					
Yes	48.7 (309)	45.4 (127)	51.3 (182)	1.267 (.926-1.735)	P=.139
No (r)	51.3 (326)	54.6 (153)	48.7 (173)	1	-
Having NPSP					
No (r)	90.9 (577)	93.6 (262)	88.7 (315)	1	-
Yes	9.1 (58)	6.4 (18)	11.3 (40)	1.848 (1.035-3.301)	P=.038*
Interval between 2 coitus					
No (r)	67.4 (428)	68.6 (192)	66.5 (236)	1	-
Yes	32.6 (207)	31.4 (88)	33.5 (119)	1.10 (.787-1.538)	P=.577
Use of contraceptive					
No measure	16.2 (103)	15.0 (42)	17.2 (61)	1.623 (1.020-2.583)	P=.041*
Pill/others	44.1 (280)	37.5 (105)	49.3 (175)	1.863 (1.318-2.633)	P=.000**
Condom (r)	39.7 (252)	47.5 (133)	33.5 (119)	1	-
Meal skipping					
1-2 meals	9.8 (62)	7.5 (21)	11.5 (41)	1.610 (.928-2.794)	P=.090
No (r)	90.2 (573)	92.5 (259)	88.5 (314)	1	-
Serum Vitamin E (µ mol/L) n=107					
0.0 to 5.99 (r)	42.06 (45)	35.0 (21)	51.1 (24)	1	-
6.0 to 6.66	41.12 (44)	41.7 (25)	40.4 (19)	1.50 (.652-3.469)	P=.339
13.0 to 35.23	16.82 (18)	23.3 (14)	8.5 (04)	4.00 (1.139-14.047)	P=.031*
Immunoglobulin A (IgA) µmol/L (n=107)					
Very low (0.00 to 0.35) (r)	64.5 (69)	76.6 (36)	55.0 (33)	1	-
Low (0.36 to 0.49)	15.0 (16)	17.0 (08)	13.3 (08)	1.091 (.368-3.238)	P=.875
≥normal (0.50 to 5.75)	20.5 (22)	6.4 (03)	31.7 (19)	6.909 (1.872-25.506)	P=.004**

Legend: Descriptive (cross-tabulation) and Simple Logistic Regression (SLoR)
r=reference category.
P<0.05*, P<0.01**

Table-14: Multiple Logistic Regression (MLoR) model of 'condom use' by CSWs

Factors associated with inconsistent condom use by CSWs	Adjusted model of 'condom use'						
	All CSWs (n=635) % n	0=consistent n=280 % n	1=inconsistent TP n=355 % n	Beta coefficient (B)	standard error	Adjusted Odds Ratio (95% CI) (lower-upper)	P-value
Category of CSWs							
Hotel-based (r)	48.5 (308)	53.6 (150)	44.5 (158)	-	-	1	-
Road/perk/shrine	51.5 (327)	46.4(130)	55.5 (197)	.592	.180	1.807 (1.271-2.570)	.001**
Use of contraceptive							
No measure	16.2 (103)	15.0 (42)	17.2 (61)	.590	.246	1.805 (1.115-2.921)	.016*
Pill/others	44.1 (280)	37.5 (105)	49.3 (175)	.905	.193	2.472 (1.695–3.606)	.000**
Condom (r)	39.7 (252)	47.5 (133)	33.5 (119)	-	-	1	-
Having NPSP							
No (r)	90.9 (577)	93.6 (262)	88.7 (315)	-	-	1	-
Yes	9.1 (58)	6.4 (18)	11.3 (40)	.777	.309	2.176 (1.187-3.988)	.012*
Monthly expenditure on food (BDT)							
500-2000	67.4 (428)	66.8 (187)	67.9 (241)	.758	.350	2.135 (1.076-4.237)	.030*
2001-3500	26.1 (166)	24.3 (68)	27.6 (98)	.852	.378	2.344 (1.117-4.916)	.024*
>3500 (r)	6.5 (41)	8.9 (25)	4.5 (16)	-	-	1	-
NGO counseling on STIs							
Yes	48.7 (309)	45.4 (127)	51.3 (182)	.365	.168	1.440 (1.036–2.003)	.030*
No (r)	51.3 (326)	54.6 (153)	48.7 (173)	-	-	1	-

Legend: Backward stepwise Multiple Logistic Regression (MLoR)
Hosmer-Lemeshaw goodness of fit for "condom use" by CSWs model (n=635)l: $\aleph^2$ =4.232, df=8, P=.836
Model summary: -2 Log likelihood (-2LL)=830.584, Nagelkerke R-square=.083
Model coefficient :Chi-square ($\aleph^2$)=40.834, P=.000, df=7
Overall predicted percentage from classification table of DV (condom use) was 62.5% (cut value is 50%).
(r)= reference category, NPSP= Non-paying sexual partners.
P<0.05*, P<0.01**

Table-15: Multiple Logistic Regression model of 'condom use' by CSWs adjusted for different independent variables including serum immunoglobulin

Factors associated with inconsistent condom use by CSWs	Adjusted model of 'condom use' (with serum immunoglobulin)						
	All CSWs (n=107) % n	0=consistent TP n=47	1=inconsistent TP n=60	Beta coefficient	standard error	Adjusted Odds Ratio (95% CI) (lower-upper)	P-value
Category of CSWs							
Hotel-based (r)	17.8 (19)	31.9 (15)	6.7 (04)	-	-	1	-
Road/perk/shrine	82.2 (88)	68.1 (32)	93.3 (56)	1.849	.731	6.352 (1.516-26.618)	.011*
Monthly expenditure on food (BDT)							
500-2000	65.42 (70)	61.7 (29)	68.3 (41)	3.474	1.689	32.26 (1.177-883.688)	.040*
2001-3500	28.97 (31)	27.7 (13)	30.0 (18)	3.269	1.708	26.297 (.924-748.399)	.056
>3500 (r)	5.61 (06)	10.6 (05)	1.7 (01)	-	-	1	-
Meal skipping							
♣One-two meals	26.17 (28)	14.89 (07)	35.0 (21)	1.156	.572	3.179 (1.037–9.746)	.043*
No meal skipping (r)	73.83 (79)	85.11 (40)	65.0 (39)	-	-	1	-
Interval between 2 coitus							
No (r)	60.75 (65)	70.21 (33)	53.33 (32)	-	-	1	-
Yes	39.25 (42)	29.79 (14)	46.67 (28)	1.287	.521	3.622 (1.306–10.046)	.013*
Immunoglobulin A (IgA)							
Very low (0.00 to 0.35) (r)	64.5 (69)	76.6 (36)	55.0 (33)	-	-	1	-
Low (0.36 to 0.49)	15.0 (16)	17.0 (08)	13.3 (08)	-.132	.639	.877 (.250–3.069)	.837
≥normal (0.50 to 5.75)	20.5 (22)	6.4 (03)	31.7 (19)	2.477	.825	11.90 (2.362-59.99)	.003**

Legend: Backward stepwise Multiple Logistic Regression Model (MLoR)
Hosmer-Lemeshaw goodness of fit for 'condom use' model (n=107): $\aleph^2$=6.113, df=7, P=.527
Model summary: -2 Log likelihood (-2LL)=109.128, Nagelkerke R-square=.397
Model coefficient: Chi-square ($\aleph^2$)=37.622, P=.000, df=7
Overall predicted percentage from classification table of DV (condom use) was 74.8% (cut value is 50%).
(r)= reference category
P<0.05*, P<0.01**

3.5 Discussion

The prevalence rates of syphilis (generally 40% but floating rate higher 52.4%) were overwhelmingly higher and hepatitis B prevalence was (5.5%) comparatively low and no HIV infection was found among the CSWs. This result (i.e. zero HIV prevalence and higher STIs rate) is very consistent with present and previous reports[10] and previous Bangladeshi studies and reports (Shirin et al, 2009; World Bank and UNAIDS, 2009; Azim et al, 2000; Sarker et al 1998; Alam MK) and also in agreement with a Japanese study (Kinuko Kimoto) about 2 types of CSWs where none of the participants were HIV positive and most frequent STI was chlamydial infection (39.1%) followed by candidacies (33.7%) and genital herpes (25%). Low HIV prevalence with risky behaviors among female sex workers (FSWs) was reported in (Sindh) Pakistan (Altaf et al, 2012). However, 'zero HIV prevalence' among healthy population and Turkish soldiers in Northern Cyprus (Altindis et al, 2006) and among risk groups other than CSWs also reported in three previous studies (Gibney et al, 2001a and 2001b, 2002) conducted in Bangladesh.

STIs prevalence among CSWs showed that 52.4% of floating FCSWs contacted syphilis which is very close to the 57% syphilis prevalence found in a previous brothel-based study (Sarker et al, 1998) and in another CSW's study (Azim et al, 2000) in Bangladesh but higher than other previous studies of street-based CSWs conducted in Bangladesh (Mondal et al, 2008; Rahman et al, 2000), a brothel-based study in India (Desai et al, 2003) and sex-trafficked women in Nepal (Silverman et al, 2008). Similar higher rate of syphilis (44%) among FCSWs was also observed in a study conducted in Pakistan (Bibi et al, 2010) and in Argentina (María et al, 2006; 45.7% syphilis rate among FCSWs). However, higher prevalence rate (62%) of syphilis was also reported in a Pakistani study (Ayesha et al, 2006) among Hijras (transgender). In contrast, low sexually transmitted infection rate (*C.trachomatis 1.4%* and *T. vaginalis 0.7%*) was reported among registered female sex workers in Ankara city (Zarakolu et al, 2010), blood donors of Tanzania [Matee et al, 2006 (syphilis 4.7%)] and Cameroon [Jean et al, 2013 (syphilis 5.7%)] and among youth clients of hotel-based CSWs (2.6%) in Bangladesh (Haseen et al, 2012) which was, however, overwhelmingly high in this study (general syphilis rate 40%).

Hotel-based CSWs were reported (figure-1.1) to have significantly ($P<0.05$) higher amount of normal CSWs (77.53%) having no STIs than floating CSWs (44.48%). In addition, having slightly higher prevalence rate (4.49%) of Hepatitis B infection in hotel groups was found as compared to floating (3.15%) ($P>0.05$) which was just opposite of a study conducted in Uruguay (Russi et al, 2003). On the other hand, absolutely syphilis prevalence rate was significantly ($P<0.05$) higher among floating CSWs (50.47%) than HBCSWs (16.29%). But, co-infection rate (FCSWs=1.89% vs. HBCSWs=1.69%) was indifferent for both groups

[10] Global AIDS Response Progress Report (GARPR), Annual Progress Report Bangladesh, 2015; 3[RD] National Strategic Plan for HIV and AIDS Response , 2011-15, National AIDS/STD PROGRAM (NASP), MOHFW, GOB ; World AIDS Report, 2011; The Round 9 surveillance, 2011 and The Round 8 surveillance, 2007.

(P>0.05). Higher co-infection rate (7.5%) with syphilis and hepatitis B among female sex workers (FSWs) than this study was reported in Argentina (María et al, 2006).

In Bangladesh there is a dearth of research on the prevalence and risk factors for hepatitis B infection (Gibney et al, 2001). In this study, slightly higher prevalence rate (P>0.05) of Hepatitis B infection was found in hotel groups (6.2%) than floating CSWs (5.0%) (table-1) but both of these were slightly lower than blood donors of Tanzania [Matee et al, 2006 (8.8%)] and Cameroon [Jean et al, 2013 (10.1%)] but much lower than previous studies conducted among CSWs [Sattar et al, 1996 (HbsAg 78.7%); Mustafa et al, 1989b (11.0%)] and among impoverished urban community in Dhaka [Ashraf et al, 2010 (29%)] as well as lower than female CSWs in Argentina [María et al, 2006 (14.4%)] and male transvestite in Uruguay [Russi et al, 2003 (50.5%)]. However, lower hepatitis B rate [(3.8%), even lower than HIV (30.1%)] like this study was also reported among sex-trafficked women and girls in Nepal (Silverman et al, 2008) and among healthy population and Turkish soldiers [Altindis et al, 2006 (2.46%)]

In this study, most of CSWS (97.6% both groups) heard about the name of HIV, which was surprisingly, opposite to the national finding in general population about “have heard about HIV” (Bangladesh Demographic and Health Survey, 2011). According to BDHS (2011) only 40.3% of non-educated ever married women and 57.0% of widow/divorced/abandoned women have heard about HIV and older and widow/divorced/abandoned women were less likely to know about HIV. In contrast to this, as overwhelming majority (69.5%) of this study had no formal education, moreover, vast majority of them (221/495) were widow/divorced/abandoned and 36% (178/495) were in >30 age, so it is very unlikely that poor, destitute, illiterate study subject had had knowledge about HIV. Professional awareness and NGO counseling on STIs would be the possible reason as different report revealed about strong GOB-NGO collaboration focus on STIs by giving free counseling, condom and STI screening[12] (Naimul Haq, 2014; Sadhana and Sanghamitra, 2008). Also, it could be plausible explanation for the ‘zero’ prevalence of HIV among CSWs in this study. In addition, more than half (55.3%) of CSWs (both type) claimed that they had no risk of HIV and more FFCSWs strongly supported that than HBCSWs (P=.000**).

Bangladesh remains a low HIV prevalence country with less than 0.1% overall prevalence in general population over the years (World Bank and UNIAIDS, 2009) and <1% among key (CSWs, PWID/IDUs, MSM and Hijras) and bridging Population (clients)[11]. In this study, higher prevalence of STIs and low prevalence of HIV among CSWs in Bangladesh pointed at that it is still at an embryonic stage and suggests potentiality for the rapid spread of HIV once it is introduced in this high risk population, unless this matter is carefully and intellectually handled. In spite of lower prevalence of HIV among key and bridging Population [11] the overall HIV/AIDS cases has increased more than 25% over a decade till 2012 which is also alarming (World AIDS Report, 2011). However, for a densely populated country of 150 million,

[11] The Round 9 surveillance, 2011 and The Round 8 surveillance, 2007

Bangladesh is fortunate to have no more than about 3000 known HIV positive cases; this is despite a high incidence of STIs, a low literacy rate, and porous borders with countries like India and Myanmar. Strong political commitment, timely donor support, and effective government-NGO collaboration are behind Bangladesh's success on this front[12] (Naimul haq, 2014).

According to UNAIDS, Bangladesh is among the few developing nation which saw early intervention in combating HIV/AIDS- it was made priority in the mid nineties. Awareness lessons work like magic, which virtually costs nothing. Initially, GOB- NGO led organizations acted as catalyst to train and educate high risk groups like FCSWs, and then sex workers educate their peers for their own safety; thus promoting 'safe sex practices' and preventing 'vulnerabilities to HIV infection' among 'high risk' priority groups (CSWs, IDUs, MSMs, and transgender). Most CSWs in Bangladesh have access to Drop-in centers (DIC), which have opened with the help of NGOs and are part of the national HIV intervention program, provide free condoms, counseling and testing for STIs. The low 'HIV prevalence rate' can be credited to about 45 local and national level NGOs. The NGOs mainly concentrated on high risk groups such as 110,581 MSM, 74,300 FCSWs and 8,882 transgender[12] (Naimul haq, 2014).

According to National AIDS/STD Program (NASP) (2014) "voluntary blood tests among high risk groups show an HIV infection rate of less than 1%, thoughtful initiatives (counseling, free STD testing and free condom distribution among CSWs) that have kept the figures low, however, the figure is high among intravenous drug users (IDUs), unlike other groups, IDUs are hard to persuade as HIV among IDUs had crossed 4% in 2002, thus, IDUs have been a major concern." According to Dr. Leo Kenny, coordinator UNAIDS, Bangladesh, "the GOB and civil society have a strong partnership. NGOs have implemented around 75% of HIV/AIDS prevention, treatment and activities nationwide." Moreover, in partnership with NGOs, media and civil society more DICs, and HIV counseling and testing centers (HCTs) are in operation[10] (Naimul haq, 2014).

Though no HIV antibody was found in the studied population, the prevalence rates of other STIs (hepatitis and syphilis) were higher among the CSWs. this was well agreement with a previous Mexican study (Juarez-Figueroa, 1998) on female sex workers where o.2% of FCSWs (n=1498) were HBV (HBsAg) carriers and prevalence of syphilis and HIV was 7.6% and 0.1% respectively. The **absence** (or very few existence) of 2 major risk factors for HBV and HIV transmission in this study participant and other (Juarez-Figueroa, 1998) were 'injecting drug use' (41/635) and 'anal intercourse' (28/635) influenced lowering the prevalence rate.

The higher prevalence rate of ulcerative syphilis and hepatitis B for longer period of time among the vulnerable group of population like CSWs could make bridge to general population via their multiple, colorful clients more rapidly, as well as could considerably increase the risk

[12] "Sex workers help Bangladesh fight HIV", Asia Times online, April 29, 2014.

of HIV transmission to them and others. Different report pointed out that Syphilis, HSV and chancroid (ulcerative STIs) increase 3 times whereas gonorrhea and Chlamydia (non-ulcerative STIs) increases 2 times risk of HIV transmission. Moreover, delay in seeking appropriate STD treatment or failure to comply fully with prescribed treatment influence HIV infection as well as prolonged cases of STIs (so as anal sex) caused disruption of the surface epithelium, eliciting inflammation, which helps in attracting infected HIV susceptible lymphocytes into the genital tract[13] (WHO, 1993).

Multiple Logistic Regression model of 'syphilis'

To examine which variables positively influenced "Syphilis" multiple logistic regressions (MLoR) analysis was done by applying 'backward stepwise' MLoR analysis. For clarification 'Forward stepwise' MLoR analysis was also performed. All categorical variables which were found statistically significant (P≤ 0.05) or borderline significant (P<0.09) in 'simple logistic regression' (table-5, 6 and 7) were introduced simultaneously in the **multiple logistic regression analysis.** Eight independent variables namely 'factors behind profession', 'present age', 'education', 'monthly income', 'length of profession', 'abortion cases', 'use of contraceptive' and 'weekly coitus with PC' were remained in the 'adjusted model of syphilis' (table-8). Most of all predictors of the model (table-8) significantly (P<0.05) influenced CSWs for inflicting with "syphilis". However, 'weekly coitus with permanent client (PC)' was a strong 'covariate' of 'syphilis model' as one of its category was marginally significant (P=.056 and other's P=.295) in 'unadjusted model' (table-7) but both categories of this covariate finally remained significant (P=.018, P=.014) in MLoR model of syphilis (table-8).

Factors behind profession: multiple factors influence CSWs to involve in sex selling profession. Poverty, exploitation, tender age and illiteracy, were the main contributory factors for entering into sex industry. However, the factors that pushed women to enter into sex industry vary from person to person and also country to country but economy is the major factor all over the world and, of course, in third world's country. The causal factors for being today's CSWs tell us the story of extreme poor, unfortunate, destitute young women, additionally handicapped by illiteracy who abandoned by their husbands and getting no other ways, exploited or falling in the trap by pimps they entered into this ugly profession only for saving them and their children from hunger. These features are mostly common for women of lower economic level in our country. In this study both categories of CSWS were induced to take this nasty profession because of poverty and no significant difference (P=.235) was observed between the two groups of CSWs in this regard (socio-demography table-2) which points at our unhealthy economy and consistent with some other studies about CSWs (Mondal et al, 2010 and 2008; Alam, MK; Ullah, 2005) except a brothel-based study (Hossain KJ et al, 2010) where close to half (47.8%) of CSWs claimed broker/pimps to entice them coming into this profession.

[13] www.stds.you and me

Adjusted logistic regression model of 'syphilis' (table-8) pointed at a new finding that, different reasons other than 'economy' (namely angry on family members, sold by step mom/others, raped/tortured, pushed by husband, self motivation and drug addiction) for being a sex worker had 1.48 times higher odds for the exposure of syphilis as compare to 'economic reason' [AOR 1.48 (.944-2.307), P=.088). This may be, due to individual's "frame of mind", "awareness", "morals and formal education", "anger", "frustration", and "ethical issues" were carrying by CSWs. Being unaware of consequences, if someone did something wrong desperately would not bring good result, even doing the wrong thing one could comeback if she wills. However, there might some other reasons which could explain properly this finding.

Present age: age range of CSWs is one of the important issues in the sex market because with the increase of age diminished the demand of CSWs. In 'adjusted model of syphilis' (table-8) it was revealed that over 30 CSWs showed 4.49 times higher risk [AOR 4.49 (1.843-10.948), P=.001*] and 19-29 years of age had 2.63 folds greater risk [AOR 2.63 (1.166-5.923), P=.020*] for infecting syphilis as compare to <18 years CSWs. This finding is consistent with a street-based national study (Mondal et al, 2008) where oldest (>30 y) floating CSWs had 2.42 times higher odds ratio to inflict with STIs [AOR 2.42, P=.220] though the difference could not reach at significant level. Similar finding was also observed in a previous study in Argentina (María et al, 2006) and in Bangladesh (Rahman et al, 2000) where the oldest (>30 y) and experienced (≥10 y) CSWs had the highest prevalence of syphilis (AOR 2.6 to 4.9) and *N.Gonorrhoea* respectively. However, opposite association of age and STI was also found in a Pakistani study (Bibi et al, 2010) and in a south African study where sex workers aged ≥29 years and without having *N. gonorrhea* had significant negative association with HIV infection (OR=0.16; 95% CI: 0.07-0.38)(Dunkle et al, 2005).

Education: education not only facilitates to get job but also increases consciousness about life, social, environment and health. Literacy is another focal point for female prostitution, illiterate young women has no quality to get a job except working as a 'domestic help' which, often is subjected to physical, verbal and sexual abuse. Thus illiterate woman has limited options for job and can easily be derailed, exploited by pimps or bad people than educated one. In this study, higher illiteracy rate (82.57%) and less formal education (1-12y schooling 17.4%) of floating CSWs was noticed which significantly differ from HBCSWs (illiteracy=48.1%, formal education=51.95%). The former finding is in agreement of most of the studies in our country (Mondal et al, 2010; Hossain KJ et al, 2010; Mondal et al, 2008; Rahman et al, 2000; Alam, MK) about commercial sex workers which always elucidated that majority of CSWs were illiterate.

Multiple logistic regression (MLoR) model of syphilis revealed that illiteracy was associated with 2-folds risk of syphilis among CSWs [AOR 2.04 (1.203-3.466), P=.008].This finding was reinforced by another previous study about FCSWs in Bangladesh (Mondal et al, 2008) where literate FCSWs were 85% less likely to inflict with STDs as compared to illiterate FCSWs [AOR 0.146, P=.000]. Here literacy acted as a protective factor which could prevent STI like syphilis. Earlier study in Africa revealed that lower educational level was significantly associated with more STI prevalence (r=-0.36; F=4.2; P=0.04), and work on street (P=0.02) (Solomon et al, 2008). However, McCloskey (2003) showed that HIV/AIDS prevalence is specially correlated with socio-economic factors like female literacy rate and poverty.

Monthly income: generally, 67.3% of CSWs (n=635) earned 1000-5000 BDT (12.5-62.5 $) and they were 2.14 folds more likely to afflict with syphilis as compared to CSWs who earned >5000 taka or >62.5 $ [AOR 2.14 (1.272-3.603), P=.004*]. Plausible explanation is 'economy' the powerful driving force that drove them into this nasty profession. Study (Mondal et al, 2008) documented that if money spent on health purpose by FCSWs was less likely to infect with STDs as compare to money did not spend on health (AOR 0.71, P=.000). In this study HBCSWs earned double than FCSWs (t=12.607, P=.000) and syphilis rate was overwhelmingly higher (P<0.05) in floating community than hotel-based CSWs (FCSWs 52.4% vs. HBCSWs 18%) and also most (60.25%) of floating CSWs used condom inconsistently as compared to hotel-based CSWs (P<0.05). Thus, extreme poverty and inconsistent use of condom could influence inflicting syphilis more severely in floating community than their counterparts.

length of profession: in this study, an increased trend in the prevalence (%) of syphilis was observed with the increase of **the length of profession** of CSWs. Length of profession is a burning question for sex trade so as observed in this study in both simple logistic regression or SLoR (table-6) and MLoR model (table-8). CSWs who had professional length 1 to 5 years and ≥6 years were respectively 2.6 times [AOR=2.613 (.939−7.217), P=.066] and 5.98 times [AOR=5.98 (2.048−17.484),P=.001*] more likely to inflicted with syphilis as compare to those who had less than one year of professional experience. Professional length ≥6 years was very vital for inflicting syphilis as it was 17-folds [UOR=17.164 (6.513−45.234), P=.000*] in SLoR model (table-6) but remained significant in MLoR with 6-folds. It is noteworthy that most (51%) floating CSWs had significantly (P=.000) higher (≥6 y) professional experience than hotel-based CSWs (12.66%) and syphilis rate was (52.4%) overwhelmingly higher in floating group. This might be due to, syphilis is contagious and even "condom" alone cannot protect its transmission as it could be spread through oral sex, anal sex and infected skin. Being unaware of STIs like syphilis most of them could contact this disease from the beginning of profession and could carry over period of time. More than >10 years in sex work was associated with syphilis reported in an Argentine study (María et al, 2006) and the oldest (>30 y) CSWs had the highest prevalence of STI (*N.Gonorrhoea)* in a street-based study in Bangladesh (Rahman et al, 2000) but opposite result (low prevalence) was observed in an Indian study (Rakhi et al, 2006). However, a Tunisian study **(**Abir Znazen et al, 2010**)** showed that IgG antibodies to hepatitis B core antigen (anti-HBc) was much higher among old FSW (P<0.001) than younger one pointed at more they work in the sex industry, more they would be exposed to STIs.

Abortion cases: having ≥1 abortion cases in the past was associated with 2 folds risk of inflicting syphilis was postulated by MLoR model of syphilis [AOR 2.072 (1.272-3.376), P=.003]. An ICDDR, B[14] study about syphilis diagnosis showed 6% participants had a history of spontaneous abortion. Studies pointed at that syphilis can seriously complicated pregnancy and result in spontaneous abortion, still birth, intrauterine growth restriction and perinatal death as well as serious sequelae in live born infected children (Owusu-Edusei et al, 2011; Genc and Ledger, 2000; Peeling and ye, 2004). In the past, parts of the world where the traditional "venereal disease" have not been controlled, such as sub-Saharan Africa, the magnitude of the problems associated with congenital syphilis is reminiscent of those faced in the west during early 1900s (Genc and Ledger, 2000). In Ethiopia, an estimated 5% of all pregnancies (75000 pregnancies loses) were lost each year through syphilis induced abortion (Schulz et al, 1990).

Use of contraceptives: for most Bangladeshi, condoms are known as a means of contraception and are not widely used. It has been reported in our country that condoms purchased by the Department of Family Planning can only be used for family planning, and NGOs working on HIV/STI prevention do not get condoms unless they declare they are working on family planning (Hossain S et al, 2004). In this study, as a method of contraception, condom use rate was slightly higher (42.8%) than oral pill/other contraceptive users (40.4%) and it was revealed from MLoR model (table-8) that these oral pill/other contraceptive users were (P=.019) 53% less likely to infect with syphilis [AOR .470 95 % CI (.250-.882)] and condom users were 16% more likely to inflict with syphilis [AOR 1.156 (.637-2.095), P=.634] as compared to CSWs who did not take any measure for contraception (reference category). This may be due to condom users might not use condom consistently (with each and every commercial and non commercial partners) and correctly (from the beginning and not just before insertion or ejaculation) either as a method of contraception or a barrier against STIs which leads to infection and morbidity and cause of low income and pill users might use condom correctly as a measure of the prevention of STI. However, the effect of pill on syphilis was not clear as neither pill can protect syphilis nor condom does it. This is logical and authentic because condom does not necessarily could protect one from syphilis[15] and ulcerative infection like syphilis is caused by a spirochete (*Treponema pallidum*) which can be spread by vaginal, anal or oral sex specifically from close contact with infectious skin lesions. Acquired syphilis is grouped into three distinct stages: primary, secondary and latent syphilis. People with primary syphilis may have pain-free open sores (chancres) in the genital area or around the mouth. Thus, unprotected vaginal, anal even oral sex could increase not only the syphilis infection but also invite others bacterial (gonorrhea, Chlamydia, trichomonas V.etc) and viral (HIV, HBV, HCV, HSV-1 & 2, HPV) pathogens. However, study (Mondal et al,

[14] Field evaluation of simple rapid tests in the diagnosis of syphilis'. ICDDR, B. Health and Science Bulletin. Vol. 4 No. 4. December 2006.

[15] www.std.uandme

2008) reported that condom use negatively associated with syphilis and showed FCSWs who usually used condom had lower syphilis rate (6.66%) than who did not use condom (10.66%).

Colorful partners of CSWs: It is well documented from different studies (Deering et al, 2011; Rakhi et al, 2005; Kinuko Kimoto) that CSWs have both commercial (permanent clients or steady clients/same clients) and non-commercial partners (non-paying sexual partners or NPSP with whom did not live and cohabiting partners or husbands or boyfriends or pimps). In this study, 276/635 (43.46%) of CSWs were married but 192/635 (30.24%) lived with husband and only 3.47% (22/635) lived with cohabiting partners (socio-demography table-1 and 2). In addition, 58/635 (9.13%) CSWs had NPSP, 31.34% (199/635) had weekly permanent clients (PC), 21.58% (137/635) had fortnightly PCs and 26.61% (169/635) had monthly PCs (Chapter-1: table-3). Higher proportion of hotel-based sex workers were engaged in weekly, fortnightly and monthly coitus (P<0.05) with permanent/ regular/same customers than floating CSWs (Chapter-1: table-3).

Female CSWs and their both commercial and non-commercial partners were found to be substantially connected to other types of partnership through other sex partners. These con-current partners included wives, FCSWs or other types of female partners (Deering et al, 2011). Thus the vulnerability of FCSWs to both acquisition and transmission of HIV/STI within complex sexual networks could expand day by day and also with increasing the areas of web networks with more clients as time passed.

Weekly coitus with Permanent/regular/steady customers (PC): In multiple logistic regression model (table-8) of syphilis 'weekly coitus with permanent clients (PC)' remained as a significant predictor of syphilis, which was also significant in crude model of STIs (table-7). It was observed that CSWs engaged in either one time [AOR 1.89 (1.11-3.206), P=.018] or ≥2 times weekly coitus [AOR 2.14 (1.166-3.932), P=.014] with PCs were respectively 1.89-folds and 2.14-fold more likely to inflict with syphilis as compared to CSWs who had no PC at all. This may be due to the effect of low 'condom use' rate with regular/steady clients or extreme poverty or hunger as reported by different studies (Zeng et al, 2015; Zahra et al, 2014; Deering et al, 2011; Rakhi et al, 2005; Kinuko Kimoto).

Multiple Logistic Regression model of hepatitis B

To examine which variable positively influence "Hepatitis B" infection, multiple logistic regressions (MLoR) analysis was done by applying 'backward stepwise' MLoR analysis. In this study, STIs like HIV, syphilis and hepatitis B were screened among two types of CSWs (hotel and floating) and **generally** hepatitis B infection was 5.5% (27/495) among CSWs (n=635) but hotel-based CSWs possessed higher infection rate (6.2%) than floating group (P>0.05). In MLoR model of 'Hepatitis B' (dichotomous outcome variable) only 2 variables namely 'genital discharge' and 'ever experienced in group sex' remained in the model (table-9). CSWs who did not have vaginal discharge were 2.47 times more likely to inflicted with hepatitis B as compared to CSWs who had 'genital discharge' [AOR 2.465 (.910-6.677), P=**.076**].This is not clear because hepatitis B virus primarily attacks liver and 'vaginal discharge' is a symptom associated with bacterial STI (mainly Chlamydia and gonorrhea and lesser extent BV) and with protozoan parasite 'Trichomonas' and fungus 'Candida' but not with hepatitis B. However, in hepatitis B infected individual, the virus can be found in all body fluids (e.g. 'blood', 'semen', 'saliva', 'menstrual blood' and 'vaginal discharge'). In this study, vaginal discharge was found more in FCSWs (36.7%) than HBCSWs (P=.014) (but hepatitis rate (6.2%) was higher among HBCSWs than floating CSWs) and as this study could not manage to screen 'gonorrhea' among CSWs, so it remained unclear. Also, 'ever experienced in group sex' was significantly associated with hepatitis B infection and it was elucidated that CSWs who experienced 'group sex' had 3.36 folds risk to infect with hepatitis B virus as compared to CSWs who did not experience this risky sexual behavior [AOR 3.364 (1.531-7.395) P=.003*]. As reported earlier CSWs have multiple colorful partnerships, these partnerships may be commercial and non-commercial. Different studies (Deering et al, 2011; Rakhi et al, 2005, 2006; Kinuko Kimoto) in agreement with this finding and 'group sex' is the most practical example of multiple partnerships. In addition, inconsistent 'condom use' rate could be increased during this sexual malpractice due to lesser control over situation.

Multiple Logistic Regression model of 'STI' adjusted for nutritional variables

In this model STIs (0, 1) was 'dichotomous outcome variable' and no STIs (0) means none (56.36%) of CSWs infected with either 'syphilis' or 'Hepatitis B' and having STIs (1) means CSWs had either 'syphilis' (38.18%) or Hepatitis B (3.64%) or had co-infection (1.82%). To examine which 'nutritional variables' positively influence "STIs", multiple logistic regressions (MLoR) analysis was done by applying 'backward stepwise' analysis. It was observed from the model (table-12) that, halves of predictors of the model namely 'serum vitamin A', 'serum iron' and 'serum IgA' significantly (P<0.05) associated with "STIs" and halves of them (BMI, serum zinc & serum IgM), however, could not reach in significance level but marginally significant.

BMI of CSWs: From the fitted model of STI, it was observed that CSWs who had normal BMI were 50% less likely to infect with STIs as compared to CSWs who were underweight (BMI <18.5) though the effect could not reach significant level (P=.246). However, high BMI (>25.1 kg/m^{2}) among CSWs was associated with 7.17 folds risk to afflict with STIs and the effect was marginally significant (P=.093).

Serum vitamin A: Multiple Logistic Regression (ML_oR) Model of STIs (0= no STI, 1= having STI) analysis showed that the risk of having STIs was 4.61 folds [AOR 4.61 95% CI .693-30.68), P=.114] and 10.792 folds [AOR 10.792 95% CI (1.93-60.29), P=.007] greater for CSWs who had severe (0.0-0.34 µmol/L serum vitamin A) and sub clinical vitamin A deficiency (0.35 to 1.04 µ mol/L) respectively as compared to CSWs who had normal serum vitamin A (>1.05 µmol/L). Here, width of the 95% CI is higher due to lower sample size (n=107) as well as higher point prevalence (AOR 10.792).

Serum zinc: higher zinc level (>18.6 µmol/L) of CSWs showed 4.23 folds risk of STIs with borderline significance (P=.083).

Serum iron: low serum iron (0.11-10.9 µmol/L) of CSWs was associated with 8.55 folds risk of infecting STIs as compared to ≥normal serum iron level [AOR 8.55 (1.197-61.02), p=.032]. CSWs even having normal level of serum iron (11-29 µmol/L) had 4.2 folds risk of inflicting STIs (P=.054).

Serum IgA: ≥normal (0.50-5.75 G/l) level of serum IgA was associated with 7.49 times more risk of inflicting STIs as compared to low serum IgA level (0.0-.49 g/L) [AOR 7.49 (1.370-40.94), P=.020].

Serum IgM: it can be said that the predicted odds for CSWs having STI (Dependent Variables or DV) were 3 times higher in lower IgA ((0.02-.29 g/L) possessors (CSWs) than the odds for ≥normal (0.30-2.85 g/L) IgA holder [AOR 3.03 95% CI (.880-10.458), P=.079].

Multiple Logistic Regression model of "condom use" by CSWS

Bivariate and multivariate analysis was done to understand the association of 'no' or 'inconsistent condom use' (for penetrative vaginal sex with clients) with different characteristics to identify those may play a significant role in determining the 'use of condom'. In the MLoR model, the effect of each category of a multi-categorical variable was assessed by keeping the first or last category as reference. All categorical variables which were found statistically significant (P≤ 0.05) or borderline significant (P<0.09) in 'simple logistic regression' (table-14) were introduced simultaneously in the **multiple logistic regression analysis.** Possible interactions between different variables in the model were assessed, where necessary.

The overall level of condom use for any purpose is low even amongst high risk groups (Sadhana, and Sanghamitra, 2008). Most of CSWs are insisted to take part in sexual intercourse without condoms. Condom negotiation skills of CSWs with clients are always an important issue. Actually, safe sex is the effective way to prevent the STI as well as HIV transmission. The floating CSW frequently lacks the personal or social status to negotiate safe sexual practices being under the threat of violence or loss of clients or poverty (Zeng et al, 2015; Zahra et al, 2014; Mondal et al, 2008; Gitali et al; Rakhi et al, 2005,2006). However, study (Urada et al, 2012) reported physical-environment (traficked/coerced), economic-environment (sex without condom for money) and policy-environment (availability of condom) factors and individual risk factor (substance abuse) were independently associated with FSW's lack of condom negotiation with venue partner.

In fact, condoms are playing more effective role in preventing the majority of STI including HIV. Hence practices of condom use are very important for CSWs because of their high frequency sexual contacts with a variety of clients and concurrent partners (both commercial and non-commercial). It was noticed from study (Zeng et al, 2015; Zahra et al, 2014; Mondal et al,2008; Rakhi et al 2005) that most CSWs simply did not aware of STIs and because of competitive market and the fear of losing clients majority of them agreed unprotected sex with different types of clients when they are coerced or offered extra money. Most of the earlier studies (Nessa et al 2004, Rahman et al 2000) about CSWs indicated that condom use in Bangladesh as well as in Asia was reportedly the lowest; although the figures have been rising due to interventions by non-governmental organizations (Sadhana and Sanghamitra, 2008). Study (Haseen et al, 2012) about youth clients of Hotel-based (HBFSWs) reported that only 12% youth clients used condom regularly and 'negotiation of condom' use with FSWs was the main determinant for condom use at last sex [OR 17.95]. However, 'condom use' is not solely a matter of CSW volition, external determinants stemming from clients, brothel owners and/or managers also need to be taken into careful consideration (Kinuko Kimoto). A study (Gallo et al, 2011) on 'determinants of condom use among FCSWs' reported that correlates of consistent condom use differed by 'partner type'.

In general, consistent condom use (CCU) rate was 44.1% among study group (n=635) but compared to floating CSWs (39.75%) it was higher (P=.023) among Hotel-based CSWs (48.70%). Study (Altaf et al, 2012) reported very low (1.3%) CCU among street-based CSWs than brothel-based (68%). However, as it highlighted by these data a significant proportion of female CSWs have unprotected sex with commercial clients (occasional or permanent/steady clients) and their regular non-commercial partners (NPSP), and it is very likely that both types of sexual partners (commercial and non-commercial) were not necessarily monogamous and thus both jointly poses a great threat for STIs among commercial sex industry and at the same time for general population through this bridging population.

A qualitative study in Iran about barriers of 'condom use' among FSWs postulated that the main barriers of condom use were 'lack of awareness of the modes of transmission of HIV and prognosis of the disease', 'doubt on the role of condom in preventing STDs and HIV', 'fear of violence or rejection by male customers', 'reluctance of sexual partners to use condoms due to diminution of sexual pleasure' (Zahra et al, 2014). The dynamics of 'condom use' ranging from CSW convincing the client to use condom, the client convincing female CSWs to use condom, to condom being available with CSWs but not used at the time of sex with clients-reported by an Indian study (Rakhi *et al*, 2005). However, self reported condom use was more closely correlated with incident than prevalent STI was reported by a study (Gallo et al, 2007).

Category of commercial sex workers: 'category of CSWs' was an important determinant of inconsistent condom use by CSWs. It was observed from the MLoR model of 'condom use' by CSWs (table-14) that floating (FCSWs) or street-based CSWs were 2 fold more likely to use condom inconsistently as compared to HBCSWs [AOR 1.807 (1.271-2.570), P=.001]. This finding is in agreement with an Indian study (Rakhi *et al*, 2005) where being street-based CSWs as compared to brothel-based was associated with inconsistent condom use [OR 3.36 (1.87-6.04)]. The plausible reason for inconsistent condom use by FCSWs was 'lower economy' of street-based CSWs which was reinforced by several studies conducted either in home[16] (Mondal et al, 2010 & 2008; Ullah, 2005; Rahman et al, 2000) or abroad (Zeng et al, 2015; Deering et al 2011; Rakhi et al, 2005; McKeganey and Barnard, 1996).

Of note, similar effect of 'category of CSWs' on 'inconsistent condom' use by CSWs was observed in MLoR model of 'condom use' by CSWs including immunoglobulin A (table-15) but 6.35 folds (AOR 6.35) and range of the 95% CI was also high (1.516-26.618). This may be due to small sample size (n=107) of latter model (table-15) as compared to former model (n=495, table-14). In fact, category of CSWs' was a significant predictor of events with OR before adjustment was [table-13: UOR 1.44 (1.050-1.971), P=.023] and with adjusted odds ratio (AOR) of at least 1.51 after correction of covariates. AOR after correction of covariates is generally considered a more precise indicator. Actually, OR operates on a log scale and the CI will appear much wider just because the point estimate is higher (6.35). When the actual mean and its 95% CI are expressed in logit (by taking a natural logarithmic transformation), the width of the CI actually isn't terribly different.[17]

Use of contraceptives: multivariate analysis (table-13) of 'condom use' by CSWs (outcome variable) showed that non-contraceptive users [AOR 1.80 (1.115-2.921), P=.016*] and oral contraceptive users [AOR 2.47 (1.695-3.606), p=.000**] were respectively 1.8 times and 2.47 times more likely to use condom inconsistently as compared to CSWs who used condoms as birth control measure. This finding is substantially reinforced by a Japanese study about CSWs (Kinuko Kimoto) which indicated that contraceptive use was significantly associated with "incomplete condom use" [AOR 5.69 (1.43-22.66)]. It was also found that 'incorrect use of condom (just before insertion or ejaculation)' was more frequent among those who used 'oral contraceptives', perhaps because CSWs who do not use oral contraceptives are careful to use condoms as a measure of contraception.

Having NPSP: CSWs have colorful non-commercial partners (with whom had regular sex but did not pay) or non paying sexual partners (NPSP) or cohabiting partners such as husband, boyfriends and regular partners. In this study NPSP means with whom CSWs had sexual relation but neither paid nor lived. It was observed from the MLoR model of 'condom use by CSWs' that CSWs having non paying commercial sex partners were 2.18-folds more likely to use condom inconsistently as compared to CSWs who didn't have NPSP. This study got only few NPSP 9.1% (58/635) but higher proportion (54.8%) of regular sex partners and 94% no use of condom with them reported in neighboring country (Rakhi et al, 2005) and in other studies (Deering et al, 2011; María et al, 2006). Deering et al, (2011) reported 23.7% NPSP and 6.3% FSWs had 4 types of partners namely husband/cohabiting partners, repeat clients/permanent clients, NPSP and occasional clients. Similarly, lower (46.2%) condom use rate with non-commercial partner and higher (89.1%) with commercial clients was reported in a Japanese study (Kinuko Kimoto) and other previous studies about CSWs in abroad

[16] COMMERCIAL SEX WORKERS IN URBAN BANGLADESH www.insticeage.studies.com, published February 2, 2008.

[17] Cross Validated: http://stats.stackeschange.com/questions/147666

(Deering et al, 2011; Morris et al, 2009; María et al, 20o6; Evans et al, 1997; Gitali et al; Tchoudomirova et al, 1997; Wirawan et al, 1993). Also, Deering et al (2011) revealed a remarkable finding that consistent condom use (CCU) with FSW's non-paying partner decreased by 18% for each one year increase in the duration of the relationship [AOR 0.82 (.68-.97)] and even lower CCU rate with husband or cohabiting partners (22.6%) than NPSP (40.3%). Even Hijra (transgender) sex workers (HSWs) were more likely to use condoms with casual clients than regular clients [AOR 5.8 (.51-2.24)] reported in a Pakistani study (Siddiqui et al, 2011). Moreover, women other than CSWs (Juan et al, 2000), were also reported to use low condom with steady partners (29.9%) than casual partners (51.4%) and new partners (44.0%)

Understanding condom use in non-commercial partnership is complex (Deering et al, 2011). CSWs have varying degree of emotional closeness, intimacy or other involvement with these partners, which may influence condom use. Condoms may be used less frequently with non-commercial partners compared to commercial clients (Siddiqui et al, 2011; Rakhi et al, 2005; Kinuko Kimoto; Deering et al, 2011; Morris et al, 2009; María et al, 2006; Evans et al, 1997) in order to make a distinction between work and pleasure. Also, not using condoms in non-commercial partnership can represent positive features of a relationship, such as increased closeness and trust, and so condoms may be avoided to remove a barrier to increased intimacy. Conversely, condom may be preferred in commercial partnerships to create a barrier to intimacy and to gain a sense of control with clients and at the same time it perceived as a symbol of infidelity and foster mistrust (Sanders, 2002; Wojcicki and Malala, 2001; Varga,1997). Because the nature of non-commercial partnership is different from non-commercial relationship, and the economic support if it exists, is often non-monetary, the decision to use a condom may be more influenced by interpersonal factors related to relationship intimacy (e.g. trust, emotional closeness, power or reproductive desires) than financial dependence (Deering et al, 2011; Murray et al, 2007).

Monthly Expenditure on food: the MLoR model of 'condom use by CSWs' (table-14) elucidated those CSWs either spent 500-2000 taka or 2001-3500 taka on food both had 2 folds risks to use condom inconsistently as compared to CSWs who spent >3500 taka on food [AOR 2.135 (1.076-4.237), P=.030 and AOR 2.344 (1.117-4.916), P=.024]. This may be due to lower 'economic condition' of CSWs especially among 'floating group' which influenced to allocate small amount of money on food. This finding is consistent with a very recent study in China (Zeng et al, 2015) where low condom use was associated with family financial constraints, inadequate power to condom negotiation, low awareness and misconception of HIV risks. Couple of years ago an Indian study (Rakhi et al, 2005) also revealed that lower income, illiteracy, poor social support and no participation in FSW support groups were the predictors of inconsistent 'use of condom' with clients. Also in a Japanese study (Kinuko Kimoto) it was depicted that CSWs who earned >450 US $ per day were less likely to use condom inconsistently as compared to CSWs who earned <450 US $. It is notable that, in this study, no or inconsistent condom use rate was higher (P=.023) among floating CSWs (60.25%) than hotel-based CSWs (51.30%) and HBCSWs had double monthly income than FCSWs (t=12.607, P=.000), so it is obvious that floating CSWs used condom inconsistently due to hunger or extreme poverty which made them to keep attention on stomach rather than STI. In addition, different social, psychological, economical and environmental stress made their conscience inactive and inadequate 'food intake' aggravate the situation worst. In contrast, economic stability gives a person mental soothing to think about him/herself, possible risk surrounding him, health and overall life. A study (Kinuko Kimoto) reported that even in developed country like Japan CSWs had to endure physical and mental stress, pointed at that all CSWs all over the world share same feeling, same pain and same stress. A very recent study (Zeng et al, 2015) also pointed at that street-based sex workers faced substantial economic, social and environmental barriers to voluntary counseling and testing (VCT) and condom use. Studies either in home[18] (Mondal et al, 2010 & 2008; Ullah, 2005; Rahman et al, 2000) or abroad (Zeng et al, 2015; Deering et al 2011; Rakhi et al, 2005; McKeganey and Barnard, 1996) supported extreme poverty and drawback of street-based CSWs.

It is important to mention that, similar effect of '***Monthly Expenditure on food***' on 'inconsistent condom' use by CSWs was observed in MLoR model of 'condom use' by CSWs including immunoglobulin A (table-15) but 32.26 folds (AOR 32.26) and width of it 95% CI was also broad (1.177-883.688). This may be due to small sample size (n=107) of latter model (table-15) as compared to former model (n=495, table-14). In fact, '***Monthly Expenditure on food'*** was a significant predictor of events with OR before adjustment was [table-13: UOR 2.252 (1.119-4.533), P=.023] and with adjusted odds ratio (AOR) of at least 1.177 after correction of covariates. Also, width of the 95% CI of former model (n=495) was within acceptable range as previously mentioned and as serum IgA included in the model which had n=107, so the total number of the model (n=107). Nevertheless, as the point prevalence of the latter model (table-15) was very high; AOR was 32.26, so depicted in their 95% CI. When the actual mean and its 95% CI are expressed in logit (by taking a natural logarithmic transformation), the width of the CI actually isn't terribly different[19].

NGO counseling on STI: It was observed from the MLoR model of 'condom use' by CSWs (n=495) that CSWs having 'NGO counseling on STI' were 44% more likely to inflict with syphilis as compared to CSWs who did not counseled by NGO. This finding is not clear but it could happen that CSWs received NGO training mostly (30.7%) on 'how to use condom' (table-4) and practically did not follow it or used it irregularly. On the other hand, CSWs who did not counseled by NGO could use condom properly and consistently. As reported earlier that understanding the 'condom use by CSWs with multiple partnerships' is complex as 'one single' incident of 'no use of condom' with partner (either commercial or non commercial) could increase the probability of infecting with STI. Interestingly, '**NGO** counseling on STI' was not significantly associated (P=.139) with 'inconsistent condom' use in bivariate analysis

[18] COMMERCIAL SEX WORKERS IN URBAN BANGLADESH www.insticeage.studies.com, published February 2, 2008.
[19] Cross Validated: http://stats.stackeschange.com/questions/147666

(table-13) of this study, the effect established in multivariate analysis (Table-14) which indicated that there must be some confounders which negatively influenced **NGO** counseling on STI' for CSWs.

Meal skipping: MLoR model of 'condom use' by CSWs (n=107) postulated that skipping regular meals (one/two times) in a day significantly associated with inconsistent condom use by CSWs [AOR 3.179 (1.037–9.746), P=.039] (table-15). This effect was as similar as 'monthly expenditure on food' on 'inconsistent condom use' (table-14) and showed that CSWs who skipped meals regularly in a day had 3 folds risk to use condom inconsistently with their clients. Meal skipping or starvation is always backed by poor economic condition and thus showed similar effect of 'monthly expenditure on food' on 'inconsistent condom use'. Moreover, proportion of starvation was higher among floating group (floating 17.1% vs. HBCSWs 1.9%) than hotel-based group ($\aleph^2$=41.46, P=.000) and 'inconsistent condom' use rate (P=.023) and 'lower income' (P=.000) was also higher in this group. Thus blending these effects simultaneously, it could easily guess the overall situation of floating CSWs regarding lifestyle and HRB including 'condom use'. Different studies either in home[20] (Mondal et al, 2010 & 2008; Ullah, 2005; Rahman et al, 2000) or abroad (Zeng et al, 2015; Deering et al 2011; Rakhi et al, 2005; McKeganey and Barnard, 1996) pointed at the vulnerable economic condition and drawback of street-based CSWs.

Interval between 2 coitus: MLoR model of 'condom use' by CSWs (n=107) detailed that CSWs who gave interval between 2 coitus with clients were 3.6 times more likely to use condom inconsistently as compared to CSWs who did not give interval between 2 coitus with clients [AOR 3.622 (1.306–10.046), P=.013] (table-15). This effect is not clear, as majority (60%-67%) of the CSWs in this study did not take interval and rest of them took a break between 2 coitus. Interestingly, most of hotel-based workers (79.22%) as compared to floating group took a break (P=.000) (socio-economic table-3). This may be due to clientele demand for CSWs or clients availability. The usual norm of CSWs about customers dealing which was informed from CSWs (from unstructured questionnaire) that, if a customer was already available just after an 'episode with client' they did not refuse. In this connection, it is noteworthy that HBCSWs usually handled almost doubled customers per day than floating group (HBCSWs 8 clients vs. FCSWs 4). Thus whatever the HBCSWs told, practically they might not have time to take a break between 2 coitus due to customers flow or demand and indulged in unprotected sex without using condom. No information regarding this was available in literature.

Serum immunoglobulin A (IgA): Serum immunoglobulins are indicator of proper immune function. It was observed from the MLoR model of 'condom use' by CSWs (n=107) that having ≥normal (0.50-5.75 g/L) serum immunoglobulin A was associated with higher odds of inconsistent condom use [AOR 11.90 (2.362-59.989), P=.003] (table-15). Plausible explanation might be, those CSWs used condom inconsistently was at risk of infecting STIs

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[20] COMMERCIAL SEX WORKERS IN URBAN BANGLADESH www.insticeage.studies.com, published February 2, 2008.

and infection could increase serum immunoglobulins level than they normally have. It was also observed from ‘MLoR model of IgA’ (immunoglobulin chapter-6, table-) that consistent condom use was associated with 7 folds <normal serum IgA level as compared to irregular condom use (P=.004), which indicates ‘irregular condom use’ was associated with higher serum IgA level that could explain possible infection. Different studies reported higher serum immunoglobulins in infection.

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CHAPTER-4

Serum micro-nutrient status of commercial sex workers

4 Serum micro-nutrient status of commercial sex workers

Vitamins, minerals and trace elements constitute micronutrients profile (Way, 1999; Truswell, 1996). Among all micronutrients, three antioxidant vitamins (e.g. β-carotene, vitamin E and vitamin C) and three antioxidant micro-minerals (e.g. iron, copper and zinc) have potential immuno-modulating properties. These nutrients are essential in the maintenance of defense mechanism i.e. immunity.

Deficiency of the natural antioxidant vitamins (E,C, and β-carotene) are likely to induce oxidative stress and lipid peroxidation (Mayes, 2000a;2000b), and deficiency of micro-minerals (Cu, Zn, Fe) impair enzymatic function of endogenous antioxidants such as metallothiaonein (MT), ferroxidases (ceruloplasmin) and superoxide dismutase etc. in the body.

4.1 Serum vitamin A (retinol) and E (tocopherol) status of commercial sex workers

4.1.1 Introduction

Vitamin A is the first and parent compounds of retinoid, a variety of related compounds (retinol, its esters, retinyl palmitate and retinoic acid), various naturally occurring compounds and synthetic derivatives exhibiting vitamin A activity (Olson et al, 2000; Sommer and West 1996). The carotenoids, principal naturally occurring progenitor of vitamin A are found in fruits and vegetables and retinyl esters in animal tissues. The carotenoids that contain at least one unsubstituted β-ionone ring can be converted into vitamin A. β-carotene, which possesses two β-ionone rings, yields to molecules of vitamin A and is the major provitamin A carotenoid. Likely α-carotene, γ-carotene and β-cryptoxanthin, which has only one β-ionone ring can yield one molecule of vitamin A (Olson et al, 2000).

Retinol or vitamin A is a fat soluble nutrient needed in small amounts for the normal functioning of the visual system, growth and development, maintenance of epithelial cellular integrity, immune function and reproduction (Blomhoff et al, 1991).

Vitamin A is reported to involve in modulation of the most aspects of immune system including development, differentiation and function (Friedman and Sklan, 1993). Both vitamin A deficiency and excess have been associated with defective T-lymphocyte proliferation, impaired T-lymphocyte-mediated cytotoxicity, NK-mediated cytolysis, IL-2 and interferon production, reduced antibody production, decreased resistance to infection (Scrimshaw and Sangiovanni, 1997; Friedman and Sklan, 1993; Chandra, 1991)

Serum retinol concentrations normally are maintained with a narrow range in individuals with adequate stores of vitamin A in the liver (as retinyl palmitate). Thus, low serum retinol can be a useful indicator to identify people with low or depleted liver stores, but not people with normal ranges (WHO, 2009).

Vitamin A deficiency (VAD) occurs when body stores (liver) are depleted to the extent that physiological functions are impaired even though clinical signs may not be evident. Depletion of stored vitamin A occurs over time when the diet contains too little to replace the amount used by tissues. Vitamin A is actively recycled through the liver among tissues, and it appears that he rate of utilization by specific tissues can at least partially adapt to diminishing availability. This adaptation and recycling services to maintain relatively constant blood levels until body stores become depleted below a critical point for which adaptation can no longer compensate (Blomhoff et al, 1991).

Levels of serum retinol are affected by infections and subclinical inflammation. Also, data can be disaggregated by age groups, parity, reproductive status (pregnant, lactating, and non-pregnant, non-lactating), trimester of pregnancy, levels of severity of VAD, and where available, by relevant socio-economic and demographic factors such as education, income, and urban/rural residence. Additionally, seasons of the year and land ownership have been found to influence vitamin A levels in some low-income populations through access to vitamin A and β- carotene rich foods (IVACG, 2004).

VAD contributes to children and maternal mortality, and other poor outcome of pregnancy and lactation. It's diminishes the body's ability to fight infections and contribute to anemia[1]. Vitamin A supplementation reduces child mortality in populations at risk of vitamin A deficiency and may also reduce maternal mortality. One possible explanation for this is that vitamin A deficiency is associated with altered immune function and cytokine dysregulation (Cox et al, 2006).

Based on serum retinol levels from 406 surveys conducted between 1995 to 2005, WHO (2009) estimated 19.1 million pregnant women (15.3%) worldwide are at risk for VAD. The main underlying cause of VAD is a diet that is chronically insufficient in vitamin A and β-carotene that can lead to reduced body stores and fail to meet basic physiologic needs, such as, supporting tissue growth, normal metabolism and resistance to infection.

The integrity of epithelial barriers and the immune system are compromised before the visual system is impaired. Damage to the eyes is the most obvious and dramatic result of vitamin A deficiency, but these signs may in fact be only the later and more readily observable effects. However, it is now recognized that the health is compromised by vitamin A deficiency, even at moderate and possibly mild, sub-clinical levels (Sommer and West 1996).

Vitamin A deficiency is known to reduce epithelial barriers and host immune mechanisms, which leads to an increase in infections along epithelial surface such as conjunctiva, respiratory, gastrointestinal and genitourinary tracts (Sommer and West, 1996). Study (Neggers et al, 2007) showed the severity of BV was increased among those US women with lower intake of folate, vitamin E and calcium. Weekly vitamin A supplementation reduced the

[1] http://www.cpc.unc.edu/measure/prh/rh_indicators/specific/womens-nu. MEASURE Evaluation. Percent of women with low serum vitamin A concentration.

risk of maternal BV in rural Bangladeshi population reported by another recent study (Christian et al, 2011). Enhancement of vitamin A status before and during pregnancy may reduce the risk of BV in areas with vitamin A deficiency. Vitamin A deficiency might increase the susceptibility to BV by reducing either epithelial integrity or host immune response to infection, or both, particularly during a nutrition sensitive period such as pregnancy (Christian et al, 2011).

Vitamin A deficiency and infections aggravate each other, as the deficiency predisposes the host to infection, which in turn decreases the intestinal absorption of the vitamin (WHO)[2]. Vitamin A deficiency also impairs iron status; its supplementation has been shown to increase hemoglobin concentration (Semba et al, 1992).

The term vitamin E[3] describes family of eight fat soluble molecules with antioxidant activities: four tocopherol isoforms (α-, β-, γ-, and δ-tocopherol) and four tocotrienol isoforms (α-, β-, γ-, and δ-tocotrienol). Only one form, α-tocopherol, meets human vitamin E requirements. In the human liver, α-tocopherol is the form of vitamin E that is preferentially bound to α-tocopherol transfer protein (α-TTP) and incorporated into lipoproteins that transport α-tocopherol in the blood for delivery to extrahepatic tissues (Traber, 2012).

Vitamin E is a lipophilic chain-breaking anti-oxidant that prevents lipid peroxidation (Richard et al, 2005).The main function of α-tocopherol in humans is that of a 'fat-soluble antioxidant' and maintained the integrity of cell membranes throughout human body and also protects the fats in LDL from oxidation. Fats, which are an integral part of cell membranes, are vulnerable to damage through lipid peroxidation by free radicals. Serum α-tocopherol is uniquely suited to intercept peroxyl radicals and thus prevent a chain reaction of lipid oxidation. When a molecule of α-tocopherol neutralizes a free radical, it is oxidized and its antioxidant capacity is lost. Other antioxidants, such as vitamin C is capable of regenerating the antioxidant capacity of α-tocopherol (Traber, 2012).

Vitamin E is known to help in 'immune synapse' of cell-mediated immunity. In cell culture study, α-tocopherol was found to improve the formation of an adhesive junction (known as immune synapse) between naïve T lymphocytes and antigen-presenting cell (APC), which eventually prompted T cell activation and proliferation (Marko et al, 2007; Molano and Meydani, 2012).

Alpha tocopherol has been shown to enhance specifically the T cell-mediated immune responses that declined with advancing age. T-cell impaired response has been partly associated with a reduced capacity of naïve T-cell to be activated during antigen presentation. and to produce IL-2 and proliferate as a result (Molano and Meydani, 2012).

[2] http://unu.edu retrieved 2016

[3] lpi.oregonstate.edu/mic/vitamins/vitamin-E retrieved in 2016

4.1.2 Methods and Materials

4.1.2a Selection criterion of serum samples

After screening of all blood samples (n=495) for STIs (HIV[4], hepatitis B, and syphilis), a subsample of (n=107) comprising 68.2% (n=73) infectious samples (having both infections and any single infection) and 31.8% (n=34) non-infectious samples were purposively selected.

4.1.2b Method of vitamin A, E estimation

Serum retinol (vitamin A) and α-tocopherol (vitamin E) were estimated simultaneously by slight modification of the method of the Bieri *et al.* (1979) using RP-HPLC (High Performance Liquid chromatographic) system (Shimadzu, Japan).

4.1.2c Instrumentations

High Performance Liquid chromatographic (HPLC) system (Shimadzu-UFLC prominence), equipped with an auto sampler (SIL 20AC HT) and UV-Visible detector (Model-SPD 20A) was used for the analysis. The data was recorded using LC-solutions software. Phenomenex C_{18} (4.6 mm x 250 mm; 5 µm) column was used for the analysis.

4.1.2d Glassware and equipments

All equipments used in HPLC analysis were soaked in 5% HNO_3 for 48 hours and then washed with double distilled water (glass distilled).

4.1.2e Reagents

Analar grade Ethanol (Merck, Germany) and HPLC grade n-hexane (Merck, Germany) was used for extraction. The eluting solvent (mobile phase) was HPLC grade methanol (Merck, Germany) and water in the ratio of 98:2.

4.1.2f Standards (internal and external)

All-trans Retinol (Sigma chemical company, Saint Louis, Missouri, USA) was used as the "external standard" and Retinol Acetate (Sigma, USA) were used as the "internal standard" for the detection and quantification of serum vitamin A. Similarly, Alpha tocopherol (Sigma Aldrich, 2.96% HPLC, Lot-MKBN3635V) was used as the "external standard" and α-tocopherol acetate (Sigma Aldrich, 2.96% HPLC, Lot-MKBP2667V) was used as the "internal standard" for identification and quantification of serum vitamin E.

[4] No HIV positive serum was detected in this study

4.1.2g Standard stock solutions

a) All-trans Retinol (stock)

Approximately 5 mg of *all-trans* retinol standard was dissolved in 100 ml of HPLC grade Ethanol (concentration 5 mg/dl or 0.05 mg/ml) and stored at -20^{o}C. Actual concentration of the solution was 4.8 mg/dl as calculated by measuring the extinction co-efficient of the solution.

b) Retinyl Acetate (stock)

Retinyl Acetate standard (5 mg) was dissolved in 100 ml of HPLC grade Ethanol (concentration 5 mg/dl or 0.05 mg/ml) and stored at -20^{o}C. To determine vitamin A from serum "Retinyl acetate" was used as an internal standard during the extraction of vitamin A and E simultaneously.

c) α-Tocopherol (stock)

Approximately 10 mg of α-tocopherol standard was dissolved in 100 ml of HPLC grade Ethanol (concentration 10 mg/dl or 0.1 mg/ml) and stored at -20^{o}C.

d) Tocopherol acetate (stock)

Tocopherol Acetate standard (10 mg) was dissolved in 100 ml of HPLC grade Ethanol (10 mg/dl Tocopherol acetate standard) and stored at -20^{o}C. To determine vitamin E from serum "Tocopherol acetate" was used as an internal standard during the extraction of vitamin A and E simultaneously.

4.1.2h Working standard solutions

The standard stock solutions of the above four stocks were diluted to prepare working standard solutions. These solutions were also kept at -20^{o}C.

a) All-trans Retinol

To measure serum vitamin A, Retinol standard (stock) was diluted to different concentrations i.e. 12.5, 25, 50, 60 and 100 µg/dl with HPLC grade Ethanol. To obtain 12.5 µg/dl, 25 µg/dl, 50 µg/dl, 60 µg/dl and 100 µg/dl concentrations of *all-trans* retinol, 0.25 ml (or 250 µl), 0.5 ml (or 500 µl), 1.0 ml (or 1000 µl), 1.2 ml (1200 µl) and 2.0 ml (or 2000 µl) retinol standards (from stocks) were respectively diluted to each 100 ml of HPLC grade Ethanol (5 drum vials each containing 100 ml of HPLC ethanol).

b) Retinol Acetate

As "Retinyl acetate" was 'internal standard', so a constant (50 µg/dl) amount of this 'internal standard' was added to each of the concentration of retinol solution. To make 50 µg/dl concentration of 'retinyl acetate' working standard, 1.0 ml (or 1000 µl) retinyl acetate from stock solution was diluted to 100 ml of HPLC grade Ethanol and stored at -20^{o}C.

c) α-Tocopherol

To measure serum vitamin E, tocopherol standard (stock) was diluted to different concentrations i.e. 1.0 mg/dl, 1.5 mg/dl, 2.0 mg/dl and 2.5 mg/dl with HPLC grade Ethanol. To obtain 1000 µg/dl (or 1 mg/dl), 1500 µg/dl, 2000 µg/dl and 2500 µg/dl concentration of α-Tocopherol, 10 ml, 15 ml, 20 ml and 25 ml of α-tocopherol standard (stock) was respectively diluted to 100 ml of HPLC grade Ethanol (4 drum vials each containing 100 ml of HPLC ethanol).

d) Tocopherol acetate

As "Tocopherol acetate" was an 'internal standard' of vitamin E for simultaneous estimation of vitamin A and E from serum, so a constant (concentration 1400 µg/dl or 1.4 mg/dl) amount of this 'internal standard' was always added to each of the concentration of tocopherol solution. To obtain 1400 µg/dl concentration of 'tocopherol acetate' working standard, 14.0 ml (or 1400 µl) "tocopherol acetate" from stock solution was diluted to 100 ml of HPLC grade Ethanol and stored at -20°C.

4.1.2i ***Storage of working STDs***

- Always stored in dark (wrapped by aluminum foil or solutions kept in amber bottle) at -20ºc. Retinol & Retinyl acetate (RA) stable for 7 days, α-tocopherol and tocopherol acetate stable for 15 days.
- By applying following formula "concentrations of the STDs" were always checked by 'Spectrophotometer' before analysis.

$$\textbf{Concentration of the STDs } (\mu g/dl) = \frac{\text{Absorbance of the STDs}}{\textbf{Extinction coefficient of the STD}} \ (g/dl) \times 10^6$$

Standards (STNDs)	Extinction coefficie	Wave length (nm)	♦Absorbance of the standards/Extinction Coefficients = concentration of the STNDs (g/dl) x10^6 = µg/dl	Concentration measured by spectrophotomete
All-trans retinol	1780	325	0.107/1780 =0.00006011 g/dl x10^6	60.11 ~60
Retinol Acetate	1510	328	0.076/1510 =0.00005033 g/dl x10^6	50.33 ~50
α-Tocopherol	75.8	292	0.152/75.8 = 0.00200527 g/dl x10^6	2005.27 ~ 2005
Tocopherol Acetate	43.6	285	0.062/43.6 = 0.00142201 g/dl x10^6	1422.01 ~ 1422

♦Absorbance measured by spectrophotometer (UV-1201, UV-VIS, Shimadzu, Japan)

4.1.2j Determination of retention time by working standards

To determine the retention time of *all-trans* retinol, retinyl acetate, α-tocopherol and tocopheryl acetate, each of the standard was injected into the HPLC instrument separately. The separate retention times were obtained from chromatographs. A mixture of four compounds at a ratio of 1:1:1:1 was also injected to check the resolution and the elution were also recorded.

Filtration and Injection Procedure of working standards

- About 70 µl (concentration: 60 µg/dl) of '*all-trans* retinol' working standard was filtered (through o.45 µm Millipore filter tips) and 20 µl injected into HPLC (Shimadzu, Japan) and retention time was recorded in chromatograph.
- After that, 70 µl (con-50 µg/dl) of 'retinyl acetate' or internal standard (of vitamin A) was filtered and 20 µl injected into HPLC and retention time was also recorded in chromatograph.
- Then 70 µl (con-2000 µg/dl) of 'α-tocopherol' working standard was further filtered and 20 µl injected into HPLC and retention time was recorded in chromatograph.
- Similarly, 70 µl (con-1400 µg/dl) of 'tocopheryl acetate' or internal standard (of vitamin E) was also filtered and 20 µl injected into HPLC system and retention time was recorded in chromatograph.
- Finally, each 20 µl of above four working standards in the ratio of 1:1:1:1 volume were mixed (concentration ratio were 60 µg/dl: 50 µg/dl: 2000 µg/dl: 1400 µg/dl) and then injected into HPLC to check the resolution and chromatograph was also recorded.

4.1.2k Preparation for the STD curve

Vitamin A and E Standards of different concentrations were prepared according to the following procedure, where concentration of internal standards of both remained same and finally, the mixtures were evaporated (and concentrated) under a stream of nitrogen gas, then reconstituted with HPLC grade methanol and injected into HPLC to obtain different peak areas from different concentrations.

Reagent mixture of different concentrations

1. 2 ml (2000 µl) (each 500 µl) mixture of 4 reagents (all-trans retinol: retinyl acetate: α-tocopherol: tocopherol acetate= amount 1:1:1:1(500 µl: 500 µl: 500 µl: 500 µl) = Concentration of 20 µg/dl: 50 µg/dl: 1000 µg/dl: 1400 µg/dl was made.
1. 2 ml (2000 µl) (each 500 µl) mixture of 4 reagents (all-trans retinol: retinyl acetate: α-tocopherol: tocopherol acetate= amount 1:1:1:1(500 µl: 500 µl: 500 µl: 500 µl) = Concentration of 40 µg/dl: 50 µg/dl: 2000 µg/dl: 1400 µg/dl

2. 2 ml (2000 µl) (each 500 µl) mixture of 4 reagents (all-trans retinol: retinyl acetate: α-tocopherol: tocopherol acetate= amount 1:1:1:1(500 µl: 500 µl: 500 µl: 500 µl) = Concentration of 60 µg/dl: 50 µg/dl: 3000 µg/dl: 1400 µg/dl
3. 2 ml (2000 µl) (each 500 µl) mixture of 4 reagents (all-trans retinol: retinyl acetate: α-tocopherol: tocopherol acetate= amount 1:1:1:1(500 µl: 500 µl: 500 µl: 500 µl) = Concentration of 80 µg/dl: 50 µg/dl:4000 µg/dl :1400 µg/dl

Peak area ratios of retinol to retinyl acetate and α-tocopherol to tocopheryl acetate were plotted against the respective concentrations-which gave a straight line for each of the analytes (figure-).

$$\text{Standard curve of Retinol} = \frac{\text{Retinol STD concentration}}{\text{Retinyl acetate concentration}} \times \frac{\text{Retinol Peak area}}{\text{Retinyl acetate peak area}}$$

$$\text{Standard curve of Tocopherol} = \frac{\text{Tocopherol STD concentration}}{\text{Tocopherol acetate concentration}} \times \frac{\text{Tocopherol Peak area}}{\text{Tocopherol acetate peak area}}$$

4.1.2l Analysis of Serum Retinol and Tocopherol

4.1.2l-1 Extraction of vitamin A and E from serum with internal standards: about 500 µl (required 5 times higher serum than manually injectable HPLC) of serum was used in this analysis as reconstituted sample was filtered through o.45 µm (Millipore) filter tips (after reconstitution of the sample) before placed on the sampling tray (auto sampler), so much of serum sample remain soaked in the filter tips and also at least 50 µl sample should remain in the HPLC vial incase of injector could not reach lower to pick serum sample. At first, 500 µl serum (previously thawed) was taken in a centrifuge tube and vortexed for few seconds then 500 µl each of both internal standard (both was working standard) retinyl acetate (concentration 50 µg/dl) and tocopherol acetate (concentration 1.4 mg/dl) was added and mixed in a vortex mixer for 30 seconds. For extraction of lipids, about 1 ml (1000µl) of n-hexane was added and mixed for 30 seconds in a vortex mixer. The samples were then centrifuged at 3000 rpm for 5 minutes and the supernatant (top hexane layer) was taken in a screw cap vial. The adding of n-hexane (1 ml) and vortexed and then centrifuged as well as hexane layer was transferred to vial- this process was carefully (not disturbing the aqueous layer beneath the hexane layer) done one more time. Finally, the extracted hexane layer (which contains fat soluble vitamin A and E) was evaporated (and concentrated) under a stream of nitrogen gas.

4.1.2I-2 Reconstitution of the dried vitamin A and E samples and Injected into HPLC

The extracted vitamin A and E (contained in vial as dry form) was re-dissolved in 500 µl HPLC grade methanol (which was also used in mobile phase). The solution was filtered into HPLC vial through o.45 µm (Millipore) filter tips and placed on auto sampling tray of HPLC (SIL-20AC HT Prominence auto sampler) for injection. A solvent flow rate of 1.2 ml/min was used and elution was monitored at 291 nm .The concentration of vitamin A and E was obtained from the integrator based on the peak area ratios of "Retinol to Retinyl acetate" and "α-Tocopherol to Tocopherol acetate" respectively.

4.1.2I-3 Preparation of mobile phase for HPLC

Before analysis of the serum samples by HPLC, mobile phase was prepared. To prepare mobile phase, 490 ml analar grade methanol was taken in a 500 ml volumetric flask. About 10 ml of double distilled water was added into the flask. The mixture was sonicated for 10 minutes and then filtered through a 0.22 µm Millipore filter.

4.1.2I-4 Chromatographic conditions

For simultaneous determination of serum vitamin A and E by RP-HPLC method, the mobile phase was comprised of 99% HPLC grade methanol (Merck, Germany) and 2% doubled distilled water (glass distilled) in the ratio of 99:2 (V/V) at a constant flow rate of 1.2 ml/minute over C18 bonded silica column (ODS)(250x4.6 mm, 5µm). The injection volume was 20 µl for both standard and samples. The run time was set for 30 minutes. The identification was based on the retention time and the quantification was based on the peak area matched against standards of vitamin A and vitamin E.

Prior to injection into the chromatographic system (LC-20AD-ProminenceLiquid Chromatography), all analytical solutions including every standard and sample were degassed by sonication and filtered through o.45 µm (Millipore) filter tips. The mobile phase was also filtered, sonicated and degassed before use. The column eluate (both vitamin A and E)/ the effluents were monitored with a UV detector (SPD-20A Prominence UV/VIS Detector) at 325 nm and 292 nm and retention times were found to be **5.2** minutes for retinol, **6.3** minutes for retinyl acetate and **13.6** minutes for tocopherol and **18.2** minutes for tocopherol acetate. All analysis was done at ambient temperature under isocratic condition. All the prepared sample solutions were first chromatographed to ensure interfering peaks were not present.

4.1.2I-5 Sample Analysis and calculation

To quantify the vitamin A and E (simultaneously) concentration in CSW's sera, successive analysis of the serum samples were done by slight modification of the method of Bieri at el (1979) using High Performance Liquid chromatographic (HPLC) system (Shimadzu-UFLC prominence) and the contents were determined by using the calibration curves of the standards vitamin A and vitamin E (sigma chemicals), and the formula, y=mx+ c.

Where, y=peak area of the analyzed sample, m= slope of the calibration curve, c=intercept/constant of the calibration curve, x=concentration of the analyzed sample.

Basic principal of using internal standard

Concentrations (retinol/α-tocopherol) =peak area ratio of sample and internal STD × value of one Peak area ratio of external & internal STD µg/dl

Retinol concentration= (obtained value µg/dl) × 0.03491 µ mol/ L

α-tocopherol concentration= (obtained value µg/dl ×23.22) µ mol/ L

0.03491 and 23.22 are conversion factors respective for retinol and α-tocopherol for STD International Unit or IU (young, 1996)

Quality control: day to day variations for vitamin A assay were determined using pooled serum sample. The inter assay variation for vitamin A and E was 5.3% and 6.4% respectively. The intra assay variation was performed within samples by using 2 replicates of the same sample randomly (as it was costly procedure).

Cautions taken were

- Machine was re-equilibrated with mobile phase for 5 minutes before next injection
- Samples were injected twice to have replicate chromatograph
- Standards were run for every 15 test.
- Prior to analysis all glass wares were soaked in 5% HNO_3 for 48 hours and then washed with double distilled water (glass distilled).

4.1.2m Data Analysis

Normality test: various statistical tools were used employing SPSS version for the analysis of serum 'vitamin A and vitamin E'. Before performing statistical analysis, "Normality" test of these two serum data set was done by "Shapiro-Wilk (S-W)" goodness of fit test (vitamin A: statistic=.827, df=107, P=.000; vitamin E: statistic=.862, df=107, P=.000) as well as by doing descriptive statistics. It was observed from 'descriptive statistics' that both 'vitamin A and vitamin E' data were positively as well as highly skewed (vitamin A: skewness=3.683, SE=.234; vitamin E: skewness=2.895, SE=.247) and both "logarithmic or Log_{10}" (vitamin A: skewness=.736, SE=.234; vitamin E: skewness=.940, SE=.247) and "Square root transformation" (vitamin A: skewness= -.194, SE=.234; vitamin E: skewness= -.706, SE=.247) was performed to see which process could eliminate the skewness in a better way. And thus, approximation to a normal distribution was greatly improved for both 'vitamin A and vitamin E' data by "Square root transformation". After the data were transformed by "Square root", 'mean' as well as other parametric tests on the transformed scale data was performed and then back transformed by taking the "square" and at the same time previous "Square root transformed" values were backed in the usual vitamin A and vitamin E units (µmol/L). However, the Square root transformed **'geometric mean'** was always less than the mean of raw data (Bland and Altman, 1996). In fact, extreme observations of a “highly skewed data set” have a large influence on the arithmetic mean, making it more prone to sampling error. Thus, lessening this influence is one advantage of using transformed data (Bland and Altman, 1996).

4.1.2m-1 Statistical Analysis

All statistical analyses of Vitamin A and E were assessed by using Statistical Package for Social Science version 17.0 (SPSS Inc, Chicago, IL, USA). Two-tailed P values <0.05 were considered statistically significant.

Univariate analysis of the samples: Descriptive statistics [Proportions, means (with 95% CI) and ranges] were used to calculate baseline information. Descriptive data were presented as the mean ((with 95% CI)) for continuous variables and as percentages for categorical variables.

Bivariate analysis: in bivriate analysis Pearson’s correlation (2-tailed) was applied to assess extent of linear correlation between two continuous variables. For participant characteristics analysis, differences in serum vitamin A and E levels (dependent continuous variables) were examined in respect to socio-demographic, lifestyle and sexual variables (independent categorical variables) using ANOVA (comparing mean vitamin A and E among categories).

Multivariate analysis: multivariate analysis was done to understand the association of different independent variables with dichotomous dependent variable. Multiple logistic regression models (MLoR), with the outcome variables dichotomized (0, 1) as normal [vitamin A: (>1.05 µmol/L); Vitamin E: (13-35.23 µmol/L)] or lower [vitamin A: (<1.05 µmol/L); Vitamin E: (<13 µmol/L)] was developed to examine the strength of relationship between dependent variables (vitamin A and vitamin E) with independent categorical variables. In the MLoR model, generally, dummy variables were created for non-binary categorical variables; the effect of each category of a multi-categorical variable was assessed by keeping the first or last category as reference category. All Covariates (socio-demographic, lifestyle and sexual variables as well nutritional indices) which were found statistically significant (P≤0.05) or borderline significant (P<0.09) in Simple logistic regression (SLoR) analysis were introduced simultaneously in the **multiple logistic regression analysis.** Possible interactions between different variables in the model were assessed, where necessary. The model was developed by ‘backward stepwise elimination’, removing the covariate with largest P value at each step until the remaining variables were significant at the 0.05 level in the final adjusted model. Also, forward stepwise technique applied in same way to confirm the model. Thus relative odds (ORs) and 95% CI were calculated to identify potential risk factors associated with serum ‘vitamin A’ and ‘vitamin E’ deficiency [0=normal to higher and 1=lower]. Hosmer-Lemeshaw goodness of fit and Nagelkarke-pseudo R^2 of the models were also observed.

4.1.3 Results

4.1.3a Serum vitamin A and E level of CSWs (n=107)

Mean serum A and Vitamin E were 0.66 µmol/L (95% CI: 0.56−0.75) and 6.29 µmol/L (95% CI: 5.36−7.20 µmol/L) respectively. From serum vitamin A and E analysis, it was depicted that mean serum A was vulnerably lower than standard normal (WHO/VACG) value of vitamin A (>1.05 µmol/L) and mean vitamin E was almost 3 times lower in study population (CSWs) than the lower tail of standard normal values of vitamin E (vitamin E: 18-29 µmol/L; Young, 1998; Edwards et al 1996). Most (63.6%) of CSWs had subclinical vitamin A deficiency (<0.7 µmol/L retinol or ≥ 0.35 µmol/L retinol) and around 19.6 % had severe vitamin A deficiency (< 0.35 µmol/L retinol) and only 12.1% (n=13) had normal vitamin A level (>1.05 µmol/L retinol). Frequency distribution of vitamin E showed that most (41.2%) CSWs had vitamin E within 6.0 to 6.66 µmol/L ranges while 14% had undetectable and 28% had extremely low vitamin E. There was no participant having vitamin E in between 6.67 to 12.99 µmol/L and 17 samples had within 13.0 to 13.45 µmol/L serum vitamin E and only one sample had 35.23 µmol/L (table-1).

4.1.3b Comparison of serum vitamin A of CSWs with National Data

While comparing serum vitamin A of CSWs (table-2) with national data (NMSS, 2011-12), severe vitamin A deficiency (<0.35 µmol/L), subclinical vitamin A deficiency (<0.7 µmol/L retinol or ≥ 0.35 µmol/L retinol, WHO/VACG), and mild vitamin A deficiency (0.7-1.04 µmol/L retinol) was observed among CSWs. In all cases except mild deficiency data regarding CSWs were overwhelmingly higher (severe-19.6%, sub-clinical-63.6% & mild-4.7%) than national data (severe-0.1%, sub-clinical-5.2% & mild-34.3%). In addition, overall 87.9% of CSWs had lower than normal serum retinol (<1.05 µmol/L) which was more than double than national level (39.7%) as well as than slum level (41.3%). Only 12.1% (13/2107) CSWs had normal serum level (>1.05 µmol/L) of vitamin A while it was 60.3% at national level that means 5 times lower prevalence of '**normal serum retinol'** among CSWs than national was noticed (table-2).

4.1.3c Influence of socio-demographic variables, business location and STI status on Serum vitamin A and E level

To examine the influence of different socio-demographic factors (age, education, income-expenditure etc.), STI status and business location as well as various lifestyle and risky sexual variables on serum vitamin A status of CSWs, one-way ANOVA was performed. In fact, no significant (P>0.05) influence of age, education, marital status, monthly income-expenditure, STI treatment cost and sex business location of CSWs on serum **vitamin A and E** level was noticed except **'STI status'** of CSWs. '**STI status of CSWS**' significantly (F (1,105)=5.544, P=.020*) and negatively associated with only serum **'vitamin A level'** and those CSWs who were inflicted with either syphilis or hepatitis 'B' or both had significantly lower level of vitamin A than those who were not infected with STIs. As mean serum vitamin A

(0.66 µmol/L) and E (6.29 µmol/L) was severely lower in the study population (CSWs) than standard normal values, so as reflected in their influential effects. Nevertheless, CSWs who had comparatively better (better of the worst) serum vitamin A [F (1,105) =5.544, P=.020*] and vitamin E [F (1,105) =.791, P=.454] concentration did not have any STI. In other words, CSWs who had no STI had higher vitamin A (0.81 µmol/L) and E (6.52 µmol/L) level though in case of vitamin E, the difference was not got statistical significance (P=.454). Interestingly, floating CSWs had higher vitamin A (0.68 µmol/L) and E (6.48 µmol/L) concentration than hotel-based CSWs although the differences were not significant (P=.773) (table-3).

4.1.3d Serum vitamin A and E and Lifestyle and risky sexual variables of CSWs

Most of lifestyle factors of CSWs were not significantly (P>0.05) influenced serum immuno-nutrient vitamins A and E except “Habit of smoking”. It was observed in this study that CSWs who did not smoke had higher level of both vitamin A and E though in case of vitamin A the difference was not statistically significant (P=.103) and vitamin E was found in borderline significant [F (1,105)=3.927, P=0.05]. However, couples of risky sexual variables of CSWs such as condom use, group sex (Gr.sex) experience, and number of persons in gr. Sex etc. were found negatively influence serum vitamin A and E levels in this study. Irregular condom users had significantly higher serum vitamin A than consistent users [F (1, 105) = 6.133, P= .015*]. CSWs who used to perform Gr. sex or ever experienced in this risky sexual act had both vitamin A and E levels lower than who did not but the differences were significant only for vitamin E (F (1,105)=7.014, P=.009) and vitamin A was insignificant (P=.740). Similar results also depicted in case of ‘having history of Gr. Sex last month’ (P=.002) and number of persons involved in gr. Sex (P=.008) (table-4 & 5).

4.1.3e Influence of different nutritional variables on serum vitamin A and vitamin E level

Influence of different nutritional variables (especially serum copper, iron, total protein and albumin) on serum vitamin A and vitamin E level of CSWs was postulated in table-6. ANOVA pointed at that [F (2, 104) =4.077 P=.046] serum copper level of CSWs positively influenced serum vitamin E and those CSWs had >normal serum copper (>22.1 µmol/L) had higher level of vitamin E (7.87±0.08 µmol/L) and vice versa. Similarly, serum iron also positively influenced serum vitamin A and those CSWs who had higher serum iron (>29.1 µmol/L) had higher level of vitamin A (0.89±0.06 µmol/L) and vice versa. It was observed that only normal ‘total protein’ level (60-80 g/L) influenced serum ‘vitamin A’ positively while >normal TP (>80.1 g/L) negatively influenced [F (2,104) =4.11, P=.019]. Similarly, normal serum albumin level (40-60 g/L) positively influenced on serum vitamin A but >60.1 albumin negatively influenced vitamin A (P=.055). Serum zinc,IgG, IgA, IgM and BMI had no significant influence on serum vitamin A and vitamin E level (table-6).

Table-1: serum vitamin A and vitamin E levels of CSWs (n=107)

Serum Micro nutrients		All CSWs % n	Range Min-max	Mean ±SD	95% CI Lower-upper
Levels	Amount (µmol/L)				
Vitamin A (µmol/L retinol) [5]					
Severe	0.0 to o.34	19.6 (21)			
Moderate	0.35 to 0.69	63.6 (68)	0.0 (ND) to 4.18	0.66±0.09	0.56–0.75
Mild	0.7 to 1.05	4.7 (05)			
Normal	>1.05	12.1 (13)			
Vitamin E (µmol/L) [6]					
Not detected or zero	0.0 to 1.0	14.0 (15)			
Very low	1.1 to 5.99	28.0 (30)	0.0 (ND) to 35.23	6.29±0.06	5.36–7.20
Low	6.0 to 6.66	41.2 (44)			
Mild to normal	13.0 to 35.23	16.8 (18)			

Human normal serum Vitamin A and E are 0.35–1.75 µmol/L and 18-29 µmol/L respectively (Young, 1998; Edwards et al 1996). ND=Not detected

Table-2: Comparison of serum vitamin A of CSWs with National Data

Grades of vitamin A deficiency		This study (CSWs, aged 14- 55 y)	NMSS, 2011-12 (NPNL women, aged 15-49 y)	
Serum vitamin A (µmol/L)	**Categories**	**Sub group n=107 % n**	**Slums n=290**	**National n=933**
0.0 to o.34 (<0.35)	Severe deficiency	19.6 (21)	0.2%	0.1%
0.35 to 0.69 (>0.35-<0.7)	Moderate (subclinical deficiency)	63.6 (68)	6.6%	5.1%
>0.7 to 1.04	Mild deficiency	4.7 (05)	34.5%	34.3%
Total <1.05 µmol/L	**Total low serum retinol**	**87.9% (n=94)**	**41.3%**	**39.7%**
Total >1.05 µmol/L	**Normal serum retinol**	**12.1% (n=13)**	**58.7%**	**60.3%**

[5] About 6.54% (n=07/107) **vitamin A** were not detected (ND) or were below detection limit.

[6] About 11.2% (n=12/107) of the CSWs had serum **vitamin E** too low to detect (not detected/ND) and rest (33/107) were found very low concentration (0.69–5.99 µmol/L).

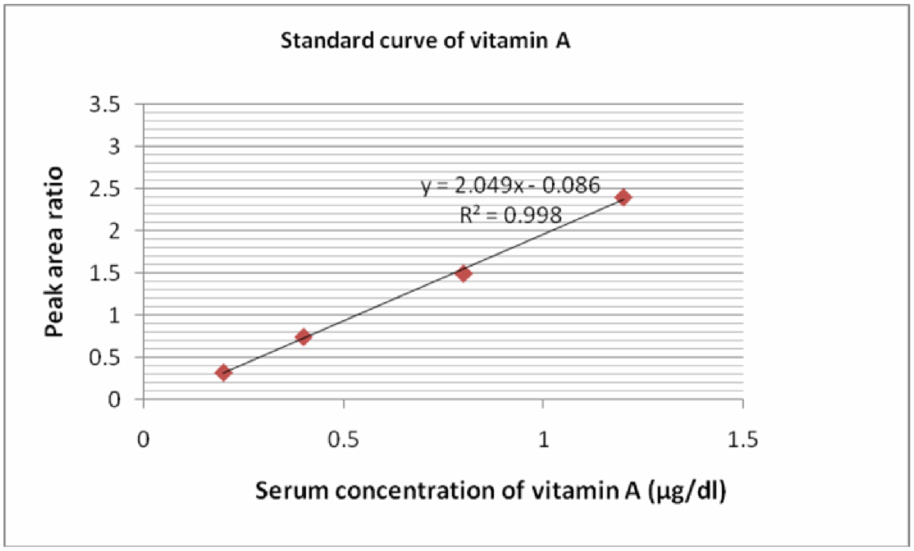

Figure-4.1a: Standard curve of vitamin A

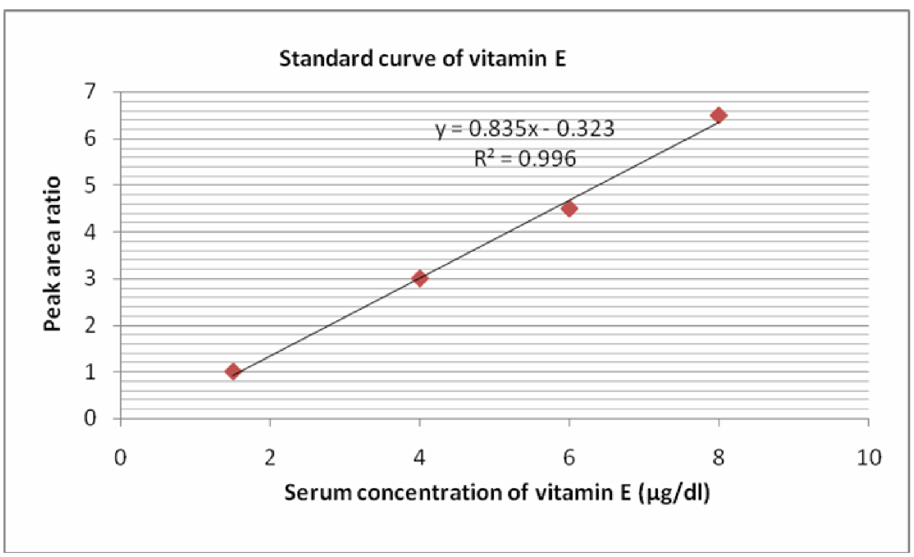

Figure-4.1b: Standard curve of vitamin E

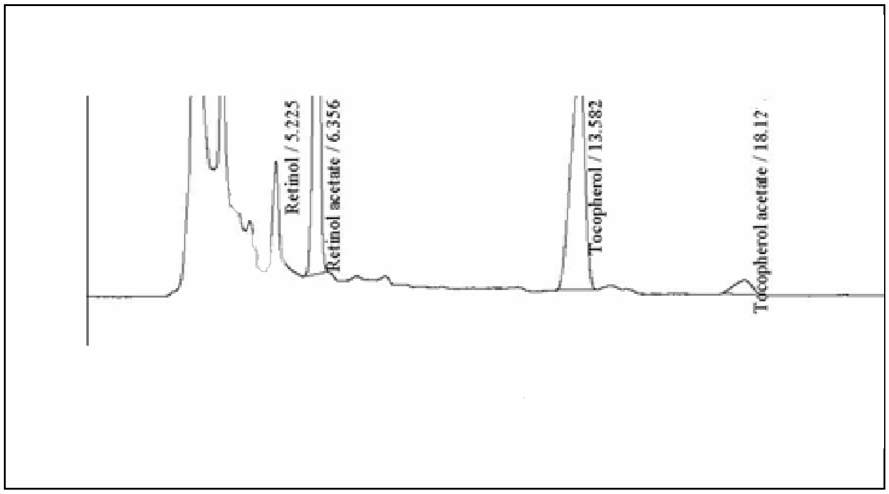

Figure-4.1c: Chromatographs of the standard vitamin A and vitamin E with internal standards Retinyl acetate (RA) and tocopherol acetate (TA)

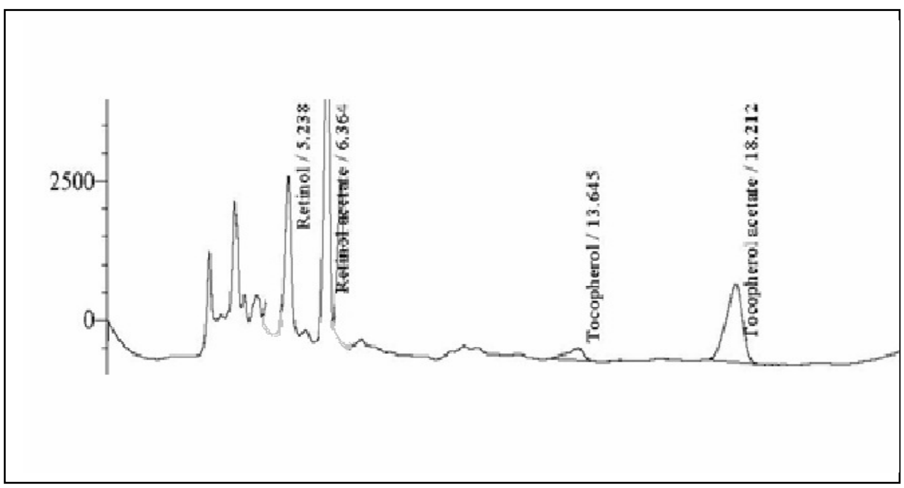

Figure-4.1d: Chromatographs of the CSW's serum sample with internal standards showing retention time 5.2 minutes for retinol, 6.3 minutes for retinyl acetate and 13.6 minutes for tocopherol, 18.2 minutes for tocopherol acetate.

Table-3: Influence of socio-demographic variables, STIs status and business location of CSWs on serum vitamin A and vitamin E

Sociological factors	All CSWs n=107 % n	Vitamin A µmol/L	ANOVA	Vitamin E µmol/L	ANOVA
Present age (y)					
≤18	12.15 (13)	0.59±0.09	F (2,104)=.137	5.79±0.08	F (2,104)=.391
19–29	45.79 (49)	0.68±0.01	P=.872	6.80±0.09	P=.677
≥30	42.06 (45)	0.65±0.05		5.88±0.01	
Education					
No literacy/can sign	77.57 (83)	0.65±0.03	F (1,105)=.117	6.33 ±0.02	F (1,105)=.282
1-5 class (primary)[7]	22.43 (24)	0.69±0.04	P=.734	6.13 ±0.04	P=.597
Marital status					
Married	41.6 (38)	0.69±0.07	F (2,104)=.088	6.46 ±0.06	F (2,104)=.376
Unmarried	13.7 (09)	0.67±0.09	P=.916	7.17 ±0.09	P=.688
Widow/divorced /abandoned	44.7 (60)	0.64±0.06		6.05 ±0.04	
Monthly income (BDT/$)					
0.0 – 2500 BDT (00- 31.25 **$)**	49.53 (53)	0.54±0.06	F (2,104)=.763	5.71 ±0.05	F (2,104)=1.583
2500.1- 5000 BDT (31.26 -62.5 **$)**	29.91 (32)	0.70±0.09	P=.469	6.77 ±0.06	P=.210
>5000.1 BDT (>62.6 **$)**	20.56 (22)	0.66±0.07		6.98 ±0.04	
Last month's Total expenditure					
0.0 – 2500 BDT (00- 31.25 **$)**	21.50 (23)	0.66±0.09	F (2,104)=.251	5.40 ±0.07	F (2,104)=.182
2500.1- 5000 BDT (31.26 -62.5 **$)**	52.33 (56)	0.68±0.07	P=.778	6.59 ±0.03	P=.834
>5000.1 BDT (>62.6 **$)**	26.17 (28)	0.60±0.06		6.41 ±0.06	
Treatment cost of STIs (last ailment, n=60/107)[8]					
Self treatment	30.0 (18)	0.52±0.09	F (2,104)=1.341	5.87± 0.01	F (2,235)=.372
5-250 taka	45.0 (27)	0.81±0.05	P=.270	5.29± 0.06	P=.691
251-5000 taka	25.0 (15)	0.59±0.08		6.33± 0.01	
STIs status					
STIs (either syphilis+ or HBV+ or both)	68.22 (73)	**0.58±0.09**	**F (1,105)=5.544**	5.79±0.06	F (1,105)=.791
No STIs	31.78 (34)	**0.81±0.03**	**P=.020***	6.52±0.08	P=.454
Places of sex trade					
Hotel	17.76 (19)	0.54±0.04	F (1,105)=1.249	5.40±0.04	F (1,105)=.084
Floating (road/perk/shrine)	82.24 (88)	0.68±0.01	P=.266	6.48±0.05	P=.773

[7] None had secondary education (1-12 class).
[8] No symptoms appeared in 37.38% (40/107) of CSWs.

Table-4: Influence of lifestyle variables of CSWs on serum Vitamin A and Vitamin E

Lifestyle Factors	All CSWs n=107 % n	Vitamin A µmol/L	ANOVA	Vitamin E µmol/L	ANOVA
Factors behind profession					
Lower Economic status	44.86 (48)	0.70±0.05	F (2,104)=3.461	6.67 ±0.04	F (2,104)=1.287
Exploited by pimps/other	25.23 (27)	0.77±0.07	P=.063	5.30 ±0.09	P=.280
Other reasons[9]	29.91 (32)	0.50±0.06		6.54 ±0.05	
Working days (last week)					
Did not work	5.61 (06)	0.52±0.05	F (2,104)=1.559	6.05±0.00	F (2,104)=1.559
1–3 days	44.86 (48)	0.75±0.06	P=.303	6.20±0.09	P=.303
4–7 days	49.53 (53)	0.59±0.02		5.42±0.04	
Age entering into sex business					
10-17 y	46.73 (50)	0.61±0.07	F (2,104)=.552	6.22 ±0.06	F (2,104)=.246
18-24	33.65 (36)	0.67±0.01	P=.577	6.89 ±0.02	P=.782
≥25 years	19.63 (21)	0.74±0.00		5.42 ±0.08	
Experience in sex business (years)					
Less than one year	6.54 (07)	0.55±0.01	F (2,104)=1.208	6.09 ±0.08	F (2,104)=.845
1-5 y	47.67 (51)	0.74±0.03	P=.303	5.83 ±0.03	P=.432
≥6 y	45.79 (49)	0.59±0.08		6.79± 0.00	
living with					
Own	57.01 (61)	0.64±0.07	F (2,104)=.118	5.90 ±0.09	F (2,104)=.358
Husband	30.84 (33)	0.70±0.00	P=.889	6.81 ±0.05	P=.700
Others[10]	12.15 (13)	0.63±0.09		6.78± 0.04	
Use of contraceptives					
No measure	23.37 (25)	0.74±0.08	F (3,103)=.409	6.78 ±0.09	F (3,103)=.301
Pill	17.76 (19)	0.68±0.00	P=.747	5.59 ±0.06	P=.824
Condom	42.98 (46)	0.62±0.06		6.35 ±0.06	
Others [11]	15.89 (17)	0.63±0.03		6.18± 0.04	
Cases of abortion					
No abortion cases	68.22 (73)	0.67±0.05	F (2,104)=.024	6.11 ±0.08	F (2,104)=2.325
1-2 cases	27.11 (29)	0.64±0.02	P=.977	7.37 ±0.03	P=.103
>3 cases	4.67 (05)	0.62±0.02		2.60 ±0.06	
Habit of smoking					
No	47.66 (51)	0.74±0.00	F (1,105)=2.705	**7.11 ±0.00**	**F (1,105)=3.927**
Yes	52.34 (56)	0.58±0.03	P=.103	**5.54 ±0.01**	**P=0.50**
Substance abuse					
No	70.09 (75)	0.70±0.04	F (1,105)=2.305	6.79±0.00	F (1,105)=.101
Yes	29.91 (32)	0.56±0.01	P=.132	6.08±0.01	P=.752
Types of substances					
No substances abused	70.09 (75)	0.70±0.04	F (3,103)=1.207	6.08± 0.01	F (3,103)=.075
Alcohol (maod)	7.48 (08)	0.71±0.08	P=.328	7.49± 0.01	P=.973
Gaza	10.28 (11)	0.54±0.01		5.28± 0.09	
Others[12]	12.15 (13)	0.45±0.00		7.12± 0.08	
Client profile					
Businessmen (small)	36.45 (39)	0.58±0.03	F (5,101)=.838	6.88± 0.00	F(5,101)=.477
Rickshaw puller	29.91 (32)	0.73±0.02	P=.526	6.53 ±0.04	P=.792
Service holder	12.15 (13)	0.78±0.04		5.64 ±0.06	
Student	6.54 (07)	0.71±0.05		5.55 ±0.05	
Bus driver	2.80 (03)	0.75±0.03		5.81 ±0.08	
Didn't know	12.15 (13)	0.55±0.05		6.10 ±0.00	
NGO counseling on STIs					
No	42.90 (46)	0.68±0.02	F (1,105)=.093	6.25 ±0.06	F (1,105)=1.110
Yes	57.10 (61)	0.64±0.08	P=.761	6.32 ±0.06	P=.295

[9] Angry with family members, sold by step mom/others, raped/tortured, pushed by husband, self motivation and drug addiction.
[10] Parents, siblings, sexual partners etc.
[11] Injection, ligation/tubectomy, menopause etc.
[12] Cannabis (afim, charush), phensedyl, injection (pethidin) and Heroin etc.

Table-5: Influence of Risky sexual variables of CSWs on serum vitamin A and vitamin E

Risky sexual behaviors of CSWs	All CSWs n=107 % n	Vitamin A µmol/L	ANOVA	Vitamin E µmol/L	ANOVA
Client turnover (last week)					
Did not work	5.61 (06)	0.52±0.09	F (2,104)=1.578	6.05 ±0.07	F (2,104)=.269
1– 14 C	61.68 (66)	0.72±0.06	P=.211	6.37 ±0.02	P=.765
≥15 C	32.71 (35)	0.56±0.09		6.17 ±0.07	
Usual no. of clients per day					
1-7	88.79 (95)	0.66±0.00	F (1,105)=.137	6.12 ±0.07	F (1,105)=.009
≥8 clients	11.21 (12)	0.61±0.07	P=.712	7.60 ±0.07	P=.924
Condom use					
No/ irregular use	56.08 (60)	0.63±0.09	F (1,105)=.477	**7.28 ±0.04**	**F (1,105)=6.133**
Consistent use	43.92 (47)	0.69±0.09	P=.491	**5.02 ±0.04**	**P=.015**
Hygienic measure after coitus					
No measure	35.51 (38)	0.72±0.06	F (2,104)=.640	7.47 ±0.07	F (2,104)=1.692
Used savlonl/soap	33.65 (36)	0.63±0.09	P=.529	5.89 ±0.00	P=.189
Others[13]	30.84 (33)	0.61±0.02		5.35 ±0.04	
Interval between 2 coitus					
No	60.75 (65)	0.70±0.07	F (1,105)=1.352	5.86 ±0.09	F (1,105)=1.388
yes	39.25 (42)	0.59±0.01	P=.266	6.95 ±0.07	P=.259
Usual coital frequency per client					
One time	48.60 (52)	0.71±0.01	F (1,105)=1.390	6.48 ±0.09	F (1,105)=1.079
≥2 times	51.40 (55)	0.61±0.03	P=.241	6.10 ±5.09	P=.301
Weekly coitus with permanent client (PC)					
No weekly PC	61.68 (66)	0.68±0.06	F (2,104)=.081	6.65 ±0.07	F (2,104)=.296
Yes 1 time/week	16.82 (18)	0.63±0.04	P=.922	4.66 ±0.08	P=.744
2 times/week	21.50 (23)	0.63±0.06		6.54 ±0.08	
Fortnightly coitus with PC					
No fortnightly PC	80.37 (86)	0.66±0.02	F (2,104)=.000	6.54 ±0.09	F (2,104)=.206
Yes 1-2 times	17.76 (19)	0.65±0.05	P=1.00	5.10 ±0.02	P=.814
>3 times	1.87 (02)	0.66±0.00		6.60 ±0.00	P (THV)=.702
Monthly coitus with PC					
No monthly PC	74.77 (80)	0.69±0.03	F (2,104)=.761	6.37 ±0.02	F (2,104)=.327
Yes 1-2 times	21.50 (23)	0.56±0.01	P=.470	6.27 ±0.09	P=.722
>3 times	3.73 (04)	0.52±0.07		4.70 ±0.02	
Having NPSP[14]					
No	91.59 (98)	0.64±0.09	F (1,105)=1.097	6.27 ±0.06	F (1,105)=.003
Yes	8.41 (09)	0.82±0.06	P=.297	7.13 ±0.06	P=.954
Ever experienced in group sex (gr. sex)					
No	74.1 (73)	0.68±0.06	F (1,105)=.111	**7.10 ±0.02**	**F (1,105)=7.014**
Yes	25.9 (34)	0.65±0.06	P=.740	**4.54 ±0.08**	**P=.009**
Gr. Sex history (last month)					
No	71.03 (76)	0.73±0.03	F (1,105)=1.276	**7.19 ±0.01**	**F(1,105)=10.140**
Yes	28.97 (31)	0.63±0.04	P=.261	**4.07 ±0.07**	**P=.002**
No of persons in the last gr. Sex					
No Gr. sex	71.03 (76)	0.63±0.03	F(1, 29)=.045	**7.19 ±0.00^t**	**F(1, 29)=5.113**
2-5 clients	20.56 (22)	0.72±0.06	P=.834	**3.86 ±0.06^t**	**P=.008**
>6 clients	8.41 (09)	0.78±0.04		**4.60 ±0.07**	P (THV)=.419
Ever experienced in Anal sex					
No	96.4 (105)	**0.63±0.05**	**F (1,105)=35.930**	6.3±0.07	F (1,105)=.794
Yes	3.6 (02)	**2.40±0.06**	**P=.000**	3.3 ±0.07	P=.787

^{t} In post Hoc tests (TUKEY/multiple comparisons) this category (within individual variable) was significantly different than other categories (of that variable) for having the lowest or highest mean value.

[13] Washed by water, cotton, urine etc.

[14] NPSP means Non paying sexual partner

Table-6: Influence of nutritional variables on serum vitamin A and vitamin E level of CSWs

Serum Micronutrients status of CSWs (n=107)	All CSWs n=107 % n	Vitamin A µmol/L	ANOVA	Vitamin E µmol/L	ANOVA
Copper (µ mol/L)					
8.18 to 22.0	68.3 (80)	0.63±0.07	F (2,104)=1.021	**5.75±0.07**	**F (2, 104)**
22.1 to 63.27	25.2 (27)	0.74±0.02	P=.315	**7.87±0.08**	**=4.077 P=.046**
Zinc (µ mol/L)					
5.97 to 11.5	37.4 (40)	0.61±0.04	F (2,104)=.453	7.0±0.09	F (2, 104)=.940
11.6 to 18.5	37.4 (40)	0.66±0.05	P=.637	6.0±0.08	P=.394
18.6 to 99.30	25.2 (27)	0.73±0.05		5.5±0.07	
Iron (µ mol/L)					
0 .11 to 10.9	15.0 (16)	**0.58±0.07**	**F (2,104)=3.459**	6.07±0.08	F (2, 104)
11.0 to 29.0	64.5 (69)	**0.66±0.09** [t]	**P=.035***	6.81±0.04	=1.523
29.1 to 70.74	20.5 (22)	**0.89±0.06** [t]		4.80±0.03	P=.223
					P (THV)=.585
Immunoglobulin G (µ mol/L)					
0 .04 to 0.49	66.3 (71)	0.71±0.03	F (2,104)=1.426	5.97±0.08	F (2, 104) = .563
0.50 to 4.99	18.7 (20)	0.60±0.08	P=.245	6.61±0.04	P=.571
5.0 to 33.0	15.0 (16)	0.50±0.03		7.31±0.03	
Immunoglobulin A (µ mol/L)					
0.00 to 0.35	64.5 (69)	0.71±0.06	F(2,104)=1.115	5.57±0.09	F(2,104)=2.282
0.36 to 0.49	15.0 (16)	0.63±0.03	P=.332	7.41±0.09	P=.107
0.50 to 5.75	20.5 (22)	0.53±0.04		7.74±0.08	
Immunoglobulin M (µ mol/L)					
0.02 to 0.29	67.3 (72)	0.70±0.04	F (2,104)=.930	6.24±0.03	F (2,104)=.884
0.30 to2.30	25.2 (27)	0.57±0.07	P=.398	6.98±0.07	P=.416
2.31 to 2.85	7.5 (08)	0.56±0.07		4.42±0.04	
Total protein (g/L)					
1.14 to 59.9	44.86 (48)	**0.68±0.05**	**F (2,104)=4.111**	44.23±0.07	F (2,492)=.840
60.0 to 80.0	21.50 (23)	**0.86±0.05** [t]	**P=.019***	30.05±0.09	P=.435
>80.1	33.64 (36)	**0.50±0.05** [t]		35.05±0.09	
Albumin (g/L)					
0.01 to 39.9	48.60 (52)	**0.72.± 0.07**	**F (2,104)=2.849**	5.43±0.07	F (1,105)=1.650
40.0 to 60.0	25.23 (27)	**0.73 ± 0.04**	**P=.055**	7.07±0.09	P=.197
>60.1	26.17 (28)	**0.47 ± 0.06**		7.13±0.02	
BMI (kg/m^2)					
<16 to 18.4 (CED)	31.78 (34)	0.79±0.09	F (2,104)=2.169	5.81±0.03	F (2,104)=.783
18.5 to 25 (Normal)	51.40 (55)	0.63±0.05	P=.119	6.84±0.08	P=.460
>25.1 (Overweight)	16.82 (18)	0.51±0.06		5.50±0.09	

[t] In post Hoc tests (TUKEY/multiple comparisons) this category (within individual variable) was significantly different than other categories (of that variable) for having the lowest or highest mean value. THV= P-value of the 'Test of homogeneity of Variance'.

4.1.3f Predictors associated with lower serum retinol and tocopherol of CSWs (Crude model)

Unadjusted logistic regression models of “vitamin A” and “vitamin E” for different independent variables are resulted in table-7. In the Unadjusted model of “vitamin A” and “vitamin E”, different predictors (categorical variables) were included separately in simple logistic regression analysis (SLoR). In crude model of “vitamin A” (table-7) ‘condom use’, ‘group sex experience’, ‘smoking habit’ and ‘serum copper’ did not significantly associated with lower serum retinol but ‘STI status’, ‘BMI’ and ‘serum Iron’ were significantly associated with lower serum retinol and former two remained significant in adjusted model (table-8) while latter one, no longer remained in adjusted model of vitamin A. In crude model of “vitamin E majority of variables did not significantly (p≤0.05) associated with lower serum tocopherol levels except ‘group sex experience’ and ‘smoking habit’, which also remained in adjusted model of vitamin E (table-9). Although, ‘serum copper’ was not significant (P=.150) in crude model of vitamin E but remained in adjusted model with borderline significance (P=.077).

4.1.3g Multiple Logistic Regression analysis of serum vitamin A and E level

To examine which variables associated with lower serum ‘retinol’ and ‘tocopherol’ multiple logistic regressions (MLoR) analysis was done by applying ‘backward stepwise’ MLoR analysis. For clarification ‘Forward stepwise’ MLoR analysis was also performed. Generally, all categorical variables which were found statistically significant (P≤ 0.05) or borderline significant (P<0.09) in ‘simple logistic regression’ (table-7) were included together in the **multiple logistic regression analysis.** Thus **“adjusted model of vitamin A”** is presented in table-8 and **“adjusted model of vitamin E” is** presented in table-9. “Hosmer-Lemeshaw goodness of fit” test was performed for both of the models.

MLoR model of serum vitamin A: two independent variables namely ‘STI status’ and ‘BMI’ were remained significant in the ‘adjusted model of vitamin A’ (table-8). It was depicted that, those CSWs who were inflicted with STIs (syphilis/hepatitis B or both) were 6 times more likely to have lower (<1.05 ≥mol/L) serum vitamin A as compared to CSWs who were not infected with STIs [AOR 6.043 (1.552-23.540), P=.009*]. Underweight (<16.5 BMI) CSWs were almost 6 times more likely [AOR 5.73 (11.468-22.329), P=.012] to have lower (<1.05 ≥mol/L) serum vitamin A as compared to CSWs who were overweight/obese (>25.1 BMI) (table-8).

MLoR model of serum vitamin E: three predictors namely ‘serum copper’, ‘experienced in group sex’ and ‘smoking habit’ were remained in the ‘adjusted model of vitamin E’ (table-9). However, ‘serum copper’ was a strong ‘covariate’ as it was not significant (P=.150) in crude model of vitamin E (table-7) but remained in adjusted model with borderline significance (P=.077). In contrast to this, ‘smoking habit’ was significant (P=.028) in crude model of vitamin E but in adjusted model it remained with borderline significance (P=.069). And it was observed that smokers CSWs had 3 times higher odds ratio having lower vitamin E as compared to non-smokers CSWs, other things remained constant [AOR 2.942 (.918-9.427), P=.069]. In addition, CSWs even having normal serum copper had 2.9 times more likely to get low serum vitamin E as compared to CSWs having >normal serum copper [AOR 2.923 (.891-9.583), P=.077] (table-9).

Table-7: Predictors associated with lower serum retinol and tocopherol of CSWs (crude model).

Explanatory variables	Unadjusted model of serum **vitamin A**				Unadjusted model of serum **vitamin E**			
factors associated with lower serum vitamins	All CS Ws n= 107	0=normal or >1.05 µmol/L % n	1=<1.05 µmol/L % n	Unadjusted Odds ratio (95% CI) (lower-upper) P-value	All CS Ws n= 107	0=normal or >13.0 µmol/L % n	1=<13.0 µmol/L % n	Unadjusted Odds Ratio (95% CI) (lower-upper) P-value
STI status								
Having STIs	73	30.8 (04)	73.4 (69)	6.21 (1.76-21.97) **P=.005****	73	72.2 (13)	67.4 (60)	.796 (.259-2.45) P=.690
No STIs (r)	34	69.2 (09)	26.6 (25)	1	34	27.8 (05)	32.6 (29)	1
Condom use								
Consistent	47	30.8 (04)	45.7 (43)	1.90 (.546-6.59) P=.314	47	22.2 (04)	48.3 (43)	3.27 (.10-10.72) **P=.050**
Irregular (r)	60	69.2 (09)	54.3 (51)	1	60	77.8 (14)	51.7 (46)	1
Experienced in Gr. Sex								
No (r)	73	76.9 (10)	67.0 (63)	1	73	94.4 (17)	62.9 (56)	1
Yes	34	23.1 (03)	33.0 (31)	1.64 (.421-6.39) P=.476	34	5.6 (01)	37.1 (33)	10.02 (1.27-78.77) **P=.029***
smoking habit								
No (r)	51	69.2 (09)	44.7 (42)	1	51	72.2 (13)	42.7 (38)	1
Regularly	56	30.8 (04)	55.3 (52)	2.79 (.801-9.68) P=.107	56	27.8 (05)	57.3 (51)	3.49 (1.146-10.63) **P=.028***
BMI (kg/m^2)								
Underweight	34	69.2 (09)	26.6 (25)	4.59 (1.29-16.35) **P=.019***	34	22.7 (04)	33.7 (30)	0.938 (.155-5.69) P=.944
Normal	55	30.8 (04)	54.3 (51)	5.81 (.002-2.02) P=.998	55	66.7 (12)	48.3 (43)	0.448 (.090-2.226) P=.326
Overweight (r)	18	0.0 (00)	19.1 (18)	1	18	11.1 (02)	18.0 (16)	1
Serum Albumin								
Below normal	52	76.9 (10)	44.7 (42)	.156 (.019-1.285) **P=.084**	52	27.8 (05)	52.8 (47)	3.13 (.891-11.02) **P=.075**
Normal	27	15.4 (02)	26.6 (25)	.463 (.040-5.462) P=.840	27	33.3 (06)	23.6 (21)	1.17 (.335-4.06) P=.809
>Normal (r)	28	7.7 (01)	28.7 (27)	1	28	38.9 (07)	23.6 (21)	1
Serum copper								
Normal	80	84.6 (11)	73.4 (69)	1.99 (.413-9.62) P=.391	80	61.1 (11)	77.5 (69)	.455 (.156-1.328) P=.150
>Normal (r)	27	15.4 (02)	26.6 (25)	1	27	38.9 (07)	22.5 (20)	1
Serum iron								
Below normal	16	15.4 (02)	14.9 (14)	2.63 (.454- 15.16) P=.281	16	16.7 (03)	14.6 (13)	0.963 (.185-5.06) P=.964
Normal	69	38.5 (05)	68.1 (64)	4.80 (1.30-17.74) **P=.019***	69	61.1 (11)	65.2 (58)	1.172 (.332-4.13) P=.805
>Normal (r)	22	46.1 (06)	17.0 (16)	1	22	22.2 (04)	20.2 (18)	1

Legend: Simple Logistic Regression (SLoR)

r=reference category, P<0.05*, P<0.01**

Table-8: Multiple Logistic Regression (MLoR) model of serum vitamin A.

Vitamin A Model	Adjusted model of serum vitamin A focusing on **lower serum retinol** level of CSWs.						
factors associated with lower serum vitamin A	**All CSWs (n=107)** **% n**	**0=>1.05** µmol/L **% n**	**1=<1.05** µmol/L **% n**	**Beta coefficient (B)**	**standard error**	**Adjusted Odds Ratio (95% CI) (lower-upper)**	**P-value**
STI status							
Having STIs	31.8 (73)	30.8 (04)	73.4 (69)	1.799	.694	6.05 (1.552-23.540)	.009
No STIs (r)	61.2 (34)	69.2 (09)	26.6 (25)	-	-	1	-
BMI (kg/m^2)							
Underweight	31.8 (34)	69.2 (09)	26.6 (25)	1.745	.694	5.73 (11.468-	.012
Normal	51.4 (55)	30.8 (04)	54.3 (51)	1.034	1.18	2.81 (.278-28.439)	.998
Overweight (r)	16.8 (18)	0.0 (00)	19.1 (18)	-	-	1	-

Legend: Backward stepwise Multiple Logistic Regression (MLoR)
Hosmer-Lemeshaw goodness of fit for vitamin A model: $\aleph^2$=2.490, df=3, P=.477
Model summary: -2 Log likelihood=60.42, Nagelkerke pseudo R-square=.307
Model coefficient :Chi-square ($\aleph^2$)=18.74, df=3, P=.000
Overall predicted percentage from classification table of serum vitamin A (DV) was 87.9% (cut value is 50%).
(r)= reference category

Table-9: Multiple Logistic Regression analysis of serum vitamin E of CSWs

Vitamin E Model	Adjusted model of serum vitamin E (0=normal or >13.0 µmol/L, 1=<13.0 µmol/L tocopherol) focusing on **lower serum vitamin E** of CSWs.						
factors associated with lower serum vitamin E	**All CSWs (n=107)** **% n**	**0=>13.0** µmol/L **% n**	**1=<13.5** µmol/L **% n**	**Beta coefficient (B)**	**standard error**	**Adjusted Odds Ratio (95% CI) Upper-lower**	**P-value**
Serum copper							
Normal	74.8 (80)	61.1 (11)	77.5 (69)	1.073	.606	2.923 (.891-9.583)	.077
>Normal (r)	25.2 (27)	38.9 (07)	22.5 (20)	-	-	1	-
Experienced in Gr. Sex							
No (r)	68.2 (73)	94.4 (17)	62.9 (56)	-	-	1	-
Yes	31.8 (34)	5.6 (01)	37.1 (33)	2.429	1.086	11.351 (1.351-25.393)	.025
smoking habit							
No (r)	47.7 (51)	72.2 (13)	42.7 (38)	-	-	1	-
Regularly	52.3 (56)	27.8 (05)	57.3 (51)	1.079	.594	2.942 (.918-9.427)	.069

Legend: Backward stepwise Multiple Logistic Regression (MLoR)
Hosmer-Lemeshaw goodness of fit for vitamin E model: $\aleph^2$=5.181, df=5, P=.395
Model summary: -2 Log likelihood= 81.420, Nagelkerke pseudo R-square=.227
Model coefficient :Chi-square ($\aleph^2$)=15.54, df=3, P=.001
Overall predicted percentage from classification table of serum vitamin E (DV) was 84.1% (cut value is 50%).
(r)= reference category

4.1.4 Discussion

Only few studies dealt with biochemical indices of vitamin A & E level of different Bangladeshi population groups as well as in global. National micronutrient survey data (NMSS, 2011-12) did not address this segment of population except slum population (women and children). Nevertheless, previous study in Bangladesh focused on the nutritional status of garments workers, a vast majority of young women migrated from all over the country and studies reported their poor nutritional status, vitamin A status (Ahmed et al, 1997), and poor socio-economic condition elsewhere. Sometimes garments workers were sexually abused and many of young girls took sex selling as 'secondary occupation' to make some extra money.

Most (63.6%) of CSWs, in this study, had subclinical vitamin A deficiency (<0.7 µmol/L retinol or ≥ 0.35 µmol/L retinol, WHO/VACG) followed by 19.6 % (21/107) had severe vitamin A deficiency (< 0.35 µmol/L retinol) and only 12.1% (13/2107) CSWs had normal serum level of vitamin A. On the other hand, the national prevalence of subclinical vitamin A deficiency (NMSS, 2011-12) of non pregnant non lactating women (NPNL), measured by estimation of the prevalence of low serum retinol (<0.7 µmol/L), was only 5.4% (n=918), with the proportion appeared a shed higher in the slums (6.9%, n=286) (table-1). Even a two decade earlier study on garments workers showed (Ahmed et al, 1997) 56.2% of girls of garments factory had low serum vitamin A (<1.05 µmol/L) and 14.2% had sub-clinical vitamin A deficiency (<0.7 µmol/L retinol or ≥ 0.35 µmol/L retinol, WHO/VACG) and 43.8% of young adolescent garment workers had normal serum level. Thus vitamin A (vitamin E as well) status of the study participants was worse than anything else. Moreover, about 87.9% of CSWs had lower than normal serum retinol (<1.05 µmol/L) which was more than double than national level (39.7%) as well as slum level (41.3%) and 5 times lower prevalence of normal serum retinol among CSWs than national level was also noticed (NMSS,2011-12) (table-2).

In this study, serum iron levels of CSWs were positively and marginally correlated (r=.176, P=.070) with serum vitamin A which also reflected in ANOVA (F (2,104) =3.459, P=.035). It was observed from ANOVA that, iron status of CSWs positively associated with serum vitamin A status. Semba et al (1992) showed that Vitamin A deficiency also impairs iron status and its supplementation has been shown to increase hemoglobin concentration.

The huge discrepancy (table-2) regarding vitamin A deficiency between CSWs and national survey may be due to (i) high rate of sexually transmitted infections (syphilis/HBV/or both) or **STIs** (generally prevalence of syphilis and hepatitis B was 40.0% and 5.5% respectively) which could lower serum vitamin A (ii) ANOVA (table-3) showed that 'STI status of CSWS' significantly [F (1,105)=5.544, P=.020*] and negatively associated with serum **'vitamin A level'** and those CSWs who were inflicted with either syphilis or hepatitis 'B' or both had significantly lower level (0.58 µmol/L) of vitamin A than those (0.81 µmol/L) who were not infected with STIs (iii) Multiple Logistic Regression (ML_oR) of 'vitamin A' depicted (table-8) that those CSWs who were inflicted with STIs (syphilis/hepatitis B or both) were 6 times more likely to have lower (<1.05 ≥mol/L) serum vitamin A as compared to CSWs who were not infected with STIs [AOR 6.043 (1.552-23.540), P=.009*]. (iv) moreover, Multiple Logistic Regression (ML_oR) of 'STIs' for CSWs (Chapter-3: table-12) also showed sub-clinical vitamin A deficiency was associated with STI [AOR 10.792 (1.93-60.29), P=.007] (v) in addition, STI status (having syphilis or HBV and/or no STIs) showed significant negative correlation (r= -.224, P=.020) with serum vitamin A concentration of CSWs. (vi) extremely lower amount of dietary intake of vitamin A by CSWs i.e. only 189.96 µg/CSW/day (chapter-3.2: table-2, 3) (vii) extremely lower intake of dietary fat (only 8.05 g) especially cooking oil (5.39 g/person/day) (chapter-3.2: table-2), which might hampered fat-soluble vitamin A absorption (viii) malnutrition of CSWs: all serum nutrients including vitamin A and E were lower than normal level viz. mean serum total protein and albumin were 50.84 g/L and 31.81 g/L respectively (Chapter-2.3: table-1), however more than one third (37.4%) of CSWs had <normal serum zinc (chapter-5.2: table-1) (ix) **low immunity probably** due to lower serum TP and ALB, mean serum IgG (2.73 g/L), IgA (.50 g/L) and IgM (.42 g/L) were also found lower among CSWs, thus infection was also high and malnutrition, infection synergistically lowered serum vitamin A. Thus, infection could lower serum vitamin A level of CSWs transiently and lower dietary intake of both fat and vitamin A by CSWs aggravate the situation and subsequently serum retinol level fall at severe rate.

Multiple Logistic Regression (ML_oR) of 'vitamin A' depicted (table-8) that Underweight (<16.5 BMI) CSWs were almost 6 times more likely [AOR 5.73 (11.468-22.329), P=.012] to have lower (<1.05 ≥mol/L) serum vitamin A as compared to CSWs who were overweight/obese (>25.1 BMI). Also, in this study, vitamin A negatively and insignificantly correlated with BMI (r= -.130, P=.481). However, the inverse relationship of vitamin E and beta carotene with obesity was observed in different studies (P Galan et al, 2005; Wallstrom et al, 2001). Study also reported (Strauss, 1999) that the basis for lower concentrations of nutrients in obese people compared with non obese people remains speculative, but it has been suggest that dietary differences and variability in body compartmental sizes are likely explanations.

Multiple Logistic Regression (ML_oR) of 'vitamin E' postulated (table-9) that CSWs even having normal serum copper had 2.9 times more likely to get low serum vitamin E as compared to CSWs having >normal serum copper [AOR 2.923 (.891-9.583), P=.077]. This may be due to copper-zinc containing enzyme superoxide dismutase (SOD) activity which is an 'antioxidant' and secure 'vitamin E' from oxidation and >normal serum copper might play an important role. Moreover, in this study serum vitamin E was significantly and positively correlated with serum copper (r=.235, P=.015*) and ANOVA (table-6) also pointed at that [F (2, 104) =4.077 P=.046] serum copper level of CSWs positively influenced serum vitamin E and those CSWs had >normal serum copper (>22.1 µmol/L) had higher level of vitamin E (7.87±0.08 µmol/L) and vice versa.

It was observed that CSWs who did not smoke had higher level of both vitamin A (P=.103) and vitamin E [F (1,105) =3.927, P=0.05] in ANOVA (table-4) and ML_oR model of vitamin E also revealed that smokers were almost 3 times more likely to have lower vitamin E (<13.5 µmol/L) than non smokers [AOR 2.942 (.918-9.427), P=.069]. Both Leonard et al (2004) and Richard et al (2005) were in agreement with this finding and showed Cigarette smoking increases human vitamin E requirements. An old study (Duthie et al, 1991) also supported the finding. However, study (P Galan et al, 2005) reported current smokers had significant lower vitamin E than nonsmokers in case of only women subjects.

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CHAPTER-4.2

Serum micro-minerals (Cu, Zn & Fe) status of Commercial Sex Workers

4.2.1 Introduction

Micro minerals such as copper, zinc and iron can act as immuno-enhancers or immuno-modulators. Trace elements regulate key metabolic pathways, modulate the immune response, and suppress the incidence of various diseases (Olivares et al, 2003). The most important vital elements in human body are copper, zinc and iron. Zinc is necessary for the immune system functions. Zinc deficiency is associated with decline in lymphocytes, which leads to form acute diarrhea (Culha and Sangun, 2007). Zinc also helps in production of antibodies and T-cell and blood cell activity.

Zinc, an almost omnipresent metal ion, is not only a vital element in various physiological processes but also a drug in the prevention and management of many diseases. The importance of the element has taken much time for recognition, with just a few years since it has been accorded its due status. Nearly half of the world's population is at risk for inadequate zinc intake, suggesting that public health programs are urgently needed to control zinc deficiency. Zinc's anti-copper action is unique. It also limits the bioavailability of vitamin A (Kaur et al, 2014).

Zinc plays a role in growth, building and repair of muscle tissue, energy production, and immune status. Diets low in animal protein, high in fiber and vegetarian diets, in particular, are associated with decreased zinc intake. Zinc status has been shown to directly affect thyroid hormone levels, BMR, and protein use, which in turn can negatively affect health and physical performance. Depletion of Zinc strongly destroys membrane barrier function and integrity, induces an increase in neutrophil accumulation, and starts a positive regulation of chemokines that plays an important role in neutrophil migration and inflammatory development (Dovhanj et al, 2009; Christudoss et al, 2010)

The levels of some trace elements, such as selenium, iron, copper, and zinc, and copper: zinc ratios, might serve as biomarkers for the increased severity of viral hepatic damage (Lin 2005). Zn induces oxidative stress via the activation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and depletion of glutathione (GSH) which, in turn, activate the apoptotic machinery leading to dopaminergic neurodegeneration similar to paraquat (Kumar et al, 2012).

T-cell functions impairment is observed with a decrease in zinc concentration. Thymic hormones are known to increase or decrease in zinc supplements. Zinc is also capable of increasing or decreasing lympholytic activity (kaur et al, 2014). The immune function is deftly synchronized by zinc, since both increased and decreased zinc levels result in a disturbed immune function (Chandra 1990; Rink and Gabriel 2001). Zinc deficiency when associated with an immunodeficiency, results in an amplified propensity to a range of pathogens (Shankar and Prasad 1998). Iron and zinc deficiencies influence both T and B-lymphocyte function by protein kinase C-dependent mechanisms (Klecha, 2005).

Zinc also inhibits the production of tumor necrosis factor, which is implicated in the pathophysiology of cachexia and wasting in acquired immune deficiency syndrome (Baum et al, 2000). The development of acquired immunity is affected by zinc deficiency due to prevention of both, the outgrowth and certain functions of T lymphocytes such as activation, Th1 cytokine production, and B-lymphocyte help (Shankar and Prasad 1998).

Zinc is an important trace element for immune function with both stimulatory and inhibitory effects on immune cells (Campo et al, 2001). In particular, zinc may either stimulate or inhibit several signaling pathways that interact with the signal transduction of pathogen-sensing receptors, called Toll-like receptors, which sense pathogen-derived molecular structures and, upon activation, lead to secretion of pro-inflammatory cytokines (Haase and Rink 2007). The immune system is the 1st system to be affected by changing zinc levels, due to its high cell turnover (Mariani et al, 2006). In patients with diminished serum zinc levels due to chronic liver disease, zinc supplementation appears to influence cell growth and cytokine production (McClain, 1997; Wellinghausen et al, 1997). In animal models, there is a reduction in the activity of thymulin and altered release of cytokines, and an imbalance of cell-mediated and antibody-mediated immunity may occur, even with mild levels of zinc deficiency (Brown et al, 2001).

Zinc deficiency has been linked with delayed wound healing (Keenan and Morris 1993; Andrews and Gallagher-Allred 1999). It is also crucial for healing of gastric ulcers, especially at the early stage (Watanabe 1995). The low plasma erythro- poietin concentration might account for depressed hematopoiesis coupled with zinc deficiency (Konomi and Yokoi 2005). Equivalent cellular and intestinal uptake of inorganic and organic forms of zinc was revealed when plasma erythropoietin concentration in zinc-deficient rats was investigated (Beutler et al, 1998).

Whenever there is an excess of zinc in the body, it may result in deficiency of copper since zinc is able to competitively inhibit gastro intestinal attachment of copper. The gene expression of upregulation of metallothionein may play an important role in this phenomenon (kaur et al, 2014). Zinc homeostasis is primarily maintained via the gastrointestinal system by the processes of absorption of exogenous zinc and gastrointestinal secretion and excretion of endogenous zinc (Krebs and Hambidge 2001). While the fractional absorption of zinc from a meal depends on the quantity of exogenous zinc and on such dietary factors as phytic acid, the fractional absorption does not appear to be dependent on the size of the rapidly exchanging pool of the host. In contrast, the quantity of endogenous zinc excreted via the intestine is positively correlated with both the quantity of absorbed zinc and the zinc status of the host, and thus this process has an equally critical role in maintaining zinc homeostasis (Krebs 2000).

Copper is an essential trace element and is one of the substances forming metallo-enzymes and proteins (Sedighi et al, 2006). The main transporter of copper in blood is a α2-globulin protein called ceruloplasmin which is a multifunctional enzyme and can convert the toxic "Ferrous" iron to its non-toxic form, "Ferric" (Sedighi et al, 2006; Patel et al, 2002). Copper and iron level showed marked increase with age and variations in serum copper concentration occur because of age, sex, diet, hormonal state and geographical factors (Sedighi et al, 2006). Copper is essential for the production of red blood cells, hemoglobin formation and absorption of iron, and for the activities of various enzymes (Culha and Sangun, 2007).

Copper is an essential nutrient in human and acts as a critical cofactor when incorporated into specific cupro-enzymes that catalyze electron transfer reactions required for cellular respiration, iron oxidation, pigment formation, neurotransmitter biosynthesis, antioxidant defense, peptide amidation and connective tissue formation (Madsen et al, 2007). Both overt copper deficiency and excess are associated with specific clinical manifestations. In developed countries like USA where almost all processed food products generously fortified with zinc and iron, thus, people who consume large amount of processed foods and regularly take vitamin minerals (which also have iron and zinc) supplements may actually get too much iron and zinc, thereby putting themselves at risk of copper deficiency.

Copper deficiency causes an accumulation of arterial lipid peroxides, possibly due to decreased activity of the copper-zinc superoxide dismutase (CuZnSOD). Many of the pathological effects of copper overload are consistent, however, with oxidative damage to membrane or macromolecules. Copper intake in vivo has shown both pro-oxidant and antioxidant effects; ceruloplasmin, the major copper-containing plasma protein, may act as either an antioxidant or pro-oxidant, depending on ambient conditions (Ferns et al, 1997). Thus, the definitive role of copper in oxidative process is still a matter of debate. Epidemiological data regarding the possible role of copper on metabolic abnormalities and atherogenesis are conflicting (Simona Bo et al, 2008).

Iron is crucial to human's biological functions and cellular biochemical process (Anderson, 2009). Although it is essential for life, but both severe iron deficiency (Dickey W, 2002) and iron overload (WeiLin Mao et al, 2015; Ying shan et al, 2005) pose significant and potentially fatal health risks, and the homeostasis of iron is tightly regulated. When this regulation is disrupted, for example because of hereditary hemochromatosis, excessive absorption of iron by the intestine leads to cellular toxicity, tissue injury and organ fibrosis (WeiLin Mao et al, 2015). The liver is a major storage organ for iron. Approximately one-third of the body's total iron is deposited in hepatocytes, sinusoidal mesenchymal cells, and reticuloendothelial cells. The liver also plays a fundamental role in recycling iron as the organ synthesizes both transfesrrin (main transporting protein) and ferritin (major storage protein). Hepatic injury and dysfunction can disturb iron homeostasis

The average iron content in a healthy adult is only about 3 to 4 gm, yet this relatively small quantity is vital. Iron is an element that is neither used up nor destroyed in the properly functioning body. Unlike some minerals, it is not required for excretion, and only very small amounts appear in urine and sweat. Minute quantities are lost in desquamated cells from the skin and intestine, in shed hair and nails and in the bile and other body secretions. The body is, however, efficient, economical and conservative in the use of iron. Iron released when the erythrocytes are old and broken down is taken up and used again and again for the manufacture of new erythrocytes. This economy of iron, unlike some minerals, is important. In normal circumstances, only about 1 mg of iron is lost from the body daily by excretion into the intestine, in urine, in sweat or through loss of hair or surface epithelial cells. Evidence indicates that iron absorption is regulated to some extent by physiological demand. Persons who are iron deficient tend to absorb iron more efficiently and in greater quantities than do normal subjects. Healthy subjects normally absorb only 5 to 10% of the iron (0.75 to 1.5 mg from 15 mg of iron contained in diet) in their foods, whereas iron deficient subject may absorb twice (~3mg from 15 mg of iron contained in diet) that amount (Hunt, 2003; Hunt and Roughead, 2000; Cook, 1990). Some factors (tannins, calcium, phosphates and phytates) reduce iron absorption whereas ascorbic acid enhances it (Hunt, 2003; Hallberg, 1981) and also haem iron of animal origin (meat, fish & poultry) has higher absorption rate than non-haem iron of plant origin. However, the interesting aspect 'the complement' effect of iron absorption is when people eat meals (not a single food), if a small amount of heme iron consumed with a meal where most of the iron is non-heme iron will enhance the absorption of all the iron. Thus, the addition of a quite small amount of heme iron from perhaps fish or meat to a large helping of rice or maize containing non-heme iron will result in much greater absorption of iron from the cereal staple (Hunt, 2003; Roughead and Hunt, 2000; Cook, 1990)

Studies showed evidences of increasing serum iron levels and altered iron metabolism in chronic Hepatitis infections (weiLin et al, 2015; Ying shan et al, 2005) or decreasing in drug addiction (Hossain KJ et al, 2007). Increased iron levels contribute to the progression of hepatic injury and fibrosis (Ying shan et al, 2005). Iron deficiency has often been found to be associated with chronic infections and complex relation of iron metabolism to infection than any other study (Fiona et al, 1977). Another previous study (Jacobs, 1975) commented that iron treatment was effective even when coexisting immunological defects remain unchanged.

4.2.2 Methods and Materials

Biochemical indicators are an objective and quantitative means of assessing the micro minerals such as copper, zinc and iron status of a population. For the correct use of serum zinc concentration as an indicator of zinc status, there are several important technical issues that were considered regarding sample collection, laboratory analysis and interpretation of the data (IZiNCG, 2007).

4.2.2a Collection of the blood samples

All commercial sex workers (CSWs) were interviewed by trained social interviewer including food frequency data (FFQ) and one day 24-hour recall method was applied to obtain dietary information (chapter-2.2). And 5 ml venous blood were collected (n=495) into trace element free evacuated blood collection tubes using stainless steel needle by trained paramedics and tried not to contaminate specimens with ambient zinc in air and water (IZiNCG, 2007). All blood specimens were collected in the morning and CSWs were not fasted on the very day of blood collection. Once blood was collected, it was stored in cool box or refrigerator until centrifuged to separate the serum. Following centrifugation, the serum or plasma was then be transferred to a screw cap vial for storage and frozen until analysis.

4.2.2b Selection criterion of the serum samples for micro-mineral analysis

After screening of all blood samples (n=495) for STIs (HIV[1], hepatitis B, and syphilis), a subsample (n=107) comprising of 68.2% (n=73) infectious samples (having both infections or any single infection) and 31.8% (n=34) non-infectious samples were purposively selected for micro-mineral analysis.

4.2.2c Instrumentation and Calibration of the standard curves

Atomic Absorption Spectrophotometer (AAnalyst 200, Version 8.0, 2013 PerkinElmer, Inc. USA) was used which has minimum detection limit of 0.03 mg/L for cu, 0.04 mg/L for Fe and 0.01 mg/L for Zn in the flame method. Copper, Iron and Zinc specific hollow cathode lamps of wavelengths 324.8 nm, 248.3 nm, and 213.9 nm were respectively used to analyze the samples. Samples were aspirated through nebulizer and the absorbance was measured with a blank (only 2% HNO_3) as a reference. Micro mineral concentrations were estimated from their respective calibration curves those were constructed by plotting absorbencies (y-axis) against respective concentrations (x-axis). Calibration curves were obtained (figure-1, 2, and 3) using 3 micro-mineral (Cu, Zn & Fe) standards (Perkin Elmer Pure, USA, Copper, Iron & Zinc Standards with 2% HNO_3, 1000 mg/L, VoL-125 ml) of different concentration for Copper (0.2, 0.4, 0.8 and 1.6 mg/L), for Iron (0.2, 0.5, 1.0, 2.0 and 3.0 mg/L) and for Zinc (0.1, 0.2, 0.4 and 0.8 mg/L). The correlation coefficient was found for copper, Iron and zinc were 0.998, 0.999 and 0.998 respectively. Ten fold (10) dilutions were used for the samples to keep the results in the analytical range.

4.2.2d Preparation of the Standards

Copper: to obtain 0.2 mg/L concentration of copper, 20 µl **copper** standard were pipetted into a volumetric flask (UK) and then it was made up to 100 ml volume with nano pure water. Similarly, 0.4, 0.8 and 1.6 mg/L concentration of copper standards were prepared by mixing 40 µl, 80 µl and 160 µl copper standards respectively with each 100 ml nano pure water.

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[1] No HIV positive serum was detected in this study

Iron: to obtain 0.2 mg/L concentration of Iron, 20 µl **iron** standard was pipetted into a volumetric flask (UK) and then it was made up to 100 ml volume with nano pure water. Similarly, 0.5, 1.0, 2.0, and 3.0 mg/L concentration of Iron standards were prepared by mixing 50 µl, 100 µl and 200 µl and 300 µl Iron standards respectively with each 100 ml nano pure water.

Zinc: to obtain 0.1 mg/L concentration of copper, 10 µl **Zinc** standard was pipetted into a volumetric flask (UK) and then it was made up to 100 ml volume with nano pure water. Similarly, 0.2, 0.4 and .8 mg/L concentration of Zinc standards were prepared by mixing 20 µl, 40 µl and 80 µl Zinc standards respectively with each 100 ml nano (18 ohm) pure water.

4.2.2e Processing and assay procedure

1. CSWs serum samples were first thawed at room temperature.
2. Centrifuged at 3000 rpm for 10 minutes to obtain a clear supernatant of the serum.
3. A volume of 200 µl (for each micro mineral) serum was diluted to 2ml (10 fold dilutions, 200 µl serum+1800 µl nano-pure water) with nano-pure with electrical resistance >18 micro ohm for copper /Zinc/ iron in acid washed test tubes or in acid washed falcon tubes (for estimating 3 or 2 minerals consecutively) separately and vortex for well mixing.
4. Within 2 hours of mixing, absorbance was read at 324.8 nm, 248.3 nm, and 213.9 nm respectively for Cu, Fe and Zn.
5. Machine was calibrated with standard preparation (Cu/Zn/Fe Standards with 2% HNo_3) at every 5-7 sample interval.
6. Triplicate samples of each serum were run to get the Mean and SD of each micro mineral.

Cares taken were:

1. All equipments (other than disposable) used in data collection and laboratory were soaked for 24 hours in ultra pure 10-20% HCL or HNO_3 solution and rinsed 3-4 times in de-ionized water.
2. All materials and equipments were stored, covered or sealed to avoid dust.
3. Stainless steel needles were used.

4.2.2f Data Analysis

Normality test: various statistical tools were used employing SPSS for the analysis of micro minerals (copper, zinc and iron). Before performing statistical analysis, "Normality" test of the micro minerals data set were done by "Shapiro-Wilk (S-W)" goodness of fit test (Cu-statistics=.761, df=107, P=.000; Zn-statistics=.646, df=107, P=.000; Fe-statistics=.899, df=107, P=.000) as well as by doing descriptive statistics. It was observed from 'descriptive statistics' that micro minerals data were **positively** '**skewed'** (Cu-skewness=2.963, SE=.234; Zn-skewness=3.571, SE=.234, Fe-skewness=1.467, SE=.234) and both "logarithmic or Log_{10}" (Cu-skewness=.704, SE=.234; Zn-skewness=1.115, SE=.234; Fe-skewness= -1.495, SE=.234) and "Square root transformation" (Cu-skewness=1.737, SE=.234; Zn-skewness=2.106, SE=.234; Fe-skewness= .146, SE=.234) was performed to see which process could eliminate the skewness in a better way. And thus, approximation to a normal distribution was greatly improved for copper and zinc data by **"logarithmic or Log_{10}"** transformation and iron data by "**Square root transformation**". After "logarithmic or Log_{10}" and "Square root transformation", mean as well as other **'parametric tests'** on the transformed scale data was performed and then back transformed by taking the "antilog" for copper and zinc data and "square" for iron data and at the same time "log transformed" and "Square root transformed" values were backed in the usual Cu, Zn and Fe units (µmol/L). However, either the 'Square root' or 'log transformation' '**geometric mean'** was always less than the "arithmetic mean" of raw data (Bland and Altman, 1996). In fact, extreme observations of a "highly skewed data set" have a large influence on the **arithmetic mean**, making it more prone to sampling error. Thus, lessening this influence is one advantage of using transformed data (Bland and Altman, 1996).

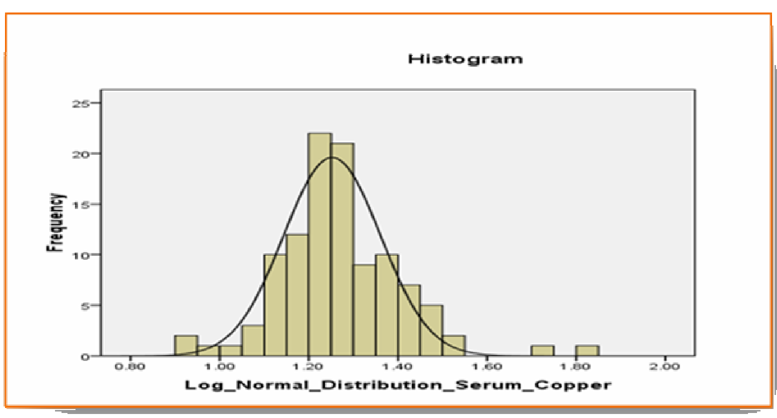

Figure-4.2.1: Log Normal Distribution of Serum Copper

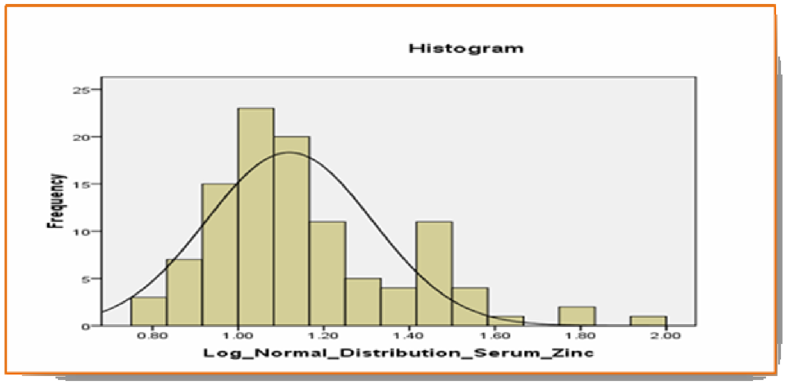

Figure-4.2.2: Log Normal Distribution of Serum zinc

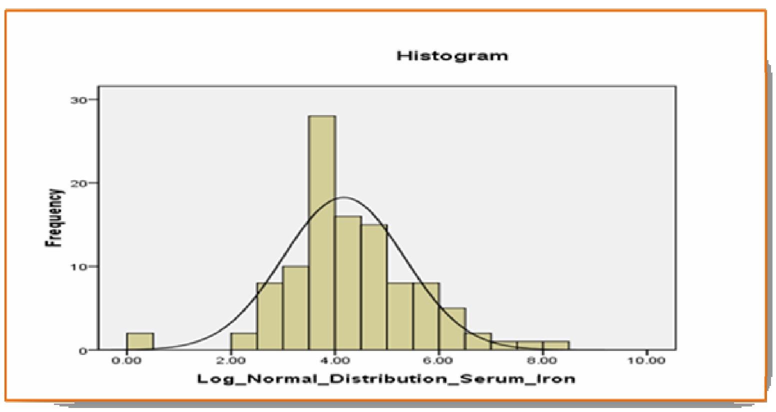

Figure-4.2.2: Log Normal Distribution of Serum Iron

4.2.2f-1 Statistical Analysis

All statistical analyses of micro mineral data (Cu, Zn and Iron) were assessed by using Statistical Package for Social Science version 17.0 (SPSS Inc, Chicago, IL, USA). Two-tailed P values <0.05 were considered statistically significant. Comparisons between continuous variables were performed by Student's t-test.

Univariate analysis of the samples: Descriptive Statistics including mean (with 95% CI), Standard deviation, range, percentage and frequency distribution of data were used to calculate baseline information. Descriptive data were presented as the mean (SD) for continuous variables and as percentages for categorical variables.

Bivariate analysis: **in** bivriate analysis Pearson's correlation (2-tailed) was applied to assess extent of linear correlation between two continuous variables. One-way analysis of variance was used for multiple comparisons and socio-demographic, dietary, bio-chemical and immunological Influences on serum microminerals (Cu, Zn, and Fe) were performed by 'ANOVA'.

Multivariate analysis: multivariate analysis was done to understand the association of different independent variables with dichotomous dependent variable. Multiple logistic regression models (MLoR), with the outcome variables dichotomized (0, 1) as <normal [Cu*(normal): (8.18-22.0 µmol/L) Zn: (5.97 to 11.4 µmol/L) Fe: (.11-10.9 µmol/L] or ≥normal [Cu* (>normal): (22.1- 63.27 µmol/L) Zn: (11.5 to ≥99.30 µmol/L) Fe: (11.0-70.74 µmol/L)] was developed to examine the strength of relationship between dependent variables (Cu, Zn, and Fe) with independent categorical variables. In the MLoR models, generally, dummy variables were created for non-binary categorical variables; the effect of each category of a multi-categorical variable was assessed by keeping the first or last category as reference category. All Covariates (socio-demographic, lifestyle and sexual variables as well nutritional indices) which were found statistically significant ($P \leq 0.05$) or borderline significant ($P < 0.09$) in Simple logistic regression (SLoR) analysis were introduced simultaneously in the **multiple logistic regression analysis.** The models were developed by 'backward stepwise elimination', removing the covariate with largest P value at each step until the remaining variables were significant at the 0.05 level in the final adjusted model. Also, forward stepwise technique was applied in same way to confirm the model. Thus relative odds (ORs) and 95% CI were calculated to identify potential positive deviants associated with higher serum 'copper*', 'zinc' and 'iron' [0=<normal and 1=≥normal]. Hosmer-Lemeshaw goodness of fit and Nagelkarke-pseudo R^2 of the models were also observed.

* in case of copper, the 'dichotomized category' was 0=normal and 1=>normal

4.2.3 Results

4.2.3a Serum micro-minerals content of CSWs

Micro minerals copper, zinc and iron were investigated in this study. From table-1, it is clearly noticed that mean serum micro-mineral levels of CSWs were within normal range. Frequency distribution of serum copper, zinc and iron level of CSWs (n=107) revealed that 24.3% (n=26) CSWs have been suffering from serum zinc deficiency (<10.1 µmol/L). Besides this, about 68.2% and 64.5% of CSWs had normal range of serum copper (11-22 µmol/L) and iron (11-29 µmol/L) respectively whereas only 37.4% had normal serum zinc level (11.5-18.5 µmol/L). However, in this study, one fourth (25.2%, n=27/107) of CSWs had elevated level of serum copper and interestingly, one fourth (25.2%, n=27/107) of CSWs had elevated level of serum zinc too and 20.6% (n=22) had elevated level of serum iron. Moreover, mean serum zinc to copper ratio was pretty low, 0.94±0.72.

4.2.3b Influence of Socio-demography, lifestyle and risky HRB of CSWs on micro-minerals

From the table-2 it was noticed that serum copper level of CSWs was significantly influenced by their present age [P=.018, F (2,104) =4.160]. In addition, education [P=.006, F (1,105) =7.943) had positive influence and marital status [P=.016, F (2,104) =4.296] had negative impact on serum zinc status. Moreover, treatment cost of last ailment [P=.010, F (2,104) =4.960] was associated with serum zinc status and also STI status significantly, negatively associated with serum Iron status [P=.001, F (1,105) =12.486]. However, monthly income, expenditure and location of sex business did not affect serum micro mineral levels of CSWs.

Associations of 'lifestyle factors' and 'risky sexual behaviors' of commercial sex workers (CSWs) with serum micro mineral status are outlined in table-3 and table-4 respectively. In case of lifestyle factors, no significant associations with serum micro minerals were found except 'substance abuse', 'types of substances' and 'NGO counseling on STIs'. Serum iron was negatively associated with substance abuse [F (1, 105) = 3.258, P=.059], those CSWs abused substances had lower serum iron (13.49 µmol/L) than those who did not abuse substances (19.77 µmol/L). Both zinc and iron status of drug addict CSWs were found significantly low as compared to non-drug abusers and alcoholic CSWs in post Hoc test (Tukey) for multiple comparisons [zinc: F (2, 104) = 6.975, P=.001; iron: F (2, 104) = 3.232, P=.043]. In addition, NGO counseling on STIs resulted in significant [F (1, 105) =5.413, P=.022] positive association with serum zinc level (table-3).

No association of risky sexual behaviors of commercial sex workers (CSWs) with serum copper was noticed. Similarly, no association of 'Risky sexual behaviors' of CSWs with zinc and iron status was revealed except 'usual per day clients' of CSWs [P=.022, F (1,105) =5.403] and 'weekly coitus with permanent clients (PC)' had positive association [P=.054, F (1,105) =3.637] with serum zinc. Although, CSWs who performed (1 time/week or 2 times/week) weekly coitus with permanent clients (PC) had higher mean zinc level (16.22 µmol/L) than those who did not have weekly PC (13.38 µmol/L), but this finding scored borderline significant (P=.054). Also, having 'non paying sexual partner' (NPSP) positively correlated with serum iron level [P=.023, F (1,105) =5.352] (table-4).

4.2.3c Dietary micro-mineral intake and related other parameters

Dietary intake of CSWs (table-5) was lacked in all nutrients except copper and iron. Calorie, fat, calcium, zinc, vitamin A, vitamin C and protein were severely deficient in the Diets of CSWs. In contrast to this, iron consumption exceeded RDA limit (110.17%) and level of copper intake (met 320.62% RDA) was apparently abnormally high as RDA is set only 900 mcg by IOM, USA. Moreover, mean iron consumption (19.97 g) as well as both plant (total plant 29.4 g) and animal (3.81 g) intake of this study group was higher than national average (National micronutrient survey 2011-12).

Importantly, in this study, mean zinc intake was only 3913.24.06 µg /person/day which was below the IZiNCG (2007) recommendations as well as IOM for dietary zinc. Although zinc intake of CSWs was slightly lower but very close to zinc intake (National Micronutrient Status Survey, 2011-12) of national average (4230.0 mcg /day/person). Besides these, mean 'phytic acid' content (492.86 mg) and 'phytate to zinc molar ratio' (7.70) was observed remarkably low in CSW's diet than national average (12.0) and a remarkable portion (41.4%) of CSWs consumed <5 grades 'phytate to zinc molar ratio' containing food while it was only 2.1% for national level (table-5).

4.2.3d Influence of dietary intake of Micro minerals on serum micro-mineral status

Influences of the dietary intake of micro-minerals on serum micro-mineral levels were depicted in table-6 which showed both dietary copper and zinc positively correlated with serum copper (r=.225, P=.020) and zinc (r=.781, P=.000) that also resulted in one way ANOVA. From the ANOVA it was observed that, CSWs who consumed less dietary copper and zinc had lower serum copper and zinc respectively and vice versa. Thus, when dietary copper consumption of CSWs was 115.03 to 1800 mcg (Adult's RDA of cu 900 mcg, IOM), serum Cu level of CSWs was observed 16.57 mmol/L, and when mean intake level increased from double (1800.1 mcg/day/person) to fourfold (3600 mcg/person/day) serum copper also concomitantly increased into 18.54 micro mol/L and so on (F (2.104) =3.221, P=0.040). Similarly, when CSWs consumed about 50% RDA of dietary zinc i.e. 1000-4000 mcg (adult's zinc RDA 8000 mcg, IOM), serum level of zinc was 10.49 (±3.35) mmol/L and when consumption was within RDA (i.e. 4000.1–7999.0 mcg) serum zinc level increased correlatively into 13.92 mmol/L and so on (F(2.104)=30.110, P=.000). However, dietary copper intake by CSWs was apparently higher as 'mean dietary copper' intake was 2885.62 mcg /person/day which exceeds RDA limit (met 320.62%) and 55.14% (most) of CSWs consumed double (1800.1 mcg/day/person) to fourfold (3600 mcg/person/day) dietary copper and interestingly, 22.43% (n=24) CSWs ingested more than four times dietary cu than RDA and other 22.43% (24/107) consumed 115.03 to 1800 mcg (double RDA) dietary cu as well (table-6).

In this study, significant positive correlations (table-6) were observed between dietary zinc and serum zinc (r=.625, P=.000) as well as dietary copper and serum copper (r=.225, P=.020). However, dietary iron and serum iron showed insignificant (P=.258) negative correlation (r=-.110) which is a remarkable indication of iron homeostasis and physiological demand of iron.

4.2.3e MLoR models of serum microminerals showing Positive predictors

Multiple Logistic Regression (MLoR) models of copper, zinc and iron, focusing on predictors positively associated with higher micro minerals (positive deviants), adjusted for different types of variables (socio-demographic, lifestyle, risky sexual behaves, dietary and biochemical etc.).

4.2.3e-1 MLoR model of copper

It revealed from the adjusted MLoR model of copper (table-7) that “19-29 year age group” [AOR 6.286 (95% CI 1.950-20.26), P=.002] and higher (3601.0-9451.59 mcg/day/person) dietary intake of copper [AOR 4.352 (95% CI 1.032-18.34), P=.045] were associated with higher serum copper level of CSWs.

4.2.3e-2 MLoR model of zinc

In MLoR model of zinc (table-8) it was observed that “1-5” class primary education, no drug habit, ≥normal RDA of dietary zinc intake (>8000 mcg/day/person) and ≥normal serum IgG (5.0-33 g/L) were associated with the outcome of ‘≥normal’ level of serum zinc. However, 19-29 age groups were less likely to be associated with ≥normal zinc as compare to ≥30 age group. The predicted odds for CSWs having ≥normal serum zinc (DV) were 9.46-folds higher in ≥normal serum IgG possessor than the odds for <normal IgG possessor.

4.2.3e-3 MLoR model of iron

19-29 age groups were 86% less likely to be associated with ≥normal iron level as compare to ≥30 age group was noticed in adjusted model of iron (table-9). Moreover, having no STI (either syphilis or hepatitis B or both infection) and NGO counseling on STIs had almost 4 times higher odds of “outcome” that is higher level of ‘serum iron’. However, CSW possessing even normal or 11.5-18.5 µmol/L zinc level had 93% less likely to have higher iron level as compare to CSWs possessing >normal or 18.6-99.30 µmol/L of serum zinc.

Table-1: Serum micro-minerals content of CSWs

Serum micro minerals (n=107)		CSWs	Range	Micro-Minerals	95% CI
Levels	Amount (µ mol/L)	% (n)	min–max	G. Mean ± SD	lower–upper
Copper (µ mol/L)					
Low	8.18 to 10.9	6.5 (07)			
Normal	8.18 to 22	68.2 (73)	8.13 to 63.10	18.34±1.37	17.46–19.71
High	22.1 to 63.27	25.3 (27)			
Zinc (µ mol/L)					
Zn deficiency	[2]5.97 to 10.0	24.3 (26)	5.37–99.30	14.41±1.67	13.15– 15.91
Low	10.1 to 11.4	13.1 (14)			
Normal	11.5 to 18.5	37.4 (40)			
High	18.6 to 99.30	25.2 (27)			
Serum Zinc to copper ratio					
	0 .179 to 0.997	72.9 (78)	.179 to 5.932	0.94 ±0.72	0.80-1.07
	1.03 to 5.932	27.1 (29)			
[3]Iron (µ mol/L)					
Low	0.11 to 10.9	14.9 (16)	0.11 to 70.73	18.49±1.67	16.43–20.69
Normal	11.0 to 29.0	64.5 (69)			
High	29.1 to 70.74	20.6 (22)			

Normal serum cu= 11.0-22.0 µmol/L, Zn=11.5-18.5 µmol/L and Fe (female) = 11.0-29.0 µmol/L (young, 1987)

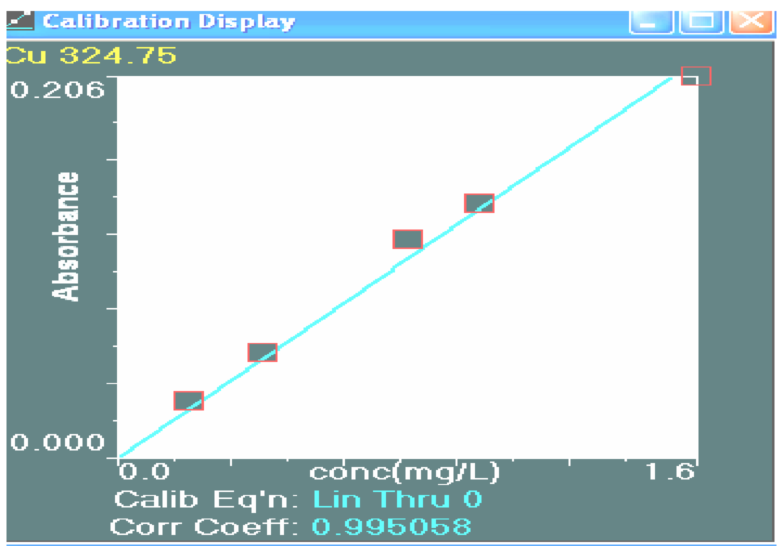

Figure-1: Standard calibration curve of serum copper

[2] Zn deficiency (NMSS, 2011-12): lower cut off for serum zinc concentration (IZiNCG) <10.1 mmol/L was 24.3% (40/107) noticed among CSWs.

[3] Serum iron and zinc was significantly (P=.025) correlated (r=.217) but not iron and copper or copper and zinc.

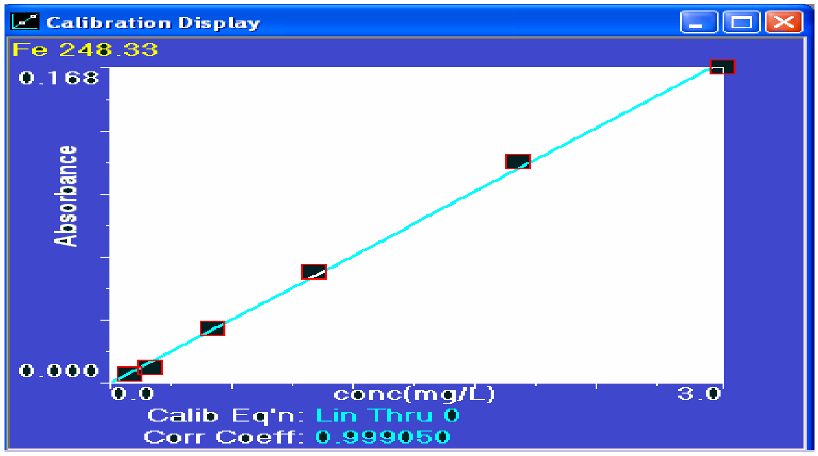

Figure-2: Standard calibration curve of serum iron.

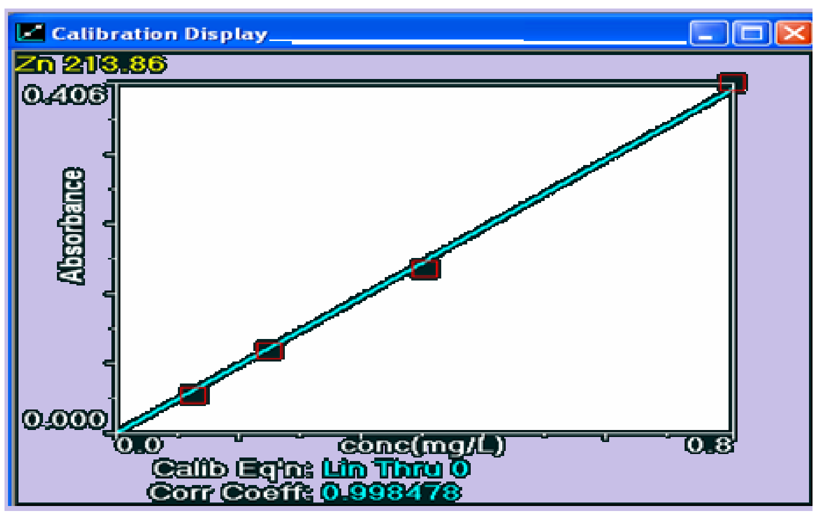

Figure-3: Standard calibration curve of serum zinc

Table-2: Influence of Socio-demography, STIs and working place on serum micro- minerals

Independent variables	Dependent variables				ANOVA
Socio-demography	All CSWs n=107 % n	Copper [1] µmol/L	Zinc [2] µmol/L	Iron [3] µmol/L	P-Values
Present age (y) [a]					
14-18	12.15 (13)	**16.22±1.32**	12.21±1.70	18.43±1.88	[a1]**F (2,104) =4.160, P=.018*,**
19–29	45.79 (49)	**19.95±1.30**[t]	18.50±1.45	19.72±1.61	[a2]F (2,104) =1.321, P=.271.
≥30	42.06 (45)	**17.38±1.38**	16.52±1.72	17.13±1.99	[a3] F (2,104) =.324, P=.724
Education [b]					
No literacy/can sign	77.57 (83)	16.84±1.21	**13.49±1.59**	18.08±1.27	[b2]**F (1,105) =7.943, P=.006***
[4]1-5 class (primary)	22.43 (24)	15.70±1.6	**18.62±1.82**	18.41±1.99	[b1]F (1,105) =.417, P=.520
					[b3]F (1,105) = .014, P=.904
Marital status [c]					
Married	41.6 (38)	18.70±1.7	**15.85±1.78**	17.39±1.45	[c2]**F (2,104)=4.296, P=.016***
Unmarried	13.7 (09)	16.32±1.30	**19.95±1.70**	18.80±1.06	[c1]F (2,104) =.063, P=.939
Wi/Di/Aba	44.7 (60)	17.40±1.03	**12.88±1.55**	19.06±1.41	[c3]F (2,104) =.013, P=.987
Monthly income [d]					
0.00-2500 taka	49.53 (53)	19.89±1.43	15.85±1.62	15.85±1.62	[d1]F (2,104) =.177, P=.838
2501-5000	29.91 (32)	19.66±1.07	19.86±1.60	19.86±1.60	[d2]F (2,104) =1.283, P=.282
>5001	20.56 (22)	18.75±1.88	15.13±1.67	15.13±1.67	[d3]F (2,104) =.556, P=.575
Last month's Total expenditure [e]					
1000-2500 taka	21.50 (23)	15.55±1.56	14.59±1.49	19.78±1.14	[e1]F (2,104) =1.797, P=.171
2501-5000	52.33 (56)	17.45±1.07	17.49±1.01	18.62±1.74	[e2]F (2,104) =.489, P=.615
>5001	26.17 (28)	19.53±1.67	17.64±1.21	19.70±1.52	[e3]F (2,104) =.119, P=.868
Treatment cost[5] **of STIs (n=60/107)** [f]					
5-250 taka	25.3 (27)	17.71±1.05	**16.98±1.62**[t]	19.85±1.07	[f2]**F (2,57) =4.960, P=.010***
251-5000 taka	14.0 (15)	14.34±1.14	**12.35±1.45**	16.32±1.78	[f1]F (2, 57) =1.300, P=.280
Self treatment	16.8 (18)	19.06±1.41	**11.60±1.51**[t]	15.33±1.78	[f3]F (2,57) =.787, P=.460
STIs status [g]					[g3]**F (1,105)=12.486, P=.001**
*STIs	68.22 (73)	17.90±1.09	17.75±1.54	**24.16±1.95**	[g1]F (1,105) =.925, P=.431
No STIs	31.78 (34)	16.90±1.71	15.08±1.83	**16.16±1.51**	[g2]F (1,105) =.411, P=.746
Places of sex business [h]					[h1]F (1,105) =.168, P=.683,
Hotel	17.76 (19)	20.23±1.59	18.18±1.81	21.20±1.35	[h2]F (1,105) =.235, P=.629
Floating (road/perk/shrine)	82.24 (88)	19.44±1.45	16.63±1.85	19.93±1.89	[h3]F (1,105) =.181, P=.671

Wi/Di/Aba=Widow/divorced/abandoned. [t]These categories were significantly (P<0.05) differ than other categories in Post Hoc Test (TUKEY test of multiple comparisons). *STIs= having (syphilis/or HBV/or both)

[4] None had secondary education (1-12 class) in this blood sample (n=107).
[5] No symptoms appeared in 37.38% (40/107) of CSWs.

Table-3: Influence of lifestyle factors of CSWs on micro-mineral status

Lifestyle factors	All CSWs n=107 % n	Copper [1] (µmol/L)	Zinc [2] (µmol/L)	Iron [3] (µmol/L)	ANOVA P-Values
Causes of profession[a]					
Lower Economy	44.86 (48)	**20.32±1.54**	18.70±1.23	21.86±1.8	**[a1]F (2,104) =.614, P=.543**
Exploitation	25.23 (27)	**18.31±1.54**	17.56±1.24	19.02±1.23	[a2]F (2,104) =1.604, P=.206
Other reasons[1]	29.91 (32)	**19.55±1.39**	13.65±1.64	18.58±1.99	[a3]F (2,104) =.922, P=.401,
Working days (last week)[b]					
Did not work	5.61 (06)	22.19±1.14	16.37±1.97	15.76±1.28	[b1]F (2,104) =.684, P=.507
1–3 days	44.86 (48)	20.05±1.60	19.24±1.53	20.70±1.93	[b2]F (2,104) =1 .542, P=.219
4–7 days	49.53 (53)	18.87±1.15	14.85±1.26	20.16±1.06	[b3]F (2,104) =.467, P=.628,
Age entering into S. trade[c]					
10-17 y	46.73 (50)	19.22±1.40	17.46±1.35	19.16±1.29	[c1]F (2,104) =.363, P=.696
18-24	33.65 (36)	20.45±1.02	17.07±1.38	22.11±1.94	[c2] F (2,104) =.221, P=.802
≥25 years	19.63 (21)	18.97±1.99	15.29±1.39	19.17±1.77	[c3]F (2,104) =.747, P=.476
Length of profession[d]					
<1 y	6.54 (07)	25.21±1.71	14.97±1.29	25.02±1.68	[d1]F (2,104) = 2.275, P=.108
1-5	47.67 (51)	18.71±1.82	17.00±1.82	19.61±1.54	[d2]F (2,104) = .087, P=.917
≥6	45.79 (49)	19.60±1.57	17.08±1.79	20.02±1.60	[d3]F (2,104) = .653, P=.108
living with[e]					
Own	57.01 (61)	19.02±1.04	15.30±1.94	18.78±1.33	[e1]F (2,104) =.557, P=.570
Husband	30.84 (33)	19.94±1.17	19.69±1.15	21.33±1.62	[e2]F (2,104) =1.306, P=.275
Others[2]	12.15 (13)	21.35±1.13	17.36±1.82	23.66±1.20	[e3]F (2,104) =1.164, P=.316
Use of contraceptives[f]					
No measure	23.37 (25)	20.47±1.14	12.58±1.43	17.01±1.15	[f1]F (3,103) =.180, P=.910
Pill/ Others[3]	33.65 (36)	19.20±1.45	17.76±1.59	21.71±1.92	[f2] F (3,103) =2.106, P=.104
Condom	42.98 (46)	19.01±1.09	16.91±1.52	17.87±1.72	[f3] F (3,103) =1.364, P=.258
Cases of abortion[g]					[g1]F (2,104) =.542, P=.58
No abortion cases	68.22 (73)	19.30±1.35	16.32±1.43	18.76±1.36	[g1] F (2,104) =1.039, P=.357
1-2/>3 cases	31.78 (34)	20.67±1.72	17.04±1.52	18.13±1.00	[g3] F (2,104) =.403, P=.670
Habit of smoking[h]					[h1]F (1,105) =.227, P=.635
No	47.66 (51)	19.95±1.47	18.64±1.99	20.47±1.34	[h2]F (1,105) =1.852, P=.176
Yes	52.34 (56)	19.25±1.68	15.33±1.89	19.87±1.29	[h3]F (1,105) =.068, P=.795
Substance Abuse[i]					**[i3] F (1,105) =3.258, P=.059**
No	70.09 (75)	20.03±1.59	17.91±1.27	**19.77±1.51**	[i1]F (1,105)=.907, P=.343
Yes	29.91 (32)	18.52±1.14	14.56±1.84	**13.49±1.56**	[i2] F (1,105) =1.582, P=.211
Types of substances[j]					
No drug	70.09 (75)	20.04±1.59	**17.91±1.27**	**19.77±1.51**	**[j2] F (2,104) =6.975, P=.001****
Drugs[4]	19.63 (21)	18.51±1.32	**14.19±1.45**[t]	**13.47±1.44**[t]	[j1]F (2,104) =.449, P=.639
Alcohol (local maod)	10.28 (11)	18.53±1.89	**21.11±1.53**	**20.34±1.80**	**[j3] F (2,104) =3.232, P=.043**
Client profile [k]					
Businessmen (small)	36.45 (39)	18.37±1.58	18.69±1.72	17.85±1.81	[k1]F (2,104) =.787, P=.562
Rickshaw puller	29.91 (32)	20.22±1.84	14.18±1.07	20.09±1.50	[k2]F (2,104) =.738, P=.597
Service holder	12.15 (13)	19.77±1.04	17.85±1.30	14.59±1.01	[k3]F (2,104) =.936, P=.461
Student/Bus driver	9.34 (10)	20.51±1.85	15.48±1.94	19.64±1.85	
Didn't know	12.15 (13)	18.20±1.38	10.61±1.27	21.86±1.14	
NGO counseling on STIs[l]					[l2] F (1,105) =5.413, P=.022
Yes	42.90 (46)	18.42±1.21	**16.60±1.74**	21.54±1.92	[l1]F (1,105) =1.937, P=.167
No	57.10 (61)	20.46±1.86	**13.18±1.66**	19.11±1.62	[l3] F (1,105) =1.119, P=.292

[1]Angry with family members, sold by step mom/others, raped/tortured, pushed by husband, self motivation and drug addiction.
[2] Parents, siblings, sexual partners etc.
[3] About 15.89% (n=17) used other methods i.e. Injection, ligation/tubectomy, menopause
[4] Cannabis (ganja, afim, charush etc.), phensedyl, injection (pethidin) and Heroin etc.
[t]This category was significantly differ (lower/higher) than other categories of the variable (within variable).

Table-4: Influence of risky sexual behaviors of CSWs on micro-mineral status

Risky sexual behaviors (n=107)	All CSWs n=107 % n	Copper [1] µmol/L	Zinc [2] µmol/L	Iron [3] µmol/L	ANOVA P-Values
Client turnover (last week) [a]					[a1]F (2,104) =.459, P=.633
Did not work	5.61 (06)	22.19±1.14	16.37±1.97	15.76±1.28	[a2] F (2,104) =.039, P=.962
1– 14 C	61.68 (66)	19.65±1.54	16.70±1.14	20.40±1.91	[a3]F (2,104) =.440, P=.645
≥15 C	32.71 (35)	19.00±1.42	17.39±1.08	20.45±1.17	
Usual client per day [b]					**[b2]F (1,105) =5.403, P=.022**
1-7	88.79 (95)	19.81±1.88	**13.80±1.59**	20.51±1.31	[b1]F (1,105) =.753, P=.387
>8	11.21 (12)	17.80±1.88	**19.95±1.99**	17.39±1.9	[b3]F (1,105) =.748, P=.389
Use of condom [c]					[c1]F (1,105) =1.342, P=.266
No use/occasional	56.08 (60)	20.30±1.13	16.17±1.27	18.34±1.15	[c2]F (1,105) =.433, P=.650
Consistent use	43.92 (47)	18.67±1.04	17.84±1.85	22.48±1.21	[c3]F (1,105) =.559, P=.573
H. measure after coitus [d]					[d1]F (2,104) =1.342, P=.266
No measure	35.51 (38)	20.56±1.64	15.38±1.60	18.53±1.28	[d2]F (2,104) =.433, P=.650
Used savlonl/soap	33.65 (36)	20.18±1.71	17.91±1.92	20.99±1.33	[d3]F (2,104) =.559, P=.573
Others[6]	30.84 (33)	17.82±1.02	17.56±1.59	21.12±1.49	
Interval between two coitus [e]					[e1]F (1,105) =.052, P=.820
No	60.75 (65)	19.72±1.42	17.05±1.00	20.15±1.02	[e2]F (1,105) =.023, P=.880
yes	39.25 (42)	19.38±1.83	16.67±1.01	20.17±1.47	[e3]F (1,105) =.000, P=.994
Coital frequency/client [f]					[f1]F (1,105) =1.162, P=.284
One time	48.60 (52)	20.39±1.42	16.27±1.70	20.18±1.56	[f2]F (1,105) =.252, P=.616
≥2 times	51.40 (55)	18.82±1.18	17.50±1.30	20.13±1.04	[f3]F (1,105) =.000, P=.984
Weekly coitus with PC [g]					**[g2]F (1,105) =3.637, P=.054**
No weekly PC	61.68 (66)	18.86±1.62	**13.38±1.55**	16.23±1.55	[g1]F (2,104) =.147, P=.863
1-2 times or more/week	38.32 (41)	17.79±1.90	**16.22±1.83**	18.46±1.37	[g3]F (2,104) =.853, P=.429
Fortnightly coitus with PC [h]					[h1]F (2,104) =.067, P=.935
No fortnightly PC	80.37 (86)	17.72±1.88	16.45±1.78	17.69±1.03	[h2]F (2,104) =.361, P=.698
1-2 times	19.63 (21)	16.08±1.22	15.30±1.87	19.97±1.63	[h3]F (2,104) =.506, P=.604
Monthly coitus with PC [i]					[i1]F (2,104) =.618, P=.541
No monthly PC	74.77 (80)	19.22±1.49	15.13±1.52	15.87±1.16	[i2]F (2,104) =.289, P=.750
1-3 times	25.23 (27)	18.22±1.45	16.79±1.18	16.61±1.27	[i3]F (2,104) =.451, P=.638
Having NPSP [J]					**[j3]F (1,105) =5.352, P=.023**
No	91.59 (98)	19.60±1.73	16.45±1.19	**17.81±1.56**	[j1]F (1,105) =.002, P=.961
Yes	8.41 (09)	19.47±1.50	21.83±1.83	**27.45±1.83**	[j2]F (1,105) =1.500, P=.223
Ever experienced in group sex (gr. Sex) [k]					[k1]F (1,105) =.019, P=.892
No	74.1 (73)	19.52±1.59	15.89±1.02	19.38±1.62	[k2]F (1,105) =1.497, P=.224
Yes	25.9 (34)	19.73±1.40	19.09±1.78	21.82±1.04	[k3]F (1,105) =.999, P=.320
Gr. Sex history (L.month [l]					[l1]F (1,105) =2.147, P=.146
No	71.03 (76)	20.26±1.23	15.70±1.77	19.25±1.53	[l2]F (1,105) =2.405, P=.124
Yes	28.97 (31)	17.92±1.29	19.85±1.30	22.38±1.19	[l3]F (1,105) =1.576, P=.212
No of persons in the last gr. Sex (n=31) [m]					[m1]F (1,105) =1.110, P=.334
2-5 clients	83.04 (22)	18.18±1.76	20.18±1.13	21.57±1.46	[m2]F (1,105) =1.217, P=.300
>6 clients	16.96(09)	17.28±1.15	19.06±1.56	24.36±1.35	**[m3]**F (1,105) =.962, P=.385,
Experienced in Anal sex [n]					[n1]F (1,105) =1.693, P=.196
No	96.4 (105)	19.45±1.56	16.97±1.74	19.98±1.71	[n2]F (1,105) =.171, P=.680
Yes	3.6 (02)	26.44±1.67	13.23±1.79	29.55±1.18	[n3]F (1,105) =1.306, P=.256

PC=permanent clients, NPSP=Non paying sexual partners,

[6] Washed by water, cotton, urine etc.

Table-5: Dietary micro-mineral intake and related other parameters

Daily Mean foods and Nutrients intake gm/person/day n=635	Mean Plant sources		Plant Total (sum)	Animal sources (Mean)	Total dietary intake (Mean)	% of RDA[1] met	National surveys[2,3] 2011-12
	Total cereals	pulses/vegetables/fruits/others					
Calorie (K cal/day/person)	1289.92	345.19	1635.11	430.96	1362.79	*61.1%	[2]Dhaka-female: 2055.2 kcal
Protein (gm/day/person)	27.34	12.35	39.69	47.74	40.07	87.11%	[2]Dhaka-female: 56.3 g
Fat (gm/day/person)	2.31	6.93	9.24	23.05	8.05	*32.2%	[2]Dhaka-female: 33.6 g
Calcium (mg/day/person y)	58.61	181.44	240.05	425.7	237.63	23.76%	[2]Dhaka-female: 499.9 g
Copper (mcg/day/person)	1829.39	943.53	2772.92	4982.6	2885.62	320.62%	-
Zinc (mcg/day/person)	2336.47	2494.27	4830.74	3757.5	3913.24	43.48%	[3]Slums: 4230 mcg/day (2900 mcg for plant, 1330 animal, RDA met 47.0%)
Iron (mg/day/person)	19.2	10.2	29.4	3.81	19.83	110.17%	[3]Slums: 7.83 mg/day (6.74 mg plant, 1.09 animal sources)
Vitamin A (µg/day/person)	1.03	422.74	423.77	228.72	189.96	27.14%	[2]Dhaka-female: 899.9 µg
Vitamin C (mg/day/person)	0.0	106.6	106.6	11.74	32.82	43.76%	[2]Dhaka-female: 70.2 mg
Other parameters related to micro-minerals							
Mean serum zinc level in study population 14.41 (CI:13.15–15.91) mmol/L							[3]Slums: 9.67 (CI:9.29-10.05) mmol/L. National: 10.04 (9.81-10.27) mmol/L
Anti-nutrients Phytic acid (mg/day/person)	567.72	165.55	733.27 (sum)	NA	492.86 (mean)		-
Mean Phytate to zinc molar Ratio 95% CI	7.53±7.11 (6.22–8.78)	1.18±1.38 (.92–1.41)	7.70±8.35 (7.05–8.35)	NA	7.70		[3]Slums:13.45 (CI 12.7–14.1) National 12.0 (10.6-13.5)
Grades[1] of phytate-Zinc molar ratio in CSWs	**(% n)**						[3]Grades[1] of phytate-Zinc molar ratio in slums
<5 ratio	41.4 (263)						**<5 ratio** 2.1%
5–15	42.2 (268)						**5-10** 64.1%
>15.0	16.4 (104)						**>15.0** 33.7%
Dietary Zinc to Copper ratio	1.277	2.64	1.74	1.08	1.71±0.88		-

[1]IOM
[2]Nutrition, Health, Demographic Survey, Bangladesh, 2011 (NHDS, BD, 2011)
[3]National Micronutrients status survey, Bangladesh, 2011-12 (NMSS, 2011-12)
*ICMR, India

Table-6: Influence of the dietary intake of Micro minerals on serum Micro minerals status.

Micro mineral consumed by CSWs/ Dietary Microminerals	CSWs % (n)	Serum Micro-minerals Mean ±SD	95% CI lower–upper	Statistical analysis ANOVA	Correlation coefficient
Copper (mcg)		**Serum Cu (μmol/L)**			
115.03 to 1800.0	22.43 (24)	16.57±1.34[t]	14.62–18.73	F(2.104)=3.22	r=.225
1800.1 to 3600.0	55.14 (59)	18.54±1.32	17.26–19.90	P=0.040*	P=.020*
3600.1 to 9451.59	22.43 (24)	20.83±1.49[t]	17.60–24.67		
Dietary Zinc (mcg) n=107		**Serum zinc (μmol/L)**			
1000.0 to 4000.0	42.99 (46)	10.49±1.29	9.74–11.30	F(2.104)=30.110	r=.781
4000.1 to 7999.0	27.10 (29)	13.92±1.43	12.15–15.95	P=.000**	P=.000**
8000.1 to 9000.0	15.89 (16)	19.98±1.49	16.16–24.71		
9000.1 to 21000.0	14.02 (16)	27.60 ±1.90[t]	19.60–38.86		
Iron (mg)		**Serum Iron (μmol/L)**			
.03 to 18.0	53.27 (57)	19.99±1.63	17.08–23.13	F(1.105)=2.123	r= -.110
18.1 to 113.84	46.73 (50)	16.88±1.68	13.99–20.04	P=.148	P=.258

P<0.05*. [t]This category was significantly differ (lower/higher) than other categories of the variable (within variable).

Table-7: Multiple logistic regression models (MLoR) of serum Cu

factors associated with high serum copper level	All CSWs n=107 % n	0=normal values n=80	1=>normal values n=27	Unadjusted Odds Ratio (95% CI) (lower-upper)	Adjusted model of Cu	
					Beta coefficient (B), SE	Odds Ratio (95% CI) (lower-upper)
Present age (Y)						
<18	12.15(13)	13.8 (11)	7.4 (02)	1.455 (.248-8.54) P=.678	.316 SE=.944	1.371 (.216-8.73) P=.738
19-29	45.79 (49)	36.3 (29)	74.1 (20)	5.517 (1.854-16.42) **P=.002****	1.838 SE=.597	6.286 (1.950-20.26) **P=.002****
≥30 (r)	42.06 (45)	50.0 (40)	18.5 (05)	1	-	1
Dietary intake of *Cu* (µg/person/day)						
115–1800 (r)	22.43 (24)	25.0 (20)	14.8 (04)	1	-	1
1801–3600	55.14 (59)	60.0 (48)	40.7 (11)	1.146 (.326-4.030) P=.832	-.250 SE= 691	.779 (.201-3.018) P=.718
3601–9451	22.43 (24)	15.0 (12)	44.5 (12)	5.00 (1.311-19.074) **P=.018***	1.471 SE= 734	4.352 (1.032-18.34) **P=.045***

Legend: Simple Logistic Regression (SLoR).
Backward stepwise Multiple Logistic Regression Model (MLoR)
Hosmer-Lemeshaw goodness of fit for Cu model (n=107): $\aleph^2$=5.387, df=5, P=.370
Model summary: -2 Log likelihood (-2LL) ratio=99.34, Nagelkerke R-square=.269
Model coefficient: Chi-square ($\aleph^2$)=21.541, df=4, P=.001
Overall predicted percentage from classification table of DV (serum Cu) was 89.4% (cut value is 50%).
(r)= reference category. P<0.05*, P<0.01**

Table-8: Multiple logistic regression models (MLoR) of serum Zn

factors associate d with (normal to higher) serum zinc level	All CSWs % n	0=<normal values n=40	1=≥normal values n=67	unadjusted odds ratio (95% CI) (lower-upper)	Adjusted Odds Ratio Beta coefficient (B) SE	(95% CI) (lower-upper)
Present age (Y)						
<18	12.15(13)	10.0 (04)	13.4 (09)	1.241 (.329-4.678) P=.749	.173 SE=.781	1.189 (.257-5.497) P=.825
19-29	45.79 (49)	50.0 (20)	43.3 (29)	.800 (.347-1.844) P=.600	1.370 SE= .627	.254 (.074-.867) **P=.029***
≥30 (r)	42.06 (45)	40.0 (16)	43.3 (29)	1	-	1
Education						
No literacy/can sign name (r)	77.57 (83)	92.5 (37)	68.7 (46)	1	-	1
1-5 class	22.43 (24)	7.5 (03)	31.3 (21)	5.630 (1.558-20.348) **P=.008****	1.607 SE=.848	4.988 (.946-26.28) P=**.027***
Drug habit						
No	70.09 (75)	57.5 (23)	77.6 (52)	2.562 (1.095-5.997) **P=.030***	.993 SE=.603	2.699 (.829-8.79) **P=.050**
Regularly (r)	29.91 (32)	42.5 (17)	22.4 (15)	1	-	1
Dietary intake of zinc *(mcg/person/day)*						
1000 to 4000 (r)	42.99 (46)	70.0 (28)	26.2 (18)	1	-	1
4001 to 7999	27.10 (29)	27.5 (11)	26.9 (18)	2.545 (.979-6.619) P=.055	.507 SE=.567	1.660 (.546-5.041) P=.372
>8000	29.91 (32)	2.5 (01)	46.3 (31)	48.22 (6.040–385.028) **P=.000****	3.976 SE=1.12	53.30 (5.94–478.24) **P=.000****
@Serum IgG (g/L)						
<normal (0.0–4.99) (r)	85.00 (91)	95.0 (38)	79.1 (53)	1	-	1
≥Normal (5.0–33.0)	15.0 (16)	5.0 (02)	20.9 (14)	5.019 (1.077-23.389) **P=.040***	2.247 SE=.926	9.46 (1.54-58.069) **P=.015***

Legend: Simple Logistic Regression (SLoR).
Backward stepwise Multiple Logistic Regression Model (MLoR)
Hosmer-Lemeshaw goodness of fit for zinc model: $\aleph^2$ =6.259, df=7, P=.510
-2 Log likelihood (-2LL) ratio=90.813, Nagelkerke R-square=.514
Model coefficient :Chi-square ($\aleph^2$)=50.634, P=.000, df=7
Overall predicted percentage from classification table of DV (serum zinc) was 77.6% (cut value is 50%).
(r)= reference category. P<0.05*, P<0.01**
@For obtaining greater cell numbers and small magnitude (or range) of 95% CI, serum immunoglobulin (IgG) categories were rearranged by adding “very low” and “low” frequencies together (namely <below normal) and also “normal” and “high” frequencies (≥normal) together.

Table-9: Multiple logistic regression (MLoR) models of serum iron of CSWs

factors associated with (normal to higher) serum iron level	All CSWs n=107 % n	0=<normal values n=16	1=≥normal values n=91	Unadjusted Odds Ratio (95% CI) (lower-upper)	Adjusted Odds Ratio Beta coefficient (B) SE	Adjusted Odds Ratio (95% CI) (lower-upper)
Present age (Y)						
<18	12.15(13)	6.2 (01)	13.2 (12)	.857 (.082-9.009) P=.898	-.041 SE=1.240	.960 (.084-10.905) P=.974
19-29	45.79 (49)	75.0 (12)	40.7 (37)	.220 (.058-.841) **P=.027***	-1.966 P=.757	.140 (.032-.617) **P.009****
≥30 (r)	42.06 (45)	18.8 (03)	46.1 (42)	1	-	1
STI status						
No STI	31.78 (34)	18.8 (03)	34.1 (31)	2.239 (.593-8.450) P=.234	1.384 SE=.776	3.989 (.871-18.273) **P=.075**
Syphilis/HBV/both (r)	68.22 (73)	81.2 (13)	65.9 (60)	1	-	1
NGO counseling on STI						
Yes	42.90 (46)	25.0 (04)	46.2 (42)	2.571 (.771-8.574) P=.124	1.433 SE=.701	4.190 (1.060-16.556) **P=.041***
No (r)	57.10 (61)	75.0 (12)	53.8 (49)	1	-	1
Serum Zinc (µ mol/L)						
<Normal (5.97–11.4)	37.4 (40)	37.5 (06)	37.3 (34)	.218 (.025-1.924) P=.170	-1.695 SE=1.16	.184 (.019-1.785) P=.144
Normal (11.5–18.5)	37.4 (40)	56.3 (09)	34.1 (31)	.132 (.016-1.116) **P=.063**	-2.614 SE=1.17	.073 (.007-.721) **P=.025***
>Normal (18.6–99.30) (r)	25.2 (27)	6.2 (01)	28.6 (26)	1	-	1

Legend: Simple Logistic Regression (SLoR)
Backward stepwise Multiple Logistic Regression Model (MLoR)
Hosmer-Lemeshaw goodness of fit for iron model: $\aleph^2$ =4.610, df=8, P=.798
-2 Log likelihood (-2LL) ratio =70.02, Nagelkerke R-square=.303
Model coefficient :Chi-square ($\aleph^2$)= 20.261, P=.002, df=6
Overall predicted percentage from classification table of DV (serum iron) was 86.0% (cut value is 50%).
(r)= reference category. P<0.05*, P<0.01**

4.2.4 Discussion

Serum Copper: mean serum copper level of the study subjects was found 18.34 µmol/L (95% CI 17.46−19.71) was within normal limits (Young, 1998'), which was below copper level of females subjects (19.50 mmol/L) in a previous study (Buxaderas and Farré, 1986) of healthy human subjects (240 males & 217 females) but higher than Bangladeshi healthy subjects used as controls (13.38 µmol/L) for the study of Panic Disorder (PD) patients (Zabun Nahar, 2012), as well as healthy European subjects (17.63 µmol/L) (Terrés-Martos et al, 1997; Moreno et al, 1998) and also than Iranian women (15.6 ±3.39 µmol/L) (Parizadeh et al, 2011). Much higher level of copper than this study subjects (19.58 µmol/L) was also reported in Iraqi healthy control groups [(Zarebavani et al, 2012) (39.92 µmol/L)], [(Iham et al, 2009) (21.73 µmol/L)].

ANOVA revealed that serum copper level of CSWs was significantly influenced by their present age [P=.018, F (2,104) =4.160]. And marital status was found to influence only serum zinc (P=.016) but not serum copper (P=.939). However, Just opposite finding of this, reported in a large Iranian study (Parizadeh et al, 2011) where widow and widowers had higher serum copper than single and married subjects (P<0.01).

In this study, one fourth (25.2%, n=27/107) of CSWs had elevated level of serum copper which might be the influential factor of 'mean copper' level (18.34 µmol/L) of CSWs to be remain within normal range. In this mineral study, 68.2% (73/107) of CSWs had infection (either syphilis or hepatitis B or both) and elevated level of serum copper is a common phenomenon in different infectious disease including AIDS (Graham et al, 1991; Moreno et al, 1998). Serum copper enhancement produced by virus action (Graham et al, 1991) is additionally reinforced by the associated bacterial infection (Moreno et al, 1998). In contrast to serum zinc, 90% of serum copper is stored in the bound form to the ceruloplasmin (protein that binds with copper). Copper is known to be an acute phase reactant and its level in serum have been shown to change significantly in a range of acute and chronic infective, inflammatory and neoplastic process. Elevations of serum copper levels are observed in most of the infections in relations to fluctuations of ceruloplasmin as an acute phase reactant (Hasan Yari et al, 2015; Zarebavani et al, 2012). Moreover, copper level in infection and neoplasia owing to increased reproduction of ceruloplasmin (Beisel, 1976; Powanda and Beisel, 1982). Studies showed evidences of increasing serum Copper level in infection (Hasan Yari et al, 2015; Zarebavani et al, 2012; Abdoreza Ghoreishi et al, 2015) or drug addiction (Hossain KJ et al, 2007). In addition, elevations of serum copper levels were observed in most of the acute and chronic parasitic infections (Culha and sangun, 2007; Zarebavani et al, 2012).

MLoR model of serum copper: in "multiple logistic regression (MLoR) model of serum copper", 2 predictors namely '19-29 age group' and 'higher daily dietary intake (3601-9451 µg/day) of copper' were found to influence serum copper level of CSWs positively. It was postulated that (table-7), 19 to 29 years age group had 6.29 times higher odds of having ≥ normal serum Cu (22.1 to 63.7 µmol/L) as compared to CSWs who were ≥30 years of age [AOR 6.289 (1.950-20.26), P=.002). Serum copper was negatively correlated with age at the onset of 30 years which is in agreement with an old study (Buxaderas and Farré, 1986) in case of whole blood copper level (P=0.045) and with another Iranian study (Parizadeh et al, 2011). Other study (Risto Laitinen, 1990) supported the relation between copper and age. Higher (3601.0-9451.59 mcg/day/person) dietary intake of copper by CSWs [AOR 4.352 (95% CI 1.032-18.34), P=.045] were associated with higher serum copper level of CSWs. Sex, hormonal state, diet and geographical differences are reported to affect serum copper levels (Sedighi et al, 2006; Johnson et al, 1992). Socio-cultural and genetic factors may also affect serum copper and ceruloplasmin levels indirectly (Sedighi, 2006).

Serum zinc: Mean serum zinc level (14.41±1.67 µmol/L) of CSWs was higher than Indian women [(Herbst et al, 2014) (10.8±1.6 µmol/L)], Korean healthy adults [(Hee et al, 1999) (11.06 µmol/L)], French adult women [Galan et al, 2005, 12.9 µmol/L] and Iraqi healthy control [(Iham et al, 2009) (9.08 µmol/L)] but lower than Iranian healthy control [(Zarebavani et al, 2012) (22.15 µmol/L)].

In this mineral study (n=107), about 37.4% (40/107) CSWs had low serum zinc (of them 26/107 or 24.3% had <10.1 µmol/L serum Zn) and 37.4% CSWs had normal serum zinc than standard level but, one fourth (25.2%, n=27/107) of CSWs had elevated level (18.6-99.30 µmol/L) of serum zinc (like copper) that could influence to persist or withstand normal serum 'mean zinc' level of CSWs. Moreover, most (73/107=68.2%) of CSWs suffering from either syphilis (n=46) or hepatitis B (n=8) or both (n=19) and. According to IZiNCG (2007, Tech brief no.2) underlying infection or tissue damage reduces serum zinc which can be measured by C-reactive protein (CRP) or by α-1 acid glycoprotein (AGP). Unfortunately this study could not measure these indicators and low serum zinc could be explained by high infection (68.2%) rate. However Pearson's correlation (r=.781, P=.000) as well as Multiple Logistic Regression (ML_oR) Model of zinc (table-8) supported the relation between dietary zinc and serum zinc. In this study, "dietary intake of zinc" is an important and strong predictor of serum zinc level and could explain for having normal serum status of 37.4% CSWs. In this study, 9.8% CSWs skipped meal (one or two times) in a day or starved, studies showed starvation or muscle breakdown during weight loss (IZiNCG, 2007) increase serum zinc by liberating zinc from muscle to the circulation and increase serum zinc concentration. Zinc is an element which cannot be stored in the body (Linus Pauling Ins.) and therefore, it can easily decline in infective diseases (IZiNCG, 2007; Culha and sangun, 2007, Zarebavani et al, 2012). Studies showed evidences of decreasing serum zinc level in infection (Zhang et al, 2012; Culha and sangun, 2007) or increasing in drug addiction (Hossain KJ et al, 2007).

'Education' (1-5 class) had significant impact on having higher zinc to copper ratio [F (1,105) =8.569, P=.004] and higher serum zinc level [F (1,105) =7.943, P=.006] in this study which is in agreement of a Persian study in case of men (P<0.001 for only men but not for women) of general population (Parizadeh et al, 2011) as well as for both Bangladeshi drug addicts and controls (Hossain KJ et al, 2007).

ANOVA showed (table-2) marital status [P=.016, F (2,104) =4.296] had negative impact on serum zinc status which is in agreement with a recent Pakistani study of women of child bearing (Akhter et al, 2014) where a significant (P<0.03) decrease in zinc concentration was found in married as compared to unmarried women. Similar finding of serum zinc was observed in healthy control of drug addicts (Hossain KJ et al, 2007).

Mean serum zinc of the study participants was remarkably better from National Micronutrient Status Survey (NMSS, 2011-12) in Bangladesh where mean serum zinc level was 9.67 µmol/L in slums and that was 10.04 µmol/L at national level. It is noteworthy that, in this study, 24.3% (n=26) CSWs have been suffering from serum zinc deficiency (<10.1 µmol/L) which is risk for any population or subgroup according to IZiNCG (2007). Similar zinc deficiency (27.8%) among women was also reported in Pakistan (Akhter et al, 2014). In contrast to this finding, prevalence of serum zinc deficiency (NMSS, 2012) predominantly high in National level (57.3%) as well as in slums (66.4%) and in an Indian study (Herbst et al, 2014). This discrepancy could be explained clearly if one draws attention to the dietary intake of micro minerals by CSWs (table-5) and correlations of dietary minerals with serum minerals which are depicted in table-6. Mean 'phytic acid' content (492.86 mg) and 'phytate to zinc molar ratio' (7.70) was observed remarkably low in CSW's diet than national average of 'phytate to zinc molar ratio' (12.0) and than in an Indian study where 'phytate to zinc molar ratio' was 26 (Herbst et al, 2014). Also, a remarkable portion (41.4%) of CSWs consumed <5 grades 'phytate to zinc molar ratio' containing food while it was only 2.1% for national level (table-5). About 52% of the Indian participants (Herbst et al, 2014) had a low serum zinc concentration according to the International Zinc Nutrition Consultative Group (IZiNCG, 2007). Diets low in energy with poor bioavailability of dietary zinc is likely to be the primary cause of the high proportion of Indian women with zinc deficiency (Herbst et al, 2014). Discrepancy of serum zinc might be due to methodical variation, sex related variation, and small population effect. Furthermore studies used different methods [atomic absorption, calorimetric methods and polarography (Hasan Yari et al, 2015) etc.] could explain the variation of serum zing among different subjects.

In this study, ANOVA showed that 'drug habit' of CSWs as well as 'types of substances' had negative influence on all serum micro minerals mainly on serum zinc (P=.046) and iron (P=.059). ANOVA (table-3) showed the influence of the types of substance abuse on serum micro minerals that serum zinc status of drug addict CSWs were found significantly low as compared to non-drug abusers and alcoholic CSWs in post Hoc test (Tukey) for multiple comparisons [zinc: F (2, 104) = 6.975, P=.001]. This discrepancy was probably due to the presence of smaller cell value (only n=11) of alcoholic group, although significant, does not thought to have much practical meaning.

In this study of CSWs, ANOVA revealed that (table-4) ‘Per day clients’ (normal) of CSWs had positive association with serum zinc [P=.022, F (1,105) =5.403] and showed that CSWs who had more than >8 clients/day had higher serum zinc concentration than who had less clients (1-7) per day, the explanation of this situation is unknown, moreover, data regarding sexual activity and serum zinc level of Female Commercial Sex Workers (FCSWs) were scare. Nevertheless, drug addict, Bangladeshi subjects (Hossain KJ et al, 2007) exhibited just opposite (i.e. negative) influence on serum zinc for multiple sexual partnerships. The discrepancy of this outcome regarding serum zinc and number of sexual partners may be due to the ‘sex differences of the study subjects’, as all ‘subjects’ of Hossain KJ et al (2007) were male as well as “drug addicts”. However, a previous research pointed at (Risto Laitinen, 1990) 15 to 18 years Finnish girl who had low serum levels of zinc had physically matured most slowly compared with their age mates.

MLoR model of serum zinc: multiple logistic regression (ML_oR) analysis was employed to identify which non-dietary (socio-economic, lifestyle biochemical and more) and dietary factors associated with high serum zinc level of CSWs (table-8). Although, ANOVA showed insignificant (P=.271) association with ‘serum zinc’ and ‘present age of CSWs’ (P=.271) like an Persian study (Parizadeh et al, 2011) but this categorical variable (3 categories of the age of CSWs) was proved to be a strong covariate (primarily having P≤0.05 in crude logistic regression model) as whenever it was adjusted with other variables the chi-square ($\aleph^2$) value of Hosmer-Lemeshaw goodness of fit marker raised to 50.924 (-2log, Cox and Snell value also raised), but whenever it was not included in the model the value of Hosmer-Lemeshaw goodness of marker was downed to 1.375. Thus, “19-29” age category of the CSWs remained significant (P=.029*) after adjusting for other variables such as ‘education’, ‘drug habit’, ‘dietary zinc intake’ and ‘serum IgG level’ in the ‘Multiple Logistic Regression (ML_oR) Model of zinc’ (table-8). According to ML_oR model of zinc (0=<normal serum Zn, 1= ≥normal to high Zn), CSWs who were in between 19-29 years of age (P=.029*) were less likely (~75%) to have [AOR .254 (.074-.867), P=.029] normal to higher serum zinc as compared to ≥ 30 age category (reference category). In contrast, <18 years CSWs had 1.19 times (or 19%) higher odds (P=.825) of having normal to higher serum zinc in compare to reference category (≥ 30 age group). Similar increasing trend of serum zinc with increasing age was found in a recent study conducted in Pakistan (Akhter et al, 2014).

‘Education’ remained significant (P=.027) in Multiple Logistic Regression (ML_oR) model of serum zinc (0=<normal serum Zn, 1= ≥normal to high Zn) (table-8). It was outlined that 1-5 classes educational attainment (primary education) had 4.99 times higher odds of having normal to higher serum zinc status of CSWs [AOR 4.988 (.946-26.284), P=.027] and the concept is consistent with other micro mineral study (Hossain KJ et al, 2007, Parizadeh et al, 2011). However, no clear relationship between educational levels with serum Zn and Cu concentrations and Zn/Cu ratio was reported in Zhang et al (2009). Similarly, serum copper

and iron level of CSWs did not influenced by education in this study and negatively influenced by education in drug dependent subjects (Hossain KJ et al, 2007).

Drug habit of CSWs was remained marginally significant (P=.050) in Multiple Logistic Regression (ML_oR) model of zinc (0=<normal serum Zn, 1= ≥normal to high Zn). It was observed that only 29.91% of CSWs of this study, abused substances and those (70.09%) who did not abuse substances were 2.699 times (95% CI .829-8.791) more likely to have higher serum zinc status (P=.050) compared to CSWs who abused drugs (reference category). 'Period of drug abuse' had negative impact on serum zinc concentration of Bangladeshi drug addicts as reported by Hossain KJ et al (2007) though was not significant (P>0.05) (table-8).

In this study, "dietary intake of zinc" is strongly correlated (table-6) with 'serum zinc' (r=.781, P=.000) and also an important and strong predictor (in both SLoR and MLoR it was significant) of 'serum zinc' level which remained finally in the Multiple Logistic Regression (ML_oR) Model of zinc. Even consuming 44% to 88.88% of RDA of dietary zinc (4001-7999 µg) CSWs could not be able [AOR 1.66 (.546-5.041), P=.372] to achieve ≥normal serum zinc as depicted in 'MLoR model of serum Zn' (table-8), instead CSWs those (n=32) consuming ≥8000 µ/person/day (>88.88%-100% of RDA) were 53.30 times more likely to get ≥normal serum zinc [AOR 53.30 (5.940-478.236), P=.000**] other variables remained constant. As 95% CI was much higher, so data should have interpreted cautiously, it might be due to very small cell number (01) as well as small samples (n=107) of the zinc model. However, higher CI was the result of high point prevalence[7].

'Serum IgG', another predictor of 'MLoR model of serum Zn' (table-8) showed similar trend like "dietary intake of zinc". CSWs possessing ≥normal serum IgG level (5.0-33.0 µmol/L) were 9.46 times more [AOR 9.46 (1.540-58.069), P=.015*] likely to possess ≥normal zinc level was resulted in Multiple Logistic Regression (ML_oR) Model of zinc (0=<normal serum Zn, 1= ≥normal to high Zn). Conversely (inverting the odds ratio), CSWs having lower serum IgG (0-4.99 mmol/L) had 89% higher risk of having below normal level (11.5-18.5 µmol/L) of serum zinc than who had higher serum IgG. In fact, Zinc is critical for normal development and function of cells that mediate both innate and adaptive immunity (Prasad, 2008). Zinc is an element which cannot be stored in the body (Culha and sangun, 2007; Zarebavani et al, 2012; Linus Pauling Ins.), regular dietary intake of the mineral is important in maintain the integrity of the immune system (Linus pauling Inst). Thus, inadequate intake can lead to zinc deficiency and compromised immune response (Ibs and Rink, 2004). Zinc and copper are believed to be important in maintaining immune function (Turnlad, 1988).

Serum iron: mean serum iron level of CSWs was 18.49±1.67 µmol/L which was lower than both case (19.1µmol/L) and control group (22.0µmol/L) of an old iron study (Fiona et al, 1977). In this study, STIs positively correlated with both serum copper (P=.431) and zinc

[7] Cross validated: http://stats.stackeschange.com/questions/147666

(P=.746) level except for iron which was found significantly, negatively associated with STI status of CSWs [P=.001, F (3,103) =12.486], CSWs those were inflicted with STIs had lower serum iron (16.16 µmol/L) than who did not (24.16 µmol/L). Serum iron status of drug addict CSWs were found significantly low as compared to non-drug abusers and alcoholic CSWs in post Hoc test (Tukey) for multiple comparisons [F (2, 104) = 3.232, P=.043] which is in agreement with a study of Bangladeshi drug dependent subjects (Hossain KJ et al, 2007). 'Non paying sexual partner' (NPSP) of CSWs positively influenced serum iron level [P=.023, F (1,105) =5.352] of CSWs. Due to low cell value (n=09) of NPSP holder this effect should have interpreted cautiously.

MLoR model of serum iron: Serum iron level was weakly correlated (r=.067, P=.490) with 'present age' of CSWs but did not reach in significant level but 'present age' was a strong 'predictor of serum iron' level as it remained in both simple logistic regression (SLoR) and Multiple Logistic Regression (ML_oR) model of serum iron (0=<normal serum Fe, 1= ≥normal to high Fe). According to ML_oR model of iron, <18 year (P=.974) and 19-29 age (P=.009) category was less likely to have normal to high iron level compare to >30 age group (reference category). The odds of having normal to higher iron level for CSWs who were in 19-29 age group (P=.009) was 86% lesser than the odds of having ≥normal iron level for CSWs who were at 30 years or more, other variables remained constant (table-9).

Moreover, STI remained exist with borderline status (P=.075) in Multiple Logistic Regression (ML_oR) model of iron. CSWs without STIs were 3.99 times [OR 3.989 (95% CI .871-18.273), P=.075] more likely to have normal to high serum iron compared to CSWs who inflicted with STIs. A unit change in the independent variable "NGO counseling on STIs" (Yes to No) increases the odds of receiving a normal to higher serum iron level (dependent variable) by a factor of 4.19 (95% CI 1.06-16.56, P=.041)(table-9). Iron deficiency has often been found to be associated with chronic infections and complex relation of iron metabolism to infection than any other study was reported (Fiona et al, 1977).

As serum zinc positively correlated with serum iron level (r=.217, P=.025), so reflected in 'Multiple Logistic Regression (ML_oR) model of iron'. According to ML_oR model of iron CSWs who had <normal (P=.144) or normal serum zinc levels (P=.025) were less likely to have normal to high iron level as compared to CSWs who had more than normal serum zinc (18.6 to 99.3 µmol/L). Conversely (inverting odds), the predicted odds for CSWs having <normal serum iron were 13.69 times higher even in normal serum zinc (Independent variables) possessor (CSWs) than the odds of higher zinc possessor. Thus, zinc plays an important role in facilitating to boost up serum iron level. Altogether, CSW should have possessed more than normal serum zinc to have higher iron level-according to the Multiple Logistic Regression (ML_oR) model of iron for CSWs (table-9). An impaired iron supply for erythropoiesis show increased zinc protoporphyrin concentrations despite increased storage in bone marrow and inflammatory diseases will increase ZPP concentration due to its limiting effect on circulating iron and not as a specific effect on erythropoiesis (Hastka et al, 1993).

Dietary copper, zinc and iron: data regarding dietary intake of micro minerals (copper, zinc and iron) were scanty. In this study, significant positive correlations (table-6) were observed between dietary zinc and serum zinc (r=.625, P=.000) as well as dietary copper and serum copper (r=.225, P=.020). However, dietary iron and serum iron showed insignificant (P=.258) negative correlation (r=-.110) which is a remarkable indication of iron homeostasis and physiological demand of iron (table-6).

Dietary copper: mean intake of copper by CSWs was 2885.62 mcg/day/person which was pretty higher than a large study (median copper intake for both men and women was only 1500 µg/day/person) conducted in Italy among adults (Simona Bo et al, 2008) and this study also showed that by taking 2080 µg/day/man (female dietary copper level was assessed but serum copper was not assessed) dietary copper, correspondent serum copper level was found 18.2+1.9 µmol/L for 54.9±5.4 years men which is in agreement with this study (CSW's dietary and serum copper were 2885.62 µg/day/CSW and 18.34 µmol/L respectively) as other studies (Buxaderas and Farré, 1986; Parizadeh et al, 2011) showed copper level is always (P=<0.05) higher in women than man due to hormonal effect. Furthermore, serum copper level is more likely to sex specific (significantly higher levels in women than men) (Buxaderas and Farré, 1986; Gámez et al, 1997, Johnson et al, 1992, Parizadeh et al, 2011). This is related to the higher absorption of copper by female (patients or normal) probably due to the influence of the female sex hormone on copper metabolism (Gámez et al, 1997, Moreno et al, 1998, Johnson et al, 1992). This is so, despite the fact that the daily energy requirements of women are ~20% lower than those of men (Moreno et al, 1998).

In contrast to dietary zinc, mean intake of copper (2885.62 mcg) apparently seems to be higher than RDA (Adult's RDA of cu 900 mcg) as RDA was set by IOM only 900 mcg per day. But, in reality, maximum foods with few exceptions contain higher level of zinc than copper and these two micro-minerals always maintain a natural balanced ratio, for example among all Bangladeshi green leafy vegetables (GLVs/ shak) most have higher zinc contents than copper except Kalmi shak (swamp morning glory) which contain lesser zinc than copper (Islam SN et al, 2012). On the other hand, high copper rich foods are mainly oysters, crabs, Lobsters, mushrooms, sesame seeds, cashew nuts, and chick peas etc. and this study population ate none of this but 9.64% (n=307) of CSWs consumed lentils (not chick peas) and interestingly, lentils contains 1620.91 mcg copper and 4166 mcg zinc (Islam SN et al, 2012) . Thus, calculative dietary copper did not necessarily exceeds limit, but other sources of copper like drinking water source, cooking pot made of copper could easily take into account. In our study, most of the drinking water sources were Municipal Corporation's public water supply and respondents did not use copper pots as these are not available in our country, so there was no possible reason to exceed dietary copper intake of CSWs. As copper is an essential micro mineral, so tolerable upper limit (TUL) of dietary copper (only intake through foods) is set to 10000 mcg (DRI; Linus Pauling institute; WHO). Thus, dietary intake of this study subjects' was not only within permissible limit of Cu of DRI (890-900 µg, IOM) but also reflected in serum copper level of CSWs which was within range of normal level (11.0-22.0

micro mol/L: Young, 1998). Furthermore, a controlled study (Hunt and Vanderpool, 2001) using females (USDA Nutrition Center in Grand Forks, North Decota), was observed that lacto-ovo vegetarians took in 1.45 mg of coppers daily of which 33% (0.48 mg) was taken into the body. In contrast, the non-vegetarian took 0.94 mg of copper daily of which 42% (0.39 mg) was taken into the body, thus vegetarians have higher levels of copper in the diet than non-vegetarians.

Dietary zinc: the important thing about dietary zinc was that, the amount of plant and animal sources of this micro mineral were pretty good and also very close (mean 4248.06 mcg=plant 4830.74 mcg+ animal 3757.51 mcg), both of which were higher than national average (NMSS, 2011-12) (table-5). As mean amount of animal origin was quite satisfactory, so it would easily be absorbed. In addition, calcium and phytic acid contents were very low and at the same time, mean phytate to zinc molar ratio (CSWs: 7.70 vs. national: 13.45) was also 1.7 times lower than national which obviously did not hamper zinc absorption or could not make zinc lesser bio-available. At last, all of these criteria also reflected in serum zinc level, which was within normal range (14.41±1.67 mmol/L) though dietary intake of zinc by CSWs fulfilled only 43.48% of RDA attainment (IOM)

In this study, mean dietary zinc intake was only 3913.24 µg /person/day which was below the IZiNCG (2007) recommendations as well as IOM for dietary zinc. Although zinc intake of CSWs was slightly lower but very close to zinc intake (NMSS, National Micronutrient Status Survey, 2011-12) of national average (4230.0 mcg /day/person) but much lower than a previous Korean study ([(Hee et al, 1999) (6410 µg/day/person, did not have phy/zn molar] and a recent Indian study [(Herbst et al, 2014) (median zinc intake 5300 µg/person/day)] where (Herbst et al, 2014) median phytate/zinc molar ratio was remarkably very higher (26) than mean phytate/zinc molar ratio (7.70) of this study. As a result, by taking comparatively lower amount of zinc (3913.24 µg /person/day vs. 5300 µg/person/day) than Indian women (Herbst et al, 2014) CSWs managed to absorb more bio-available dietary zinc than Indian women which also reflected in both of their serum zinc level (14.41 µmol/L vs.10.8 µmol/L) because of low phytic acid (492.86 mg) content and lower phytate to zinc molar ratio than them (7.70 vs. 26.0) (table-6). Furthermore, Pearson's correlation (r=.781, P=.000) as well as Multiple Logistic Regression (ML_oR) Model of zinc (table-8) supported the relation between dietary zinc and serum zinc. In this study, "dietary intake of zinc" is an important and strong predictor of serum zinc level. Discrepancy of mean dietary zinc intake (among different studies) obtained from one 24-h recall might be due to economic variation, inadequate zinc intake due to poor diet quality and low consumption of flesh foods rich in zinc (Herbst et al, 2014).

Dietary Iron: although, iron intake of CSWs was up to the mark of RDA, but most of the iron came from cereals (19.2 mg) and then non-cereal plant sources (10.2 mg), only 3.81 mg of Iron provided by animal origin, and animal foods are the richest sources of "Heme Iron". Nevertheless, mean iron consumption (19.97 g) as well as both plant (total plant 29.4 g) and animal (3.81 g) intake of this study group was remarkably higher than national average (NMSS, 2011-12).

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CHAPTER-5

Immunoglobulins profile (serum IgG, IgA, IgM) of commercial sex workers

5.1 Introduction

Immune system is vast, complex and interlinked. It has two greater arms like innate (or natural/short term) and Adaptive (acquired/long term) immunity. The immune system plays a leading role in fighting off the constant bombardment of our bodies by invading pathogenic organisms, such as bacteria, fungi, viruses, toxins and allergic compounds. Also, Immune system is intimately linked to the quality and quantity of nutrient intake (Fernandes, 1991). The host is defense-regulated and maintained by two branches of immune system. One is cell-mediated immunity, carried out primarily by $CD4^+$ Th-1 (produce IL-2, IFN-γ) and $CD8^+$ T lymphocytes, which plays a pivotal role in cytotoxic responses against malignant cell infected with intracellular bacteria and viruses. The other branch is humoral (antibody-mediated) immunity, of which the B-lymphocyte plays a dominant role, to ward off and/ or destroy extracellular pathogens. The T lymphocytes may also influence humoral immunity, as the Th-2 $CD4^+$ T-lymphocyte subset secretes a wide variety of antibody-inducing cytokines (i.e. IL-4, IL-5 & IL-6). Unfortunately, the vast array of defense mechanisms is not impregnable. Primary causes of breakdown in these lines of defense (i.e. serious breakdown of our immune system) is the either chronic or acute under nutrition and malnutrition (Jolly and Fernandes, 2000).

The immune system comprises of immune cells [lymphocytes (i.e. B-cells, T-cells and natural killer or NK cells), phagocytes [i.e. monocytes, macrophages & polymorphonuclear granulocytes (neutrophil, eusonophil and basophils)] & accessory cells (i.e. APC or antigen presenting cells, platelets, mast cells & endothelial cells)], antibodies [i.e. Immunoglobulins IgG, IgA, and IgM] cytokines (interleukins and interferones; mediators) and complements (or amplifier i.e. β-globulin). Functional network of the immune system is like that of nervous system. It has learning and memory function based on cell to cell communication and it shares mediators, receptors and antigens (Lydyard and Grossi, 1966).

Immunoglobulins play a key role in the body's immune system. They are proteins produced by specific immune cells called plasma cells in response to cancer cells and foreign pathogens i.e. bacteria, viruses, fungi and other microorganisms as well as substances that are recognized as "non-self" and harmful antigens. The first time a person is infected or otherwise exposed to a foreign substance (antigen), their immune system recognizes the microorganism or substance as "non-self" and stimulates plasma cells to produce specific immunoglobulin(s) or antibodies that can bind to and neutralize the threat. With subsequent exposures, the immune system "remembers" the antigen that was encountered, which allows for the rapid production of more antibodies and helps prevent re-infection. Deficiencies in immunoglobulin (Igs) do not always lead to infectious complications. IgG is the major type of antibody found in the blood that can enter tissues. It coats germs, helping other cells to seek and destroy them[1]

Immunoglobulins (Igs) or antibodies are glycoprotein molecules produced by the lymphocytes located in the bone marrow, a class known as ‘Beta lymphocytes’. Main functions of these multifunctional tools involve enhancing humoral immunity (Ab-mediated) against bacterial, viral and other pathogens (therapeutic roles) and prevention of superimposed infections (prophylactic roles). Immunoglobulins are formed by plasma cells as a humoral immune response when the immune system comes into contact with antigens. At first contact, antibodies of the IgM class appear first as a primary reaction, followed by the formation of IgG and IgA antibodies. The quantitative determination of immunoglobulin can provide important information on humoral immune status. Depressed immunoglobulin concentrations in the serum occur in both primary and secondary immune deficiencies.

Immunoglobulin is produced in response to the presence of antigen by B-cells with the help of T-cell. Hence, antibody responses (help and suppression) are regulated by mainly T-cells (other cells i.e. macrophage also produced mediators) by producing different mediators (or cytokines) i.e. IL-2 (T-cell growth factor), IL-4 (B-cell growth factor) and IL-5 (B-cell differentiation factor). Also, IL-2 acts synergistically with IL-4 to stimulate the growth of B-cells and other important mediators’ important involvement are also usually required for normal and healthy immune system (Levinson and Jawetz, 2005). The increase or decrease in various immune proteins from basal level represents viral host interaction in the immune cells and used as the alternative prognostic markers for defense immunity of the host and clinical outcome of the diseases. Viral copies, CD4 and CD8 T lymphocytes cell counts are specific markers for host immune system failure but other proteins involved in immune system such as β-2 micro globulin, neopterin, many cytokines, chemokines and immune cell binding proteins also counterpart in balancing between virus replication and virus destruction by host immunity (Chanida, 1999).

Quantitative immunoglobulin tests measure the total amount of each primary immunoglobulin class, IgA, IgM, and IgG, without distinguishing between subclasses. Separate testing can be performed to measure immunoglobulin subclasses and/or to detect and measure specific antibodies. A variety of conditions can cause an increase (hypergammaglobulinemia) or decrease (hypogammaglobulinemia) in the production of immunoglobulins. Some cause an excess or deficiency of all classes of immunoglobulin while others affect only one class. Some of the conditions are passed from one generation to the next (inherited) and others are acquired.

[1] (Lymphomation. org).http://www. Lymphomation. org/tests-immunoglobulins.htm

5.2 Structure and classes of Immunoglobulins

Plasma cells produce immunoglobulins which are composed of heavy and light chains. Each plasma cell produces only one type of heavy chain (IgA, IgD, IgG, IgM and IgE) and one type of light chain (either kappa or lambda [κ or λ]). After the chains are produced they are assembled within the plasma cell to form a whole Immunoglobulin. Serum protein electrophoresis separates serum proteins into the following fractions: albumin, alpha, beta and gamma globulins. Approximately 60% of the total protein in the serum is albumin, while the remaining fractions are composed mainly of globulins, predominantly Immunoglobulins. The gamma fraction contains the largest portion of immunoglobulins; hence an increase in gamma globulin is referred to as a gammopathy (Busher, 1990).

Each immunoglobulin is composed of two heavy chains that are of the same class and two light chains that are also alike. Each heavy chain has a variable region, in which amino acid substitutes make each chain different from the next and a constant region, in which there are very few amino acid differences from the constant region of any other immunoglobulin of that heavy chain type. Light chains are of either γ or κ type and have constant and variable regions. There are five classes of immunoglobulins are named by capital letters that correspond to their heavy chain types: IgG (γ-heavy chain), IgA (α-chain), IgM (μ-chain), IgE (ε-chain) and IgD (δ-chain). Each class represents a group of antibodies and has a slightly different role. Three-fourths of the immunoglobulins in normal serum is of the IgG type and has also several subclasses.

5.2a Immunoglobulin G (IgG) – About 70-80% of the immunoglobulin in the blood is IgG. IgG antibodies form the basis of long-term protection against microorganisms. Specific IgG antibodies are produced during an initial infection or exposure, rising a few weeks after it begins, then decreasing and stabilizing. The body retains a catalog of IgG antibodies that can be rapidly reproduced whenever exposed to the same antigen. In those with a normal immune system, sufficient IgG is produced to prevent re-infection. Vaccinations use this process to prevent initial infections and add to the catalog of IgG antibodies, by exposing a person to a weakened, live microorganism or to an antigen that stimulates recognition of the microorganism. IgG is the only immunoglobulin that can pass through the placenta. The mother's IgG antibodies provide protection to the fetus during pregnancy and to the baby during its first few months of life. Immunoglobulin G (IgG) or gamma globulins (with it 4 sub classes viz. IG1, IG2, IG3 & IG4) carry long term immunities. Approximately 67% of serum IgG is IgG_1, 20-25% is IgG_2, 5-10% is IgG_3 and 5% is IgG_4. The concentrations of IgG subclasses are physiologically varied with age; IgG_1 reached adult levels by 1 to 4 years of age, whereas IgG2 level normally begins to rise later in childhood compared to other subclasses[2]. It has been shown that low IG_1 concentration is associated with primary and secondary immune deficiency status. Low IG2 is associated with an increased risk of bacterial infection. Furthermore, IG1 and IG_3 are known to activate complement system efficiently whereas IG2 and IG4 are poor complement activator and even IG4 may itself inhibit complement activation.

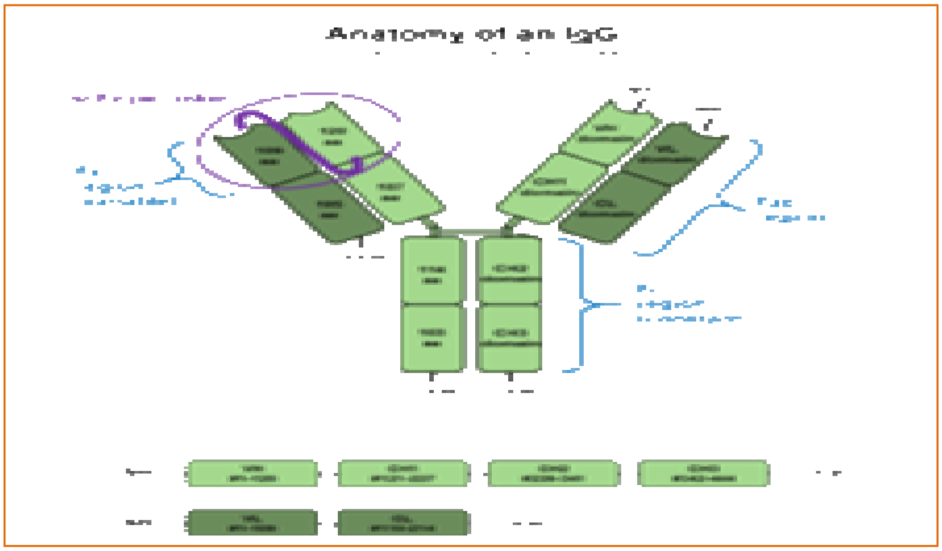

Figure-5.1: Immunoglobulin G (IgG)

***5.2b Immunoglobulin A (IgA)* –** IgA comprises about 15% of the total serum immunoglobulins but is also found in saliva, tears, respiratory and gastric secretions, and breast milk. IgA provides protection against infection in mucosal areas of the body such as the respiratory tract and the gastrointestinal tract. When passed from mother to baby during breast-feeding, it helps protect the infant's gastrointestinal tract. Significant amounts of IgA are not present until after 6 months of age. There are two IgA subclasses: IgA_1 and IgA_2. Infants who are breast-fed acquire IgA from breast milk. The IgA in breast milk can be protective against infections, particularly in the time between the decrease of mother's antibodies and the production of the baby's own antibodies.

5.2b-1 Clinical importance of IgA

Our body is protected at surfaces (i.e. mucosal surfaces which include mouth, ears, sinuses and nose, throat, airways within the lung, gastro intestinal tract, eyes and genitalia) that come into contact with the environment. IgA antibodies are transported in secretions to mucosal surfaces and play a major role in protecting these surfaces from infection though other immunoglobulin classes are also found in secretion at mucosal surface, but not in nearly the same amount as IgA.

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[2] http://www.hawaii.edu/medicine/pediatrics/pedtext/s05c05.html 29-08-2015); Nobel medicus

This is why IgA is known as the “secretory Antibody”. If our mucosal surface spread out they would cover an area equal to one and one half tennis courts, so the importance of IgA in protecting our mucosal surfaces cannot be overstated[3].

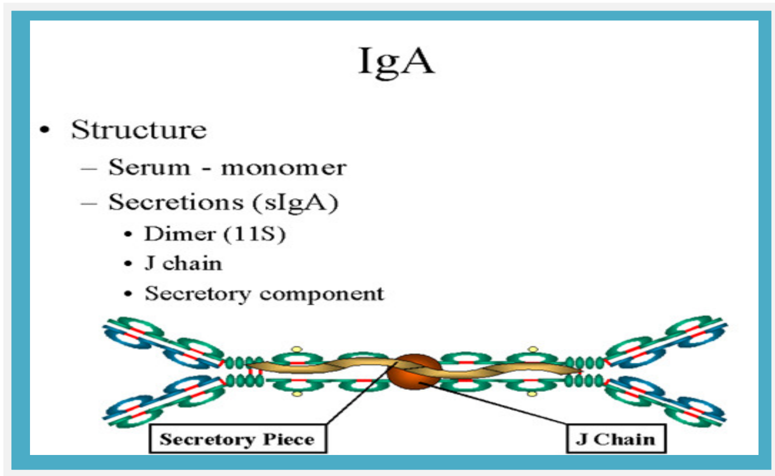

Figure-5.2: Immunoglobulin A (IgA)

5.2b-2 Special chemical characteristics of IgA

IgA has special chemical characteristics. It is present in secretions as two antibody molecules attached by a component called the J chain (‘J’ for joining). In order for these antibodies to be secreted, they must also be attached to another molecule called the secretory piece. The IgA unit that protects the mucosal surfaces is actually composed of two IgA molecules joined by the “J chain” and attached to the “secretory piece”

***5.2c Immunoglobulin M (IgM)* –** Immunoglobulin M, or IgM for short, is a basic antibody that is produced by B cells as a body's first response to a new infection, providing short-term protection. They increase for several weeks and then decline as IgG production begins. IgM can be produced by a fetus but, due to the size of the IgM molecule, it does not pass through the placenta. IgM antibodies in a newborn can indicate an infection that began during pregnancy.

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3 http://primaryimmune.org/about-primary-immunodeficiency-diseases/types-of-PIDD/selective. Immune Deficiency Foundation (IDF).

IgM remains in the bloodstream where it can kill bacteria that enter the bloodstream. IgM in normal serum is often found to bind to specific antigens, even in the absence of prior immunization. For this reason IgM has sometimes be called a "natural antibody". This phenomenon is probably due to the high avidity (strength of binding immunoglobulin or Ab to an antigen) of IgM that allow it to bind detectably even to weakly cross-reacting antigens that are naturally occurring in nature[4] .

IgM (Immunoglobulin M) antibody molecule consisting of 5 units. **1: Base unit**. **2: Heavy chains**. **3: Light chains** **4: J chain**. **5: Intermolecular disulfide bonds**.

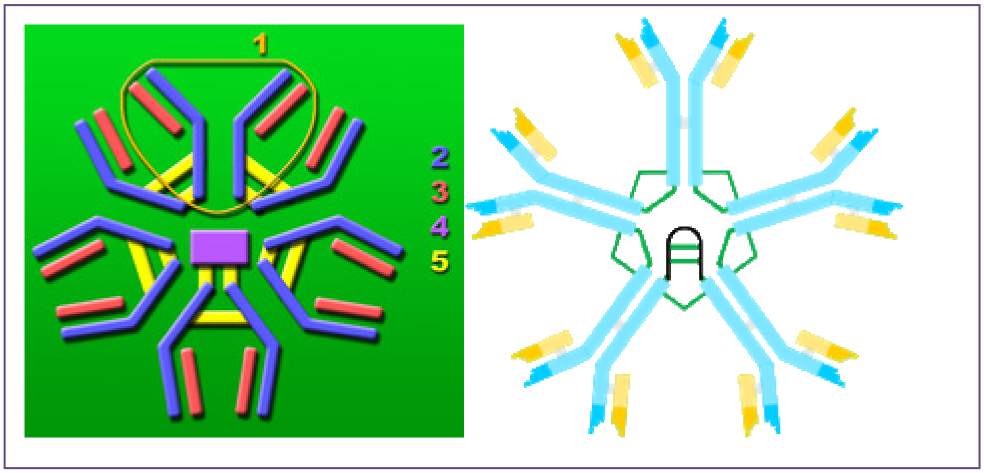

Figure-5.3: IgM Heavy **chains are blue**; **light chains are orange**.

5.2d Immunoglobulin D (IgD) – the role of IgD is not well defined and IgD is not routinely measured.

5.2e Immunoglobulin E (IgE) – IgE is associated with allergies, allergic diseases, and with parasitic infections. It is almost always measured as part of an allergy testing blood panel, but typically is not included as part of a quantitative immunoglobulins test.

5.2f Malnutrition and immunity

Nutritional status is critically important for immune cell function (Valerie et al, 2014). Nutritional deficiencies or malnutrition is the most common cause of immunodeficiency (Varella et al, 1997). Nutritional deficiencies such as CED and PEM have been reported to impair essentially all forms of immune function (Andrew et al, 2012; Varella et al, 1997; Chandra, 1997; Scrimshaw and SanGiovanni, 1997; Islam et al, 2004), making susceptible to infection including hepatitis, HIV and STIs. Immunodeficiencies further produce favorable conditions for triggering other clinical

[4] Lymphomation. org http://www. Lymphomation. org/tests-immunoglobulins.htm

complications (Varella et al, 1997). T cell immunity is much more adversely affected by malnutrition than is B cell immunity (Torosian, 1995).

5.2f-1 Role of T cells in malnutrition: T cells play a central role in regulating both the humoral (antibody) and cell-mediated arms of the adaptive (acquired) immune system (Levinson and Jawetz, 2005). Malnutrition can result in immune cell detects and increased risk of mortality from infectious disease. T cells play an important role (and also opposite) in the immune adaptation to both obesity and malnutrition. T cell in obesity have been shown to have an early and critical role in inducing inflammation, accompanying the accumulation of inflammatory macrophages in obese adipose tissue, which are known to promote insulin resistance. Conversely, T cell numbers is decreased in malnourished individuals and T cells in the setting of malnutrition have decreased effector function and proliferative capacity. The **adipokine leptin** which is secreted in proportion to adipocyte mass, may have a key role in mediating adipocyte-T cell interactions in both obesity and malnutrition, and has been shown to promote effector T cell function and metabolism while inhibiting regulatory T cell proliferation. Additionally, key molecular signals are involved in T cell metabolic adaptation during **nutrient stress**; among them, the metabolic regulator AMP kinase and the mammalian target of rapamycin have critical roles in regulating T cell number, function, and metabolism. In summary, understanding how T cell number and function are altered in obesity and malnutrition will lead to better understanding of and treatment for diseases where nutritional status determines clinical outcome (Valerie et al, 2014).

5.2g Fat and immune system: Several experimental study has consistently pointed to serum 'fatty acid' as an important inducing molecule of the innate immune system through activation of toll-like receptors (TLR)[5]. Recent study (Arai et al, 2013) has also suggested that fatty acid activates B cell TLR4 and that there is a requirement for B cell TLR stimulation by fatty acid for increased IgM.

5.2h Factors influence serum immunoglobulin

There are numerous conditions and diseases that can cause increased or decreased production of immunoglobulins (Igs). Determining the distribution of immunoglobulin levels in general populations is important for interpreting reference values. The common guidelines for definition and determination of reference intervals in the clinical laboratory note that partitioning should be considered when there are significant differences among subgroups defined by age, sex and common exposures such as smoking or alcohol consumption (Wayne PA, 2000. Horn and Pesce, 2003. Sasse, 1992). Earlier (Shulman et al, 1975) Study on serum immunoglobulin, emphasized to consider the ranges of values for any individual's age, sex, race and environment when interpreting results of serum immunoglobulin assay. Environmental conditions and other factors,

[5] www.plosone.org

persistent or changing, had also been suggested as reason for the variation. However, studies focusing upon the possible influencing factors i.e. demographic factors (age, sex), common environmental exposures (i.e. alcohol consumption, smoking), and metabolic abnormalities and inflammation markers (IL-6) on serum immunoglobulin levels of normal population are scarce (Gonzalez-Quintela et al, 2008). Immunoglobulin levels were influenced by age, sex, environment, race and genetic factors (Lugada et al, 2004).

Age: A half century old study (Veys and Claessens, 1968) substantiated the fact that serum Immunoglobulin levels were influenced by age and recommended to compare the Immunoglobulin levels in individuals of the same age but not with age category. Old study Shulman et al (1975) also showed a progressive rise in IgA with increasing age. Immunity is less than normal at both ends of life, i.e. in the newborn and the elderly (Lervinson and Jawetz, 2005)

Sex: Sex differences in immunoglobulin concentration especially high IgM levels in females have been attributed to hormonal (stimulatory effect of estrogen) effects on B lymphocytes (Gonzalez-Quintela et al, 2007; Bouman et al, 2005). Sex difference with higher IgM levels in women was also confirmed by Shulman et al (1975).

Race: race was independently associated with alterations in serum IgG levels (but not IgA & IgM) and blacks had significantly higher IgG levels (15.87 vs. 12.09 g/L; $P<0.001$) than White (Tollerud et al, 1995). All serum Immunoglobulin values in black adults were much higher than in whites reported previously by Shulman et al (1975). Milner (1971) also reported racial variation.

Genetic and other factors: The mean levels of IgM, IgG and IG1 in HIV-negative and positive African subjects were generally higher than those in comparable western subjects (Lugada et al, 2004). Variation in immunological profile may be genetically determined. Ethnic variations were found in a study conducted in the USA. Variations may arise from numerous antigenic challenges especially in the tropics from chronic viral and parasitic antigenic exposure. This may result in chronic stimulation of B cells and increased production of Igs even in HIV-negative individuals. Common viral infections start with local invasion of epithelial surfaces which initially induces production of interferon and secretary IgA from these surfaces (Roitt, 1994). Study indicated that resistance to HIV infection in exposed sero-negative persons involved cell-mediated and HIV-specific mucosal and systemic immune responses (Chris et al, 1999).

5.3 Methods and Materials

5.3a Selection criterion of serum samples for the estimation of serum IgG, IgA, and IgM

After screening of all blood samples (n=495) for STIs (HIV[6], Hepatitis B, and syphilis), 107 blood samples (hotel-based 19/107 and floating 88/107) comprising 68.2% (n=73) infectious samples and 31.8% (n=34) non-infectious samples were purposively selected for the detection of serum IgG, IgA, and IgM.

5.3b Instrumentation and reagents

"Turbi-quick compact semi-automated Analyzer"[7] (Vital Diagnostics, Foril, Italy) was employed for the quantitative immunoturbidimetric estimation of serum IgG, IgA and IgM of CSWs. Standard Kits (Immunoglobulins IgG, IgA, and IgM, Ref ACC16-009, Lot 179, 2014-01, Vital Diagnostics S.r.l. via Balzella 41/G/4, 47122 Foril, Italy) were used for the analysis of immunoglobulins in the serum samples of CSWs.

Image-1: Semi-automated 'Turbiquick' machine and Cuvette

[6] No HIV positive serum was detected in this study

[7] pdf.medicalexpo.com/pdf/vital-diagnostics/tur ; www.akralab.es>vital_diagnostics>catalogo ; elamallab.com; www.biotec co.com>pdf>turbiquick ; www. antonioserioprodottichimici.it>files; www.frankshospitalworkshop.com>vital

Image-2: Smart card' of Semi-automated 'Turbi-quick' machine and 'Turbi-kits' for the estimation of serum immunoglobulin M.

Image-3: One 'Pre-Incubation hole' (arrow) and 4 reading channels of the 'Turbi-quick machine'.

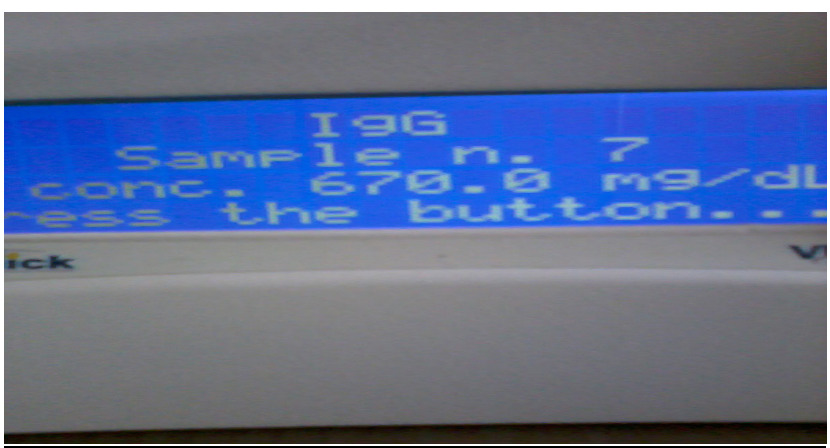

Image-4: Displaying result (serum concentration of IgG mg/dl) by 'Turbi-quick machine'

5.3c Principle and description of the immunoglobulin kits

Quantitative immunoturbidimetric estimation of serum IgG, IgA and IgM were based on Turbidimetry principle: the attenuation of the intensity of the light through a solution containing Antigen-Antibody aggregates is proportional to the antigen concentration.

Reagent R1: Tris buffer 20 mmol/L, PEG 8000, P^H 8.2. Sodium azide 0.95 g/L

Antibodies R2: Goat serum, Anti–human IgG, IgA and IgM, PH 7.5. Sodium azide 0.95 g/L (for each respective immunoglobulin)

Saline solution: Nacl 9% (for samples pre dilution)

Smart card: 'Turbi-quick' reader works only in association with 'Turbi-kits'. 'Turbi-kits' is the reagents needed to perform analysis on the instrument and inside each turbi-kit box, there is a 'Smart Card'. The digital 'smart card' must be inserted in the 'smart card slot', located in the rear panel of the instrument. Without the smart card, the instrument doesn't work. In the **smart card's chip** in fact, the following data are stored:

Calibration curve: in each card the 'calibration curve' of the test is stored, and hence this test doesn't need to construct 'calibration curve'.

Test settings: reading time, mixer behavior, and kind of filter are already stored in the card in order to avoid incorrect readings due to wrong filter, mixing or reading time setting errors

Expiry date: the expiry date of the kit is stored on the card in order to avoid the use of expired kits

Number of the test: the number of the tests performable with kit, is stored in the card, this counter will decrease each time the user performs one test.

5.3d Estimation procedure of serum IgG

1. Before switching on the turbiquick machine the Filter[8]♦ was set on position "A" according to the instruction leaflet supplied with kit.
2. "Digital smart card" was inserted (supplied with kit) into the machine before switch on which was showed first the 'expiry date' of the kit whenever the switch was on.
3. Serum sample was vortexed before pipetting 25 µl serum into respective ependorp tube
4. About 1000 µl 9% normal saline (supplied with kit) was then added into ependorp tube [25 µl serum+1000 µl Nacl 9 g/L (1:41)] and vortex for well mixing.

♦ Filter "A" was used for the detection of both IgG and IgA and filter "B" was used for only IgM detection.

5. About 400 µl of R_1 was pipetted into another fresh, plastic Cuvette with tiny magnetic stirrer (supplied from respective kit's company for one time use only).
6. Now 50 µl of pre-diluted serum sample was pipetted from ependorp tube to respective plastic cuvette with stirrer.
7. Then plastic cuvettes with stirrer (bearing sample numbers) were placed (maximum 1-4 positions) in the respective 'incubation holes' of the semi-automated machine and incubated for at least 1 minute.
8. After ≥ 1 minutes incubation one by one plastic cuvette with stirrer was transferred in the 'reading channel' (other than 4 holes for incubation), after slightly pressing the switch the screen of the machine was then counting for 5 seconds, and when requested on the display "pour R2", 100 µl R2 was pipetted immediately into the cuvette with very intensive care followed by the stirrer began to stirred for few seconds (which was also displayed on the screen by the message "stirring") and after mixing with R2 the amount of the respective serum immunoglobulin G (IgG) in the blood serum was displayed digitally (mg/dl) within 120 seconds of time.

5.3e Estimation procedure of serum IgA

1. Filter was set on position A according to the instruction leaflet supplied with kit.
2. "Digital smart card" was inserted (supplied with kit) into the machine before switch on which was showed first the 'expiry date' of the kit whenever the switch was on.
3. Serum sample was vortex before pipetting 25 µl serum into respective ependorp tube
4. About 500 µl of 9% normal saline was then added into ependorp tube [25 µl serum+500 µl Nacl 9 g/L (1:21)] and vortex for well mixing.
5. About 400 µl of R_1 was pipetted into another fresh, plastic Cuvette with tiny magnetic stirrer (supplied from respective kit's company for one time use).
6. Now 50 µl of pre-diluted serum sample was pipetted from ependrop to respective plastic cuvette (with stirrer).
7. Then plastic cuvettes with stirrer (bearing sample numbers) were placed (maximum 1-4 positions) in the respective 'incubation holes' of the semi-automated machine and incubated for at least 1 minute.
8. After ≥ 1 minutes incubation one by one plastic cuvette with stirrer was transferred into the 'reading channel' (other than 4 holes for incubation), after slightly pressing the switch the screen of the digital machine was then counting for 5 seconds, and when requested on the display "pour R2", 100 µl of R2 was immediately pipetted into the cuvette with very intensive care followed by the stirrer began to stirred for few seconds (which was also displayed on the screen by the message "stirring") and after mixing with R2 the amount of the respective serum immunoglobulin A (IgA) in the blood serum was displayed digitally (mg/dl) within 120 seconds of time.

5.3f Estimation procedure of serum Ig M

1. At first, Filter of the machine was set on position “B” according to the instruction leaflet supplied with kit.
2. “Digital smart card” was inserted (supplied with kit) into the machine before switch on which was showed first the ‘expiry date’ of the kit whenever the switch was on.
3. Serum samples were vortex before pipetting 25 µl serums into respective ependorp tube.
4. About 250 µl 9% normal saline was then added into ependorp tube [25 µl serum+250 µl Nacl 9 g/L (1:11)] and vortex for well mixing.
5. About 400 µl R_1 was pipetted into another fresh, plastic Cuvette with tiny magnetic stirrer (supplied from respective kit’s company for one time use).
6. Now 50 µl pre-diluted serum sample was pipetted from ependorp to respective plastic cuvette (with stirrer).
7. Then plastic cuvettes with stirrer were placed (maximum 1-4 positions) in the respective ‘incubation holes’ of the digital machine and incubate for at least 1 minute.
8. After ≥ 1 minutes incubation one by one plastic cuvette with stirrer was transfered in the ‘reading channel’ (other than 4 holes for incubation), after slightly pressing the switch the screen of the digital machine was then counting for 5 seconds, and when requested on the display “pour R2”, 100 µl of R2 was immediately pipetted into the cuvette very carefully followed by the stirrer began to stirred for few seconds (which was also displayed on the screen by the message “stirring”) and after mixing with R2 the amount of the respective serum immunoglobulin M (IgM) in the blood serum was displayed digitally (mg/dl) within 120 seconds of time.

5.3g Data analysis

Normality test: various statistical tools were used employing SPSS for the analysis of serum Immunoglobulins. (IgG, IgA & IgM). Before performing statistical analysis, “Normality” test of these data set were performed by “Shapiro-Wilk (S-W)” goodness of fit test (IgG-statistics=.477, df=107, P=.000; IgA-statistics=.482, df=107, P=.000; IgM-statistics=.544, df=107, P=.000). It was observed that serum “Immunoglobulins” data were **“positively”** and **“extremely”** skewed (IgG-skewness=3.569, SE=.234; IgA-skewness=4.276, SE=.234, IgM-skewness=2.728, SE=.234) and both “logarithmic or Log_{10}” (IgG-skewness=2.344, SE=.234; IgA-skewness=2.506, SE=.234, IgM-skewness=2.137, SE=.234) and “Square root transformation” (IgG-skewness=1.436, SE=.234; IgA-skewness=2.007, SE=.234, IgM-skewness=1.833, SE=.234) was performed to see which process could eliminate the skewness in a better way. And thus, approximation to a normal distribution was greatly improved for serum IgG, IgA & IgM data by **“Square root transformation”**. After “Square root” transformation, mean as well as other **“parametric tests”** on the transformed scale data was performed and then back transformed by taking the “square” for all IgG, IgA & IgM data and at the same time previous “Square root transformed” values were backed in the usual

IgG, IgA & IgM units (µmol/L). However, either the 'Square root' or 'log transformation' '**geometric mean**' was always less than the **"arithmetic mean"** of raw data (Bland and Altman, 1996). In fact, extreme observations of a "highly skewed data set" have a large influence on the arithmetic mean, making it more prone to sampling error. Thus, lessening this influence is one advantage of using transformed data (Bland and Altman, 1996).

5.3g-1 Statistical analysis

All statistical analyses of serum immunoglobulins data were assessed by using Statistical Package for Social Science version 17.0 (SPSS Inc, Chicago, IL, USA). Two-tailed P values <0.05 were considered statistically significant.

Univariate analysis of the samples: Descriptive statistics (Proportions, means, and SDs) were used to calculate baseline information. Descriptive data were presented as the mean (range) for continuous variables and as percentages for categorical variables.

Bivariate analysis: Bivriate analysis using Pearson's correlation to assess extent of linear correlation between two continuous variables. For participant characteristics analysis, differences in serum Immunoglobulin levels (dependent continuous variables) were examined in respect to socio-demographic, lifestyle and sexual variables (independent categorical variables) using ANOVA (comparing mean Immunoglobulin among categories).

Multivariate analysis: multivariate analysis was done to understand the association of different independent variables with dichotomous dependent variable. A multiple logistic regression model, with the outcome variable dichotomized (0, 1) as higher IgG (12.1 to 33.0 g/L) or lower IgG (00-4.99 g/L) was developed to examine the strength of relationship between dependent variable IgG with independent categorical variables. In the MLoR model, generally, dummy variables were created for non-binary categorical variables; the effect of each category of a multi-categorical variable was assessed by keeping the first or last category as reference category. All Covariates (socio-demographic, lifestyle and sexual variables as well nutritional indices) which were found statistically significant ($P \leq 0.05$) or borderline significant ($P < 0.09$) in simple logistic regression (SLoR) analysis were introduced simultaneously in the **multiple logistic regression analysis.** The model was developed by 'backward stepwise elimination', removing the covariate with largest P value at each step until the remaining variables were significant at the 0.05 level in the final adjusted model. Also, forward stepwise technique applied in same way to confirm the model. Thus relative odds (ORs) and 95% CI were calculated to identify potential risk factors associated with immunoglobulin deficiency [0=higher IgG (12.1 to 33.0 g/L) and 1=lower IgG (00-4.99 g/L)]. Hosmer-Lemeshaw goodness of fit and Nagelkarke-pseudo R^2 of the model was also observed.

5.4 Results

5.4.1 Immunglobulin profile of commercial Sex workers (CSWs)

Mean IgG, IgA, and IgM values were 2.73 g/L (95% CI 1.52–3.93), 0.50 g/L (95% CI 0.33–0.68) and 0.42 g/L (95% CI 0.28–0.55) respectively. In fact, mean serum Immunoglobulin G levels (IgG) was observed remarkably low in its lower tail value (almost half) whenever compared with the standard normal values (IgG=5.0-12.0 g/L) (Young, 1998) and IgA level has just touched the lower tail of the standard normal values (IgA=0.5-3.5 g/L). However, IgM was the only serum antibody (0.42 g/L) that remained within normal range (IgM=0.3-2.3 g/L). Moreover, Frequency distribution' of serum Immunoglobulin levels showed that most CSWs (IgG=85.1%, IgA=79.5% & IgM=67.29%) had very low serum Immunoglobulin level and serum IgG was predominantly affected, IgA and IgM was the next affected Immunoglobulins. Conversely, 6.5% (07/107), 2.8% (n=03/107) and 7.5% (n=08/107) CSWs had elevated serum IgG (12.1-33 g/L), IgA (3.51- 5.75 g/L) and IgM level (2.61-2.85 g/L) respectively (table-1).

Comprehensive analysis of serum Immunoglobulin (Igs) including IgG (table-2) outlined that 69.16% CSWs (n=74) had profoundly reduced serum IgG (<1.00 g/L), 11.12% (n=12) had mild to moderate (3.0-5.0 g/L) serum IgG and 8.4% (n=16) had normal IgG level.

5.4.2 Influence of Socio-demography, business location and STIs status on serum immunoglobulin levels

Serum IgM of CSWs was affected significantly by their marital status (F (2.104) =4.545, P=.013*), STI status (F (1,105) =5.729, P=.018*) and sex business location (F (1,105) =6.204, P=.014*). Unmarried CSWs had higher level of serum IgM than married and married were more likely to have serum IgM than widowed/divorced/abandoned. Both serum IgA and IgM was positively influenced by CSW's 'STI status' but serum IgM level achieved only statistically significant status (P=.018*) and in case of IgA, it's significant status was borderline (P=.053, F(1,105)=3.830). Also, sex business location influenced serum IgM level, hotel-based CSWs (0.78 g/L) had higher level (P=.014*) of serum IgM than floating CSWs (0.35 g/L). Moreover, serum IgA was affected by "money spent on STI treatment", it was observed that CSWs who spent more taka (251-5000 BDT) had higher serum IgA than who spent less taka (5-250 taka) on STI treatment (F (2,104) =5.155, P=.009*). However, no influences of present age, education, monthly income-expenditure, STI status, sex business location and BMI of CSWs on serum Immunoglobulin G (IgG) were noticed (table-3).

5.4.3 Influence of lifestyle factors of CSWs on serum immunoglobulin levels

Most of the lifestyle factors of CSWs did not influence serum Immunoglobulins status. However, a trend of decreasing immunoglobulin M (IgM) with increasing professional length of CSWs was exhibited borderline significance (P=.058). Similar finding was also noticed in case of “habit of smoking” (P=.060) (table-4).

5.4.4 Influence of risky Sexual Behaviors of CSWs on serum immunoglobulin levels

“Condom use” and “experienced ever in group sex” was negatively related with serum IgA status, in both cases, CSWs who either used condom consistently (F (1,105) =5.929, P=.017*) or experienced Gr. Sex (F (1,105) =4.597, P=.034*) had lower serum IgA. Similarly, ‘condom use’ (F (1,105) =3.961, P=.049*) and usual “coital frequency per client” were negatively influenced serum IgM level though latter one was borderline significant [F (1,105) =2.860, P=.054] (table-5).

5.4.5 Multiple logistic regression (MLoR) models of serum immunoglobulin

To observe the association of different variables with three types of serum immunoglobulins (IgG, IgA and IgM) multiple logistic regressions (MLoR) analysis was done by applying ‘backward stepwise’ MLoR analysis. All categorical variables which were found statistically significant (P≤ 0.05) or borderline significant (P<0.09) in ‘simple logistic regression’ model or in ANOVA were included together in the **multiple logistic regression analysis.** “Hosmer-Lemeshaw goodness of fit” test was done.

5.4.5a MLoR model of IgG: According to MLoR model of IgG (table-6), having children, ‘<normal serum zinc’ and ‘below normal serum IgM’ were significantly remained in both models and associated with (all P<0.05) higher odds of the outcome (i.e. less than normal serum immunoglobulin G).

5.4.5b MLoR model of IgM: MLoR model of IgM (table-7) postulated that ‘infected with syphilis’, ‘<normal serum IgG’ and ‘below normal serum IgA’ were significantly associated with lower serum IgM of CSWs.

5.4.5c MLoR model of IgA: According to MLoR model of IgA (table-8), ‘STI status’, ‘use of condom’ and ‘ever experienced in group sex’ were associated with low serum IgA level of CSWs.

Table-1: serum immunoglobulin level of CSWs (n=107)

Immunoglobulin (n=107)		All CSWs	Range	Mean ±SD	Mean (95%
Levels	Amount (g/L)	% n	Min-max		Lower–upper
	$^{\phi}$IgG (g/L)				
Very low	0 .00 to 0.49	66.4 (71)			
Low	0.50 to 4.99	18.7 (20)	0.04 to 33.0	2.73±1.14	1.52–3.93
Normal	5.00 to 12.0	8.4 (09)			
High	12.1 to 33.0	6.5 (07)			
	IgA (g/L)				
Very low	0.0 to 0.35	64.5 (69)			
Low	0.36 to 0.49	15.0 (16)	0.00 to 5.75	0.50±0.19	0.33–0.68
Normal	0.50 to 3.50	17.8 (19)			
High	3.51 to 5.75	2.8 (03)			
	IgM (g/L)				
Very low	0.02 to 0.29	67.29(72)			
Normal	0.30 to 1.25	25.2 (27)	0.02 to 2.85	0.42±0.16	0.28–0.55
High	2.61 to 2.85	7.5 (08)			

Human normal serum IgG, IgA, and IgM are 5.0-12.0, 0.5-3.5 and 0.3-2.3 g/L respectively (Young, 1998)

$^{\phi}$ Strong Correlation was found between serum IgG and IgM (r=.488, P=.000) but not in case of serum IgG and IgA or IgA and IgM.

Table-2: Comprehensive analysis of serum immunoglobulins including IgG

♣level of Serum IgG (g/L)		Immunoglobulin level possessed by FCSWs n (%)
Mild-moderate (3.0-5.0 g/L)		12 (11.22)
Significant (2.00-2.99 g/L)		01 (0.94)
Reduced (<2.0 g/L)		04 (3.74)
Profoundly reduced (<1.00 g/L)		74 (69.16)
Adequate level of IgG	6.1-8.0 g/L	06 (5.61)
	9.0-12.0 g/L	03 (2.80)
Elevated	12.1 -15 g/L	05 (4.67)
	17.0-33.0 g/L	02 (1.87)
Combined interpretation of IgG and total Igs (IgG + IgA+ IgM)♣		
♣Level of Total immunoglobulin (IgG + IgA + IgM) g/L		**Mean 3.64 (95% CI 2.34-4.95)**
No Humoral immunodeficiency (total Immunoglobulin >6.0 g/L)		10 (9.35%)
Adequate level (4.0-6.0 g/L)		18 (16.82%)
Low level <4.0 g/L		79 (73.83%)
Contain adequate amount of Abs (Total Immunoglobulin 4.0-6.0 g/L and or IgG levels 2.0 to 4.0 g/L)		n=16 (14.9%) CSWs
Low level (Total Immunoglobulin<4.0 g/L and or serum IgG <2.0 g/L)		n=91 (85.1%) CSWs
	Total	n=107 (100%)

♣Reference: Agarwal and Cunningham-Rundle, 2007

Table-3: Influence of socio demography, STIs status, business places, and BMI of CSWs on serum Immunoglobulins.

Socio-demographic factors	All CSWs n=107 % n	IgG [1] (gm/L)	IgA [2] (gm/L)	IgM [3] (gm/L)	ANOVA
Present age (y) [a]					
<18	12.15 (13)	2.42±1.14	0.49±0.02	0.56±0.09	a1 F (2,104) =.655, P=.521,
19–29	45.79 (49)	3.47±1.07	0.50±0.17	0.50±0.13	a2 F (2,104) =.006, P=.994
≥30	42.06 (45)	2.0 ±1.15	0.51±0.16	0.28±0.12	a3 F (2,104) =1.393, P=.253
Education [b]					b1 F (1,105) =1.130, P=.290
No literacy/can sign	77.57 (83)	2.38±1.00	0.54±0.12	0.39±0.10	b2 F (1,105) =.518, P=.473
# 1-5 class (primary)	22.43 (24)	3.93±1.05	0.38±0.13	0.52±0.07	b3 F (1,105) =.619, P=.433
Marital status [c]					c1 F (2,104) =.262, P=.770
Married	41.6 (38)	3.27±1.07	0.68±0.17	**0.39±0.10**	c2 F (2,104) =1.499, P=.228
Unmarried	13.7 (09)	3.01±1.02	0.17±0.12	**1.08±0.04** [t]	**c3 F(2,104) =4.545, P=.013***
Widow/divorced /abandoned	44.7 (60)	2.34±1.01	0.44±0.17	**0.34±0.11**	
Monthly income [d]					d1 F (2,104) =.213, P=.808
0.00-2500 taka	49.53 (53)	2.52±1.14	0.46±0.10	0.40±0.13	d2 F (2,104) =.108, P=.898
2501-5000	29.91 (32)	3.33±1.09	0.54±0.14	0.38±0.16	d3 F (2,104) =.190, P=.827
>5001	20.56 (22)	2.34±1.05	0.54±0.16	0.50±0.13	
Last month's Total expenditure [e]					
1000-2500 taka	21.50 (23)	2.27±1.01	0.23±0.15	0.53±0.13	e1 F (2,104) =.183, P=.833
2501-5000	52.33 (56)	3.08±1.11	0.57±0.19	0.39±0.14	e2 F (2,104) =.990, P=.375
>5001	26.17 (28)	2.39±1.08	0.56±0.15	0.38±0.09	e3 F (2,104) =.390, P=.678
♣Treatment cost of STIs [f] (last ailment, n=60/107)					f1 F (2, 57) =.149, P=.862
Self treatment	30.0 (18)	3.26±1.12	**0.28±0.09**	0.32±0.09	**f2 F (2, 57)=5.155, P=.009***
5-250 taka	45.0 (27)	2.21±1.11	**0.20±0.07**	0.43±0.08	f3 F (2, 57) =3.199, P=.045
251-5000 taka	25.0 (15)	2.39±1.14	**1.00±0.16** [t]	0.39±0.16	
STIs status [g]					g1 F (1,105) =.777, P=.380
♦STIs	68.22 (73)	2.36±1.11	**0.62±0.19**	**0.30±0.09**	**g2 F (1,105) =3.830, P=.053**
No STIs	31.78 (34)	3.51±1.06	**0.25±0.09**	**0.65±0.16**	**g3 F(1,105) =5.729, P=.018***
Places of sex business [h]					h1 F (1,105) =.705, P=.403,
Hotel	17.76 (19)	3.83±1.03	0.52±0.09	**0.78±0.09**	h2 F (1,105) =.007, P=.933
*Floating	82.24 (88)	2.49±1.08	0.50±0.12	**0.34±0.08**	**h3 F(1,105) =6.204, P=.014***
BMI (kg/m²) [i]					i1 F (2,104) =1.881, P=.158
CED <16-18.4	31.78 (34)	1.40±1.12	0.48±0.13	0.30±0.11	i2 F (2,104) =.072, P=.931
Normal 18.5-25.0	51.40 (55)	3.86±1.05	0.53±0.17	0.46±0.16	i3 F (2,104) =.697, P=.500
Overweight >25.1	16.82 (18)	1.78±1.01	0.45±0.13	0.50±0.15	

[t] In post hoc test (TUKEY), 'MULTIPLE COMPARISON' showed this category (within respective variables) was significantly (P<0.05) different than other categories for having higher or lower value.

[#] None had secondary education (1-12 class) among 107 CSWs.

♣ No symptoms appeared in 37.38% (40/107) of CSWs.

♦ Syphilis Positive (+ve) / Hepatitis B Positive (+ve) / both Positive (+ve)

*Floating CSWs mainly worked on road or in perk or in shrine etc.

Table-4: Influence of lifestyle variables of CSWs on serum Immunoglobulins

Lifestyle factors	All CSWs n=107 % n	IgG [1] (gm/L)	IgA [2] (gm/L)	IgM [3] (gm/L)	ANOVA
Factors behind profession[a]	44.86 (48)	2.57±1.06	0.58±0.14	0.42±0.08	[a1]F (2,104) =1.728, P=.183
Lower Economic	25.23 (27)	4.49±1.09	0.56±0.05	0.55±0.07	[a2]F (2,104) =.847, P=.480
Exploited by pimps/other	29.91 (32)	1.47±1.02	0.34±0.09	0.30±0.12	[a3]F (2,104) =.956, P=.388
Working days (last week) [b]					
Did not work	5.61 (06)	3.36±1.12	0.27±0.14	0.68±0.13	[b1]F (2,104) =.172, P=.842
1–3 days	44.86 (48)	3.04±1.14	0.54±0.14	0.53±0.14	[b2]F (2,104) =.248, P=.781
4–7 days	49.53 (53)	2.37±1.15	0.49±0.13	0.29±0.10	[b3]F (2,104) =1.873, P=.159
Age entering into sex trade [c]	46.73 (50)	2.70±1.00	0.48±0.13	0.38±0.13	[c1]F (2,104) =.222, P=.801
10-17 y	33.65 (36)	3.18±1.07	0.65±0.06	0.50±0.07	[c2]F (2,204) =.947, P=.391
18-24	19.63 (21)	2.02±1.06	0.31±0.14	0.36±0.08	[c3]F (1,105) =.360, P=.699
Length of profession[d]					
<1 y	6.54 (07)	0.78±0.59	0.86±0.05	**0.63±0.16**	[d1]F (2,104) =1.780, P=.174
1-5	47.67 (51)	3.89±1.07	0.44±0.14	**0.55±0.07**	[d2]F (2,104) =.642, P=.529
≥6	45.79 (49)	1.79±1.09	0.52±0.12	**0.25±0.09**	**[d3]F (2,104) =2.756, P=.058**
living with[e]					
Own	57.01 (61)	1.97±1.12	0.34±0.11	0.37±0.08	[e1]F (2,104) =1.038, P=.358
Husband	30.84 (33)	3.58±1.09	0.73±0.14	0.42±0.14	[e2]F (2,104) =2.225, P=.113
Others♦	12.15 (13)	4.09±1.10	0.67±0.14	0.65±0.05	[e3]F (2,104) =.864, P=.425
Use of contraceptives[f]					
No measure	23.37 (25)	1.32±0.18	0.50±0.14	0.20±0.14	[f1]F (3,103) =.591, P=.621
Pill	17.76 (19)	2.66±1.08	0.33±0.13	0.65±0.05	[f2]F (3,103) =.296, P=.828
Condom	42.98 (46)	3.38±1.08	0.56±0.11	0.44±0.07	[f3]F (3,103) =1.505, P=.218
Others**	15.89 (17)	3.10±1.04	0.54±0.09	0.43±0.14	
Cases of abortion[g]					
No abortion cases	68.22 (73)	2.95±1.07	0.52±0.06	0.47±0.09	[g1]F (2,104) =.311, P=.734
1-2 cases	27.11 (29)	2.49±1.06	0.50±0.05	0.50±0.05	[g2]F (2,104) =.155, P=856,
>3 cases	4.67 (05)	0.74±0.13	0.28±0.08	0.28±0.11	[g3]F (2,104) =.592, P=.555
Habit of smoking[h]					
No	47.66 (51)	2.14±1.06	0.60±0.09	**0.55±0.06**	[h1]F (1,105) =.838, P=.362
Yes	52.34 (56)	3.26±1.16	0.41±0.08	**0.30±0.13**	[h2]F (1,105) =1.180, P=.280
					[h3]F (1,105) =3.351, P=.060
Substance abuse[i]					
No	70.09 (75)	2.76±1.08	0.50±0.19	0.47±0.08	[i1]F (1,105) =.008, P=.931
Yes	29.91 (32)	2.60±1.13	0.51±0.09	0.28±0.09	[i2]F (1,105) =.001, P=.975
					[i3]F (1,105) =1.587, P=.211
Types of substances[j]					
No drug	70.09 (75)	2.76± 1.07	0.50±0.09	0.47±0.08	[j1]F (3,103) =.753, P=.523
Ganja (cannabis)	7.48 (08)	0.81± 0.17	0.26±0.12	0.14±0.13	[j2]F (3,103) =.891, P=.448
Alcohol (local maod)	10.28 (11)	1.62±.1.00	0.30±0.07	0.41±0.11	[j3]F (3,103) =.750, P=.525
Others♣	12.15 (13)	4.64± 1.05	0.83±0.08	0.27±0.05	
Client profile [k]					
Businessmen (small)	36.45 (39)	1.56±0.72	0.45±0.09	0.44±0.12	[k1]F (5,101) =1.066, P=.384
Rickshaw puller	29.91 (32)	2.99±1.09	0.52±0.09	0.31±0.11	[k2]F (5,101) =.977, P=.435
Service holder	12.15 (13)	5.25±1.08	0.45±0.13	0.74±0.15	[k3]F (5,101) =.299, P=.553
Student	6.54 (07)	1.22±1.07	1.20±0.09	0.21±0.17	
Bus driver	2.80 (03)	0.24±0.10	0.52±0.14	0.29±0.11	
Didn't know	12.15 (13)	4.44±1.16	0.30±0.09	0.41±0.16	
NGO counseling on STIs[l]					[l1]F (1,105) =.853, P=.358
Yes	42.90 (46)	2.08±1.13	0.44±0.08	0.29±0.16	[l2]F (1,105) =.384, P=.537
No	57.10 (61)	3.21±1.08	0.55±0.11	0.51±0.09	[l3]F (1,105) =2.426, P=.122

*Angry with family members, sold by step mom/others, raped/tortured, pushed by husband, self motivation and drug addiction (2.1%).
♦Parents, siblings, sexual partners etc.
**Injection, ligation/tubectomy, menopause
♣Other abused cannabis (afim, charush), phensedyl, injection (pethidin) and Heroin etc.

Table-5: Influence of risky sexual behaviors of CSWs on serum Immunoglobulin.

Risky sexual behaviors	All CSWs n=107 % n	IgG [1] (gm/L)	IgA [2] (gm/L)	IgM [3] (gm/L)	ANOVA
Client turnover (last week)[a]					
Did not work	5.61 (06)	3.36±1.07	0.27±0.14	0.68±0.11	^{a1}F (2,104) =2.099, P=.128
1– 14 C	61.68 (66)	1.77±1.08	0.46±0.08	0.36±0.11	^{a2}F (2,104) =.511, P=.602
≥15 C	32.71 (35)	4.42±1.11	2.73±.0.07	0.49±0.16	^{a3}F (2,104) =.802, P=.451
Usual client per day[b]					
1-7	88.79 (95)	2.20±1.00	0.51±.07	0.37±0.16	^{b1}F (1,105) =6.104, P=.015
>8	11.21 (12)	6.86±1.07	0.43±0.14	0.78±0.06	^{b2}F (1,105) =.086, P=.771
					^{b3}F (1,105) =3.478, P=.065
Use of condom[c]					^{c1}F (1,105) =.470, P=.494
No use/occasional	56.08 (60)	3.10±1.09	**0.69±0.16***	**0.57±0.15**	**^{c2}F (1,105) =5.929, P=.017***
Consistent use	43.92 (47)	2.25±1.06	**0.26±0.12**	**0.30±0.12**	**^{c3}F (1,105) =3.961, P=.049***
Hygienic measure after coitus[d]					
No measure	35.51 (38)	3.93±1.04	0.33±0.13	0.37±0.33	^{d1}F (2,104) =1.216, P=.301
Used savlonl/soap	33.65 (36)	2.44±1.06	0.69±0.12	0.60±0.51	^{d2}F (2,104) =1.433, P=.243
Others*	30.84 (33)	1.65±1.11	0.49±0.09	0.27±0.13	^{d3}F (2,104) =1.984, P=.148
Interval between two coitus[e]					^{e1}F (1,105) =.016, P=.901
No	60.75 (65)	2.79±1.06	0.49±0.08	0.45±0.32	^{e2}F (1,103) =.011, P=.916
yes	39.25 (42)	2.63±1.06	0.51±0.09	0.36±0.21	^{e3}F (1,105) =.381, P=.538
Coital frequency per client[f]					
One time	48.60 (52)	2.67±1.09	0.44±0.11	**0.54±0.15**	^{f1}F (1,105) =.008, P=.929
≥2 times	51.40 (55)	2.78±1.06	0.56±0.08	**0.31±0.05**	^{f2}F (1,105) =.510, P=.477
					^{f3}F (1,105) =2.860, P=.054
Weekly coitus with •PC[g]					
No weekly PC	1.68 (66)	2.43±1.01	0.46±0.35	0.39±0.27	^{g1}F (2,104) =.349, P=.706
Yes, 1 time/week	16.82 (18)	3.83±1.11	0.55±0.33	0.31±0.23	^{g2}F (2,104) =.177, P=.838
2 times/week	21.50 (23)	2.71±1.05	0.58±0.44	0.59±0.49	^{g3}F (2,104) =.891, P=.413
Fortnightly coitus with PC[h]					
No fortnightly PC	80.37 (86)	2.47±1.06	0.49±0.13	0.41±0.10	^{h1}F (2,104) =.404, P=.669
Yes 1-2 times	17.76 (19)	3.91±1.09	0.60±1.13	0.32±0.12	^{h2}F (2,104) =.222, P=.801
>3 times	1.87 (02)	2.27±1.11	0.21±0.14	1.42±1.06	^{h3}F (2,104) =2.172, P=.119
Monthly coitus with PC[i]					
No monthly PC	74.77 (80)	2.37±1.13	0.51±0.17	0.35±0.06	^{i1}F (2,104) =.705, P=.497
Yes, 1-2 times	21.50 (23)	4.10±1.06	0.49±0.09	0.58±0.12	^{i2}F (2,104) =.005, P=.995
Yes, >3 times	3.73 (04)	1.93±0.92	0.48±0.17	0.83±0.13	^{i3}F (2,104) =1.670, P=.193
Having ♦NPSP[J]					
No	91.59 (98)	2.84±1.13	0.52±0.15	0.43±0.14	^{j1}F (1,105) =.372, P=.543
Yes	8.41 (09)	1.49±0.94	0.34±0.12	0.30±0.08	^{j2}F (1,105) =.304, P=.583
					^{j3}F (1,105) =.282, P=.596
Ever experienced in Group sex[k]					^{k1}F (1,105) =.055, P=.816,
No	74.1 (73)	2.82±1.07	**0.63±0.18***	0.46±0.15	**^{k2}F (1,105) =4.597, P=.034***
Yes	25.9 (34)	2.52±1.12	**0.23±0.09**	0.32±0.13	^{k3}F (1,105) =.299, P=.553
Gr. Sex history (last month)[l]					^{l1}F (1,105) =.885, P=.349
No	71.03 (76)	6.41±1.07	**0.60±0.16**	0.45±0.14	**^{l2}F (1,105) =3.009, P=.066**
Yes	28.97 (31)	6.03±1.08	**0.26±0.08**	0.33±0.06	^{l3}F (1,105) =.590, P=.444
Clients (n=31) in the last Gr. Sex[m] 2-5 clients	83.04 (22)	2.52±1.08	0.23±0.10	0.38±0.06	^{m1}F (1, 29) =1.008, P=.324
>6 clients	16.96(09)	0.13±0.11	0.34±0.11	0.40±0.07	^{m2}F (1, 29) =1.044, P=.315
					^{m3} F (1, 29) =.430, P=.517
Ever experienced in Anal sex[o]					^{o1}F (1,105) =.359, P=.550
No	96.4 (105)	2.78±1.05	0.51±0.13	0.42±0.12	^{o2}F (1,105) =.411, P=.523
Yes	3.6 (02)	0.07±0.00	0.09±0.03	0.03±0.00	^{o3}F (1,105) =.584, P=.446

* Washed by water, cotton, urine etc.

♦NPSP means non-paying sexual partner

•PC means Permanent client

Table-6: Multiple Logistic regression (MLoR) model of serum IgG

Explanatory variables	All CSWs	Outcome variable (IgG)		Logistic regression models of serum IgG		
Factors associated with lower serum IgG level	n=107 % n	0=≥normal serum IgG (µmol/L) n=16	1=<normal Serum IgG (µmol/L) n=91	Unadjusted Odds ratio (95% CI) (lower-upper)	Beta-coefficient (B) SE	Adjusted Odds ratio (95% CI) (lower-upper)
Having children						
No (r)	49.53 (53)	81.2 (13)	44.0 (40)	1	-	1
Yes	50.47 (54)	18.8 (03)	56.0 (51)	5.53 (1.47-20.72) **P=.011***	1.964 SE=.829	7.125 (1.404-36.167) **P=.018***
Serum Zinc (µ mol/L)						
< Normal (5.97 to 11.4)	37.4 (40)	21.4 (06)	43.0 (34)	6.65 (1.262-35.05) **P=.025**	2.294 SE=1.113	9.915 (1.120-87.753) **P=.039***
Normal (11.5 to 18.5)	37.4 (40)	39.3 (11)	36.7 (29)	1.65 (.504-5.40) P=.408	1.461 SE=.919	4.310 (.711-26.725) P=.112
>Normal (r) (18.6 to 99.30)	25.2 (27)	39.3 (11)	20.3 (16)	1	-	1
@*Serum IgM (g/L)*						
Below normal (0.02 to 0.29)	67.3 (72)	6.2 (01)	78.0 (71)	53.25 (6.63-428.02) **P=.000**	4.134 SE=1.143	62.442 (6.650-586.346) **P=.000****
≥Normal (0.30 to 2.85) (r)	32.7 (35)	93.8 (15)	22.0 (20)	1	-	1

Legend: Simple Logistic Regression (SLoR)
Backward stepwise Multiple Logistic Regression (MLoR)
Hosmer-Lemeshaw goodness of fit for IgG model: $\varkappa^2$=28.823, df=6, P=.947
-2 Log likelihood (-2LL)=46.046, Nagelkerke R-square=.594
Model coefficient :Chi-square ($\varkappa^2$)= 44.240, df=4, P=.000
Overall predicted percentage from Classification table of IgG (DV) was 94.9% (cut value is 50%).
(r)= reference category
P<0.05*, P<0.01**
@For obtaining greater cell numbers and small magnitude (or width) of 95% CI, serum immunoglobulins (IgG, IgA) categories were rearranged by adding “very low” and “low” frequencies together (and named below or <normal) and made ≥normal by adding “normal” and “high” frequencies together.

Table-7: Multiple Logistic regression (MLoR) model of serum IgM

Explanatory variables factors associated with low serum IgM level	All CSWs n=107 % n	Outcome variable (IgM) 0=≥normal IgM (µmol/L n=35	1=<normal Serum IgM (µmol/L) n=72	Logistic regression model of serum Immunoglobulin M: Unadjusted Odds ratio (95% CI) (lower-upper)	Beta coefficient (B) SE	Adjusted Odds ratio (95% CI) (lower-upper)
Infected with Syphilis						
No (r)	48.60 (52)	71.4 (25)	37.5 (27)	1	-	1
Yes	51.40 (55)	28.6 (10)	62.5 (45)	4.416 (1.74-9.99) **P=.001****	2.759 SE=.830	15.78 (3.103-80.28) **P=.001****
@*Serum IgG (g/L)*						
Below normal (0.0 to 4.99)	85.0 (91)	57.1 (20)	98.6 (71)	53.250 (6.63-428.06) **P=.000****	4.405 SE=1.261	81.864 (6.919-968.53) **P=.000****
≥Normal (5.00 to 33) (r)	15.0 (16)	42.9 (15)	1.4 (01)	1	-	1
@*Serum IgA (g/L)*						
Below normal (0.0 to 0.49)	79.44 (85)	54.3 (19)	91.7 (66)	9.263 (3.18-26.95) **P=.000****	3.123 SE=.870	22.710 (4.13-125.0) **P=.006****
≥Normal (0.50 to 5.75) (r)	20.6 (22)	45.7 (16)	8.3 (06)	1	-	1

Legend: Simple Logistic Regression (SLoR)
Backward stepwise Multiple Logistic Regression (MLoR)
Hosmer-Lemeshow goodness of fit for IgM model: $\aleph^2$ =2.183, df=3, P=.535
-2 Log likelihood (-2LL) =72.01, Nagelkerke R-square=.622.
Model coefficient :Chi-square ($\aleph^2$)=63.259, df=3, P=.000
Overall predicted percentage from classification table of IgM (DV) was 86.9% (cut value is 50%).
(r)= reference category, P<0.05*, P<0.01**
P<0.05*, P<0.01**

@For obtaining greater cell numbers and small magnitude (or width) of 95% CI, serum immunoglobulins (IgG, IgA) categories were rearranged by adding "very low" and "low" frequencies together (and named below or <normal) and made ≥normal by adding "normal" and "high" frequencies together.

Table-8: Multiple Logistic regression (MLoR) model of serum IgA

Explanatory variables factors associated with low serum immunoglobulin level	**All CSWs** **n=107** **% n**	0=≥normal serum IgA (µmol/L) n=22	1=<normal Serum IgA (µmol/L) n=85	**Logistic regression models of serum IgA** Unadjusted OR (95% CI) (lower-upper)	Beta	Adjusted Odds Ratio (95% CI) (lower-upper)
STI status						
No STI	31.78 (34)	13.6 (03)	36.5 (31)	3.636 (.996-13.3) **P=.051***	1.	4.633 (1.186-18.10) **P=.027***
Yes (Syphilis/HBV/ both) (r)	68.22 (73)	86.4 (19)	63.5 (54)	1		1
Use of condom						
Irregular (r)	56.08 (60)	86.4 (19)	48.2 (41)	1		1
Consistent	43.92 (47)	13.6 (03)	51.8 (44)	6.797 (1.871-24.7) **P=.004****	1.	4.937 (1.292-18.87) **P=.020***
Ever experienced in Group sex						
No (r)	74.1 (73)	90.2 (20)	62.4 (53)	1		1
Yes	25.9 (34)	9.1 (02)	37.6 (32)	6.038 (1.323-27.6) **P=.020***	1.7	6.036 (1.24-29.48) **P=.026***

Legend: Simple Logistic Regression (SLoR)
Backward stepwise Multiple Logistic Regression (MLoR)
Hosmer-Lemeshaw goodness of fit for IgA model: $\aleph^2$ =2.297, d.f=4, P=.681
-2 Log likelihood=86.616, Nagelkerke R-square=.293
Model coefficient :Chi-square ($\aleph^2$)=22.113, P=.000, df=3
Overall predicted percentage from classification table of IgA (DV) was 79.4% (cut value is 50%).
(r)= reference category
P<0.05*

5.5 Discussion

There is a dearth of data regarding immunoglobulin profile in Bangladesh, even ‘reference’ or normal immunoglobulin level of Bangladeshi ‘men’ and ‘women’ was not available, let alone ‘commercial sex workers’. As no reference value of serum immunoglobulin level of Bangladeshi people exists to compare with, so it is daunting to interpret the serum antibodies values as many factors (age, sex, environment, race and genetic factors) influenced serum immunoglobulin level (Lugada et al, 2004; Bouman et al, 2005; Gonzalez-Quintela et al, 2007). However, some remarkable studies regarding immunoglobulin level of Bangladeshi men and women especially drug addicts (Islam et al, 2004), dockyard labors (Sayeed et al, 2010), Panic Disorders (PD) patients (Zabun et al, 2012), eclampsia and pre-eclampsia patients (Ahsan *et al*, 2006) and Somatization disorders patients (Hossain *et al*, 2007) were carried out, but no study focused on immunological status of CSWs in Bangladesh. Nevertheless, a recent study (Sultana *et al*, 2012) on garments worker’s immune function revealed that their immunological status was worse than age matched control. Different studies in Bangladesh regarding serum immunoglobulin status which used age matched healthy controls of Bangladeshi nationals (to compare with the study population) showed different ranges of normal serum immunoglobulin level. Healthy controls of dockyard labourers (Sayeed *et al*, 2010) had 11.94 g/L serum IgG, 1.38 g/L IgM and 2.61 g/L serum IgA, healthy controls of PD patients had 1.24 g/L serum IgG, .096 g/L IgM and .194 g/L serum IgA and healthy controls of (Islam *et al*, 2004) drug addicts (all men n=100) had 6.93 g/L serum IgG, 1.72 g/L IgM and 2.90 g/L IgA. Also, normotensive pregnant control (Ahsan et al, 2006) of eclampsia/preeclampsia had 7.10 g/L serum IgG, 2.16 g/L IgM and 3.65 g/L IgA, and healthy controls of somatization disorders patients (Hossain *et al*, 2007) had 26.34 g/L serum IgG, 2.36 g/L IgM and 2.76 g/L serum IgA. Notably, study (Zabun et al, 2012) revealed mean (IgG, IgA and IgM) immunoglobulin levels even in ‘healthy control’ (‘PD patients’ as well) were lower than this study subjects, commercial sex workers (Table-1). However, low serum IgG (Zabun et al, 2012) and IgA (Hossain *et al*, 2007) than healthy control ($P<0.05$) were also reported among psychiatric patients in Bangladesh. Thus, normal serum immunoglobulin levels of Bangladeshi individual as reported earlier were quite speculative.

In this study 495 sera of CSWs were screened for STIs but due to resource constraints (both economic and laboratory) all sera could not be afforded to analyze for immunoglobulin, rather purposively selected 107 serum samples comprising 68.2% (n=73) infectious sera (having both HBV/syphilis infections or any single infection[♣]) and 31.8% (n=34) non-infectious samples were analyzed for the estimation of serum IgG, IgA, and IgM. Mean serum IgG, IgA, and IgM values of CSWs were 2.73 g/L (95% CI 1.52–3.93), 0.50 g/L (95% CI 0.33–0.68) and 0.42 g/L (95% CI

[♣] No HIV positive serum was detected in this study among 495 sera

0.28−0.55) respectively. Serum IgG was less than half of the lower tail (or <50% than lower tail) and IgA has just touched the lower tail of the standard normal value. In contrast, only mean serum IgM was within normal range (0.42 g/L) and just above the lower tail of the standard normal value (0.30-2.3 g/L; Young 1998). However, only IgM levels in females have been attributed to hormonal (stimulatory effect of estrogen) effects on B lymphocytes reported by different studies (Gonzalez-Quintela et al, 2007; Bouman et al, 2005; Shulman, 1975). Normally serum IgM is elevated at acute infection but serum IgG is elevated in Individual suffering from chronic infections. The high serum level of IgG antibodies than normal in chronic infection may simply represent an inflammatory reaction to the external microbial challenge. It was observed from a previous study about immunologic profile of CSWs in India (Babu et al, 1997) that compared to normal control the CSWs had significantly elevated serum IgG, IgA and IgM levels but no significance difference was established between CSWs who tested positive for HIV and those who did not.

Plausible explanations of lower mean serum IgG, IgA, and IgM values of CSWs than normal level could be (i) from biochemical analysis it was revealed that mean serum total protein (53.22 g/L) and albumin level (37.22 g/L) of this segment of CSWs (n=107) was far away from standard normal values [(Standard[9] total protein=60-80 g/L and albumin=40-60 g/L), Young 1998]. Actually, total protein is measured in serum to give an indication of total immunoglobulin concentration since [(total protein-albumin) =globulins] of which the major component is immunoglobulin. Some chronic liver diseases cause increases in immunoglobulins which ultimately increases total protein though this may be offset by a decrease in albumin[10]. However, serum total globulins may be low in patients with humoral immunodeficiency (primary or secondary) but because IgG is the major component, deficiencies of IgA or IgM may not significantly affect total globulins[10]. Of note, mean serum total protein (56.98 g/L) and albumin level (36.78 g/L) of total CSWs (n=495) was also below the normal value. In addition, ANOVA depicted (Chapter-2.3, table-5) that serum IgA [F (1, 105) =8.712, P=.004] and IgM [F (1, 105) =5.256, P=0.24] positively influence serum TP level (dependent variables) of CSWs though serum IgG had no effect (P=.197) and previous study (McGee and McMurray, 1988) using animal models determined that malnutrition, including protein and protein-calorie malnutrition, suppressed the IgA immune system at mucosal surfaces. (ii) It was also noteworthy, dietary analysis (Chapter-2.2, table-2, 3) of all CSWs (n=635) revealed that their diet was severely deficient in calorie (1362.79 kcal/person/day), protein (40.0 g), fat (8.05 g), zinc (3913.24 µg), vitamin A (189 µg) and calcium (237.63 mg). (iii) it was observed that (Chapter-2.2, table-8) dietary protein intake >50g was associated with higher serum IgG [F(2,104) =3.130, P=.048] .

[9] Association for Clinical Biochemistry, 2012: www.acb.org.UK>Total Protein and Albumin

[10] Association for Clinical Biochemistry, 2012: www.acb.org.UK>Albumin

Also, lower dietary protein and energy intake as different studies conducted both in human (Najera et al, 2001) and animal model (Iyer et al, 2012. Valerie et al, 2014) focused that dietary protein is critical for maintaining a functional pool of memory T cell (Iyer et al, 2012) and together, these data suggest that malnutrition decreases CD8+ T cells number and function and predisposes to infection (Valerie et al, 2014). In fact, memory T cells secret interleukin that enhance antibody production by the memory B cells (Levinson and Jawetz, 2005) (iv) furthermore, research showed calorie-restriction and fasting prevent autoimmunity, likely by decreasing T cell responses and inflammatory cytokine production (Valerie et al, 2014) which could possibly partly explain this finding. As T-cell possesses the regulation capacity of antibody responses i.e. help and suppression (Levinson and Jawetz, 2005), so malnutrition could also alter B cell normality through affecting T-cell immunity. (v) Malnutrition shifts the balance of pro-inflammatory Th1 versus anti-inflammatory Th2 cytokines, and may offer an explanation as to how malnutrition predisposes to infection (Valerie et al, 2014) (vi) role of laptin in mediating nutritional effects on T cells: laptin is an adipokine secreted in proportion to adipocyte mass. In addition to its well-described role in regulating appetite, energy expenditure and body weight, leptin is also a pro-inflammatory cytokine. Leptin has direct and indirect effects on T cell number and function, promoting Th1 and Th17 cell number and cytokine production while inhibiting Th2 cytokine production and Treg proliferation (vi) malnutrition especially ‘protein energy malnutrition’ and specific micro-minerals like ‘iron and zinc deficiency’ cause of secondary immune deficiency (Marshall, 2010; Ress, 2008). Previously Busher (1990) also reported that malnutrition and congenital immune deficiency can cause a decrease in total globulins due to decreased synthesis. Moreover, malnutrition leads to decreased adipocyte mass, which results in decreased circulating leptin. Concurrently malnourished individuals show changes in CD4+ (T regulatory cells) and CD8+ (T-effector cells) T cell number and function, leading to increased susceptibility to infection and protection against certain forms of autoimmunity. Leptin may mediates a subset of these malnutrition-related changes to T cell number and function, as leptin is known to alter CD4+ T cell number and cytokine production. Conversely, obesity heightens immune reactivity increases inflammatory T cell numbers in visceral Adipose tissue (VAT) and promote systemic inflammation (Valerie et al, 2014)(vii) this study observed that lower serum zinc status attributable for lower serum IgG (dependent variable in MLoR model of IgG) and showed 9.9-folds risk (P=.039) for having lower serum IgG (this chapter 5: table-6) and similarly higher serum IgG (Chapter-4.2: table-8) influenced 9.46 times (P=.015) for acquiring higher serum zinc (dependent variable in MLoR model of zinc). Serum immunoglobulins level (i.e. IgG, IgA and IgM antibodies) of the study subjects clearly depicted the altered B-cell functions. Considering the small segment (n=107, 73 infectious samples and 34 non infectious samples of CSWs), inference should have drawn with caution. Lower IgG may have implications as regards malnutrition and infection and thereby altered immune function especially B cell functioning. Moreover, immunoassay methods also influence serum immunoglobulin level (Agarwala and Cunningham-rundles, 2007; Maddison and Reimer, 1976). Further studies are required to provide better insights on the altered immune system in CSWs and to understand how malnutrition, stress, social isolation and environment may affect immunoglobulin level of CSWs by modifying both cell-mediate and humoral immunity.

Serum IgM of CSWs was affected significantly by their marital status [F (2.104) =4.545, P=.013*], STI status [F (1,105) =5.729, P=.018*] and sex business location [F (1,105) =6.204, P=.014*]. Unmarried CSWs had higher level of serum IgM than married and married were more likely to have serum IgM than widowed/divorced/abandoned. In spite of lower (than normal level) mean immunoglobulin status of our study participants, unmarried CSWs had the highest igM level (1.08 g/L) than either married or divorced/abandoned/widowed CSWs in post hoc test (Tukey) which also reflected normal serum igM level of unmarried group. In this study, unmarried CSWs were more prevalent (χ^2=152.533, P=.000) in hotel-based group than floating and their mean monthly income (HBCSWs: 7357.19 vs. Floating CSWs: 3142.42 BDT) also significantly higher (χ^2=115.096, P=.000) than their counterpart. Thus, plausible explanation for "normal IgM status" of this small segment (13.7%) of unmarried CSWs is "better income" which drives them to better nutrition as well as normal immune status than other group. Different studies (Iyer et al, 2012; Valerie et al, 2014) confirmed that nutritional status (either under or over nutrition) influence normal functioning of the immune system.

Economy also played an important role in restoration of immune function especially serum IgA, it was observed that CSWs (n=60) who spent 251-5000 BDT for the last STI related ailment had the normal igA level (1.0 g/L) in post Hoc test (tukey) than who spent nothing (self treatment) [F (2,104) =5.155, P=.000]. Treatment for infectious disease relates normal functioning of the immune system. Economy plays a dual role for treatment as well as good diet thus ultimately gained normal immunity.

Frequency of Coitus with each client, a sexual act of CSWs had borderline (P=.054) significant association with serum IgM antibody level. Serum IgM level markedly decrease with more than 2 times coital frequency (with per client) as compared to one time coitus with each client. This may be due to the severe physical, psychological and social stress and isolation as well as mental torment of CSWs, which are associated with impaired immune cell proliferation and antibody production (Islam et al, 2004. Stefanski and Engler, 1998. Herbert and Cohen, 1993. Pariante et al, 1997. Castle et al, 1995). Sexual act with a large number sex partners associated with decreased serum immunoglobulin and eosinophils was observed among drug addicts in Bangladesh (Islam et al, 2004).

The most important aspect of the immune system is T-cells found in the form of T-helper and T-suppressor cells. During stress related emotion, increased stressor hormone secretion and cortisol, once released into the bloodstream, immediately begins to cause division of T-

Suppressor cells. This rapid cell division increases the number of T-Suppressor cells while at the same time suppressing T-helper cells. This reduces immune protection and leaves the body vulnerable to disease and infection.

A very recent qualitative study (Zhang et al, 2015) among female sex workers in China examined 'psychological stress' in the context of commercial sex through the lens of occupational health and found that FSWs faced a continuum of stressors that resulted from poverty, limited employment, lack of social protection, violence perpetrated by clients and limited social support from peers and stable partners. This obviously pointed out socio-economic status as well as comparatively good health and nutritional status and lesser-duration stress faced by very young and unmarried CSWs than older one. In sex industry, poor and young are always welcomed and as time passed they faced various physical, mental, psychological, social and also verbal stress and trauma additionally than their previous poor life. Their previous lives were full of starvation and grief but their hazardous and stigmatized occupational involvement generates stress that can only be understood by them. Moreover, victimization, difficult clients and police harassment also add torment to these daily stressors (Alam and Hussain, 2013).

No single study regarding stress on immunoglobulin status of CSWs was available but one immune system's function study in 'garments workers' [n=45 (14 males+31 females), control=41 (20 men+21 females)] was carried in Bangladesh which claimed that "stress-related suppression" of the "complement system" would be the explanation of the significantly ($P<0.01$) lower complement (C_3 and C_4) mediated bactericidal activity of garments workers than healthy control though concentration of C_3 and C_4 were not differed significantly between two study groups (Sultana et al, 2012). In addition, serum IgG level (13.5±1.7 g/L) of the garments workers was significantly higher ($P<0.001$) than that in the control subjects (11.4± 2.0 g/L) which seems that occupational exposure to fiber particles that are not pathogenic might stimulate antibody production. Psychological stress and social isolation (Islam et al, 2004; Stefanski and Engler, 1998; Herbert and Cohen, 1993; Pariante et al, 1997. Castle et al, 1995) develop severe immunodeficiency with depletion of TH cells and suppression of cell-mediated immunity. Stress itself is associated with a lower percentage of T lymphocytes, TH and TS cells (Herbert and Cohen, 1993).

WHO Reported on occupational Hazards on women that an increased may represent a source of psychological stress. Under stress, complex adaptive mechanisms are activated and several parts of the endocrine system react simultaneously. Prolonged activation of the adaptive mechanism is believed to be involved in the genesis of various chronic disorders such as cardiovascular, gastrointestinal and musculoskeletal diseases (WHO, 1994). The immune response is also regulated by the neuroendocrine system and event occurring in the central nervous system also modulate immune functions (sultana et al, 2012).

Michael et al, (1997) examined “the effect of academic examination stress on serum immunoglobulins, complements and acute phase proteins on volunteers” showed that “academic examination stress” induced significant increase in serum IgG, IgA and IgM and α^2 macroglobulin (acute phase protein) in students who had higher level of Perceived Stress Scale (PSS) but not with student of low-stress perception. These finding suggest that psychological stress is accompanied by an altered secretion of serum immunoglobulins, compliment factors and some acute phase proteins (Michael et al, 1997). Thus, our study subjects who were professionally commercial sex workers, earned their livelihood (as well as for their children/families) by selling human flesh endured several kinds of pressure like physical, mental, psychological, economical, social and also verbal stress and trauma at a time. No profession creates such a volume and Intensity of stress like CSWs, which could also, altered their normal immune system to vulnerable stage that was reflected in our study. If short duration “academic examination stress” (Michael et al, 1997) could altered immunological functioning, then what could be the “long endurance stress” do on “immunological condition” of CSWs can easily perceivable. As serum IgG is the main for adaptive immune function, so mean IgG level of CSWs (2.73 g/L) was half of the lower tail of normal value, reflected unhealthy, malnourished (also their mean serum TP and albumin level were well below normal level and energy intake was only 1391 Kcal/person/day) and stressful lives of CSWs.

As previously mentioned, mean serum immunoglobulins level of CSWs (n=107) was found remarkably low among CSWS. CSWs who were STIs positive (HBV or Syphilis or both) had significantly lower serum IgM (no STIs: 0.65 g/L vs. STIs: 30 g/L) than normal indicated possible ‘malnutrition’ especially protein [$F(1,105) = 5.729$, $P = .018$]. As dietary protein is critical for maintaining a functional pool of memory T cells (Iyer et al, 2012) and T cells secret interleukin that enhance antibody production by the memory B cells (Levinson and Jawetz, 2005), so studies reported malnutrition in human (Najera et al, 2001) and animal model (Iyer et al, 2012. Valerie et al, 2014) decreases CD8+ T cells number and function and predisposes to infection (Valerie et al, 2014). Conversely, higher serum IgA level (STIs: 0.62 g/L vs. no STIs 25 g/L) of STI positive (HBV or Syphilis or both) CSWs than STI negative CSWs pointed at chronic infection though the difference was at borderline significance ($P = .053$).

Business location of CSWs is an indicator of economy and social position as well whenever considered their socio-economical status of hierarchy. Different studies (Rakhi et al, 2006; Ullah, 2005; McKeganey and Barnard, 1996) outlined street-based or floating CSWs as lowest class so as reflected in this study, ANOVA showed floating CSWs had significantly lower level of serum IgM (floating vs. hotel= 0.34 g/L vs. 0.78 g/L) antibody (compare to normal) than their counterpart [$F(1,105) = 6.204$, $P = .014$], this may be, due to their lower income (only 3156 taka/month) as well as nutritional status of floating CSWs (floating CSW’s mean BMI: 20.93±4.07 vs. hotel-based CSWs: 21.11±3.63). Of note, floating CSWs were inflicted mostly ($P < 0.05$) with syphilis (52.4%)

than HBCSWs (18%). In contrast, HBCSWs had slightly higher prevalence of hepatitis B (6.2%) than floating (5.0%) CSWs (P>0.05).

Professional length of CSWs showed an influential influence on serum IgM level but the differences did not reach statistically significant level (P=.058). Serum antibody level diminishes as time passes and (Lavinson and Jawetz, 2005) so as was noticed in this study, CSWs who just entered into this ugly profession had normal level of IgM antibody but it began to diminished over cascade of time. This may be due to physiological reason (WHO, 1994. Michael et al, 1997) as well as severe stress endured by CSWs (Islam et al, 2004. Stefanski and Engler, 1998. Herbert and Cohen, 1993. Pariante et al, 1997; Castle et al, 1995).

In this study (n=107) more than half (52.63%, 56/107) of CSWs were smokers and smoking negatively associated with serum normal immunoglobulin M level. Although nonsmokers CSWs had comparatively higher level of serum immunoglobulin M (0.55 g/L) than smokers (0.30 g/L), but the difference showed borderline significant (P=0.60). It is noteworthy that, this study showed lower mean serum immunoglobulin status of CSWs especially for IgG and IgA but ANOVA where smoking was an independent variable and serum IgM was a dependent variable revealed that nonsmokers possessed a pretty good amount of IgM antibodies compared to smokers. Old study (Hersey et al, 1983) is in agreement with this finding and another study (Tollerud et al, 1995) showed only serum IgG (not IgA and IgM) was negatively altered in current smokers than never smokers. A study (Gonzalez-Quintela et al, 2007) showed smoking was negatively associated with only serum IgG level (P<0.001, IgA and IgM were not reached significant level). A very recent study revealed (Shrestha & uppoor 2014) that cigarette smoking may be associated with the suppression of B-cell function and immunoglobulin production and the alteration of antibody levels (mainly IgGs) further explains the potential mechanism by which smoking exacerbates disease progression. Smoking decreases the proliferative capacity of T-cell. However study showed (Chanida et al 1999) that serum IgG and IgM had significant close correlation (r=0.72, P=0.001) as immune marker of HIV positive industrial workers but IgG seemed to be more relevant than IgM.

Consistent use of condom was negatively associated with serum IgA and IgM antibody which indicate malnutrition as well as lower serum antibody than normal. CSWs who used to maintain “use of condom” consistently had significantly lower IgM (P=.049) and IgA (P=.017) than normal, on the other hand, higher level of IgM and IgA pointed at possible current and past infection respectively.

Plasma levels of antibodies are regarded as indices of immunity. In serological analysis, no HIV was detected by ELISA among two types (hotel-based and floating) of CSWs (n=495) and hotel-based CSWs were reported to have less STIs including syphilis (18%, n=32, P<0.05) and hepatitis B (6.2%, n=11, P>0.05) than floating CSWs (syphilis 52.4%, hepatitis B 5.0%). After screening of all blood samples (495) for STIs (HIV, hepatitis B, and syphilis), 107 blood samples (hotel-based 19/107 and floating 88/107) comprising 68.2% (n=73) infectious samples (having both infections and any single infection) and 31.8% (n=34) non-infectious samples were purposively selected for the detection of serum IgG, IgA, and IgM. In short, in this study only 18 CSWs were infected with hepatitis B but syphilis rate was remarkably high (38.18%, n=189/495). In this study, serum immunoglobulin levels (IgG, IgA, IgM) were insignificantly (for all immunoglobulins P>0.05) and negatively correlated with serum vitamin A [r (IgG) = -.130, r (IgA) = -.081), r (IgM) = -.114].

In fact, there are numerous factors that are associated with reduced levels of serum immunoglobulins (hypogammaglobulinaemia). Hypogammaglobulinaemia is more frequently due to secondary rather than primary causes (Jaffe et al, 2011. Richoard et al, 2013; Gupta, 2001). ‘Primary or inherited immunoglobulin deficiency’ is less common disorders in which the body is not able to produce one or more class of immunoglobulin and ‘secondary or acquired’ is the most common causes of Hypogammaglobulinaemia result from an underlying condition/factors that either affects the body’s ability to produce immunoglobulin [nutritional i.e. malnutrition (Marshall, 2010; Niedzwiecki and Rath), alcoholism, radiation therapy and certain drugs including nonsteroidals, anticonvulsant agent viz. phenytoins and carbamazepine and immunosuppressive or chemotherapy agents, hematological malignancies i.e. CLL, MM, Rheumatological diseases and viruses i.e. HIV, CMV, rubella and Epstein-Bar) or increases the loss (catabolism) of immunoglobulin (i.e. renal disease such nephritic syndrome, severe burns, sepsis, protein-losing enteropathy etc.] from the body (Richoard et al, 2013).

Other factors[11] associated with lowered levels of IgG including intense exercise, excessive physical stress (Islam et al, 2004. Stefanski and Engler, 1998. Herbert and Cohen, 1993. Pariante et al, 1997; Michael et al, 1997; Castle et al, 1995), smoking (Shrestha & uppoor 2014; Gonzalez-Quintela et al, 2007; Chanida et al 1999; Tollerud et al, 1995; Hersey et al, 1983), and aging (Lavinson and Jawetz, 2005). Research (Islam et al, 2004) also proved that illicit drugs suppressed serum Immunoglobulin level in Bangladeshi male drug addicts. Studies and researches (Marshall, 2010; Ress, 2008; Niedzwiecki and Rath) reported that biochemical and nutritional disorders (malnutrition and iron, zinc and other nutrients deficiencies) are also cause of secondary immune deficiency. However, in this study, it was obvious that CSWs suffered from secondary (acquired) immune deficiency as most primary immunoglobulin deficiencies are the result of inherited diseases.

[11] WebMD, Medscape: IgG and IgM Deficiency,

Multiple Logistic Regression models of serum immunoglobulin: In this study, mean IgG (2.73 g/L) was remarkably lower than the lower tail of the standard normal value (5-12 g/L). Multiple logistic regressions analysis was performed for Individual serum immunoglobulins (IgA, IgA,IgM) to find out which individual factor associated with lower level of these immunoglobulins.

MLoR model of IgG: having children was associated with 7 times higher odds of having <normal serum IgG (table-6) as compared to CSWs who did not have children [AOR 7.13 (1.40-36.17), P=.018]. In this study, mean IgG (2.73 g/L) was remarkably lower than the standard normal value (5-12 g/L) and most (59%) of floating CSWs had children in contrast to HBCSWs who had only 16.6% children ($\aleph^2$=120.87, P=.000). Also, as mentioned earlier monthly income of floating CSWs was just half of HBCSWs and both groups' endured lots of pressures as studies reported (Alam and Hussain, 2013; Michael et al, 1997). Studies also reported CSWs not only involved in this nasty profession for themselves but also for their children and families. Thus, to provide bread for children and families CSWs, especially street-based CSWs transformed their bodies as a commodity and having children pushed them extra pressure as compared to CSWs having no children. Different studies pointed at low serum IgG antibodies due to stressful situations (Alam and Hussain, 2013; Herbert and Cohen, 1993; Michael et al, 1997).

Possessing <normal serum zinc was associated with 10 folds risk (AOR 9.992, P=.039*) of acquiring <normal serum IgG by CSWs as compared to >normal serum zinc possessors; even the risk was 4 folds (P=.112) if CSWs had normal serum zinc level [AOR 4.31]. This finding is well agreement of researches (Marshall, 2010; Ress, 2008; Niedzwiecki and Rath) those outlined that specific mineral deficiencies could cause of secondary immune deficiency. Similarly, below normal IgM possessors were 62 times [AOR 62.44 (6.65-586.35) P=.000] more likely to have <normal serum IgG as compared to ≥normal IgM possessor. Here, width of the 95% CI was broader (6.65-586.35) due to small sample size (n=107) so as small cell values of the categorical variables. Actually, OR operates on a log scale and the CI will appear much wider just because the point estimate is also higher (62.44). When the actual mean and its 95% CI are expressed in logit (by taking a natural logarithmic transformation), the width of the CI actually would not be terribly different[12].

MLoR model of IgM: according to ***IgM model*** (table-7) CSWs infected with syphilis had 16 times higher risks (P=.001) of having lower serum (<normal) antibody IgM as compared to CSWs who were not inflicted with syphilis [AOR 15.78 (3.1-80.28)]. In this study, syphilis infection rate was (52.4%) significantly ($P<0.05$) higher among floating group than hotel groups (18%) and mean IgG was severely low (only 2.73 g/L), and IgM (0.42 g/L) which was the only serum antibody that remained within normal range (0.3-2.3 g/L) (Young. 1998). Frequency distribution (table-1) showed most (67.29%) CSWs had below normal serum IgM and ANOVA [F (1,105) =6.204,

[12] (Cross Validated: http://stats.stackeschange.com/questions/147666).

P=.014] (table-3) depicted that floating CSWs had significantly lower serum IgM antibody than hotel-based CSWs (hotel: 0.78 vs. floating: 0.34 g/L) and syphilis rate was significantly higher (52.4%) among floating group than hotel (18%) (Chapter-3: table-1). Mean TP (50.84 g/L) was also well below than 'standard normal' values and only 24.6% of CSWs (Chapter-2.3: table-1) had normal serum TP values which reflected their poor nutritional status. Thus, lower serum IgM antibody level might be due to low serum protein which also reflected in ANOVA [F (1,105) =5.256, P=.024] (Chapter-2.3: table-5). CSWs having low serum antibody than normal especially IgG and IgM means they are vulnerable for infection and so were infected with syphilis especially floating group. Of note, IgM is the first antibody of adaptive immune system that appeared in response to the invasion of microorganisms. However, previous studies reported (Pope et al, 1994; Larson et al, 1995) that although the percentages of CD4 and CD8 lymphocytes subsets for persons with syphilis were within the range of those percentages found in uninfected persons, the percentages of CD4 cells were significantly lower (<0.001) and those of CD8 cells were higher (P=.03) among patients with syphilis than the uninfected population.

Below normal IgG and IgA possessors were 82 times [AOR 81.86, P=.000] and 23 times [AOR 22.71, P=.006] more likely to have <normal serum IgM as compared to ≥normal IgG and ≥normal IgA possessors respectively. It is notable that, strong Correlation was found between serum IgG and IgM (r=.488, P=.000) but not in case of serum IgG and IgA or IgA and IgM. Also ANOVA (table-3) showed that CSWs inflicted with STIs (either syphilis or HBV or both) had lower both serum IgG (2.3 g/L) and IgM (0.30 g/L) as compared to CSWs who did not get infection and in case of IgM it was significant [F (1,105)=5.729, P=.018]. Conversely, ANOVA [F (1,105) =3.830, P=.054] revealed having STIs was positively associated with higher serum antibody A (IgA) level, similar effect was also remained in multiple logistic regression (table-8).

MLoR model of IgA: 'No STIs', 'consistent condom use' and 'ever experienced in group sex' were associated with low serum IgA antibodody, opposite of which was attributable for STIs as antibodies increased in numbers and come into action due to the body's invasion of microorganism. As previously mentioned STIs was positively associated with higher serum antibody A (IgA) level in **MLoR** model of IgA **(table-8)**. It was observed from the model, CSWs did not have STIs had 4.63 times risks of having low serum IgA as compared to CSWs, who had STIs (syphilis/HBV/or both) [AOR 4.633 (1.186-18.102), P=.027]. This may be due to sexually transmitted infections as antibodies increased in numbers and come into action due to the body's invasion of microorganism. Previous Indian study (Babu et al, 1997) addressing immunologic profile of CSWs is in agreement with this finding and showed that compared with normal control CSWs had significantly elevated serum IgG, IgA and IgM levels but no significance difference was established between CSWs who tested positive for HIV and those who did not. Moreover, more than four decades earlier study (James and Catterall, 1969) showed that both IgM and IgG globulin were raised in primary, secondary and latent syphilis (untreated syphilis) but serum IgA was raised at the secondary stage and no associations were found between Venereal Disease Research Laboratory (VDRL) titers and concentrations of immunoglobulins.

Consistent condom used by CSWs was attributable (5 times) for their low serum ‘antibody A’ (IgA) as compared to inconsistent condom users [AOR 4.937 (1.292-18.868), P=.020]. This effect was also due to ‘STIs of CSWs’ as CSWs who used condom consistently would have low STIs rate as compared to inconsistent or irregular users. And ‘irregular use of condom’ increased infection as well as antibodies substantially due to bodies faced invasion of organisms. Ever experienced in group sex was also attributable for (6 times) low serum IgA as compared to who did not perform this malpractice [AOR 6.0, 95% CI (1.236-29.483), P=.026] (table-8).

As because this is a cross-sectional study, a causal relationship between immunoglobulins and a considerable number of potentially confounding factors were although adjusted for, cannot exclude the possibility that serum IgG concentration can affected by other lifestyle variables which are intrinsically related to serum Immunoglobulins. Further prospective studies and intervention should be undertaken in this regard.

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