SELENIUM ON CHRONIC ARSENICOSIS

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Faculty of Post-graduate Medical Science & Research ,
University of Dhaka , Bangladesh.

Dedicated

To

My parents

DECLARATION

I do hereby declare that this thesis entitled "SELENIUM ON CHRONIC ARSENICOSIS" is based on the research work carried out by me. No part of it had been presented previously for any higher degree. The research work was carried out under the Faculty of Post-graduate Medical Science & Research, University of Dhaka , Dhaka with the supervision of Prof. (Dr.) Shah Mohammad Keramat Ali, Professor of Clinical Nutrition, Institute of Nutrition and Food Science (INFS), University of Dhaka, Dhaka , and Prof (Dr.) M. Mujibul Haque, Professor and ex . Head of Dermatology, Dhaka Medical College, Dhaka, Bangladesh .

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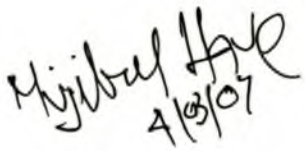
Certificate

"Selenium On Chronic Arsenicosis" submitted by Dr Abdul Momin for the award of Ph.D in Dermatology, is an independent research work done under the Faculty of Post - graduate Medical Science & Research , University of Dhaka, Bangladesh and this dissertation has not been used as the basis for the award of any degree or fellowship .



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(Dr. Abdul Momin)

Abstract

Introduction

A prospective randomized double blind phase 3 intervention trial was carried out during the period from November 2004 to November 2006 to evaluate the role of selenium in arsenicosis patients with an approval by the ethical review board of the Bangladesh Medical & Research Council (BMRC), Dhaka.

Objectives

The study was done to workout the effectiveness of selenium on chronic arsenic poisoning.

Materials and methods

Two hundred and sixty Arsenicosis patients were identified clinically and confirmed by urine tests from 11 villages of Shahpur Union, under Chatkhil Upazilla of Noakhali District (about 170 km south west from Dhaka). Urine and blood samples were collected from each patient at the beginning and at the end of 4, 8 and 12th months but hair and nail samples were collected at the beginning and at the end of study period. Samples of their drinking water were also collected at the beginning and at the end of first month.

Investigators were blinded and after randomization, 87 patients were included in group A and 87 patients in group B and another 86 patients in group C. Each patient was given an identification number and randomly assigned into one of the three treatment trial groups following a computer generated random number table . At the end of the trial the code was decoded and found 'A' for selenium alone, 'B' for placebo and 'C' for second control Vitamin ACE respectively. Selenium was given to group 'A' at a dose of 100 μg per day, Vitamin ACE was given to group 'C' at a dose of beta-carotene 6mg, alfa-tocopherol 200mg and Ascorbic acid 200 per day and potato containing sugar was given to placebo group 'B'. The study continued for 12 months. The specific treatment was provided to the patients and supervision was strictly maintained by the trained field workers at home level of the patients. None of the patients was allowed to drink arsenic contaminated water throughout the study period.

Result

Among the study subjects, majority (60%) were young adult with an age range between 30 and 49 years. Female were 64% and the rest were male. About ninety one percent suffered from the disease for more than 04 years. Out of the arsenicosis subjects 99.6% had melanosis, 97

% leucomelanosis and 92 % palmo-planter hyperkeratosis. Non-healing indolent ulcer was present in 3.1% cases, histopathologicaly which revealed Bowen's disease 2 (1.5%) and squamous cell carcinoma 6 (8.5%) respectively.

The severity of melanosis and keratosis was reduced to 76% and 80% in selenium treated group respectively. In Vitamin ACE group keratosis improved by 23% but no change was observed in placebo group. Reduction of associated symptoms was observed 68% in selenium treated group, 26% in Vitamin ACE group but only 2.8 % in placebo group.

Biochemical analysis revealed that Arsenic load in hair dropped from 43.3 % to 23.3 % (46% improved) after intervention in selenium treated group , whereas 38% improved in Vitamin ACE group and 23% improved in placebo group respectively . Seventy one percent subjects of selenium treated group showed high concentration of arsenic in their nail before intervention but after intervention it come down to 58.2% with an improvement of 18%. Urinary excretion of arsenic concentration significantly reduced in all intervention groups but trend of concentration gradually lowered in selenium treated group. It was also

observed that the study population had low concentration of selenium which rose to 41% in selenium treated group after intervention.

Conclusion

Selenium treated arsenicosis patients showed rapid and sustained improvement of their clinical signs and symptoms by 16 weeks of intervention but no toxicity was observed in the study subjects. A dose of 100 microgram of selenium as selenomethionine per day along with use of arsenic free water is safe, effective and cheap remedy for arsenicosis patients of Bangladesh.

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Glossary and abbreviations

ALT	:	Alanine aminotransferase
As	:	Arsenic
As III	:	Trivalent arsenic molecule
AsV	:	Pentavalent arsenic molecule
BAL	:	British Anti -Lewesite
DMSA	:	2,3 -dimicaptosuccinic acid
DMPS	:	Dimercaptopropane sulfone
Gpx	:	Glutathione peroxidase
GSH	:	Glutathione
H_2O_2	:	Hydrogen peroxide
HCl	:	Hydrocloric acid
HG-AAS	:	Hydride generation atomic absorption spectrophotometer
MMA	:	Monomethyl arseninic acid
Na BH_4	:	Sodium bromohydrate
NIST	:	National Institute of Standard technology
r	:	Spearman correlation coefficient
SAM	:	S-adenosylmethionine
Se	:	Selenium
SRM	:	Standard Reference Materials
TW	:	Tube well
UNICEF	:	United Nations Children's Education Fund
WHO	:	Word Health Organizations
µg	:	Microgram

Chapter 1

INTRODUCTION

1. Introduction

Bangladesh, an alluvial and deltoid land of 1, 47,570 sq. km. She is a poor and most densely populated country in the world .The country is prone to various natural disasters like cyclones, flood and droughts. She is blessed with vast surface water collection. Due to abundance rivers and high rainfall, ground water is still the major source of drinking and agricultural water for all 140 million people of Bangladesh. In recent years, ground water pollution with Arsenic (As) has become a serious public health problem. This situation began in the 1970s, when the United Nations Children's Fund, in response to epidemics of cholera, dysentery, and other waterborne infectious diseases, spearheaded an effort to switch the region's population from drinking surface waters to groundwater. Millions of tube wells were drilled into arsenic-rich sediments; as a result, in many of these wells arsenic levels reach 500 - 1,000 µg/L and even higher .Arsenic contaminated drinking ground water in the Ganges delta of India is also a recognized health problem (Katzung, 2001). Bangladesh is adjacent to West Bengal of India, having similar aquifers and the alluvial formation with close geological similarity as that of India. Contamination of drinking water with arsenic above the permissible level (0.01µg/L) has emerged as a major national crisis in

Bangladesh (Figure -1.A). More than 75 million people are at risk and 25 million are exposed population from the affected 61 out of 64 districts. More than 1.12 million tube-wells found contaminated with high level of arsenic (Elizabeth, 2000; Douglas, 1999). It is increasingly recognized that arsinocosis is a serious public health issue since the high level of arsenic in tube-well was first detected in 1993.

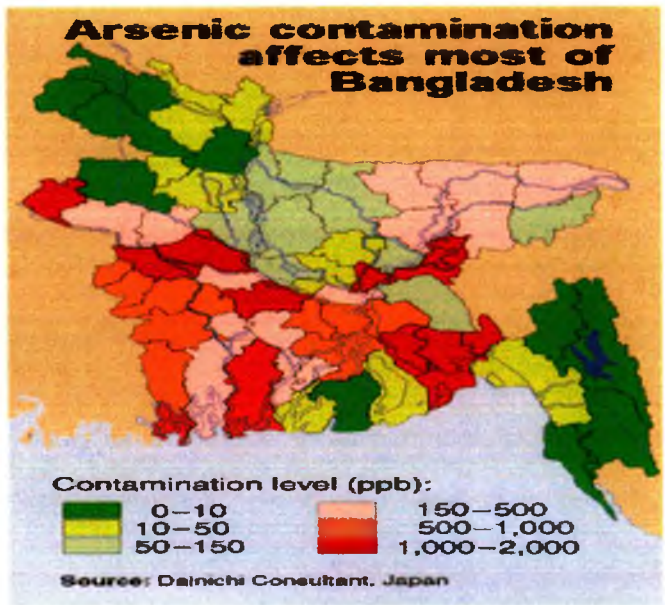


Figure -1.A: Level of arsenic contamination in different region of Bangladesh

The situation in Bangladesh is relevant to arsenic contaminated ground water in seven districts of west Bengal of India where prevalence of arsenic toxicity is about 20% (Shikder, 1999). In terms of number of people exposed, Bangladesh is the vulnerable country of the world . A public health specialist cautioned "Half of

Bangladesh population at risk of arsenic poisoning" (Ganapati, 2000). In recent times the number of patients with arsenicosis in Bangladesh is reported to be more than 44,000. The term 'arsenicosis' denoting chronic arsenic poisoning is being used in recent years. It was earlier referred to as 'arsenic toxicology' or 'arsenism'.

Hypothetically, Spallholz, Professor of education and nutrition in Texas Tech University in USA, explained that poor dietary selenium intake in rural areas in Bangladesh where arsenicosis is occurring and excessive selenium excretion owing to Se/ As complexion may add to the like hood of Arsenic being more toxic and carcinogenic over time (Spallholz, 2004). Biswas 1999 in India found cytotoxic effect of arsenic can be prevented through short-term dietary supplementation by selenium in mice in vivo, which is of significance in protecting against the widespread toxicity observed in human populations exposed to arsenic through drinking water from contaminated tube wells in West Bengal and Bangladesh.

In an animal study in the Pharmacology Department of Banghabandhu Sheikh Mujib Medical University, Bangladesh observed a significant reduction of arsenic in the different tissues and vital organs of rats that reverted back to normal after selenium

intervention was given (Nasir, 2004). As selenium is an antioxidant, administration of selenium in controlled dose may reduce the arsenic accumulation and thereby its toxicity. Scarcity of knowledge exists in selenium intervention in human subjects. Rabbinic et al, 2003 has done a controlled clinical trial with selenium and antioxidant in arsenic sis, where they found encouraging result in reducing the arsenic load from the tissue. In our phase 2 pilot study with 10 volunteer arsenic sis patients in 2004, we have found selenium enhances the arsenic excretion without any toxicity (Momin, 2004).

There is no specific treatment widely recognized for chronic toxicity of arsenic. Chelating agents, vitamins, spirulina, all these have limitations in different grounds. Above all, it should be remembered that people with clinical manifestations are not only the sufferer but millions of people who do not present with features of arsenicosis are also highly vulnerable to the more serious and delayed health effects in the long run. Thousands of people may develop lung cancer, bladder cancer, liver cirrhosis etc, if the silent accumulation of arsenic is not prevented. The aim of study is not limited to symptomatic relief or chelating therapy, but could be reversible with enhancing arsenic excretion put an end to toxicity even if exposed for a long time.

As the problem of arsenic poisoning remains at its boiling point, We found interest to evaluate the role of selenium on arsenic poisoned people of Bangladesh and for that matter we designed this randomized placebo controlled trial with Selenium on arsenicosis . We bear in mind that a dietary supplement of selenium is feasible as well as inexpensive specific therapy agents that may help to turn the heat off of a potentially massive health crisis in an already impoverished place.

1.2: Physio-pathologic Contribution on Arsenic to Human system and Selenium's role to avert it

1.2.1: Causes of Arsenic Contamination

Intermittent incidents of arsenic contamination in groundwater can arise both naturally and industrially. The natural occurrence of arsenic in groundwater is directly related to the arsenic complexes present in soils. Arsenic can liberate from these complexes under some circumstances. Since arsenic in soils is highly mobile, once it is liberated, it results in possible groundwater contamination. The alluvial and deltaic sediments containing pyrite has favored the arsenic contamination of groundwater in Bangladesh. Most regions of Bangladesh are composed of a vast thickness of alluvial and deltaic sediments, which can be divided into two major parts – the recent floodplain and the terrace areas. The floodplain and the sediments beneath them are only a few thousand years old. The terrace areas are better known as Madhupur and Barind Tracts and the sediments underlying them are much older than the adjacent floodplain. Most of the arsenic is occurring in the younger sediments derived from the Ganges Basin. The investigators found that there is a layer containing arsenic compound at a depth of 20 to 80 meters (Raman, 1998). This layer is rich in arseno-pyrite, pyrite, iron sulfate, and iron oxide as revealed by the geological investigation.

The researchers also inferred that, arsenic is perhaps in the outcrops of hard rocks higher up the Ganges catchments. These outcrops were weather-beaten in the recent geological past and then the eroded soil was deposited in West Bengal and Bangladesh by the ancient courses of the Ganges (Robinson, 1966). Arsenic in sediment or water can move in adsorbed phase with iron, which is available in plenty in the Himalayas. Here about 100 to 300 mg/kg arsenic combined with iron oxides can be found in the sediments under aerobic conditions (Bogert, 1973). When these sediments were deposited in Bengal basin under tidal environment, it came under anaerobic condition. The sulfate available in Bengal basin was reduced to hydrogen sulfide in presence of sulfur reducing bacteria. Iron minerals and hydrogen sulfide rapidly tie together to form iron sulfide. Arsenic had been absorbed on the surface of iron sulfide and produced arseno-pyrite. This mineral usually remains stable unless it is exposed to oxygen or nitrate. In aerobic environment, arseno-pyrite is oxidized in presence of oxygen and arsenic adsorbed with iron sulfide becomes mobilized.

The groundwater in Bangladesh has declined progressively due to the excessive extraction of water for irrigation and domestic water supply, lack of water management and inadequate recharge of the aquifer. The groundwater declined beyond 8 meters in 12% areas of

Bangladesh in 1986. This extent rose to 20% areas in 1992 and 25% areas in 1994. The study on forecasting groundwater level fluctuation in Bangladesh indicated that 54% areas of Bangladesh are likely to be affected up to 20 meters in some areas particularly in northern part of the country (Robinson, 1966). Excessive groundwater extraction may be the vital reason for creating a zone of aeration in clayey and peaty sediments containing arseno-pyrite. Under aerobic condition, arseno-pyrite decomposes and releases arsenic that mobilizes to the subsurface water. The mobilization of arsenic is further enhanced by the compaction of aquifers caused by groundwater withdrawal.

1.2.2 : Mechanism of Arsenic Contamination

Presently, there are two well-known theories about the mechanism of arsenic contamination in groundwater. These are oxidation and oxyhydroxide reduction theory. The oxidation theory is so far the accepted theory.

According to this theory, arsenic is released from the sulfide minerals (arseno-pyrite) in the shallow aquifer due to oxidation. The lowering of water level owing to over exploitation of groundwater for irrigation has initiated the release of arsenic. The large-scale withdrawal of groundwater has caused rapid diffusion of oxygen

within the pore spaces of sediments as well as an increase in dissolved oxygen in the upper part of groundwater. The newly introduced oxygen oxidizes the arseno-pyrite and forms hydrated iron arsenate compound known as pitticite in presence of water. This is very soft and water-soluble compound. The light pressures of tube-well water break the pitticite layer into fine particles and make it readily soluble in water. Then it seeps like drops of tea from the teabag and percolates from the subsoil into the water table. Hence, when the tube-well is in operation, it comes out with the extracted water. This mechanism is portrayed in Figure 1.B.

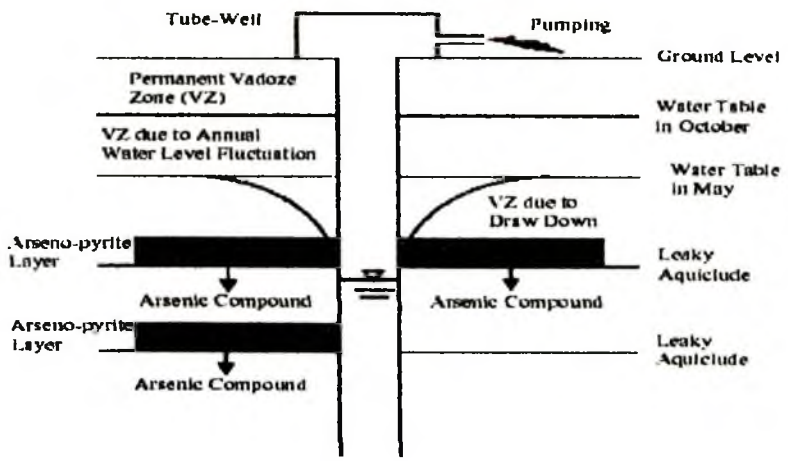


Figure 1.B: Mechanism of Arsenic Contamination

The alternative hypothesis on the arsenic contamination is the oxyhydroxy-reduction theory proposed by Nickson . Some scientists and researchers have accepted this theory recently as main the process for mobilization of arsenic in groundwater. According to this theory, arsenic is derived by desorption from ferric hydroxide

minerals under reducing conditions. Ferric hydroxide minerals are present as coatings in the aquifer sediments. In anaerobic groundwater, these sedimentary minerals release its scavenged arsenic. The oxidation of arseno-pyrite could be the main mechanism for the groundwater arsenic contamination in Bangladesh but there is not enough hydrological and geochemical data to validate the process completely. The validity of oxyhydroxy-reduction theory is also questionable due to the lack of comprehensive sampling and systematic analysis of iron oxy-hydroxides in the affected areas.

1.2.3 : Scale of the Arsenic Crisis

Arsenicosis in West Bengal in 1983 (Saha, 1984) and in 1987 in border-crossing Bangladeshi patients (Saha, 1995). During the 1980s, a Calcutta-based researchers uncovered 3,000 villages in West Bengal contaminated about 50 µg/L, (Chakraborti, 2003) prompting the 1998- 99 British Geological Survey- (BGS) and Bangladeshi government survey of 3,534 wells throughout Bangladesh. The BGS survey found wide-spread and severe arsenic contamination, (Figure 1.C, Survey BG, 1999) with estimates that 8.6% of the population drinks at 50-100µg/L As, 10.9% at 100-300µg/L As, 4.5% at 300-600µg/L As, and 0.83% at > 600µg/L As (Lokuge, 2004).

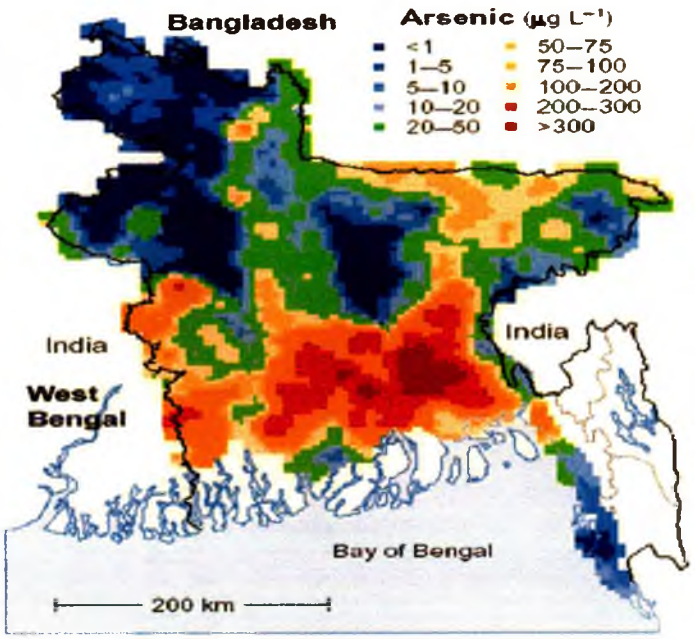


Figure 1.C: Tube well arsenic concentrations in Bangladesh, as determined by the 3,534-well Bangladeshi Government- British Geological Survey (Survey BG, 1999)

Preliminary field testing of water samples showed that there are over 05 million shallow tube wells in the country, around 80-90% of these have been contaminated and about 72 million people of 61 out of 64 districts in Bangladesh have been exposed to the risk of drinking arsenic contaminated ground water (Anwar, 2002, Shahidullah, 2001). The revised WHO and EPA drinking water limits are at 10 µg/L, and toxicity is readily apparent above 300 µg/L. In one Bangladeshi district alone, an academic group found that 358 out of 375 sampled tube wells (95.5%) were contaminated by As > 50µg/L, with 126 tube wells (33.6%) showing severe contamination by As > 300µg/L (Hassan, 2003; Yokota, 2002). Water treatment technologies and alternative water sources have met mixed success,

but they are widely constrained by costs—about 97% of villages have not received any remediation. A few small-scale clinical trials in West Bengal of arsenic chelators such as BAL, D-penicillamine, DMSA, and DMPS have not shown promising results (Rahman, 2001).

A large portion of this Ganga-Meghna-Brahmaputra plain, an area totaling 500,000 square kilometers and spanning all of Bangladesh and most of India, shows significant groundwater arsenic contamination, putting more than 500 million people at risk of chronic arsenic poisoning, says Chakraborti. He published these alarming estimates in the June 2004 issue of the Journal of Environment Monitoring. World Health Organization (WHO) has estimated that 80% of Bangladeshis are at risk of arsenic-related diseases and labeled this "the worst mass poisoning in history."

It is estimated that about 200,000 (Bagla, 1996)-300,000 (Chakraborti, 2002) patients are afflicted with skin lesions and associated cancers in West Bengal, while several times this figure in neighboring Bangladesh. Projections from the BGS survey data estimate that 3,000 (Clarke, 2003)-9,100 (Lokuge, 2004) patients die per year from arsenicosis in Bangladesh. Despite uncertainty on the precise numbers affected, there is at UNICEF, WHO, the World Bank, the Governments of Bangladesh and India, and the academic

community that the Arsenic Crisis is a "global health emergency"(Smith, 2000; Chakroborti, 2002 and Rahman, 2001)).

Traditionally, people used surface water for drinking and cooking. In the 1970s-80s, increasing incidence of infectious, waterborne diseases urged the government and rural people to switch the water source from surface water to groundwater. The Governments of Bangladesh and India used UNICEF and World Bank financing to drill 600,000 tube-wells at 20-60m depths throughout the countryside to:

a) Provide villagers with drinking water free from cholera, typhoid, and dysentery; and

b) Provide reliable, year-round irrigation water for intensive rice cultivation, as an alternative to the annual and sometimes unreliable monsoon rains.

Since then, government and private initiatives have introduced a total of 3 to 10 million more tube wells, which now provide 95% of the drinking water. Although the incidence of waterborne diseases drastically decreased, contamination of the groundwater by arsenic was discovered 10-15 years later. There is no municipal supply of drinking water, and people obtain groundwater from tube wells with hand pumps, which are either owned by one household or shared by several households. In the 1980s, it was discovered that the alluvial

sediments of the Ganges delta are highly contaminated by arsenic, particularly in the shallow aquifers (Acharyya, 1999).

The cause of arsenic contaminated drinking water in Bangladesh is geological. Borehole sediment analysis show high arsenic concentration in soil layers where it is found to be associated with iron pyrites. Dense population on a limited surface area causes vigorous extraction of ground water by thousands of borehole and tube-wells in Bangladesh as drinking water and for irrigation during drought season .Due to this heavy extraction, there is marked fluctuation in water level before and after the monsoon season. During such fluctuation, pyrites are decomposed and arsenic leaches out from pyrites (Ahamed, 1997). Bangladesh is situated in the terminal end of three great river systems, the Ganges, Brahmaputra and Meghna, a large part of the country is under normal inundation and affected by severe flood and sediments containing Arsenic have been deposited throughout the geologic time (Khan, 1997).

Though Bangladesh becomes the eye of the crisis, incidence of arsenic contamination is also documented in various countries of the world e.g. Argentina, Taiwan, Mongolia, China, Thailand, Mexico, Poland, Chili, Canada, West Bengal of India and some states of USA.But it is an irony that a large number of the people of the most

vulnerable and severe catastrophic country like Bangladesh are neither aware nor can imagine the fate of long term exposure to arsenic through drinking water. In recent times, the numbers of arsenicosis poisoned victims are reported to be over 44000 in quick succession in contrast to the measures taken to mitigate the contaminated water.

Arsenic is a naturally occurring silvery white, metalloid but brittle, tasteless, poisonous element in the earth crusts. It is also found in the atmosphere, aquatic environment, soils and sediments and in the organisms. It is mobilized in the environment through various natural phenomena like weathering, biological activity, volcanic activity, and anthropogenic activity (fossil fuel combustion, smelting operation) as well as through a range of human activities including mining, industry and agricultural use of arsenical pesticides. The main mode of transport of arsenic in the environment is through water. Intensive extraction of ground water for irrigation and application of phosphate fertilizer possibly triggered the recent release of arsenic to ground water in Bangladesh (Acharyya, 2000).

Soil- and waterborne arsenic does not readily permeate the skin, though soil can be a key source of exposure in young children who show significant hand-to-mouth activity. Exposure to arsenic

may occur through inhalation and ingestion. Inhalation occurs due to occupational exposure to smelting of Copper, Lead, Zinc and other ores, pesticides and by cigarette smoking. Ingestion of arsenic containing foodstuffs, marine fish and other seafood's contribute in arsenic exposure. People are also exposed on a more sporadic basis through a hodgepodge of human activities, such as the burning of fossil fuels, waste incineration, smelting of ores, pesticide and herbicide use, coal burning, semiconductor production, and other manufacturing processes. The public health impact of these exposures is largely unknown as the epidemiologic focus has been on exposure via drinking water.

Most of the villagers depend on rice cultivation and, to a much smaller extent, on wheat and vegetables. Studies from 2002 and 2003 have found indirect arsenic exposure through rice and vegetables irrigated with As-contaminated groundwater (Roychoudhury, 2002; Roychoudhury, 2003; Mehrag, 2003; Alam, 2003) as evidenced by significantly elevated arsenic concentrations in tube well irrigated boro (winter season) rice over monsoon-irrigated aman (summer season) rice (Duxbury, 2003). Approximately half the arsenic found in rice is inorganic, (Schoof, 1999) and dietary projections indicate that exposure through rice consumed at

500g/daily is up to one-half the exposure through water. Although it is unknown whether the arsenic levels are natural or are introduced through irrigation . In all, the BGS estimated that 21 million are exposed to arsenic-contaminated drinking water above 50 μg/L As in Bangladesh, (Survey BG, 1999) and the Indian government estimates 4 million in West Bengal, (Welfare, 1996) with similar estimates by UNICEF, the World Bank, and academic researchers (Akman, 2002).

Exposure to arsenic adversely affects multiple organ systems. Following ingestion, arsenic is absorbed and distributed widely to almost all tissues of the body e.g. liver, spleen, kidneys, heart, intestine, lungs, brain, muscle and thyroid gland .The toxicity of arsenic compounds depend on the amount, the chemical and physical form of arsenic e.g. trivalent and inorganic arsenic is more toxic than pentavalent and organic arsenic and the duration of exposure to arsenic (Khan, 1997). Arsenic can give rise to acute and chronic toxicity in the body. The symptoms of acute toxicity include vomiting, diarrhea, muscle cramp, facial edema and cardiac abnormalities. But the clinical manifestation due to chronic toxicity develops very insidiously after a long time of exposure.

Among children, chronic arsenic exposure has also been reported to cause adverse effects on the digestive, respiratory,

cardiovascular, and nervous systems. An article in the September 2004 issue of EHP reported intellectual impairment occurring when arsenic in drinking water exceeded 50 μg/L.

There is evidence that arsenic-exposed people who are predisposed to non-cancerous skin lesions may be more vulnerable to other cancers. "During our long field experience in West Bengal and Bangladesh we observed that those who are suffering from severe keratosis appear more likely to develop cancer later on," says Chakraborti, director of the School of Environmental Studies at Jadavpur University in Calcutta, India, "Not only skin cancer but internal cancers also may arise in people who show such non-cancerous lesions ."

1.2.4: A Special Population: The Very Young

Infants and children are deemed to be more susceptible than adults to the adverse effects of arsenic and other toxic substances. Chakraborti has observed that arsenical skin lesions show up sooner in children than they do in adults. If the child's nutrition is poor, outward signs of arsenic toxicity manifest even sooner and at less extreme levels of exposure. An additional concern is the potential for increased sensitivity of children to arsenic-

associated neuro-psychological effects such as reduced verbal IQ scores, as reported in the September 2004 issue of EHP.

Chakraborti speculates that infants and children may be intrinsically more susceptible than adults due to differences in metabolism, a view supported by some preliminary studies. "In one of our studies on an arsenic-affected population in Bangladesh, we found that the second step in arsenic metabolic pathways is more active in exposed children in comparison with exposed adults," he says. In the June 2005 issue of EHP, Maria and colleagues identified a developmentally restricted component of arsenic metabolism, a genetic association with urinary arsenic metabolites that applied only children.

Complicating this scenario is the special threat posed by in utero exposure to arsenic. One of the concerns here is that low-level exposures may have a greater impact if experienced in utero than if experienced in childhood or adulthood. Waalkes and colleagues were the first to identify the transplacental carcinogenic potential of arsenic. They duplicated this finding in several rodent studies, reported in the 1 August 2004 issue of Toxicology and Applied Pharmacology and the 20 May 2004 issue of Toxicology.

"The critical window of exposure for mice equates to about the middle three months of pregnancy in humans," says Waalkes. "This could lead to a fifty percent increase in the risk of hepatocellular carcinoma for adults. This is a reproducible phenomenon, and it has alarming implications for in utero exposures in humans." The first half of fetal development is a period of very high sensitivity because of a high rate of cell proliferation, cell differentiation, and gene imprinting, all of which, when disrupted, can lead to carcinogenesis.

Arsenic readily crosses the placental barrier and fetal damage has been reported. Arsenic content in human umbilical cord blood has been found to be the same as in the maternal blood. Small doses of inorganic arsenic induce mild vasodilatation and result in slight edema. This had been misinterpreted as weight gain and was recommended as 'tonic' in earlier times. · Arsenic, when absorbed into the biological system, can undergo biotransformation under in vivo conditions. Less toxic pentavalent arsenate could be reduced to the highly toxic trivalent arsenite. The half-life of this bio-transformed highly toxic arsenite is three to five days, when it can cause harmful effects to the system. The lethal dose of arsenic is 1-4 mg/kg body weight (Pontius, 1994)

Smith's studies of bladder and lung cancers also have indicated that there may be a long latency ,40 years or more for these cancers ,from arsenic exposure to the manifestation of malignant disease. For example, he has found very high lung cancer risks in Chilean adults who were exposed as children or in utero. He notes that it is critically important to study large populations with significant and well-documented arsenic exposure. Smith says Chile has the best-documented exposure in the world.

"In any country where people are exposed to high levels of arsenic, if nothing else is done, they should focus on protecting pregnant women, providing them with low-arsenic water," says Waalkes. "That would be my top priority if I could advise the governments of those countries on what to do."

1.2.5 : Pathology of Arsenicosis

Trivalent toxic arsenite is known to inhibit enzymes by reacting with biological ligands containing sulphydryl (SH) groups. Pyruvic dehydrogenase is highly sensitive to arsenic because of its interaction with two 'SH' groups of lipoic acid. Arsenic inhibits pyruvate dehydrogenase, leading to pyruvate accumulation (Alfred, 1991). It has been reported that arsenic levels in skin (0.12 ppm), nail (0.36 ppm) and hair (0.65 ppm) are relatively higher because of

its affinity for SH groups. Blood arsenic level ranges from 0.01 to 0.92 ppm/unit (Das, 1995).

Arsenic is a known carcinogen, and is responsible for cancers of the skin, lung, and bladder. Chronic arsenic exposure has been implicated in several non-cancerous conditions, including diffuse and spotted melanosis, palmo-plantar nodular hyperkeratosis, and a variety of systemic health effects (Tchounwou, 2003). Much knowledge has been acquired from the use of Fowler's solution (1% As2O3) by physicians to treat psoriasis, asthma, syphilis, and leukemia from 1786 until the late 1960s, (Cuzick, 1992;Fierz, 1965) and from large-scale epidemiological studies since the late 1970s in arsenic-afflicted villages of Taiwan, the Andes Mountains, and Bangladesh/West Bengal.

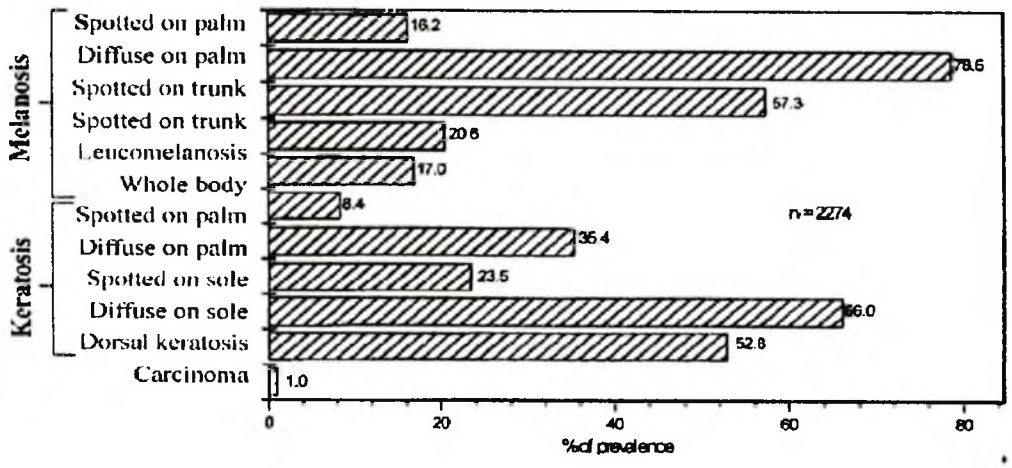


Figure 1.D: Prevalence among 2,274 arsenicosis patients from West Bengal. (Mitra, 2004)

Epidemiological arsenicosis studies in Bangladesh/West Bengal have found the following salient clinical signs, listed in decreasing frequency. (Mazumder, 2003; Saha, 2003; Rahman, 2003) (Figures 1.D).

1) Melanosis (Hyerpigmentation) (95%), diffuse (~90%) or spotted (~50%) (rain-drop patterns), esp. trunk and forearms; reported more frequently and more consistently than any other symptom of arsenic toxicity; sometimes spots have mild keratinization; develops after 6+ mos. exposure at the highest doses.

2) Leucomelanosis (Hypo pigmentation) (35%), spotty, irregular discoloration on trunk and forearms; often together with hyper pigmentation; often formed from hyper pigmentation spots after removing patient from arsenic exposure; develops after 1+ yr. exposure

3) Hyperkeratosis (60%), bilateral hardening and actinic nodules (0.4-1cm diameter.) on soles and palms; often on thenar and lateral borders of palms, on roots or lateral surfaces of fingers; sometimes coalesced into a) verrucous growths, b) diffuse keratosis, or c) papular keratotic plaques, sometimes on other parts of the body (fingers, toes, arms, legs); develops after 5+ yrs. exposure; less common in children

4) Hepatomegaly (20%), often with non-cirrhotic portal fibrosis and portal hypertension.

5) Neuropathy (20%), peripheral vascular disease, burning sensations in the eyes; similar to Guillain-Barré syndrome, with loss of touch, hyperpathia, tremor and deep tendon reflexes, often in lower limbs; dizziness

6) Skin Cancer, (1-5%) squamous and basal cell carcinomas (SCC/BCC), often with several foci, arising de novo or originating from hyperkeratosis, often in the feet and hands, sometimes on trunk, lesions 0.8-3.5cm in diameter; amputation of limb is common remedy in Bangladesh villages. Also, the arsenic-unique Bowen's syndrome (SCC in situ, often on trunk in scaly, crusted plaques 1mm-10cm diameter); develops after 10+ yrs. Exposure

7) Lung, bladder, urinary tract cancers (1%) often found in older patients; remains under-diagnosed in Bangladesh and West Bengal during due to lack of facilities and resources.

The prevalence and incidence of these non-cancer manifestations of arsenic exposure is highly variable from one country to the next. For example, whereas skin pigmentation and hyperkeratosis are common indicators of arsenic exposure in Taiwan, it may be more common in India to see respiratory stress,

polyneuropathy, and peripheral vascular disease linked with habitual ingestion of high-arsenic drinking water.

1.2.6: Arsenic-induced Melanosis and Keratosis

The link between exposure to arsenical medicines and skin cancer and hyperkeratosis has been reported (Hutchinson, 1887). Since then, the signs of arsenic dermatopathology have been clearly defined. (Shannon, 1989) In Bangladesh, hyper/hypo pigmentation and hyperkeratosis have an average latency of 5-10 years, while arsenic-induced skin cancers have an average latency 19-23 years of exposure. (Haque, 2003) Adults show signs of toxicity 4-8 times more frequently than children, men show signs about 20% more often than women. In a typical village exposed about 300 ug/L As, about 25% of the total population show signs of arsenic toxicity. Dermatosis can develop after the termination of exposure, as has been anecdotally reported from Bangladesh, as well as in historic case reports of former arsenical medicine patients. For example, in a Chilean town, residents who had been consuming As-contaminated well water between 600-800 ug/L from 1958 to 1970 were provided with arsenic free drinking water by municipal authorities. Even six years later, children between the ages of 13-14 yrs. showed 65.5% (male) and 78.3% (female) prevalence of skin lesions despite the absence of these lesions when clean water was first provided.

(Borgono, 1997) Similarly, 54 patients out of a cohort of 163 wine growers who had been heavily exposed to arsenical pesticides in the 1940s developed skin carcinomas and arsenic-associated lesions 20 to 35 years afterwards. (Luchtrath, 1983)

1.2.7 : Arsenic-induced Skin Cancers

A classic epidemiological study of 40,421 inhabitants of an arsenic-afflicted region in Taiwan found 428 cases of skin cancer; 90.2% of these cases were associated with hyper pigmentation, and 71.7% were associated with hyperkeratosis. Younger patients often only manifested hyper pigmentation, while older patients often manifested both (Yeh, 1973). A 1995-1996, epidemiological survey of 7,638 arsenicosis patients in West Bengal found a strong dose-response relationship between drinking water arsenic content and hyper pigmentation and hyperkeratosis (Figure 1.E) (Mazumder, 2003).

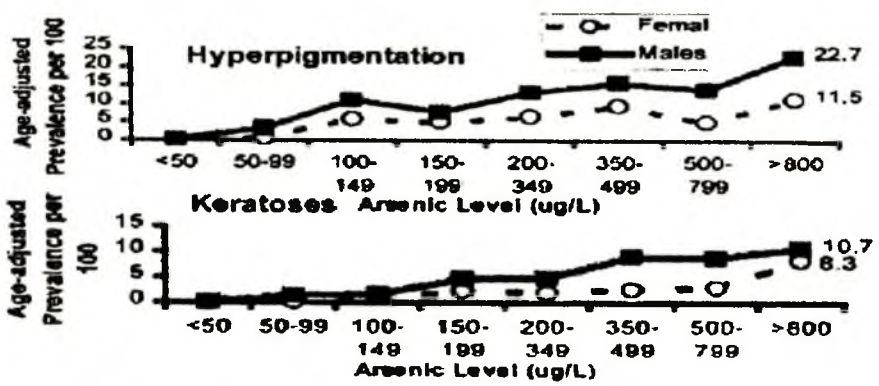


Figure 1.E: The dose response curves of melanosis and keratosis Observed in a 7,638-patient survey in West Bengal. (Mazumder, 2003).

In a 454 patient, 33-year cohort study in an arsenic contaminated village of Japan, it was found that the relative risk of skin cancer (observed) was 4.79% for hyper pigmentation, 10.18% for hyperkeratosis, and 51.07% for individuals with both (Tsuda, 1995). A 2001 New Hampshire study a Squamous cell carcinoma (SCC) risk associated with long-term As exposure [Odds: 2.07, 95% CI: 0.92-4.66] and BCC [Odds: 1.44, 95% CI: 0.74-2.81] (Karagas, 2001). Studies elsewhere have shown that arsenic in drinking water is associated skin cancer, including SCC, Basal cell carcinoma (BCC) (Guo, 2001), and Bowen's disease (Centeno, 1983) and has shown a dose response with skin cancer in Taiwan (Tseng, 1968; Tseng, 1977; Yeh, 1973; Wu, 1989; Tsai, 1999) Argentina, (Arguello, 1950) Mexico (Cebrian, 1983) and Chile (Zaldivar, 1974), Mongolia, (Guo, 2001) with cases in Oregon (Morton, 1976) and dose-response with skin lesions in Indian West Bengal (Haque, 2003) and Bangladesh (Watanbe, 2001; Tondel, 1999; Rahman,1999; Ahsan, 2000).

There is also a wealth of historic studies showing dermal and internal carcinomas in patients exposed to arsenic in the mining (Birmingham, 1965) and smelting industries. (Homlqvist, 1951; Enterline, 1982; Enterline, 1987 ; Yoshida, 2004). In a study conducted by Yu Chen indicates a more than doubling of future excess mortality in Bangladesh owing to cancer of the lung, liver, and

bladder resulting from exposure to arsenic in drinking water (i.e., a rate of 229.6 per 100 000 population vs the background overall risk of 103.5 per 100 000 population). (Yu Chen, 2004)

1.2.8 : Pharmacology of Arsenic Exposure

Although the pathology of arsenic poisoning has been well established, the pharmacology of arsenic within the body is not fully understood, despite attempts to generate human models (Mann, 1996).Rat models have been used extensively, but they do not represent human metabolism completely (Waalkes, 2004). It has been established that As (V) is taken up through phosphate transport systems (particularly in kidney tubule cells (Ginsburg, 1963; Ginsburg, 1965; Tsukamoto, 1983), while As (III) (as As $(OH)_3$) passively diffuses through the membrane, with strong evidence that GSH mediates cellular transport (particularly in liver cells and erythrocytes, discussed below). Drinking water exclusively contains inorganic arsenic, about 90% As (III) and 10% As (V) in typical Bangladeshi arsenic well. Internalized arsenic is methylated in the liver cells and to a limited extent in erythrocytes, and then rapidly excreted through urine and feces (Vahter, 2001). Residual, unreduced As (V) substitutes for phosphate in bone crystals, while residual As (III) complexes with thiol proteins in body tissues, particularly the keratin-rich skin, hair, and nails (Guy, 1999). In

addition, the binding of arsenite to thiol groups of critical enzymes is believed to contribute to its toxicity (Thomas, 2001).

1.2.9: Genetic Differences in Arsenic Methylation

Arsenic patient population studies have found that signs of arsenicosis do not correlate completely with arsenic exposure. It has been suggested that individuals have differential methylation of arsenic dependent upon genetic factors (Vahter, 2001; Goering, 1999). One particular line of evidence is the complete inability of certain a variety of primates to methylate arsenic, (Vahter, 1985; Vahter, 1995; Wildfang, 2001) and methylation differences observed between men and women, between ethnic groups, and between families in recent epidemiological studies in humans in Chile, (Chung, 2002) Argentina, (Vahter, 1995) Taiwan, (Chen, 2003; Hsueh, 1998; Yu, 2000) and Mexico. (Lofted, 2003).

1.2.10 Association Between Tissue Retention of Arsenic and Cancer

A 1975 study of arsenic carcinoma patients orally dosed with arsenicals found that arsenicosis patients retained arsenic in tissues about 100% more than control patients (Bettley, 1975). A neutron activation study of 183 skin specimens from 27 patients with past exposure to arsenic and 60 with no exposure history did not find correlation between arsenic content and development of skin

lesions, although arsenic patients had 70-100% higher arsenic content than non-arsenic patients (Domonkos, 1959) . A 1993-1996 case-control study in New Hampshire with 587 BCC patients, 284 BCC patients, and 524 controls found a link between toenail arsenic content above the 97 percent of arsenic content and skin cancer [Odds: 2.07 for SCC: 95% CI: 0.92, 4.66 and 1.44 for BCC: 95% CI: 0.74, 2.81] (Karagas, 2001). Interestingly, in vitro studies suggest that methylated arsenicals found in keratinocytes originate from the bloodstream, and are not metabolized in skin cells themselves (Patterson, 2003;Vega, 2001) .

The most alarming role of chronic arsenic exposure is carcinogenicity, which may affect even after decades of exposure. Bowen's disease, basal cell carcinoma and squamous cell carcinoma of the skin is reported as the consequences of prolong exposure to arsenic .An expert panel of the National Research Council in Washington, USA concluded that chronic ingestion of inorganic arsenic causes bladder, lungs and skin cancers (Douglas, 1999) .The adverse heath effects of arsenic may be transferred to the generations to come, as the trans-placental crossing has been demonstrated in both experimental animals and human subjects (WHO, 1981).

1.2.11: Molecular Basis of Arsenic-Induced Cancers

Arsenic is hypothesized to induce cancer through the formation of Radical Oxidative Species [ROS], which damage proteins, DNA, and lipids, and elevate AP-1, NF-kB, and tyrosine phosphorylation, as well as other cancer-associated signaling pathways (Xie, 2004). Over 40 studies in the past decade have shown ROS's generation and cellular oxidative damage by arsenic species (Shi, 2004), as well as strong links of ROS's to skin carcinogenesis (Nishigori, 2004). A 2004 study by Tchounwou found that arsenic was highly genotoxic and cytotoxic to human keratinocytes (HaCaT), melanocytes (CRL1675) and dendritic cells (THP-1 + A23187) as measured by the Comet assay and fluorescein diacetate assays (Graham, 2004). It has been reported that 64 arsenicosis patients in Taiwan had elevated ROS levels in their blood and depressed antioxidant capacity (Wu, 2001). A 2004 rat-model study (Rossman, 2004; Burns, 2004) and a 2000 mouse-model study (Yamanaka, 2000) found that UV and arsenite are strongly synergestic skin carcinogens, and it is speculated that the arsenicosis pandemic in Bangladesh is worsened by greater exposure to sunlight. Epidemiological studies among arsenicosis patients in Bangladesh suggest that general, pre-malignant arsencisosis neoplasms are genetically regulated by DNA repair gene XPD (Ahsan, 2003) and oxidative stress genes myeloperoxidase (MPO) and catalase (CAT)

(Ahsan, 2003) . Bangladeshi arsenicosis patients also showed markedly higher Sister-Chromatid Exchange rates. (Mahata, 2003) It has been reported that elevated levels of 8-OHdG (generated by hydroxyl reaction with DNA) were reported in 78% of 28 Japanese patients with arsenic-induced neoplasm's and keratosis (Matsui, 1999) . An in vitro human keratinocyte model found that As exposure induced keratosis-like proliferation, coupled with the proliferation marker Ki-67 (Klimeccki, 1997). Mouse-model studies found that As (III) strongly stimulates TGF-α and GM-CSF in keratinocytes, (Germolec, 1998;Germolec, 1996) while As (V) induces IL-α (via NF-κB and AP-1) and keratinocyte proliferation that was reversible by IL-α-neutralizing ABs (Corsini, 1999). TGF-α over expression in the epidermis elicits hyperkeratosis and SCC, (Dominey, 1993;Vassar, 1991) both symptoms commonly seen in arsenicosis patients. DNA micro array studies have also found a variety of oxidative stress genes in human keratinocytes up regulated by arsenic exposure (Hamadeh, 2002; Rea, 2003).

Researchers at Dartmouth College have discovered that arsenic may trigger endocrine disruption. The findings, published in the journal Environmental Health Perspectives, may offer important information on how arsenic causes a variety of the diseases to which it has been linked. 'This is unlikely to be the only mechanism

underlying diseases associated with low-level arsenic exposure, but we expect it will be an important contributor,' said Hamilton. (J Hamilton et al Heavy Metal: Arsenic Is An Endocrine Disruptor, Environmental News Network 2001).

Arsenic's cancer-causing properties may stem from the production of DNA-damaging particles called free radicals, researchers report. The finding supports the use of antioxidants such as vitamin C and vitamin E, which mop up free radicals, in cancer prevention. 'Having a better understanding of how arsenic causes gene mutations and cancers provides a means to design interventions both in the treatment as well as in the prevention (of cancer),'(Hei et al, Arsenic Triggers Flood Of Free Radicals - Reuters Health, 23 Feb 2001).

'This piece of research provides the first clear-cut evidence that an environmental carcinogen acts predominantly through a free-radical pathway,' says Heir ... professor of radiation oncology and public health at the Center for Radiological Research at Columbia's College of Physicians and Surgeons.... The study, which also involved P&S dermatology researchers and researchers at Albert Einstein College of Medicine and Colorado State University, showed that cells cultured in the laboratory sharply increased their free radical production within five minutes of being exposed to an arsenic

compound. The compound, sodium arsenite -- the main toxic form of arsenic in the environment -- also boosted the rate of mutations among the cells. Mutations are a key step in cancer development. The mutation rate shot up still higher when researchers added a chemical that reduced the cells' production of natural antioxidants. This was consistent with previous research suggesting that antioxidants can protect cells from arsenic-induced genetic damage (Columbia News 15 Feb 2001).

No one yet knows how this inter individual variation in arsenic metabolism actually affects cancer risk. "This is a difficult question since when you deal with the carcinogenicity of inorganic arsenic you are dealing with six or more distinct [metabolites]," says H. Vasken Aposhian, a molecular and cell biology professor at the University of Arizona. Aposhian is involved in studies in New England, Mongolia, Romania, Mexico, and Kazakhstan to identify unique or abnormal arsenic urine profiles in people who develop cancer in areas of high arsenic exposure. Once studies reveal which of these metabolites are promoters and/or carcinogens, it will be possible to better answer the riddle of inter individual variation in vulnerability to arsenic-induced effects. The metabolite MMA(III) presently is one of the leading candidates as potential cancer inducers. If MMA(III) turns out to be carcinogenic, an increased or

decreased amount in the urine might prove useful as a marker for potential future arsenic-mediated cancer.

There is substantial evidence of variation in susceptibility to arsenic toxicity. This variation is due to different ethylating capacity between individuals and population groups. There may be genetic polymorphism in the biotransformation of in organic arsenic and its role in toxicity (Vahter, 2000). Arsenic patient population studies have found that signs of arsenicosis do not correlate completely with arsenic exposure. It has been suggested that individuals have differential methylation of arsenic dependent upon genetic factors (Vahter, 2001; Goering, 1999). One particular line of evidence is the complete inability of certain a variety of primates to methylate arsenic, Vahter, 1985; Vahter, 1995; Wildfang, 2001) and methylation differences observed between men and women, between ethnic groups, and between families in recent epidemiological studies in humans in Chile, (Chung, 2002) Argentina, (Vahter, 1995) Taiwan, (Chen, 2003; Hsueh, 1998; Yu, 2000) and Mexico (Loffredo, 2003).

Latency periods of several years for the development of arsenic related health problem is one of the main discrepancies between the severity and unawareness. Many of the advanced and serious problems are incurable due to delayed clinical manifestations.

Detection at early stages can be helpful in remission of symptoms provided the supply of arsenic free drinking water is ensured. But detection of arsenic level in every source of water and to provide the alternative to those contaminated sources is almost too much for a poor country like Bangladesh. Moreover, the people already ingested arsenic contaminated water throughout the unknown years is highly vulnerable to various diseases including cancer to various extents. So, the more important issue than the number of patient has been detected, is the mass population at risk due to past and continuing exposure to arsenic. Therefore, it became obvious to search for the curative treatment to reduce accumulation of arsenic in different organs as well as mitigate water to save the people already exposed for long time.

The National Research Council in Washington, USA reported the possible roles played by cystine, folic acid, vitamin B12, Zinc and selenium deficiencies in increasing individual susceptibility to the toxic effects of arsenic. Clearly there are grounds for particular concern that malnutrition might play an important role in Bangladesh setting, and the need to know more than is currently known about the interaction between diet and susceptibility (Douglas, 1999).

It was recognized many years ago that inorganic arsenic inhibits enzyme activity and the trivalent arsenite is the main form of interacting with tissue sulfhydral (-SH) groups resulting in inhibition of many functional groups in enzymes and receptors (vahter, 1999). These enzymes are involved in the process of biotransformation of arsenic and cellular defense as antioxidant in the body. The methylation of arsenic has been considered as detoxification reaction because of the lower retaining ability of methylated arsenicals (Huges, 2000). Once ingested, all arsenic compounds are immediately converted to arsenite as a precursor to methylation and this process is coupled to the oxidation of GSH (reduced glutathione) to GSSG. Glutathione reductase plays the major role to supply the reduced form of glutathione as an electron donar. High concentration of arsenic inhibits this glutathione reductase including synthesis of glutathione peroxidase, oxidoreductase etc. which all are selenium containing enzymes participate as physiological antioxidation process.

Selenium is considered an antagonist to arsenic (Underwood, 1971). Selenium is an important element for the synthesis and activity of the enzymes inhibited by arsenicals . In both the cases selenium deficiency or high arsenic concentration, selenoprotiens are diminished resulting to the accumulation and

toxicity of arsenic in various tissues. Arsenic and selenium are metalloids with similar chemical properties and metabolic fates (Styblo and Thomas, 2001). Moreover, the interaction between selenium and arsenic was studied for many years and the antagonistic protective effects of arsenic against selenium toxicity was postulated by some researchers (Lavender, 1997) and the opposite course of events has also been observed, i.e. selenite can stimulate the excretion of arsenite in the bile of rats (WHO, 1981). Selenium is an essential trace element for the biosynthesis of many enzymes playing vital role in antioxidative function of the body . It cannot be synthesized within the body and must be supplied exogenously through foods. It is documented that arsenic, platinum and gold significantly influence the fate of exogenous selenium, whereby they may adversely affect the availability of this essential element for the synthesis of selenoenzymes (Gregus, 2000).

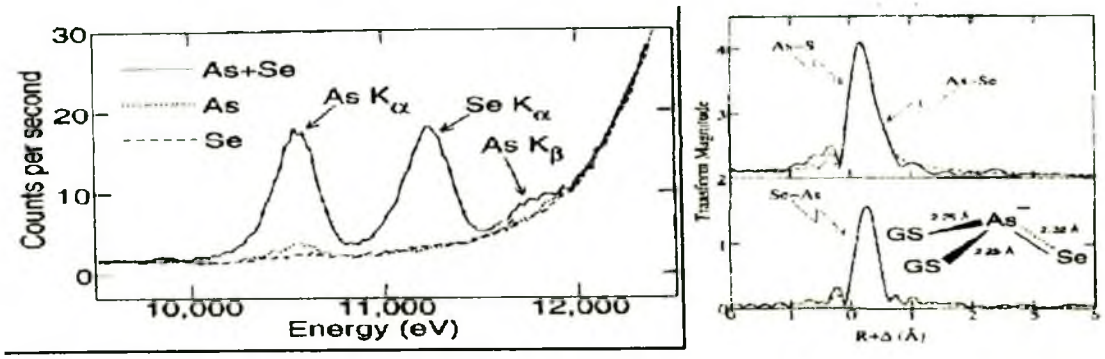


Figure 1.F : Rabbits injected with arsenite and selenite show excretion of both compounds into the bile after 25 minutes. The predominant product appears to be a [(GS)2AsSe]- complex (right), which was shown to be excreted stoichiometrically (i.e. 1:1 ratio) in rabbits injected with arsenite and selenite. (Gailer, 2002;Gailer, 2000)

Rats injected with arsenite and selenate accumulated As2Se in renal lysosomes, which was eventually excreted into urine over a period of weeks (Berry, 1994). A 2003 study in rats found that selenite reduces As levels in the liver and kidney, and that selenite induces biliary excretion of arsenic metabolites, but inhibits urinary excretion (n = 6/set, 6 sets). (Csanaky, 2003) (Rats retain arsenic in the erythrocytes significantly longer than in humans and are incomplete toxicological models, while rabbits have been shown to more closely resemble human pharmacokinetics of arsenic toxicity.)

b) Selenium reduces body arsenic load:

Rats exposed to arsenite and arsenate showed a 64%, 62%, 41%, and 39% reduction in blood As content 24, 48, 72, and 96s hrs after receiving selenite injections (n = 16/set), with lesser reductions

in liver, kidney, lung, spleen, muscle, brain, and heart tissue (Hilmy, 1991). A 2003 study in Albino rats found that selenite administered to arsenite-exposed rats for 28 days prevented arsenic-induced histopathological changes in the ovary and the uterus, and drastically reduced blood plasma arsenic levels. It was found that selenite-arsenite treated rats had blood plasma As concentrations of 0.20 ± 0.02 □g/100mL, whereas the arsenite treated rats had 2.38 ± 0.02 μg/100mL As, and the controls had an arsenic baseline of 0.37 ± 0.03 μg/100mL (Chattopadhyay, 2003). Similarly, selenite was shown to reduce arsenite-induced cytotoxicity in porcine endothelial cells in conjunction with increased activity of GPx (n = 6/set) (Yeh, 2003).

c) Selenium counters cellular toxicity of arsenic:

In one study, antagonism was noted between Se and As in toxicity assays of fish cell lines, with non-toxic amounts of selenite and selenate reducing the acute toxicity of arsenate, and to a lesser extent, arsenite (Babich, 1989) .In a 2004 human cell study, HOS cells exposed to As_2O_3 at high levels of 1mM for 6 weeks showed dramatically increased cell survival when treated with Se-methylselenocysteine, and selenomethionine (Rossman 2004,). Both selenium compounds reduced 6-week mutations by 70-80%. The protective effect of glutathione was also seen in a study by Alauddin

in 2003, in which rabbits were exposed to arsenite for 7 days, followed by treatment with vitamins/zinc/selenium or placebo (Rabbani, 2003). Arsenite administration to rabbits significantly reduced glutathione concentration (post-arsenic, 17.5 ± 0.81 mg/dL vs. pre-arsenic, 32.0 ± 0.76 mg/dL, $p<0.001$, n = 10/set). The recovery of the depleted glutathione was significantly greater in the vitamins/zinc/selenium (67.0 ± 17.0%) treatment group compared with the placebo group (36.0 ± 7.0%). Metabolic studies have attempted to explore the effect of arsenite on selenium anticarcinogenetic activities and have produced an unclear picture. Three rat-model studies found that arsenite inhibits selenium metabolism and consequently increases its cytotoxicity (Kraus, 1989; Hsieh, 1975;Hsieh, 1977). Similarly, a 2001 study in cultured rat hepatocytes found that selenite inhibited arsenite metabolism and increased cellular toxicity (Styblo, 2001) . Another rat-model study found that arsenite decreases the anti-carcinogenic activity of selenite, but boosts the anticarcinogenic activity of $(CH_3)_3Se+$ (Ip, 1988;Obermeyer, 1971).

d) Selenium prevents arsenic-induced chromosomal damage:

Similarly, selenite has been shown to inhibit arsenite-induced chromosomal aberrations in human lymphocytes (Sweins,

1983), SCEs by arsenite in human primary peripheral lymphocytes, (Beckman, 1986) DMA-induced tetraploidy by dimethylarsinic acid in hamsters, (Uden, 1997) arsenite-induced hypomethylation in vitro in human colon Caco-2 cells and in vivo in rat liver and colon, (Davis, 2000) and arsenite-induced sister chromatid exchanges in human peripheral lymphocytes (Hu, 1989). Albino mice exposed to selenite followed by arsenite showed as much as a 40% reduction in chromosomal aberrations, although mice exposed to arsenite followed by selenite and simultaneous arsenite and selenite showed smaller reductions (n = 12/set). (Biswas, 1999) In a human population study, 150 μg/Se/day for 21 days has been shown to decrease the MN and chromosomal aberrations by 46% in lymphocytes of As-exposed mineworkers in China, with the authors speculating that low local dietary Se may have exacerbated baseline cancer frequencies (Hu, 1989; Xuan, 1991).

1.2.12.2: Hypothesized Mechanisms of Arsenic-Selenium Antagonism (Figure 1.G)

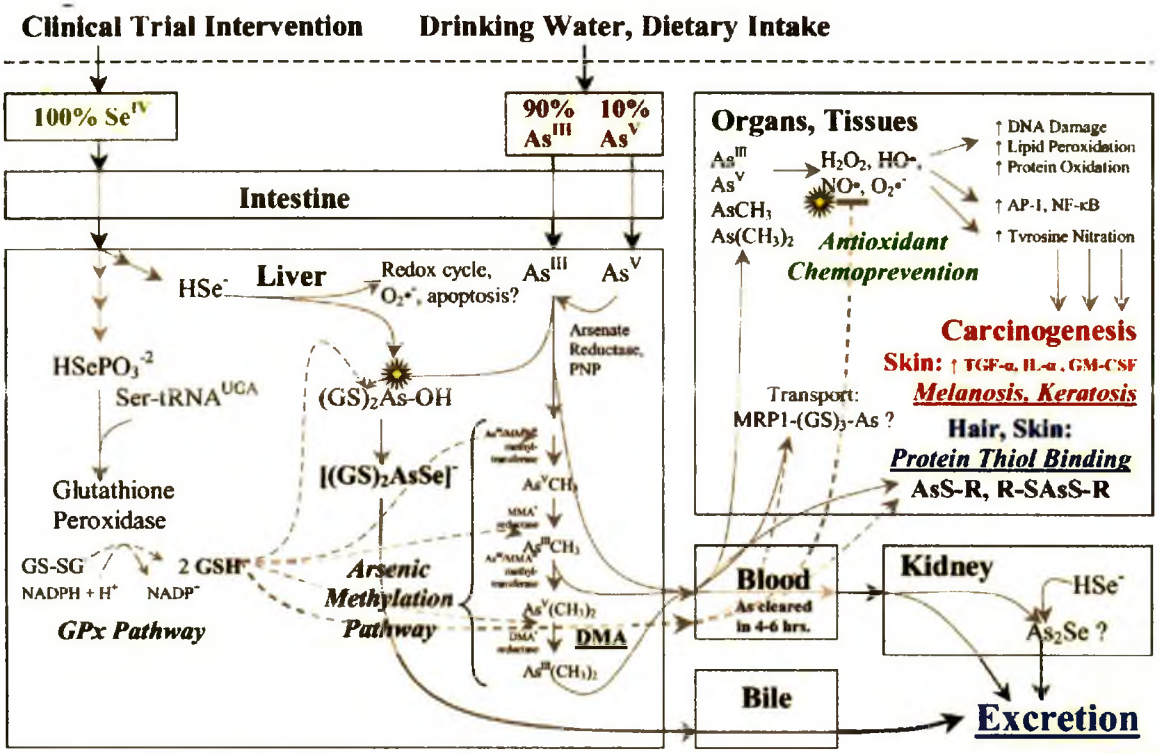


Figure 1.G: The arsenic-selenium metabolism pathway.

a) Mechanism 1: Se up regulation of GPx & GSH anti-oxidant activity:

Selenium is incorporated into Glutathione Peroxidase (GPx), and selenium supplementation in humans leads to direct increases in blood GPx levels (Thomson, 2004; Duffield, 1999; Alfthan, 1991) . GPx protects proteins, DNA, and lipids against oxidative stress by catalyzing the reaction $2GSH + H_2O_2 \rightarrow GSSG + 2H_2O$. Many studies have shown that GSH specifically provides anti-oxidant protection against ROS's generated from arsenic metabolites, as evidenced by

a) As inhibition of yeast glutathione reductase (Styblo, 1997),

b) Depletion of GSH levels and promotion GPx activity in mice within 1 hr. of arsenite injection. (Matsui, 1999), and

c) The decreased sensitivity of NBA (promyelocytic leukemia) cells (Dai, 1999) and rat lung epithelial cells (Lau, 2004) in response to increased GSH levels.

A 2003 study by Alauddin found that rabbits injected with selenite show increased GSH blood levels, with simultaneous protection against arsenite toxicity (Rabbani, 2003). Another 2003 study found that selenite reduced cytoxicity, but not apoptosis by arsenate in porcine endothelial cells, and increased GPx activity. (Yeh, 2003) Likewise, GSH depletion studies in the human UROtsa cell line (Bredfeldt, 2004) the rat myoblast L6 cell line, (Shimizu, 1998) and a variety of human cancer cell lines (Yang, 1999) have shown heightened sensitivity to arsenic. A hamster ovary cell line, xrs-5, was found to have 6-fold lower GPx activity, and the addition of GPx to these cells reduced arsenite-induced micronuclei. Moreover, a 1.6-fold increase of glutathione peroxidase activity by selenite adaptation effectively removed the arsenite-induced micronuclei in CHO-K1 cells (Wang, 1997).

b) Mechanism 2: As-Se Precipitation and Excretion:

Test-tube reactions of arsenate, selenite and GSH produced the Gailer Product, $[(GS)_2AsSe]^-$, (Gailer, 2002;Gailer2000) which is red-brown and insoluble along with as well as dimethyldiselenoarsinate anion, (Gailer, 2002) $(GS)_3As$ (Scott, 1993;Gailer1998) and trimethylselenonium ion, $(CH_3)_3Se^+$. (Sun, 1987) Rabbits injected with arsenite and selenite excrete $[(GS)_2AsSe]^-$ into bile within 25 minutes (Gailer, 2002; Gailer, 2002) while rats injected with selenite and arsenate accumulate As and Se in a 2:1 ratio in renal lysosomes, which is then excreted into urine over a period of weeks (Figure 1.G) (Berry, 1994).

B6C3F1 mice gavaged with 5 mg 73arsenate/kg showed increasing 73arsenic excretion when supplemented with 2.0, 0.2, and 0.02 ppm Se diet, in proportion to the dosage of selenium (Kenyon, 1997) .Similar to As metabolism, Se reacts with 4 mol eq. of GSH to produce GS-Se-SG and GS-SG, which can be reduced to HSe^- by glutathione reductase and NADPH. (Ganther, 1986) It has also been found that $[(GS)_2AsSe]^-$ can be formed from the nucleophilic attack of HSe^- on the As of the arsenic metabolism intermediate $(GS)_2As\text{-}OH$, followed by the loss of the –OH group, suggesting a mechanism for the formation of the excreted $[(GS)_2AsSe]^-$ "Gailer" product (Gailer, 2002).

c) Mechanism 3: As-binding, Methylation, and Transport by GSH:

Arsenic is metabolized in humans and mammals by the As (V) reductase and $AsCH_3$ reductase-catalyzed (Aposhian, 2004) pathway (Thomas, 2001) with 10-30% excreted as As III/V, 10-20% as As III/V CH_3, and 60-80% as As III/V $(CH_3)_2$. (Vahter, 2001) The pathway is shared by both arsenic and selenium, with S-adenosylmethionine (SAM) donating methyl groups, and GSH donating electron groups for reduction (Zakharyan, 1995;Csanaky, 2005). (Figure 1.H).

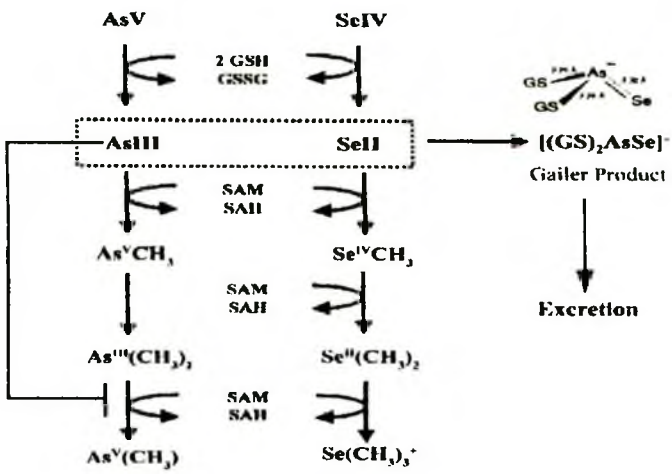


Figure 1.H : Metabolism of Se and As.

Thus, selenium could detoxify arsenic by increasing GSH levels and thereby facilitating arsenic detoxification through methylation, which has been shown to reduce tissue retention and increase excretion (Aposhian, 2004). However, there is recent, conflicting evidence that methylation of As (V) and As (III) to $AsCH_3$ and DMA actually increases arsenic cytoxocity (Styblo, 2000; Petrick,

2000; Petrick, 2001). A transport mechanism is also possible. NMR studies have clearly established that GSH complexes in vitro with As (III), $AsCH_3$, and DMA. (Scott, 1993; Hirano, 2004) As (III) readily accumulates in rabbit erythrocytes, with NMR showing up to 68% remaining bound to GSH, while 20% remaining bound to Hb, with insignificant binding by As (V), $AsCH_3$, and DMA (Delnomdedieu ,1995). There is also recent evidence that GSH mediates As (III) transport by Multidrug Resistance Protein 1 (Leslie,2004).

d) Mechanism 4: Free Radical Generation and Apoptosis:

Finally, there has been speculation that selenite also has a pro-oxidant mechanism in cancer prevention through ROS-induced caspase-3 dependent apoptosis in myelocytic leukemia cells. (Zuo, 2004) Indeed, a 1999 study by Spallholz found that methyselenol, a metabolite of selenomethionine, could potently generate superoxide in vitro, suggesting a possible apoptotic mechanism for selenomethionine anti-carcinogenesis observed in the NPC and SELECT trials (Spallholz, 2001). Nonetheless, the preponderance of evidence suggests that Mechanisms 1, 2 and 3 together are responsible for the observed anti-arsenic properties of selenium.

1.2.12.3 Association Between Low Selenium Intake and Arsenicosis Cancers in Humans

Malnourishment (Hsueh, 1995; Chen, 1988) and β-Carotene (Hsueh, 1997) deficiency in arsenic regions of Taiwan is associated with skin cancer and the arsenic- and cancer-linked Blackfoot disease. It has been found in 2004 study that poor nutrition (as represented by age and sex-matched BMIs) contributes to increase arsenic toxicity among 138 exposed vs. 144 unexposed individuals from 3 Bangladeshi villages (crude prevalence ratio: 1.92, 95% CI: 1.33-2.78), (Milton, 2004) with similar results in a separate, 2004 study of 115 exposed vs. 120 unexposed patients (Islam, 2004) . In a 2004 survey of 192 arsenic-exposed West Bengalis, the authors found a possible link between increased arsenic-induced skin lesions and low dietary intake of animal protein ($p < 0.001$) (high in Se), calcium ($p < 0.001$), fiber ($p < 0.002$), folate ($p < 0.006$), and Vit. C ($p < 0.04$) (Mitra, 2004). There is evidence that low Se intake may influence the development of arsenicosis (Valentine, 1994) and the arsenic-linked Blackfoot disease in As-contaminated areas of Taiwan (Wang, 1996). Similarly, liver biopsies from 5 severely affected arsenicosis patients admitted to a hospital in West Bengal found high levels of arsenic, while selenium was undetectable (Das, 1995).

1.2.12.4 : Human Trials of Selenium Protection Against Arsenic Toxicity

In 1989, a clinical trial study, 150μg/Se/day for 21 days was shown to decrease the MN and chromosomal aberrations by 46% in lymphocytes of As-exposed mineworkers in China, with the authors speculating that low local dietary Se may have exacerbated baseline cancer frequencies (Hu, 1989;Xuan, 1991). A statistical post-mortem analysis of 76 dead Swedish copper smelter workers and 25 controls have also found selenium protection against lung cancer induced by arsenic, lead, and other heavy metals in lung tissue ($p < 0.05$) (Gerhardsson, 1985). After the 1989 mine study, Yang 2002 is the second clinical trial to assess the efficacy of dietary selenium supplements in countering arsenic toxicity, as measured from hair and plasma arsenic content (Figure 1.I) (Yang, 2002).

Eighty-three Chinese patients who had been exposed to arsenic-contaminated well water for 19 years were simultaneously provided clean drinking water and a randomized, 200μg/Se/day or placebo regime. After 14 months, the hair arsenic content of selenium patients decreased from 2.57 ± 0.16 μg/g to 0.680 ± 0.06 μg/g (73% reduction, n = 54), whereas placebo patients dropped from 2.62 ± 0.34 to 1.25 ± 0.16 μg/g (52% reduction, n = 29). Blood arsenic content dropped from 0.051 ± 0.004 to 0.015 ± 0.001 mg/L

in the selenium group (70% reduction n = 54), while arsenic content dropped from 0.064 ± 0.008 to 0.024 ± 0.002 mg/L in the placebo group (62% reduction, n = 29).

	0 month	3rd month	9th month	14th month	No. of cases
Blood As ($\mu g\,ml^{-1}$)					
Se-group	0.051 ± 0.004	0.056 ± 0.006	0.023 ± 0.001	0.015 ± 0.001	54
Placebo-group	0.064 ± 0.008	0.041 ± 0.004	0.031 ± 0.003	0.024 ± 0.002	29
P-value	>0.10	<0.05	<0.01	<0.001	
Hair As ($\mu g\,g^{-1}$)					
Se-group	2.57 ± 0.16	2.15 ± 0.15	1.23 ± 0.10	0.680 ± 0.06	54
Placebo-group	2.62 ± 0.34	2.42 ± 0.31	2.01 ± 0.26	1.25 ± 0.16	29
P-value	>0.1	<0.1	<0.05	<0.001	

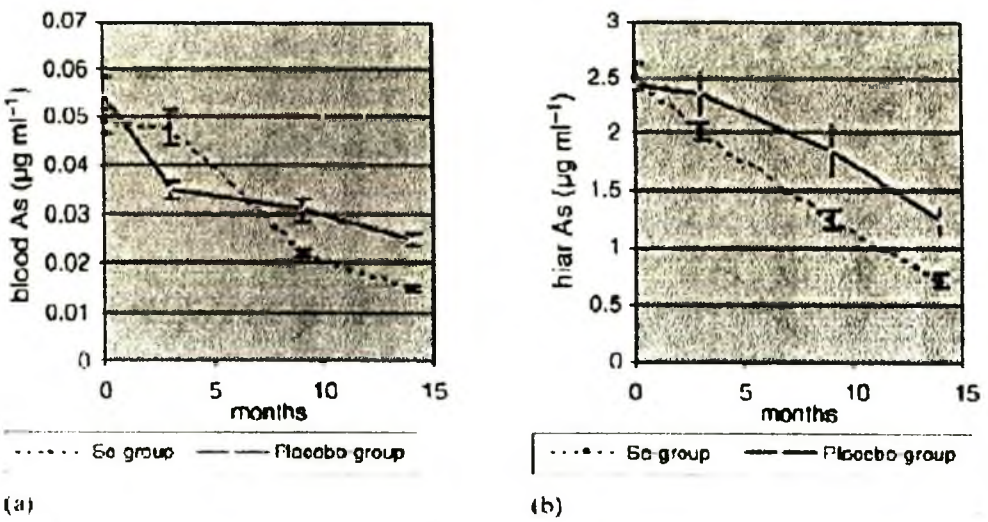


Figure 1(b). Charts show blood (a) and hair (b) As concentration using data from all farmers sampled. Values are mean ± S.E.

Figure 1.I : The findings of Yang 2002. The start of the clinical trial (t = 0 months) coincided with the replacement of the villagers' arsenic-contaminated drinking water with arsenic free water. Therefore, both placebo and selenium treatment register declines in blood and hair arsenic content.

The physicians were blinded and after 14-months, the improvement of selenium supplementation vs. placebo was greatest

among arsenicosis patients suffering from 3 (50%, $p < 0.01$) or 2 (36%, $p < 0.002$) types of lesions (palmer-plantar hyperkeratosis, hypo pigmentation patterns, hyper pigmentation patterns) than among those suffering from only 1 (0%) type of lesion. In another study Xia, 2000 in Inner Mongolia showed arsenic load was decreased in 57% from urine , 54% from hair and 59% from blood respectively after 14 months oral intervention with organic selenium at dose of 200 mg/day (Xia, 2000). In view of the rabbit, rat, mouse, and human cell line studies discussed above, we propose to validate the Yang 2002 study with an assessment of the use of selenium supplements in countering arsenic toxicity inhibiting the progression of melanosis and keratosis in the context of the Bangladesh arsenic crisis.

1.3 Juxtification of the study

Bangladesh is one of the poor countries in the world has been hit by an environmental catastrophe in recent years called "Arsenicosis." The New York times reported on 10th November, 1998 as "Bangladesh is in the midst of a mass poisoning in history, dangerous level of arsenic have been found in the ground water, entering millions of people sip by sip as they drink from over 4 million tube wells." "If this were the United States, they'd call out the National Guard and get everyone bottled water," said Chapel, a physicist who is one of the world's leading experts on arsenic contamination (New York Times, 10.11.98). Fred Pearce of The 'Guardian London' describes occurrence of arsenic is one of the biggest outbreak of poisoning this century and foreign agencies are to blame. Experts say half the population of Bangladesh is being slowly poisoned. A recent survey indicate that tube well water from 61 of the 64 districts has been contaminated with unacceptable levels of arsenic exposing an estimated 75 million people to the risk of chronic arsenic poising in the country. In recent times the number of patients with arsenicosis in Bangladesh is reported to be over 44,000. Because of the tremendous magnitude of the problem, there seems to be no easy and quick way to tackle the problem overnight.

Schmetzer on February 7, 1999 in Chicago Tribune reports that a Belgium study has found the arsenic contained in well water and used for irrigation has crept into leaves, roots and cattle fodder, and may have poisoned the entire food chain in this river-braided delta , home for 140 million people. Another potential danger is through fish consumption cultured in arsenic contaminated water. Fish is the main source of animal protein of Bangladeshi .

Arsenic produces serious health hazards when ingested in toxic amount. There are several reports on clinical and public health consequences of chronic arsenic poisoning in Bangladesh. Arsenicosis, a chronic condition due to prolonged exposure of arsenic above safe level usually manifested by characteristics skin lesions with or without involvement of internal organs and malignancies. From nutritional and metabolic point of view, arsenic is likely to adversely affect human nutrition.

Many agents have been tried to ameliorate Arsenicosis with variable results. We are yet to find an agent safe and effective remedy for Arsenicosis. Selenium is a known antioxidant and has an ability to negate the toxic effects of several heavy metals including arsenic. The interaction between arsenic and selenium was reported by Levander in 1977 and concluded that arsenic has a protective effect

against the toxicity of a variety of forms of selenium .He also documented the metabolic antagonism between arsenic and selenium as arsenite stimulated the excretion of selenium in to the bile, so did selenite stimulate the excretion of arsenic (Levander, 1977). Antagonistic interactive effects were also observed between arsenic and selenium including complete to partial alleviation of the selenium toxicity (Hoffman, 1992). Moreover, both arsenic and selenium interact extensively with sulfhydryl (-SH) groups in tissues, it is possible that arsenic elimination is delayed in Se-deficiency state because there could be more target –SH groups for arsenic to react with because selenium is low (Kenyon, 1997). On the contrary, if sufficient selenium is available to the tissues, the –SH group would not be left free for arsenic to react with and there would be abundance of antioxidant enzymes to counteract the per-oxidative stress by arsenic (Passwater, 2000). A Chinese study (Hu GG, 1989) one among the workers exposed to arsenic showed selenium found antagonizes the toxic effect of arsenic.

Selenium is a suitable agent to reduce arsenic accumulation after chronic exposure for a number of reasons including competition for the methyl donor, S-adenosylmethionine, competition for glutathione (GSH) and inhibition of glutathione reductase by a number of arseno-glutathione complex (Kenyon, 1997). Styblo and

Thomas (1995) reported that like arsenicals, selenite has been shown to react with glutathione to form a seleno-di- glutathione complex. This complex is metabolized by glutathione reductase. It has also been reported in a study that arsenic, platinium and gold containing drugs significantly influence the fate of exogenous selenium, whereby they may adversely affect the availability of selenium which is essential element for the synthesis of selenoenzymes (Gregus, 2000).

Moreover, both selenium and arsenic interact extensively with sulfhydral (-SH) groups in tissues, it is possible that arsenic elimination is delayed in Se-deficiency because there could be more target – SH groups for arsenic to react with because Se intake is low (Kenyon, 1997). Again the in vitro uptake of selenite by intact rat erythrocyte was found to be proportional to cellular glutathione concentration. Release of selenium was dependent upon a reaction catalyzed by glutathione reductase possibly the reduction of seleno-di-glutathione complex as reported by Styblo and Thomas. So, if there is excess arsenic in tissues, GSH is likely to be saturated in order to metabolize arsenic and defending cells from oxidative stress. Consequently, decreased amount of GSH would have interfered the selenium availability and as a result antioxidant selenoenzymes would be decreased. On the contrary, if sufficient selenium is available to the tissues, the –SH group would not be left free for

arsenic to react with and there would be abundance of antioxidant enzymes to counteract the per-oxidative stress by arsenic.

Population thriving on diets low in methionine is likely to suffer more from arsenic exposure (Gorge, 1981). Researcher's has identified many essential trace elements whose functions were previously unknown, and that marginal or severe imbalances can be considered risk factors for several diseases of public health importance (Mertz, 1981). In a study by Wuyi, 2001 that selenium can prevent the accumulation of arsenic in the human body and rectify the damages in the experiment. After the administration of 100-200 micrograms selenium per day for 14 months, 75.0% and 55.0% of the patients served as patients for selenium-therapy group in clinical examination and symptom, and 25.6% and 24.4% as control group showed improvement. In the selenium-therapy group, liver function, hepatic ultrasonography, electrocardiogram and electron microscope observation of erythrocyte reversed significantly than the control as 80 %, 60 %, 72.22 %, 84.78 % versus 46.15 %, 30.7 %, 0 %, 44.83 % respectively. They found that oral selenium supplementation could effectively decrease arsenic concentration in hair, urine and blood of the selenium-group much more than that of control group and reverse the arsenic related skin lesions and symptoms (Wuyi, 2001).

The most efficacious and safest form to supplement selenium is not the inorganic salt form, but the organic forms (high selenium yeast and selenomethionine). The selenomethionine is reported to be more bioavailable even than the selenium rich spirulina (Huq, 2000). Schrauzer of the university of California, San Diego concluded that ten-fold lower oral dosage of organic selenium produced two-fold greater increase in selenium levels in the blood, organically bound selenium is at least twenty-fold more effective in providing the body with the trace element. Studies in New Zealand and Finland showed that selenomethionine was at least 75 % bioavailable compared to 59 % for sodium selenite and raised blood selenium level higher, more rapidly and sustained longer than inorganic selenium (Valentine 1994).

While numerous laboratory investigations have shown that selenium may have anticarcinogenic activity, the epidemiological data have been inconsistent. In a meta - analysis Zhuo suggest that selenium may have some protective effect against lung cancer in populations where average selenium levels are low. The evidence for these findings is greater in studies of toe nail selenium than in studies involving other measures of exposure (Zhuo, 2004).

In a 1989 clinical trial study, 150μg/Se/day for 21 days was shown to decrease the MN and chromosomal aberrations by 46% in lymphocytes of As-exposed mineworkers in China, with the authors speculating that low local dietary Se may have exacerbated baseline cancer frequencies. (Hu, 1989;Xuan, 1991) A statistical post-mortem analysis of 76 dead Swedish copper smelter workers and 25 controls have also found selenium protection against lung cancer induced by arsenic, lead, and other heavy metals in lung tissue ($p < 0.05$). (Gerhardsson, 1985) After the 1989 mine study, Yang 2002 is the second clinical trial to assess the efficacy of dietary selenium supplements in countering arsenic toxicity, as measured from hair and plasma arsenic content. (Yang, 2002; Wang, 2001)

Therefore, it necessarily becomes meaningful to evaluate and justify the therapeutic role of selenium to fight back against the curse of the arsenicosis.

1.4 Review of literature

1.4.1: Background information of selenium

1.4.1.1: History

Selenium is an essential trace mineral of fundamental importance to human health. It was named after the goddess moon "Selene" by the Swedish chemist John Jacob Berzelius in 1817. Selenium first attracted the attention of biologists in the 1930s and then Schwarz established selenium as an essential nutrient for animals in 1957. Soon thereafter deficiencies of selenium and vitamin E were shown to be involved in several economically important nutritional diseases in cattle, ship, swine and poultry (Munson, 1996). Since then, selenium was studied in greater extent to evaluate its role in biological activities on human health. In 1973, Rotruck and colleagues at the University of Wisconsin demonstrated that selenium was incorporated into molecules of an enzyme called glutathione peroxidase (GPX). This vital enzyme protects RBC, cell membrane, subcellular components against undesirable reactions with peroxides (Passwater, 2000).

1.4.1.2 : Source and chemistry

As an inorganic salt and mineral, selenite and selenate are found mainly in the soil. Grains grown in the selenium rich soil and seafoods contain selenium in organic form as selenomethionine or

selenocysteine. Selenium enters the food chain through plants, which take it up from the soil. Therefore, selenium deficiency has been reported in parts of the world notable for volcanic regions, acid soils and complexion with iron or aluminum frequently reduce the uptake of selenium by plants (Rayman, 2000). Among the animal sources, lobster, shrimp, oyster, beef, ham, bacon, lamb, chicken, and egg contain selenium of which highest amount in lobster, 22 μg / oz. The forms of selenium in biological materials, both plant and animal, include selenides, selenate, selenomethionine, selenocysteine, methyl selenocysteine (Munson, 1996). The most efficacious and safest form to supplement selenium is not the inorganic salt forms but the organic forms (high selenium yeast and selenomethionine). The selenomethionine is reported to be more bioavailable even than the selenium-rich spirulina (Vacchina and Cases, 2001).

Chemically, selenium is an allotropic metal in-group six of the fourth period of the periodic table and a member of sulfur family. It is red or gray colored and does not react with water but with nitric acid, perchloric acid and metals to form selenides. Selenium has 26 isotopic forms of which four occur naturally. The principal natural form of the element is $_{34}Se^{80}$. The remaining 22 are radioactive isotopes of selenium of which $_{34}Se^{75}$ is a weak

gamma emitter widely used in biology with a half life of 118 days. The valences of selenium are + 2, + 4 and + 6 (Munson, 1996).

Symbol	:	Se
Atomic number	:	34
Molecular weight	:	78.96
Melting point	:	220 C
Boiling point	:	688 C

1.4.1.3 : High selenium yeast

Selenium yeast is produced when selenium is naturally incorporated into protein of growing yeast under optimum conditions. The resultant yeast has a high concentration of the selenium-containing proteins, selenomethionine and selenocysteine. Food yeasts are not infectious. High selenium yeast contains selenomethionine which is similar to the essential amino-acids methionine but with an atom of selenium instead of an atom of sulfur. The form of selenomethionine that the body can use is L-selenomethionine, which is a better-absorbed and incorporated into body component than any other form. Studies in New Zealand and Finland showed that selenomethionine was at least 75% bioavailable compared to 59% for sodium selenite and raised blood selenium level higher, more rapidly and sustained longer than inorganic selenium Valentine,1994).

1.4.1.4 : Selenoproteins

About 35 selenoproteins have been identified, though many have roles that have not yet been fully elucidated (Rayman, 2000). Sunder of the University of Missouri, Columbia had classified selenoproteins in four distinct groups – 1) ion-specific proteins 2) selenomethionine-specific protein, 3) selenocysteine-specific protein 4) selenium binding protein (Passwater, 2000).

Selenoproteins	Functions
Glutathione peroxidase	Maintains membrane integrity, modulate eicosanoid synthesis by removing hydroperoxides
Iodothyronine deiodinase	Responsible for production of T3 and conversion of prohormone to active hormone.
Thiredoxin reductase	Responsible for regeneration of antioxidant system and maintains intracellular redox state
Glutathione reductase	Responsible for maintaining glutathione in reduced form as electron donor in many antioxidative reactions.
Selenoprotein P	Protects endothelial cells against damage by peroxynitrites
Selenoprotein W	Prevents muscle wastage and protects against damage after over activity

As a constituent of selenoproteins, selenium has structural and enzymatic roles as redox center. The first selenoenzyme recognized, glutathione peroxidase, is a tetramer containing 4 atom selenium and plays a major role in the cell's antioxidant defense .It utilizes its highly hydrophilic electron donor substrate glutathione in order to reduce chemically very different hydro-peroxidase to alcohol (Weitzel, 1990).

1.4.1.5 : RDAs for Selenium

Selenium has been recognized as an essential dietary trace element since 1989, when a 50-200µg Se daily intake was recommended, (Sciences, 1980) later conservatively revised to 55µg Se/day on the basis of a controlled 1999 New Zealand study. The minimum intake of Se is estimated to be 20 µg/L, while toxicity is believed to occur above 500 to 700µg/day (Thomson 1993; and Rayman 2000). The RDA was based on Se's integration as selenocysteine into glutathione peroxidase (GPx-1, 2,3,4), but it has been argued that an intake of 100ug Se/day is necessary for maximal GPx activity. (Thomson 2004; and Rayman 2000) Selenocysteine is also incorporated into other critical proteins such as 1) selenoprotein-P, 2) 5' thyronine deiodinase (I, II) 3) thioredoxin reductase, and 4) selenophosphate synthetase (Figure 1.J). Genome surveys have found a total of 25 selenoproteins, although the function of the remaining proteins is still being explored (Burk 2003)

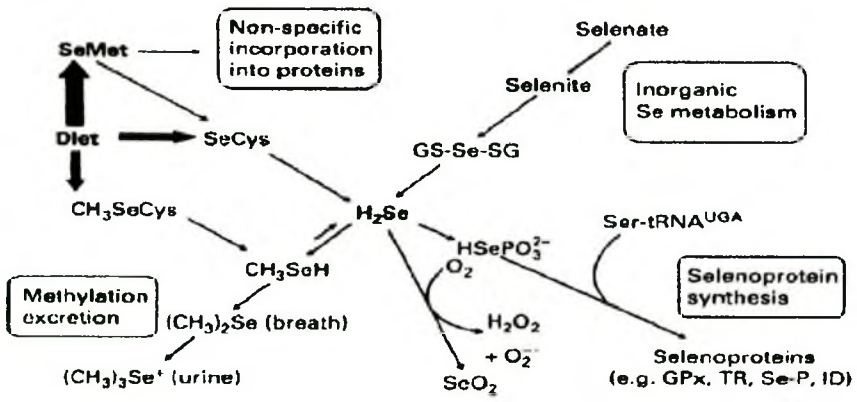


Figure 1.J: Selenium metabolism pathways. (MP, 2004)

1.4.1.6 : Selenium in the Diet

The FDA's RDA is easily fulfilled in American diets at 80-120μg/day through the consumption of 40-60g of animal protein. In the developing world and largely agrarian Bangladesh villages, animal protein intake is low, with fruits and vegetables often proving to be poor sources of Selenium (Spallholz, 2004). To our knowledge, there were no soil selenium maps of Bangladesh or the regional Delta area. Spallholz ,1978 had measured in 25 samples from Jessore district of Bangladesh and found less amount of selenium in soil which automatically produce low selenium containing crops, which in turn produces low selenium containing animal foods (Spallholz ,1978). Inorganic Se is found in dietary supplements as selenite, but in the unsupplemented dietary intake, it is found organically as L-selenocysteine, selenomethionine, and Se-methylseleno-Lcysteine (Schrauzer, 2003). Some clinical trials in cancer chemoprevention have used selenomethionine or selenium yeast (90% selenomethionine), while others have used selenite.

1.4.1.7 : Deficiency diseases

Since 1950s, selenium deficiency diseases have been identified on a wide scale in livestock being reproductive impairment, growth depression and white muscle disease, a myopathy of heart and muscle. These disorder have had such economic consequences

that top dressing of pastureland with selenised fertilizers, mineral mixes, boluses and drenches are now applied to prevent their occurrence (Rayman, 2000).

Clinical deficiency disease responsive to selenium in human beings is Keshan disease, a cardiomyopathy observed in China. Low plasma selenium levels also have been noted in colonic, gastric, pancreatic carcinoma, cirrhosis, burns, and kwashiorkor. Premature infants and adults sustained on parenteral or enteral solutions devoid of selenium are at risk of deficiency characterized by cardiomyopathy, growth retardation, cataract formation, abnormal placental retention, deficient spermatogenesis and dystrophic changes in skeletal muscle (Munson, 1996). In a selenium deficient host, harmless viruses can become virulent e.g. harmless strain of Coxsackie's virus becomes cardiovirulant by oxidative mutation occurs in the genome. In patients with Blackfoot disease, selenium concentration was reported to be decreased in comparison to the normal (Wang, 1996). Epidemiological studies have that persons with low selenium diets have two to three times greater risk of heart disease than those eating selenium rich diet (Passwater, 2000). Low selenium status is also associated with greater incidence of depression and other negative moods such as anxiety, confusion, and hostility. Deficiency of selenium is reported to reduce

testosterone biosynthesis, formation and development of spermatozoa.

In an experiment in rat, the hepatic glutathione peroxidase activity was depressed following selenium deficiency, which is a vital antioxidant enzyme in biological system. The brain and liver was particularly observed to be deficient of antioxidant enzymes (Turan, 2001). In another study, this enzyme activity was restored completely after supplementation of 40 ppm dietary selenium (Ip and Ganther, 1992). The same effect was also observed in another study on women in New Zealand after supplementation with high-selenium wheat bread (Thomson, 1985).

1.4.1.8: Selenium Deficiency & Success of Large-Scale Selenium Supplementation Programs:

Selenium deficiency in humans (< 10μg Se/day) is limited to regions of the world where the Se soil content is low, particularly in northeast China, northern North Korea, eastern Siberia, south central China, Tibet, and Nepal (Oldfield, 2002). Se deficiency combined with iodine deficiency results in several diseases, particularly 1) Keshan disease, 2) Kashin-Beck disease, and 3) Myxdematous cretinism. (Coppinger, 2004). Keshan and Kashin-Beck disease once afflicted millions in China, but government

programs that added Se to table salt to about 6 million people have led to dramatic decreases in the prevalence of these disease. (Cheng, 1990; Wang, 1996) There is no evidence of these disorders in Bangladesh.

1.4.1.9 : Selenium Toxicity

Human toxicity from selenium includes loss of hair, nails, and teeth, as well as skin inflammation, nausea, and fatigue. Subtler signs include a garlic odor, metallic taste, or dizziness. Acute selenium poisoning can lead to fever, anorexia, gastrointestinal signs, impairment of thyroid hormone production, ALS, liver and kidney impairment (Vinceti, 2001). Inorganic forms such as selenite are about 4-fold more toxic in mice than inorganic forms such as selenomethionine (Ammar, 1981). Based on previous studies, intakes of 400 µg/day and plasma selenium of 1000 ng/ml have been established by the NAS as the no observed adverse effect level (NOAEL) (Food and Nutrition Board, 2000). In a 2003-2004 study conducted by the Marshall ,24 men were randomized to either 1600 µg/day or 3200 µg/day selenomethionine for an average of 12 months. The 3200 µg/day group reported more selenium-related side effects, although blood chemistry and hematology results were all within normal limits for both treatment groups. Side-effect reports from the 3200 µg/day groups did not correspond to peaks in plasma

selenium levels. No obvious selenium-related serious toxicities were observed (Reid, 2004). These results, prior RDAs, and the New Zealand study suggest that the proposed treatment of 100μg Se/day does not represent a risk to patients.

1.4.1.10 : Selenium Anticarcinogenisis

A number of epidemiological and prospective studies since the 1970s have found inverse correlations between selenium intake and risk of cancer (Rayman 2000). The multicenter, double blind, randomized, placebo-controlled Nutritional Prevention of Cancer (NPC) trial provided 1,312 non-melanoma skin carcinoma patients 200μg Se/day from 1983 to 1996 and found that selenium had both cancer chemo preventive and carcinogenic properties. The initial analysis did not find reduced incidences of BCC, SCC, or UV-induced skin damage. However, the study found significant reductions in total cancer mortality [RR: 0.50, 95% CI: 0.47-0.8]. (Clark, 1996) In particular, selenium was shown to decrease the risk of prostate cancer by 63% . It has been suggested that the metabolites CH_3SeH(Cho, 2004; Ip, 1990) or selenite (Shen, 2000; Stuart, 1999) are responsible for the generalized, anticarcinogenic activity by selenium observed in the NPC trial through O2 --mediated apoptosis, perhaps aided by GSH (Shen, 2000) (Spallholz 2001). Exotic selenocompounds such as p-XSC have also received recent

attention for their anti-carcinogenic properties (El-Bayoumy, 2004). However, a 2003 re-analysis of the 1983-1996 NPC clinical trial data found that selenium supplementation was associated with statistically significantly elevated risk of SCC (HR = 1.25, 95% CI = 1.03 to 1.51) and of total nonmelanoma skin cancer (HR: 1.17, 95% CI: 1.02-1.34). (Duffield-Lillico, 2003; Duffield-Lillico, 2002) Adjustment for a variety of co-founders did not alter this finding, though the authors found that the highest incidence rates were concentrated among participants in the highest two tertiles of baseline plasma selenium. Further, elimination of cases from the first 2 years of treatment caused the unadjusted relative risk of total nonmelanoma skin cancer to decline from 1.27 (95% CI = 1.11 to 1.45) to 1.20 (95% CI = 0.98 to 1.47). The results of the NPC trial show that selenium is not effective against cancers induced by UV-exposure, despite six mouse-model and human skin culture studies showing otherwise (Pence1994; Stuart, 1996; Rafferty, 1998; Burke, 1992; Burke, 1992 ;Overvad, 1985) .The NPC results indicate that there is both a potential benefit and a potential risk associated with selenium supplementation. However, there are three factors that distinguish the conditions of this proposed study with those of the NPC trial: 1) Arsenic and other heavy metal exposures were not covariates in the study, as the NPC trial was concerned with UV-induced carcinomas. In contrast, this proposal seeks to assess

selenium's ability to counter arsenic toxicity in villagers exposed to arsenic at carcinogenic levels above 200 µg/L. 2) The American patients in this study had baseline selenium intakes an order-of-magnitude greater than the intakes estimated for the arsenicosis villagers in Bangladesh. It is postulated that supraddition of selenium above dietary sufficiency may have pro-carcinogenic effects whereas selenium supplementation from deficiency to sufficiency may have anti carcinogenic effects. 3) The predominantly Caucasian, Type I/II/III patients of the NPC trial had a significantly increased risk of UV-induced skin carcinoma (Dwyer, 2002) than the highly UV SCC and BCC-resistant Type IV/V pigmented Bangladeshi participants for this proposed trial (Ortonne 2002).

1.4.1.11 : Therapeutic significance

Selenium has a wide range of involvement in various pathophysiology closely related to maintaining cellular integrity by means of enzymatic and antioxidant activity. Clinical trials and studies have already revealed that selenium has specific participation as immuno-stimulant, anti-viral, psychotropic, anti-inflammatory, anti-angiopathic and anti-cancer effects. Selenium enhances proliferation of activated T cells and increases natural killer cell activity. It has ability to up-regulate the expression of receptor for cytokine interleukin – 2, thereby enhances response to

antigen. Selenium was reported to reduce epileptic seizure in children and 100 μg selenium supplement significantly decreased anxiety, depression and tiredness (Rayman, 2000). Selenium is increasingly recognized as a versatile anti-carcinogenic agent. It appears to inhibit the replication of tumor viruses and the activation of oncogens (Schrauzer, 1992). Selenium can produce anti-tumor metabolite (methyl selenol) that inhibits angiogenesis and induces apoptosis of cancer cells. GPx4 an isomer of selenium containing glutathione peroxidase shields the sperm cell from oxidative damage and polymerizes into mature spermatozoa. In studies by scott and co-workers, supplementation of sub-fertile men with 100 μg selenium per day for 3 months significantly increased sperm motility. Arthritic inflammation is produced by certain prostaglandins and selenium is involved in controlling this prostaglandin's by controlling the free radical damage that stimulates their production (Passwater, 2000). At Manchester Royal Infirmary, administration of selenium 66 μg / day to patients with chronic and recurrent pancreatitis significantly reduced pain and frequency of attack. It is also reported that, in men with coronary disease, platelet agreeability is inversely related to selenium status (Rayman, 2000). Selenium acts as an acceptor of bioorganic methyl groups, and is involved in the detoxification of metals and of certain xenobiotics (Schrauzer, 1992). Suggestive evidence was found that diets deficient or excessive in selenium

might alter arsenic disposition and methylation in mice (Kenyon, 1997).

1.4.1.12: Daily requirement and overdose

There is no global consensus or guideline for selenium intake to be followed by most countries. But the developed countries like USA and UK have their recommended allowance according to selenium status in the soil, food and population of those countries. In 1980, the National Academy of Sciences stated that a safe and effective range for selenium intake is 50 to 200 µg/day (Passwater, 2000). In 1989, the United States Recommended Dietary Allowance (USRDA) of selenium was established as70 µg/day and 55 µg/day for men and women respectively .The UK Reference Nutrient Intake (NRI) was 75µg/day for men and 60 µg/day for women. On the basis of various studies in China and New Zealand, an expert working group from WHO/FAO/IAEA recommended an intake level of only 40 µg/day for men and 30µg/day for women (Rayman, 2000).

Age group	Daily requirement
Birth to 3 years	10 to 20 mg
4 to 6 years	20
7 to 10 years	30
Adolescent and adult males	40 to 70
Adolescent and adult females	45 to 55
Pregnant females	65
Breast-feeding mother	75

As selenium is an essential trace nutrient, it must be remembered that it is a toxic mineral with fairly small therapeutic window. The condition for selenium toxicity is called "selenosis". Acute and chronic toxicity associated with excess dietary selenium has been observed in both animals and humans in the form of neurological and neuromuscular symptoms with skin lesions (Kenyon, 1997). Through the conventional amount of selenium supplementation is 50 to 200 µg /day, but in Greenland many residents consume 1300µg/day and in China consumption of selenium at the dose of 1000 µg /day in was found to be protective from "Keshan disease" which was endemic there. Again selenium poisoning from intake of 5to 50 µg / day resulted in nausea, vomiting, loss of hair and nail, tooth decay, skin lesion and neurological abnormalities (Munson, 1996). In case of selenium over dose or toxicity, some symptoms like diarrhea, unusual tiredness, itching of skin, irritability, metallic taste may be observed of which garlic odor of breath and sweat is the prominent one (Passwater, 2000). Interestingly, another toxic metalloid substance "arsenic" has a protective effect against toxicity of a variety of forms of selenium. The appealing explanation is that selenium and arsenic react in the liver to form a detoxification conjugate, which is then excreted into the bile (Levander, 1977).

1.4.2 Background information of arsenic

1.4.2.1 : History

No other element has such a complex and variegated past. As early as 500 B.C. the ancients knew about arsenic, whose name comes from the Greek word for potent. Through the centuries, this "king of poisons" was a common means of homicide. And yet, arsenic's image has not always been so morbid. People in the middle Ages wore arsenic amulets around their necks to ward off the bubonic plague, and women in Victorian times applied arsenic compounds to their faces to whiten their complexions. Hippocrates, the father of western medicine, recorded arsenic's usefulness as a topical remedy for skin ulcers. Arsenic was used more than 2400 years ago in Greece and Rome as a therapeutic agent and as a poison. It is discovered in 1250 A.D by Alberteus Magnus as a metalloid .The history and folklore of arsenic prompted intensive studies by early pharmacologists. Hippocrates, the father of medicine, recommended the use of an arsenic sulfide for the treatment of abscess. In 1908, Paul Ehrlich was honored with novel prize for developing treatment of syphilis and sleeping sickness using arsenical compound. Indeed, the foundations of many modern concepts of chemotherapy derive from Ehrlich's early work with organic arsenicals and such drugs were once a mainstay of chemotherapy.

Today, arsenic compounds are still used for pharmaceutical purposes. Arsenic trioxide is known for its use in the treatment of acute promyelocytic leukemia in patients who are unresponsive to, or have relapsed from, certain chemotherapy agents. Research published in the 1 April 2005 issue of the Journal of Clinical Oncology suggests that arsenic trioxide may have therapeutic uses in other malignancies as well, and that it may be used in combination with other chemotherapy drugs to expand their benefits.

Arsenic cannot be found as a free element. It exhibits a broad range of chemical reactivity with an ability to form alloys with other elements by covalent bonds. Arsenic participates readily in oxidation – reduction, methylation –demethylation and acid –base reactions. Arsenic not only can create compounds with oxygen, chlorine, sulfur, carbon, and hydrogen but also with lead, mercury, gold, and iron. It mainly remains as oxide, hydride, sulfide, arsenate and arsenite.

1.4.2.2 : Source and chemistry

Arsenic is found in soils and rocks, natural water and air as a ubiquitous toxicant. It is released into environment through natural processes like weathering reactions, biological activities and

volcanic emissions. A wide variety of human activities are also responsible to mobilize arsenic in the environment as a byproduct from the smelting of copper, lead, zinc and other ores. It is also present in coal at variable concentrations and released during combustion. Application of arsenical pesticides and herbicides has increased its environmental dispersion through fruits, vegetables, fish etc. Even poultry and livestock also contain arsenic as it is added in feed to promote growth.

Symbol	: As
Period status	: Group –VA, Period -4
Atomic number	: 33
Atomic weight	: 74.9216
Specific gravity	: 5.727
Boiling Point (°F)	: 1139
Melting point (°F)	:814
Melting point	: 613 C

Arsenic is termed a metalloid as it has properties of both metals and non-metals. In nature it is widely distributed in a number of minerals, mainly as arsenicals of copper, nickel, and iron or as arsenic sulfide or oxide. Arsenic is also added to the environment through the burning of arsenic containing fuels and through volcanic eruptions and other natural processes. Weathering of rocks converts arsenic sulfides to arsenic trioxides, which enter the arsenic cycle as dust or by dissolution in rain, river or ground water. Humans are exposed to inorganic and organic arsenic primarily from air, food, and water. It is mainly transported in the

environment by water (WHO, 1981). There are many different forms of inorganic and organic arsenic.

Arsenic, unlike other chemicals, exists in soluble and insoluble forms; organic and inorganic forms; and tri- and pentavalent forms in which the trivalent arsenite is highly toxic compared to the pentavalent arsenate. The most important and available inorganic arsenic compound is arsenic trioxide, molecular formula of which is As_2O_3. It is not fairly soluble in water but it is highly soluble in either hydrochloric acid (HCL) or in alkali. In oxygenated water, arsenic usually occurs as arsenate but under reducing condition arsenite predominates. An increase in pH may increase the concentration of dissolved arsenic in water readily participate in oxidation-reduction, methylation demethylation and acid-base reaction. Pentavalent arsenate species are found mostly in oxygen-rich aerobic environment, whereas the highly toxic trivalent arsenite species are found mostly in anaerobic environments such as ground water. In water, four arsenic species may exist, these are arsenite, arsenate, mono methyl arsenic acid, and dimethyl arsenic acid but in ground water arsenic occurs predominantly as tri and pentavalent form. The solubility of arsenic in water as As_2O_5 is 1.5×10^6 mg/L, at 16°C and as 3.7×10^4 mg/l at 20°C.

Name	Formula
Arsenic pentaoxide	As_2O_3
Arsenic acid	H_3AsO_4
Arsenic tri oxide	As_7O_3
Arsenous acid	H_3AsO_3
Arsenenous acid	$HasO_2$
Arsenic tri chloride	$AsCl_3$
Arsenic tri sulfide	As_2S_3

1.4.2.3: Pharmacokinetics of arsenic

Despite being a metalloid substance, arsenic is readily absorbed from lung, gastrointestinal tract and skin. The absorption of arsenic depends on the chemical and physical form of compound. Inorganic soluble arsenic compounds are rapidly absorbed from the gastrointestinal tract (WHO, 1981). For instance, arsenic uses the transmembrane carrier mediated active transport system for absorption. Dietary components can interact with arsenic, adsorbing it and limiting its dissolution kinetics (DeSesso, 1998). In humans, the extent of absorption of trivalent sodium arsenite is 96.5% and for soluble pentavalent arsenic is 94%. In contrast, gastrointestinal absorption of the less soluble trisulfide and lead arsenate was reported to be only 20%-30% in hamster (Vahter, 1999). Absorption of arsenic in the lung id dependent on particle size as well as water solubility. The respirable particles size less than 0.1 -1 μ are carried into the lungs and therefore more likely to be absorbed. High absorption of trivalent arsenic solution in man is evident from high

urinary levels of arsenic and also from studies on nephrotoxic potentials of arsenic (Hirata, 1990).

Following absorption of trivalent or pentavalent arsenic compounds, arsenic is distributed in almost all tissues. It depends upon the duration and form of arsenicals absorbed. In various studies on distribution and accumulation of arsenic both in humans and rats, few vital organs were found to be similar i.e. liver, kidney, heart, lungs, brain and testes (Gregus and Klaassen, 1986). Arsenic is also deposited extensively in the hair and nails. In humans, inorganic arsenical does not appear to cross the blood brain barrier, however, transplacental transfer of arsenic has been reported (WHO, 1981). In spite of high maternal exposure, the concentration of arsenic in the breast milk was low on average about 3 mg / kg (Vahter, 1999). Thus inorganic arsenic and its metabolites are not significantly excreted in milk.

Arsenic compounds are subject to metabolic transformation. Mainly two types of reaction have been documented for the metabolism of arsenic both in human and animal .One is oxidation-reduction (redox) reaction and another is methylation reaction. After absorption arsenic is rapidly reduced mainly in blood and methylated in liver to produce less toxic metabolites, monomehyl

arsenic acid (MMA) and dimethylarsenic acid (DMA). Pentavalent arsenic compounds are to be reduced into trivalent form for methylation .At this stage reduced glutathione (GSH) plays the vital role as an electron donor. Trivalent arsenicals are then methylated using S-adenosyl methionine (SAM) as methyl donor and GSH as an essential cofactor (Delnomdedieu , 1994).

1.4.2.4: Limit of Arsenic in Drinking Water

According to WHO, in Bangladesh and India, the permissible limit of arsenic in drinking water is 50 µg/L (Mandal, 1996). Under this guideline, the concentration of 10 µg/L arsenic water is safe and tolerable, but this concept may not valid for Bangladesh and India, the permissible limit would be 10 µg/L due to (1) the USA is expected to change its maximum permissible limit (2) the people in arsenic affected area in Bangladesh and West Bengal are suffering from malnutrition (poor nutritional status) (3) adult people of Bangladesh and West Bengal drink average 4.0 liters of water per day. The maximum admissible concentration of arsenic in drinking water of different countries is shown below in a tabular form (Table 1.A).

Location	Recommending Agency	MAC (mg/L)
United States	U.S. environmental protection agency	0.01
Canada	-	0.05
Europe	European Union	0.05
Great Britain	-	0.05
Japan	-	0.05
India	Bureau of Indian standard	0.05
Bangladesh	-	0.05
	WHO	0.01

Table 1.A: Maximum Admissible Concentration (MAC) of arsenic in drinking water (Mandal, 1996)

1.4.2.6 : Excretion of Arsenic

Both trivalent and pentavalent inorganic arsenic in solution are readily absorbed after ingestion. When arsenic is absorbed into the body, the major portion is excreted mainly through urine and a small portion through faeces. Arsenic is also eliminated through skin, hair, and nail and to some extent through bronchial secretions. After administration arsenic appears in urine within 2 to 8 hours. About 25% arsenic being excreted in 24 hours and about 75% within 7 days of exposure. The major metabolites found in the urine are mono methyl arsenic acid and dimethyl arsenic acid. A portion of the absorbed arsenic is deposited in the skin, hair and nails, where it is firmly bound to keratin. Storage in these metabolically 'dead' tissues is responsible for the slow elimination rate of arsenic. Arsenic in urine, hair and nails has thus been used as an index for monitoring the exposure of victim to arsenic and urinary arsenic is generally reported as the most reliable indicator of

recent exposure to inorganic arsenic. Blood arsenic is not considered a good indicator because it is cleared within a few hour of absorption. In unexposed persons arsenic concentration in urine range from 0.01 - 0.05 mg/ liter, in hair usually below 1 mg/kg, in nail 0.43 - 1.08 mg/kg, and in blood 0.0015 - 0.0025 mg/liter. There is no such recommend value of normal arsenic concentration in skin.

The first methylation reaction is rate limiting and is catalyzed by an enzyme different from that transfers the second methyl group (Buchet and Lauwers, 1988). Methylation is most effective in low level of arsenic exposure, while bioconversion of inorganic arsenic, arsenic decreases at higher concentration (Fischer, 1985). Some authors in humans revealed that the capacity to methylate inorganic arsenic is sequentially saturated when daily intake exceeds 0.5mg (WHO, 1996). When bio-methylation becomes saturated, increasing doses of inorganic arsenic causes accumulation in the tissues. Metabolism of inorganic arsenic in human is a sequential process As(V)to As(111) to MMA to DMA, which occurs primarily in liver. Hepatocytes exhibited the greatest methylation capacity for inorganic arsenic followed by keratinocytes and bronchial epithelial cells (Syblo, 2000). Therefore depletion of reduced glutathione in the liver inhibits arsenic methylation (Buchet and Lauwers, 1987). However organic arsenicals are not metabolized

in the body (WHO, 1981). The methylation process is dose depended and as the dose of arsenic increases, a reduction of the percentage of DMA is observed in urine while retention of arsenic is higher. Metabolism of arsenic is varies considerably between species e.g. the marmost monkey and the chimpanzee has been reported not to methylate inorganic arsenic at all (Vahter, 1999). A remarkable difference between humans and animals is that MMA is excreted in the urine to a greater extent.

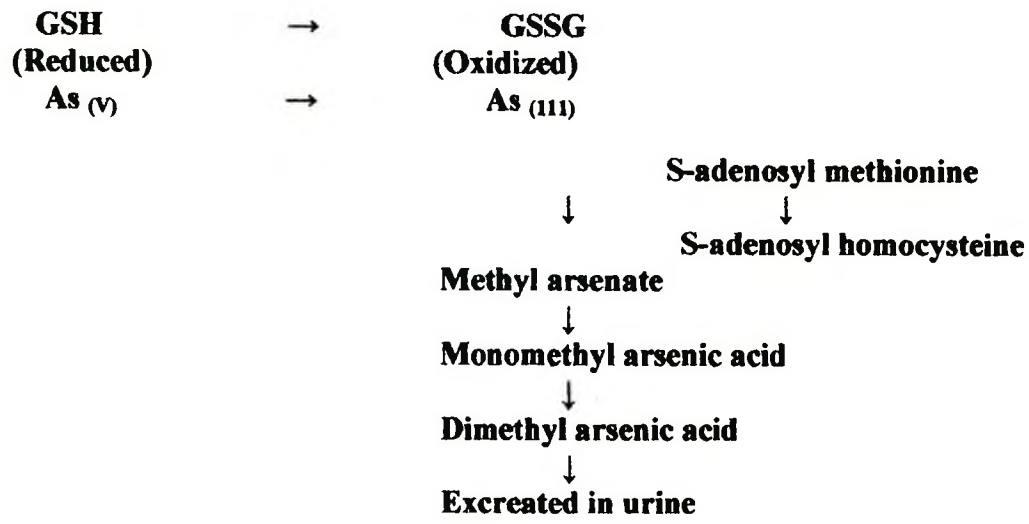


Figure 1.K Biotransformation of inorganic arsenic

Arsenic is reported to be more readily excreted as methylated metabolites. Humans and other mammalian spices inactivate inorganic arsenic by methylation, which are excreted mainly in urine along with unchanged arsenic (Rowland and Davis,

1982). Inorganic arsenic and its metabolites are eliminated primarily via kidneys, but the rate of urinary excretion depends upon the chemical form of the compound ingested, the route of exposure and the dose level (Vahter, 1999). Others secondary routes of elimination are nail, hair, sweat and faeces. Arsenic also undergoes significant biliary excretion as 24%-37% of injected arsenic in rats was reported to be excreted in bile within two hours (Gregus and Klaassen, 1986; Gyurasics, 1991). After administration, about 25% of arsenic is excreted in 24 hours and about 75% within 7 days of exposure .The significant metabolites are found in urine are DMA and MMA. A portion of the absorbed arsenic is accumulated in the skin, hair and nail where it firmly bound to keratin. The half-life of urinary excretion of arsenic is 3-5 days. Urinary arsenic is generally reported that the most reliable indicator of recent exposure to inorganic arsenic. Increased dose levels cause an increased excretion of inorganic arsenic and corresponding decrease excretion of methylated forms (Vahter, 1999). Buchet and Lauwers (1981) reported that when multiple doses are administered as steady state, 60% of the dose is excreted in the urine. Only few percent (<10%) are excreted in faeces, which is probably related to the reabsorption of arsenic by the intestine after biliary excretion. Other minor routes of excretion include perspiration, expired breath and milk as well as incorporation into skin, hair and nails since arsenic can accumulate

in keratin containing tissues (WHO, 1981; De Sesso, 1998). It has also been reported that milk of lactating women undergoing treatment with arsenic-containing drugs for syphilis had high levels of arsenic in breast milk (Gilman, 1991).

1.4.2.7 : Uses of arsenic

Arsenic (V) is considered to be essential to mammals for the maintenance of growth, blood cells and normal iron metabolism. Arsenic compounds are mainly used in agriculture, forestry and industrial processes. Arsenic trioxide is used in manufacturing of agricultural chemicals (pesticides), glass and glassware, industrial chemicals, copper and lead alloys and pharmaceuticals, in doping semiconductor chips, as arsenical war gas lewisite .In agriculture, arsenic compounds such as lead arsenate, copper acetoarsenate, sodium arsenite, calcium arsenate and organic arsenic compounds are used as pesticides. Substantial amount of methyl arsenic acid and dimethyl arsenic acid are used as selective herbicides. Chromated copper arsenite, sodium arsenate and zinc arsenate are used as wood preservatives. Some phenyl arsenic compounds such as arsenilic acid are used as feed additives for poultry and swine. In industry arsenic is used in the preparation of dyes, poisonous gas, transistor, as a component of semiconductor, as a preservative in tanning and in the textile and paper industries. As medicine arsenic

was used since the fifth century BC when Hippocrates recommended the use of arsenic sulfide for the treatment of abscess. Arsenic preparation was used for the treatment of skin disorders, tuberculosis, leukemia, asthma, leprosy, syphilis, amoebic dysentery and in dentistry etc. Homeopaths also use arsenic as drug n some cases. In current therapeutics arsenicals are important in treatment of certain tropical diseases such as African trypanosomiasis. A recent study claimed that moderate to high doses of arsenic given for a period of 30 days can induce complete remission of acute promyelocytic leukemia. Today the use of arsenic as medicine has declined sharply due to its mutagenic and carcinogenic effect.

1.4.2.8 : Mechanism of cellular toxicity

The toxicology of arsenic is complicated by its ability to convert between oxidation and organo-metalloid form (Thompson, 1993). Though the signs and symptoms of arsenic toxicity do not reflect the involvement of all physiological systems, but the magnitude of toxicity at the cellular level can be indiscriminating to almost every tissue. The severity depends on the amount of arsenic accumulated in the body, duration of exposure, host defense mechanism, nutritional status and genetic polymorphism. Individual variation in arsenic toxicity is reported to be due to the polymorphism of the genes regulating the expression of

methyltransferase, which is essential for methylation of arsenic (Vahter, 2000). But the most important and common mechanism for arsenic toxicity is postulated to be through binding to sulfhydryl containing enzymes (Scott 1993; Chouchane and Snow, 2001). All arsenicals exert their effect by reacting with sulfhydral (-SH) groups in cellular metabolism. As a result, -SH containing proteins (enzymes) essentials for oxidative metabolism are inhibited (Cullen, 1984; Li and Chou, 1992). Trivalent inorganic arsenicals are more potent inhibitors of these enzymes than that of pentavalent, which is known to capable of uncoupling mitochondrial oxidative phosphorylation (Hilmy, 1991;Styblo, 2000). This mechanism involves competitive substitution of arsenate for inorganic phosphate in the formation of ATP, with subsequent formation of an unstable arsenate ester that is rapidly hydrolyzed. The pyruvate dehydrogenase system is specially sensitive to trivalent arsenicals because of their interaction with two-SH groups of lipoic acid. The pentavalent arsenic is coupled to the oxidation of reduced glutathione (GSH) to GSSG to form the trivalent arsenic, which is methylated to form the monomethyl and dimethylarsenite, which is readily eliminated from the body (Figure1.L).

Another important key to cellular toxicity is the prolong exposure to heavy metal including arsenic can cause depletion of certain antioxidant activity, such as selenium containing glutathione

peroxidase (Pederson , 1991; Ip and Ganther, 1992). Many toxic substances associated with smoking, alcohol consumption, air pollution, pesticides and heavy metals produce their toxic effects by generating free radicals mediated by lipid peroxidation at various levels. Reduced glutathione and related enzyme system may protect the tissue from the oxidative stress by antioxidative function. But prolong exposure to arsenic causes over-use failure of this system that may result in initiation of biochemical injury to the organs (Santra, 2000; Chouchane and Snow, 2001). In a study on animal model, it was observed that, lipid peroxidation increases after arsenic exposure as the GSH, super oxide dismutage and glutathione reductase activity decreased; indicating the free radical mediated degeneration of tissues (Cadbury, 1999). The key to the toxicity of arsenic is its electrophilic nature; arsenic binds to electron-rich sulfhydryl groups of proteins and enzymes by binding to a thiol-containing active site (DeSesso, 1998).

Hamilton, program director of the Dartmouth College Superfund Basic Research Program, published papers on the latter topic in the March 2001 issue of EHP and the August 2004 issue of Chemical Research in Toxicology. "We demonstrated this with the glucocorticoid receptor and subsequently showed that arsenic has similar effects on all five steroid receptors," says Hamilton.

"Furthermore, we recently found similar effects on other members of the nuclear receptor signaling family, including retinoic acid and thyroid hormone receptors." Since these receptors are central to so many biological processes, Hamilton suggests that this may be an important way by which chronic arsenic exposure contributes to so many malignancies as well as nonmalignant diseases.

Pentavalent arsenicals	**Trivalent arsenicals**
As_{v} + ADP ↓ Formation of As- ADP (unstable arsenic ester) ↓ ATP formation decreased ↓ Oxidative phosphorylation decreased ↓ Oxidative stress occur ↓ Inevitable cell injury	As_{III} +(-SH) ↓ Inhibits (–SH) containing Proteins/enzymes ↓ Inhibits NAD links Phosphorylation ↓ ATP formation decreased ↓ H2O2 production increased ↓ Lipid peroxidation increased ↓ Free radicals/ ROS (Reactive Oxygen Spices) formed ↓ Oxidative stress occur ↓ Cell injury / cellular death

Figure 1.L: Important steps of arsenic induced cellular toxicity

1.4.2.9 : Features of arsenic toxicity

Due to chronic exposure to arsenic for prolong period, arsenic toxicity develops involving almost all organs and systems in the body termed arsenicosis. When a massive amount of inorganic arsenic is ingested, acute exposure may adversely affect various organs, including gastrointestinal tract, hepatobiliary system, cardiovascular system, peripheral and central nervous system (Hirata, 1990). Despite the alteration of biochemical parameters have been reported in many studies, there are some characteristic clinical features observed in arsenic toxicity. Major organ systems affected and clinical features arsenic toxicity (MIG, 1998) are tabulated below (Table 1.B).

Pre-clinical stage	Initial stage or Clinical Stage	Second stage or Stage of Complication	Last stage or Stage of Malignancy
No clinical manifestation	Melanosis, Keratosis, Conjunctivitis, Gastro-enteritis, Bronchitis	Leukomelanosis , (rain – drops pigmentation), Hyperkeratosis, Non-pitting edema, Hepatopathy, Peripheral neuropathy	Neuropathy, Hepatopathy, Gangrene, Skin cancer, Bladder cancer, Lung cancer,

Table 1.B : Clinical features in different stages in arsenicosis.

A new case definition of arsenicosis due to chronic arsenic toxicity has been revised by the WHO on October 2002 are stated

below in tabulated form.

1. Prolonged (at least 6 months) intake of water with arsenic levels greater than 0.05mg/L or exposure of high arsenic level from foods/air/medicine.
2. Dermatological features characteristics of chronic arsenic toxicity:

- Minor: Grade I skin lesion (mild):

a) Diffuse melanosis

b) Suspicious spotty depigmentation / pigmentation over trunk / limbs

c) Mild diffuse thickening of soles and palms

- Major: Grade II (moderate) / Grade III (severe) skin lesions

Grade II (moderate):

a) Definite spotty pigmentation/ depigmentation on the trunk and limbs, bilaterally distributed

b) Severe diffuse thickening (with or without wart like nodules of the palms and soles

Grade III (severe):

a) Definite spotty pigmentation / depigmentation as above with few blotchy pigmented / depigmented macular patches over trunks or limbs

b) Pigmentation involving the undersurface of tongue and / or buccal mucosa

c) Larger nodules over thickened palms and soles occasionally over the dorsal aspect of hands and feet. Diffuse verrucous lesions of the soles with cracks and fissures and keratotic horns over palms and soles.

3. Non cancer systemic manifestations:

- Major: Signs and symptoms of chronic lung disease, (Cough, breathlessness, chronic bronchitis, pulmonary fibrosis, chronic obstructive airway disease, bronchiectasis) Hepatomegaly (Non cirrhotic portal fibrosis) with or without splenomegaly, cirrhosis of liver / ascities, Peripheral neuropathy, Peripheral vascular disease (Raynaud's phenomenon, Gangrene of limbs, indolent ulcer)
- Minor: Weakness, Diabetes mellitus, Hypertension, Ischemic heart disease, Cardiac arrhythmia, Cerebro vascular accident, Swelling of hands / feet, Hearing defect, Dimness of vision, Headache, Mee's line in finger nails, Conjunctivitis, Chronic diarrhea, Anemia, Abdominal pain, Anorexia, Nausea, Nephritis/ Nephrosis, (Nasal septum perforation and rhino-laryngo-pharyngitis in inhalation Arsenic exposure)

4. Cancers:

- Major criteria: Bowen's disease, Squamous cell skin cancer, and Basal cell skin cancer.
- Minor criteria : Cancer of urinary bladder, Cancer of lung,

Cancer of liver, kidney cancer

5. Biomarkers of exposure: Arsenic level in urine above 0.05 mg /L or in hair more than 0.8 mg / kg or in nail more than 1.3 mg / kg.

1.4.2.10 : World Situation of Arsenic Toxicity

Arsenic contamination was detected in Argentina as early as the beginning of this century. Possibly it was the first detection of arsenic pollution in the world. The source of contamination was found to be natural due to soil composition polluting the shallow well water. Arsenic contamination incidents in ground water have been found in many countries of the world. Major incidents of arsenic toxicity occurred in Taiwan (1968), Mexico (1963), Chile (1957), and in Inner Mongolia. Inner incidents have been reported involving smaller population groups in Toroku and matshou in Japan; Xinjiang and Guizhou in China; Ronpibool in Thailand; Minnesota in USA; Ontario and Nova Scotia in Canada; Hungary; Millard country in Utah; California; and Fairbanks, and Alaska. Large areas of China also face severe arsenic exposure from groundwater contamination, with more than 3 million people affected, based on estimates in the August 2004 issue of Toxicology and Applied Pharmacology. In Shanxi Province alone, an estimated 900,000 people are at risk of arsenicosis. Among the investigated villages in Shanxi, an average of 52% of wells give water containing arsenic concentrations higher

than 50 µg/L, according to a recent report from the School of Public Health at China Medical University in Shenyang. At least 10% of Ghana's rural borehole wells have arsenic concentrations exceeding 10 µg/L. In the Terai region of Nepal, inhabited by half the country's total population, hundreds of thousands of shallow tube wells have been installed by various agencies, and groundwater is the primary source of drinking water. According to Chakraborti, around 500,000 people in Terai are at risk of arsenic poisoning from drinking this water, and up to 1 in 20 people may show skin lesions indicative of arsenicosis. In the recent past ground water arsenic contamination in seven districts of west Bengal of India was reported to be biggest arsenic calamity in the world. The next hit of arsenic calamity has set in Bangladesh, which is now considered to be the largest arsenic calamity ever reported in the world.

Table 1.C: Arsenic contamination across the Globe

Country	Origin of arsenic	First identified	Affected region	Range of contamination	Population exposed
Argentina	Natural, due to the soil composition pollutin g the shallow well waters. Also high	Beginning of the 19th century	The Chaco-Pampean Plain of Central Argentina, covering around one million sq. km.	Groundwater arsenic concentration in some places ranges from 100 to 2000 µg/L	200,000

	content in some river waters.				
Bangladesh	Natural origin, deriving from the geological strata underlying Bangladesh	1993	61 out of 64 districts	Less than 0.25 µg /L to more than 1600 µg /L	Up to 57 million are drinking water with an arsenic concentrations greater than the WHO guideline value, and up to 35 million drinking water with concentrations in excess of the Bangladesh standard
Chile	Associated with quaternary volcanism in the sparsely populated and arid Central Andean Codilleras	1962	Africa Province in north Chile	Not available	400,000 over an area of 125,000 sq. km
China	Natural, in reducing environment	First identified in Xinjiang Province in early 1980s	Inner Mongolia Shaanxi and Xinjiang	90% of the wells tested had arsenic at level higher than 50µg /L (highest concentration detected was	600,000 in China and 1.1 million in Inner Mongolia

			Provinces	2400µg /L)	
Ghana	Effects of mining activities and possibly some arsenopyrite oxidation	Not available	Obuasi	Some shallow wells and streams contain low to high concentration	100,000
Hungary and Romania	Natural	Not available	Southern part of the Great Hungarian Plain and parts of neighboring Romania	2 to 176µg /L	400,000
India	Geological origin, analogous to the problem in Bangladesh	Resultant health effects were first identified in late 1980s	West Bengal (8 out of 17 districts) Also suspected occurrence in Bihar, Gangetic and Brahmaputra plains	Not available	Over 5 million. Estimated 300,000 people are suffering from various stages of arsenicosis
Mexico	Natural. Volcanic sediment type aquifer having oxidizing, neutral to high pH	Not available	Lagunera Region of north central Mexico. Affected area expands up to 32,000 sq. km.	1 to 500 µg /L (average 100µg /L	400,000

	groundwater condition				
Nepal	Not known, but believed to be natural	Late 1990s. water experts in Nepal decided it was time to look into the quality of water supply there.	20 Terai districts in the plains of Nepal	Not available	550,000 people (2.4% of population) exposed to arsenic exceeding 50 µg/L and 3.19 million (13.6% of population) exposed to arsenic exceeding 10 µg/L

1.4.2.11: Social Costs of Arsenic Contamination

While addressing the problem of arsenic contamination, emphasis is being put on the identification, mitigation, and supply of safe drinking water. Arsenic is not only a physical but also a social phenomenon; the social fallout of arsenicosis is enormous. The arsenic hazard has a strong social dimension, affecting issues such as relationships within the family and the village, as well as the mental health of the sick.

It was observed the social costs of arsenic contamination in the following forms: social instability, superstition, ostracism, marital problems, discrimination against women, increased poverty, diminished working ability, and death.

Social Instability: Lack of proper knowledge about arsenic contamination and unavailability of arsenic-safe drinking water as well as proper treatment are creating extreme instability in the social life of the people in the arsenic-prone areas of Bangladesh. Moreover, social conflict over contaminated water contributes to destruction of social harmony and network relationships.

Superstition: Superstitions and prejudices are constructed surrounding arsenic patients. For example, in the northeastern district of Kushtia, arsenic is considered as a "curse of Allah" or the work of evil spirits. People stay away from arsenic victims, neglect them, or become scared of them because of these superstitions.

Ostracism: Arsenic patients are often identified by the society as patients of leprosy and as a result they remain ostracized, at either the household or the village level. Children of arsenic patients are not allowed to attend social or religious functions. The patients as well as their close relatives are not allowed to use public tube wells and village ponds. Often family members, like husbands or wives, abandon the arsenicosis victims.

The problem is more serious in the case of children. The entry of arsenic affected children into schools becomes restricted.

Some may be denied the opportunity to go to school. They also are subject to social ostracism by their friends and classmates.

Diminished working ability: Arsenic is a silent killer. The black spots on a victim's body slowly become nodules and even grow if the victim remains exposed to arsenic contamination. Limbs and internal organs like the liver, kidney, and lungs may be affected. Gangrene cripples the victim and makes him or her unable to do hard labor. Arsenic is carcinogenic.

Marriage related problems: Arsenic has an adverse impact on marital relationships. People are reluctant to develop marital relationships with families whose members suffer from arsenicosis. This has caused serious anxiety for parents of unmarried adult children. Many women are divorced or abandoned by their husbands due to arsenicosis.

Increased poverty: Those in poverty are the main victims of arsenic contamination as they are compelled to drink contaminated tube well water. Researchers believe that the severity of arsenicosis is very much related to nutritional deficiency. Malnutrition makes them easy victims. Due to poverty, victims are deprived of proper treatment. When seeking treatment, the costs become a burden to them.

As arsenicosis decreases the victim's ability to work, he or she often suffers from a reduced income. Due to ostracism, arsenic patients lose their jobs. Thus, arsenic negatively contributes to the poverty situation in Bangladesh.

Gender implications of arsenic contamination: In Bangladesh women perform the majority of the household work, but their work remains relatively invisible and unrecognized in society. Among many other tasks, collecting and carrying water for household use, particularly in the rural areas, is the responsibility of women and girls. Arsenic contamination in nearby drinking water often compels them to collect and carry water from a long distance, imposing an additional burden on them.

Because of socio-cultural restrictions, women often do not receive opportunities to obtain information from outside sources. Thus, they are not properly aware of the danger of arsenic. This makes arsenic mitigation activities difficult.

Women are frequent victims of ostracism due to arsenicosis. They are doubly vulnerable: from the disease itself and by being divorced, abandoned, or even forced out of the society. As gender discrimination exists in many forms in the patriarchal society of Bangladesh, women suffer more from these things than men.

1.4.2.12 : Treatment of arsenic toxicity

Treatment of arsenic toxicity was first actively accomplished by the threat of poison gas "Lewisite" in ware fare. Since then the search for a specific antidote against arsenic poison become a focal concern of the medical science. Though acute toxicity is rare in comparison to that of chronic, there is no specific treatment for chronic arsenic toxicity. For acute toxicity, British developed dimercaprol, which is known as British Anti Lewisite (BAL), a sulphonate derivative known, as Unithiole developed by former Soviet Union was the mainstay of treatment. They act by forming soluble chelate with arsenic that can be excreted in the urine. Di-mercapto succinic acid (DMSA) and Di-mercapto propane sulphonate (DMPS) were used in Europe for treatment of chronic arsenic toxicity and also for mercury poisoning. Penicillamine, an analogue of cysteine is also used as chelating agent in acute arsenic toxicity. But all these have serious unwanted effects and high dose with very narrow therapeutic index.

In chronic arsenic toxicity, the mode of treatment is different. Because chronic toxicity develops very slowly and silently for a prolong period. Chelating agents, Vitamins, high nutritional diets, spirulina, may be considered to some extent effective in combating the arsenic toxicity but all these have limitations in different grounds

(Ahmed, 1998; Huq, 2000; Yerebakan, 2002; Turan, 2001; Vacchina and Cases, 2001; Misbahuddin, 2006). Above all, it should be remembered that people with clinical manifestations are not only the sufferer but millions of people who do not present with features of arsenicosis are also highly vulnerable to the more serious and delayed health effects in the long run. Therefore, the prevention of further exposure to arsenic is to be considered at first. Prevention of accumulation, reversing the affected or altered biological functions and rapid elimination of arsenic are the further objectives of treatment in chronic arsenic toxicity.

1.5 Hypothesis

Selenium antagonizes the toxic effects of arsenic by its powerful enzymatic antioxidant effects on toxic free radicals produced by metabolic overload of arsenic relieving clinical features and sparing glutathione availability in abundance for normalizing metabolism of arsenic in tissues.

1.6. Objectives

1.6.1 : General objective:

- To determine effectiveness of selenium in counteracting the accumulation of arsenic in tissues after chronic exposure.
- To demonstrate that selenium supplements in arsenicosis patients reducing arsenic body load, as represented by hair , finger nail and urine arsenic levels.

1.6.2 : Specific objective:

1. To find out prevalence of arsenicosis in eleven villages of rural Bangladesh.
2. To estimate the selenium level in serum at the beginning and at the end of the supplementation of selenium in arsenicosis patients selected by a predetermined criteria.
3. To estimate the arsenic in the urine, hair and nail at the beginning and at the end of the supplementation of selenium.
4. To assess the clinical recovery of symptoms of arsenicosis.
5. To recommend the appropriate measure to control arsenicosis.

Chapter 2

MATERIALS AND METHODOLOGY

2. Materials and methodology

2.1: Type and period of study

A prospective randomized double blind phase 3 intervention trial was carried out in a defined rural community in chatkhil upazilla of District Noakhali, during the period from November 2004 to November 2006. The study was approved by the ethical review board of the Bangladesh Medical Research Council (BMRC) (Appendix 7.16).

2.2: Study Population

Arsenicosis patients were identified clinically in 11 villages of Shahpur union, under Chatkhil Upazilla; of District Noakhali (about 170 km south west from Dhaka) .The area is a hyper-endemic arsenic zone according to British geological survey report. The area is having more than 19,000 people. The arsenicosis cases were identified from this area. These victims were the population drinking water from shallow tube well for a long time.

Prior to this data collection , a pilot study was carried out in the same locality on 10 arsenicosis volunteer patients to observe the toxicity of selenium (Momin, 2004).

2.3 : Development of questionnaire

In order to obtain quality data, general health information including socio-demographic, information and any arsenic related problems were identified by using a standard questionnaire developed for this study. The questionnaire was pre-tested and finalized before data collection. (Appendix 7.19)

2.4: Selections of the subjects

The subjects of the study were arsenicosis patients of different age and sex. Arsenicosis patients were screened out from a large number of people who attended in an arsenic consultation health camp after a mass campaign with the help of local elite and opinion leaders.

Recruitment of the subjects was assisted by village leaders, local NGOs and staff and student of the local college. Arsenicosis were diagnosed clinically and were confirmed by spot urine examination by digital arsenitor. Arsenicosis cases were randomly selected following to the inclusion and exclusion criteria.

2.5 : Inclusion criteria

All adults, both male and female with history of exposure to arsenic contaminated drinking water from shallow tube-well for more

than 6 months and presenting signs / symptoms of arsenicosis .

Characteristics of patients :

- Patients with age group above 12 years. Patients below the age of 12 will be too young to show melanosis and keratosis.
- Both male and female sex
- Skin manifestations of arsenicosis – melanosis, hyperkeratosis and Leucomelanosis .Participants must show at least mild melanosis on the trunk, to establish a baseline of the secondary endpoints of melanosis and keratosis. .
- Presence of arsenic in urine
- Voluntary consent given for participation in the study

2.6 : Exclusion criteria

1. Patients not exposed to arsenic
2. No clinical feature of arsenicosis
3. Patients refusing consent
4. Patients not having arsenic in urine
5. Patients known to have received vitamins and minerals from local doctors for any condition
6. Patients having concurrent illness like malaria, tuberculosis or other chronic illness
7. History of smoking, alcohol intake or taking hepatotoxic drugs.
8. Pregnant and lactating mother was also excluded.

2.7: Selection of interviewer

Six interviewers were selected from the local community. The interviewers received seven day orientation training. The principal investigator provided hands on training to the interviewers during field-testing of questionnaire. The principal investigator supervised the field testing procedure and gave on the spot feedback to the trainees.

2.8 :Data collection

2.8.1: Baseline survey:

In the study area a baseline survey was also conducted to identify arsenic affected patients and their socio-demographic characteristics. The baseline survey was included the following information:

1. Age, gender, height and weight
2. Socio-economic information
3. Water use data: drinking water source, cooking water source, numbers of tube wells, duration of use.
4. Skin manifestation of arsenicosis
5. Sampling of urine for arsenic detection
6. Sampling of water from the tube well which usually used by the patients for arsenic estimation

Finally, 260 patients were selected according to the inclusion criteria. Suspected arsenicosis patients were identified by clinical examination and confirmed by urine tests. To confirm the exposure, the amounts of arsenic present in the tube well water used by the patient were also estimated. Each patient was informed about the purpose of the study and right to withdrawn from participation was assured (Appendix 7.17) . Voluntary informed written consents were taken from each arsenicosis patients .The information were kept strictly confidential and used only for research purpose (Appendix 7.18).

Anthropometric measurements such as height, weight were measured for each of the participants following standard procedure and recorded in the specific part of the questionnaire. The body weight was measured on standing position by bathroom scale on bare foot and light clothing's and recorded to the nearest 0.1 kg. Height of the subjects were measured barefoot in standing position with an especially wooden scale and recorded to the nearest 0.1 cm. In 10% (25) randomly selected cases, their 24 hours food recall was also collected. On completion of socio-demographic and anthropometric data hair, nail urine and blood samples were collected from each patient.

2.8.2: Sample collection:

Urine, and blood samples were collected before and at the end of 4, 8 and 12th months. All the samples were obtained during the first week of the month .Only hair and nail samples were collected at the beginning and at the end of study period. Sampling of their drinking water was also done at the beginning and at the end of first month. All collected urine and blood samples were kept frozen in a salt-ice mixture for 7 days during the collection of them in field. All the samples were transported to Dhaka by next 24 hours for laboratory analysis in frozen containers and stored at -20° C until analysis was carried out. Each patient was given an identification number and randomly assigned into one of the three treatment groups following a computer generated table of random numbers.

2.8.2.1: Collection of nail

Nail of the arsenicosis patients has been selected from at least 5 digits from hand and foot to collect for estimation of Arsenic. About 100 microgram of nail has been collected by a sharp nail clipper to a white paper which is then transferred to a 4" disposable sealed polyethylene bags for storage at room temperature with proper labeling such as identification number, date of collection and nature of specimen.

2.8.2.2: Collection of hair

Scalp hairs from the neck region are selected to collect for estimation of Arsenic. Three finger pinch of dry scalp hair about 300 microgram, 2 cm in length was cut from less readily contaminated sites close to the occipital area of the scalp with a pair of scissors by right hand from 1-2 mm above the scalp skin. Hair longer than 1 inch from the scalp was excluded. The cut off hair has been stored in a "4" plastic container with proper labeling such as identification number of the patient, date of collection, name of the specimen.

2.8.2.3 : Collection of urine

Freshly voided urine samples (20-30 ml) were collected from each patient on the spot in a pre-washed, 06 inch long polyethylene, screw-capped bottles. Immediately after collection, the pot has been tightly corked and was labeled with a mercury signing pen properly which include identification number of the patient, date of collection and name of the specimen and stored in an ice box containing salt-ice mixture.

2.8.2.4 : Collection of blood:

After collection of hair, nail and urine patient was asked to lie down in a bed to collect the blood samples. A tourniquet was

tightly bind over the mid arm of right hand of the patient .With hexisol lotion the area of the flexor surface of elbow joint has been cleaned properly. Patient was asked to clench his fist to tighten the area which gave a good engorgement of the vein of the area. Then by sitting besides the patient, about 03 ml blood was drawn with a gentle prick from the engorged brachial vein through a 5cc disposable sterile plastic syringe and which immediately transferred to a sterile test tube . The prick area of the patient was sealed with a band aid tape and then the patient was advised to get up and collect his allocated drugs .The blood in the test tube now labeled with identification number and centrifuged by a centrifuge machine with a 3000 rotation per minute for 7 minutes to separate RBC from plasma. The supernatant serum was collected from the centrifuge tube in a sterile 03 inch Eppendroff glass tube with screw-capped which then labeled with a mercury signing pen properly for identification which include identification number, date of collection and nature of the specimen and then kept in a frozen containers .

2.8.2.5: Collection of drinking water

Another 100 ml bottle was supplied to each patient to bring drinking water during the next visit but not the water from their contaminated tube-well water. All these samples were properly labeled with the identification number of the corresponding patients.

2.8.2.6 : Skin Biopsy

In 8 cases, the non-healing ulcerated area of the skin of foot or hand was excised under local anesthesia with all aseptic measure at National Hospital, Chandragonj, Noakhali. Patient was advised to visit the hospital in a prefixed date and taken a written consent for the biopsy. . The skin tissue was taken in each individual patient by a scalpel surgery in different sittings and preserved in screw-capped glass tube with 05 ml of 40 % formalin. After surgery the excised area was sutured properly and dressed with sterile gauge and bandage. The collected material were labeled properly and sent to Dhaka for histopathological examination. After 8 days the stitch was removed from the sutured area with minimum discomfort. The excised area was examined every time for any complication or relapse while the patient was attended the arsenic camp to take their respective study drugs.

2.8.3: Macroscopic Photographs & Clinical Analysis

Participants were digitally photographed in the field by the principal investigator using standardized lighting, scales, and positioning to assess the progression or regression of melanosis and keratosis. The patient's torso, palms, and soles were photographed every 16 weeks using a high-resolution digital SLR camera. At the trial conclusion, the photographs were randomized, blinded and

submitted to a panel of 3 dermatologists from the University of Dhaka and Dhaka Medical College for assessment .

2.9 :Treatment procedure

After randomization, the attending patients were grouped in three groups , 87 patients were included in group 'A' , 87 patients in group 'B' and the rest 86 patients in group 'C' . Each group of participants was provided one of the three intervention drugs throughout the study period. All the drugs were identical in appearance being blindly coded as 'A' 'B' 'C' respectively. The drugs were delivered to each participant in a sealed air tight plastic bottle. Only code number was written on each bottle. Each bottle was packed earlier with 15 tablet of the respective group of drugs by pharmacists which were kept confidential. Neither the investigator nor the patients knew the intervention groups. Each patient was instructed to visit the camp every monthly in order to receive the drug. While receiving the drug, the patient had to bring the previously used containers in order to check the compliance. Each patient was instructed to swallow half of the tablet daily orally with a glass of water. The specific treatment was provided and supervised by the trained field workers at home level of the patients. The overall supervision both in clinic and in field was maintained by principal investigator, over 12 months follow-up.

At the end of 12 months, the collected samples were analyzed in the laboratory of Institute of Nutrition and Food Science (INFS), University of Dhaka , Dhaka . After the laboratory analysis was complete, the code of drugs 'A' 'B' 'C' were decoded and data were analyzed along with clinical , biochemical and social data .

No patients were allowed to drink arsenic contaminated water throughout the study period. All the study subjects were encouraged to taken boiled surface water throughout the study period. A local office –cum- clinic was setup in the area to facilitate diagnostic and patients care activities. A registered medical officer was recruited to monitor patients care activities. Adverse Events (AEs) were reported by the physicians to the Data Safety Monitoring Board, headed by Professor Keramat Ali, Professor of Nutrition, University of Dhaka, and Dhaka.

2.10 :The intervention agents

A) Selenium in a solid tablet form, named 'Selenium' (Manufactured by Schiff and distributed by Schiff products, Salt Lake City, UT, USA 84104) packed as 100 tablets in a sealed bottle. Each tablet containing 200 µg selenium as high selenium yeast rich L selenomethionine, without any artificial color or preservatives.

B) Placebo preparations containing potato starch in each as a tablet form (manufactured by The Acme laboratories ltd, Satmasjid road, Dhanmondi, Bangladesh) which were also identical in appearance and color to the tablet selenium.

C) Antioxidant vitamin A, C and E containing beta-carotene 12 mg, ascorbic acid 400 mg and alpha-tocopherol 200 mg in each as a tablet form (manufactured by The Acme laboratories ltd , Satmasjid road, Dhanmondi, Bangladesh) which is identical in appearance and color to the tablet selenium .

2.11: Field Working Schedule

SL No	Specimen	0	1	2	3	4	5	6	7	8	9	10	11	12
1	Drug dispensation	●	●	●	●	●	●	●	●	●	●	●	●	●
2	Blood sampling	●				●				●				●
3	Urine sampling	●				●				●				●
4	Health examination	●	●	●	●	●	●	●	●	●	●	●	●	●
5	Nail sampling	●												●
6	Hair sampling	●												●
7	Water sampling	●	●											
8	Photograph	●				●				●				●
9	ECG	●				●				●				●
10	Skin Biopsy		●	●										

2.12 :Laboratory work

2.12.1: Determination of total arsenic in biological samples

The effect of medication was assessed in the different study group through clinical and biochemical examination. Selenium concentration was estimated from their serum .Total Arsenic concentration were estimated from hair, nail and corresponding urine of each participants .Estimation of arsenic was also done from their drinking water to confirm that patients were not re-exposed to arsenic during the study period. In randomly selected 10 % cases, their 24 hours food recall also noted to assess the arsenic consumption.

Other laboratory tests were performed which included liver function test (ALT, Alkaline Phosphatase), Urine for albumin and microscopical exam, random blood glucose of each patient. Electrocardiogram (ECG) was done in selective cases where it was found high blood pressure or high pulse rate in clinical examination.

2.12.1.1: Hair and Fingernail Arsenic Content Analysis

Fingernail and hair samples were washed twice with acetone and then with deionized water, weighed (wet weight), air dried, and dried in the oven at 60°C, then weighed again (dry weight). The dry

hair samples were weighed 100 mg and transferred into 50 ml polyethylene digestion tube, 02 ml high purity nitric acid was added and the samples were soaked in acid for at least 12 h before the tube was heated in a preheated hot block heater (Environmental Express Co., USA) at 120°C for 1 hour. After initial digestion a few ml of 30% hydrogen peroxide was added and the sample was heated for additional half an hour to give a transparent solution. Finally, the solution was diluted quantitatively to a fixed volume 50 ml and the solution was analyzed for arsenic by continuous flow hydride generation atomic absorption spectrophotometer (HG-AAS).

Nail samples were washed in the same manner as the hair samples to get rid of external dirt. The dry nail samples (100 mg) were weighed into polyethylene digestion tube. The samples were dissolved in acid in the same manner as hair. Digested hair and fingernail samples were measured for Total As, by continuous-flow HG-AAS.

2.12.1.2 : Hair and Fingernail Arsenic Analysis QA/QC

QA/QC measures were including calibration with NIST reference samples, use of blanks and replicates. For method validation, standard water from NIST (SRM 3103a), standard fish tissue from NIST (SRM 1946), and standard hair from IAEA (IAEA-

085, IAEA-086) was periodically analyzed for As. Calibration of the machine were made daily with fresh standard solutions of analytical-grade As from chemservice (coefficient of variation < 5%). For each specimen, three replicates were taken. Non-detects were assigned a value one-half the detection limit. (Liu, 1997).

2.12.1.3 : Urine samples Arsenic Content Analysis:

The Urine samples were stored in the refrigerator at – 4 °C before analysis. A few samples were split into two portions. One portion was digested in acid and then analyzed; another portion was directly analyzed by hydride generation atomic absorption spectrophotometer. In most cases, there were no significant differences in the arsenic level obtained by these methods. Eventually all the urine samples were directly analyzed without acid digestion. Pre-treatment with L-cystine and reduction with Potassium iodide and ascorbic acid was done for 45 minutes prior to analysis. (Guho, 1997) .

All biological tissues hair, nails are digested in high purity acids and pretreated with reductants prior to analysis. A quartz T-tube mounted on a burner in an atomic absorption spectrophotometer (Buck Scientific, USA, Model 210 VGP) was served as an atomization cell for arsine vapor. The hydride generation

method in this study using a reductant such as, sodium borohydride (in presence of acid and other reagents) to bring down all arsenic to As^{3+} state in the ground water. The primary reaction of tetrahydroborate (BH_4^-) anion from sodium borohydride with acids (H^+) and arsenic in ground water, generates free hydrogen (H_2) gas, covalent metal hydride AsH_3 (arsine) gas.

$$As^{5+}, As^{3+}\,(sample) + NaBH_4 \rightarrow As^{3+}$$
$$BH_4^- + H^+ + H_2O \rightarrow B(OH)_3 + H_2\,(g)$$
$$As^{3+} + H_2\,(g) \rightarrow AsH_3\,(g)$$

A peristaltic pump is utilized in mixing the sample, acid (hydrochloric acid) and the reactant ($NaBH_4$) in a reaction vessel. The effluent then passes through a gas-liquid separator. The arsine gas AsH_3, is purged from the reaction vessel by a flowing argon gas and air to the atomization source for continuous mode detection. The atomization source in our AAS instrument is a quartz T-tube heated in air-acetylene flame.

$$AsH_3(g) + \text{Heated Quartz Tube} \rightarrow As^0\,(\text{ground state})$$
$$As^0\,(\text{ground state}) + \text{light (HCL)} \rightarrow As^*\,(\text{excited state})$$

Absorbance of light is proportional to the concentration of arsenic in water

The atomic vapor in the heated cell absorb the resonance energy from the arsenic hollow cathode lamp (HCL) beam (wavelength λ = 193.7 nm) to provide the analytical signal. Prior to introduction of the vapor for thermal decomposition, the vapor passes through a moisture trap. The peristaltic pump and the set up allow on-line continuous hydride generation and fast arsenic detection in all samples.

2.12.1.4 :Urine Arsenic Analysis QA/QC Check

The atomic absorption spectrophotometer is calibrated everyday with standards prepared from high purity As_2O_3 (Aldrich Chemical, USA) or stock solutions from Perkin-Elmer Co. (USA). For quality control (QC), the QC standards and reagent blanks are analyzed at regular intervals. The QC checks are achieved by inserting the QC standards and reagent blanks during the continuous on-line analysis of samples. All standards and the reagents are prepared fresh daily. Distilled deionized water is used in all steps of reagent preparation and analysis. Standard reference materials (SRM 1643d, SRM 1640) from the National Institute of Standards and Technology (NIST, USA) are used as controls for precision and accuracy check of data and validation of analytical methodology

HG-AAS analysis of sample: hair, nail, urine

Analysis	:	Arsenic
Instrumentation	:	Continuous-HG-AAS (Buck Scientific, USA , Model 210 VGP)
Lamp	:	Arsenic Hollow Cathode Lamp (HCL),
Wavelength	:	193.7 nm
Slits	:	0.7 nm
Background Correction	:	Deuterium Continuous Background Correction
Reductant	:	1.5% $NaBH_4$ (basic medium)
Acid	:	10% HCl / 3% H_2SO_4
Purging Gas	:	Argon
Detection Limit	:	1.00 ppb
%RSD	:	± 2.5% at 1.00 ppb level
Standard stock Solution	:	ICP Standard solution from SPEX (USA) and/or Perkin-Elmer Standard solution (USA)

2.12.2: Selenium analysis

Estimation of total selenium, as described in (Le 2004 Le, 2000; Ma, 2000) and as further developed by Alauddin (Alauddin, 2003) and J. Feldmann (Raab, 2004). For the analysis of selenium, the selenium hydride gas passes directly into the Atomic florescence spectrophotometer (AFS) with selenium cathode at 196.1 nm wavelength.

2.12.2.1 : Procedure

250 microgram of serum was transferred into 50 ml polyethylene digestion tube, 02 ml high purity nitric acid was added and the samples were soaked in acid for at least 30minutes, before the tube was heated in a preheated hot block heater (Environmental Express Co., USA) at 115°C for half an hour. After initial digestion 06 drops of 30% hydrogen peroxide was added and the sample was heated for additional half an hour to give a transparent solution. Then, 06 ml of high purity hydrochloric acid was added to the digested materials and the sample was again heated for half an hour more at 115°C. Finally, the solution was diluted quantitatively to a fixed volume (10 ml) and the solution was analyzed for selenium by continuous flow hydride generation Atomic florescence spectrophotometer (HG-AFS) (PS analytical 10.055 Millennium Excalibur, England) with millennium software version 1.52.

2.12.2.2 : Serum Se Analysis QA/QC Check

The atomic florescence spectrophotometer is calibrated everyday with standards prepared from high purity stock solutions from Perkin-Elmer Co. (USA). For quality control (QC), the QC standards and reagent blanks are analyzed at regular intervals. The QC checks are achieved by inserting the QC standards and reagent blanks during the continuous on-line analysis of samples. All standards and the reagents are prepared fresh daily. Distilled deionized water is used in all steps of reagent preparation and analysis. Standard reference materials (SRM 1643d, SRM 1640) from the National Institute of Standards and Technology (NIST, USA) are used as controls for precision and accuracy check of data and validation of analytical methodology.

2.13 : Other biochemical parameters

For biochemical parameters such as random blood glucose, serum alkaline phosphatase, serum Alanine transaminase measurement various method has been adopted. Serum glucose level was estimated by glucose oxidase method .Glucose oxidase catalyses the oxidation of glucoseto glutonic acid. The formed hydrogen peroxide was detected by a chromogenic oxygen acceptor, phenol-aminophenazone in the presence of peroxidase.

Serum ALT level was determined as described by Bergmeyer. ALT catalyses the transfer of amino group from L-alanine to 2-oxogluterate resulting in the formation of pyruvate and L-glutamate .The puruvate thus formed undergoes reduction with simultaneous oxidation of NADH to NAD in the lactatedehydrogenase – catalyze indicator reaction . Serum alkaline phosphatase was measured by colorimetric method .

Urine was examined for presence of albumin and microscopical examination for any cast and RBC which denotes as abnormal findings. ECG was done in selective cases when there was high blood pressure (either > 160 in systolic or > 90 in diastolic or in combination) or high pulse rate (> 100 per minute) found in physical examination.

2.14 : Statistical analyses of the data

Data analyses were carried out using Statistical Package for Social Science (SPSS), (version 12.0 for windows, SPSS Inc, Chicago, USA). Data were presented as mean ±SEM (standard error of mean) and in graphic forms .The statistical methods used were Chi-square test (x^2) and Student's t-test (two tailed). Differences were considered significant with * $p<0.05$.

2.15 : Clinical outcome evaluation

Clinical improvement was assessed on the basis of skin manifestation and patient's wellbeing according to the checklist schedule. The patients were examined in every follow-up visit to evaluate the prognosis in terms of change in severity of keratosis, melanosis through comparing the state of lesions which as recorded in the checklist of previous visits. (Appendix 7.4). The evaluation was also done through comparing the patient's perception on changing in severity of their skin lesions and their physical wellbeing. While recording the prognosis of skin lesions as observed, the patients view on prognosis was also considered. The efficacy of the intervention as indicated by prognosis of severity of keratosis, melanosis, leucomelanosis were evaluated as grade 1 = mild, grade 2 = moderate and grade 3= severe skin grading according to the standardized methods of observations and recorded in the checklist at each follow-up visit. In every follow-up visit the patients were examined and asked for any side effects and if any was also recorded in the checklist (Appendix 8.4).

2.16 : Major outcome variables

The following clinical and laboratory characteristics were used as major outcome variables to assess the treatment effects.

2.16.1 : Clinical variables:

Skin changes including melanosis, keratosis and leucomelanosis were categorized into 1 to 3 severity grades according to the standardized methods of observations. Other clinical symptoms were reported by the patients.

1. Skin manifestations : severity grades 1 to 3
2. Weakness
3. Anorexia
4. Nausea
5. Vomiting
6. Dizziness
7. Diarrhea
8. Upper Abdominal pain
9. Chest pain
10. Cough
11. Conjunctivitis
12. Hypertension
13. Hepatomegaly with or without Splenomegaly
14. Cirrhosis with or without ascities
15. Pedal non-pitting edema
16. Peripheral Neuropathy : Tingling
17. Peripheral vascular disease : Intermittent claudication and Gangrene

2.16 .2: Grading of severity of skin lesions

Based on clinical manifestations and observations according to the WHO definition, patients were categorized into three severity grades:

- Grade I skin lesion (mild) :

  a) Diffuse melanosis

  b) Suspicious spotty depigmentation / pigmentation over trunk / limbs

  c) Mild diffuse thickening of soles and palms

- Grade II(moderate):

  a) Definite spotty pigmentation/ depigmentation on the trunk and limbs, bilaterally distributed

  b) Severe diffuse thickening (with or without wart like nodules of the palms and soles

- Grade III (severe):

  a) Definite spotty pigmentation / depigmentation as above with few blotchy pigmented / depigmented macular patches over trunks or limbs

  b) Pigmentation involving the undersurface of tongue and / or buccal mucosa

  c) Larger nodules over thickened palms and soles occasionally over the dorsal aspect of hands and feet.

  d) Diffuse verrucous lesions of the soles with cracks and

fissures and keratotic horns over palms and soles.

e) Non-healing ulcer: Bowen's disease, squamous cell carcinoma, Basal cell carcinoma.

2.16.3 : Laboratory Variables

- Arsenic concentrations in nail
- Arsenic concentration in hair
- Arsenic concentration in urine (Total)
- Arsenic concentration in drinking water (during study period)
- Arsenic concentration in tube well water
- Selenium concentration in serum
- Liver function test (SGPT, Alkaline Phosphatase)
- Kidney function test (Routine urine exam)
- Random blood sugar
- Electrocardiogram
- Estimation of arsenic and selenium from their 24 hours food recall

2.16.4 : Laboratory Appliances

1. Electronic digital balance (SHIMADZU, Japan)
2. Centrifuge machine (ANDREAS HETTICH, Germany)
3. Atomic absorption spectrophotometer (Buck Scientific, USA, Model 210 VGP)
4. Atomic florescence spectrophotometer (PS analytical 10.055 Millennium Excalibur, England)
5. HPLC system (LKB, Bromma, Sweden)
6. Semi automated clinical chemistry analyzer(Microlab

300 ,Netherlands)

7. Refrigerator (DAEWOO, Korea)
8. Arsine Generator
9. Automatic micropipettes
10. Polythene tubes
11. Test tube of different size
12. Cylindrical flask
13. Heat block burner(Environmental Express Co., USA)
14. Safety rubber gloves
15. Safety gas mask

2.16.5: Drugs and chemicals

1. Hydrochloric acid (HCL) concentrated – E MERCK
2. Nitric acid (HNO_3) concentrated –E MERCK
3. Potassium iodide(KI) –BDH CHEMICALS
4. Ascorbic acid - BDH CHEMICALS
5. Sodium borohydrate ($NaBHO_4$)-ACROS Organics,USA
6. Sodium hydroxide (NaOH)- BDH CHEMICALS
7. Hydrogen Peroxide(H_2O_2)- E MERCK
8. Deionized water

2.17 : Time schedule of activities with milestones

Sl. No	Name of milestones	Starting Date	Completion date
1.	Planning and protocol review	01/11/2004	15/11/2004
2.	Preparation, Supplies, QA/QC, Calibration, Sampling Training & Testing	16/11/2004	15/12/2004
3.	Survey of Villages, Patient Recruitment	16/12/2004	15/02/2005
4.	Selection and Consent, Tabulation of Records and Randomization	16/02/2005	28/02/2005
5.	Training of supervisors and field workers	01/03/2005	31/03/2005
6.	Interview , Sample collection and intervention:		
	• Samples of hair,nail,blood & urine collection	01/04/2005	07/04/2005
	• Laboratory analysis of collected sample (Initial batch)	09/04/2005	30/07/2005
	• 1st Follow up visits with samples of blood & urine collection	01/08/2005	07/08/2005
	• Laboratory analysis of collected sample (Ist Follow-up batch)	09/08/2005	30/11/2005
	• 2nd Follow up visits with samples of blood & urine collection	01/12/2005	07/12/2005
	• Laboratory analysis of collected sample(2nd follow-up batch)	09/12/2005	31/03/2006
	• Final follow up visits with sample collection of hair,nail,blood & urine	01/04/2006	07/04/2006
	• Conclusion of Trial & Laboratory analysis of collected sample (Final batch)	09/04/2006	30/07/2006
7.	Data Analysis, their preparation & Seminar	01/08/2006	30/10/2006

Chapter 3

RESULTS

3 . Results

In this study, out of 260 patients selected, of which 45 patients could not complete one year study periodic follow-up, so the dropout rate was 17.3%. After decoding, analysis was done and data presented by graphs.

Table 1 : Distribution of age by sex in study population

Age in Group	Selenium			Placebo			Vit ACE		
	Sex		Total	Sex		Total	Sex		Total
	Male N (%)	Female N (%)	N (%)	Male N (%)	Female N (%)	N (%)	Male N (%)	Female N (%)	N (%)
10- 29	9(32.1)	12(20.3)	21(24.1)	11(32.4)	11(20.8)	22(25.3)	8(25.8)	12(21.8)	20(23.3)
30- 49	16(57.1)	38(64.4)	54(62.1)	12(35.3)	38(71.7)	50(57.5)	18(58.1)	34(61.8)	52(60.5)
50- +	3(10.7)	9(15.3)	12(13.8)	11(32.4)	4(7.5)	15(17.2)	5(16.1)	9(16.4)	14(16.3)
Total	28(100)	59(100)	87(100)	34(100)	53(100)	87(100)	31(100)	55(100)	86(100)
Mean± SD	35.8±14.0	36.0±10.8	35.9±11.8	39.5±17.3	35.1 ±9.3	36.8±13.0	38.0±11.7	36.3±11.3	36.9±11.4

Figures in parentheses are percentile

Among the study subjects, 167 (64.2 %) were young adult female and the rest were male. The majority (60%) of the study subjects were between below 50 years with female dominance in all groups (Table 1).

Table 2: Socio-demographic characteristics of study population

Characteristics	Selenium (N = 87)	Placebo (N = 87)	Vit ACE (N = 86)	p-value
Number of subjects	28(M),59(F)	34(M),53(F)	31(M),55(F)	0.63
Age in years, mean±SD	35.9±11.8	36.8±13.1	36.9±11.4	0.83
Body weight, kg, mean±SD	47. 6±9.3	48.2±9.2	48.5±10.1	
Body height, cm, mean±SD	153.0±8.3	153.1±9.2	154.0±8.7	
Literacy in %				0.09
a) Upto Primary	67.8	64.4	73.3	
b) Secondary	19.5	14.9	19.8	
c) HSC+	12.7	20.7	6.9	
Monthly household income, mean±SD	2781.6±3339.7	2458.6±2809.5	1858.1±1597.0	0.07
Family size, in %				0.17
a) 1-4 (small)	13.8	17.2	11.6	
b) 5-6(medium)	39.1	52.9	47.3	
c) 7+ (large)	47.1	29.9	38.5	

Figures in parentheses are denoting sex

Mean age of 3 groups did not differ significantly ($p<0.83$), so on height and weight .The Monthly income between groups were not significant ($p<0.07$). More than sixty eight percent of the study subjects had up to primary level education. The proportion of highly educated subjects was poor ($p<0.09$). Majority of family had 5 or more member (Table 2, Appendix 7.13).

Table 3: Distribution of arsenic contamination in drinking water of the study subjects

Study Groups	Before intervention				After intervention			
	<50µg/L N (%)	>50 µg/L N (%)	Total N (%)	Mean±SD	<50µg/L N (%)	>50µg/L N (%)	Total N (%)	Mean±SD
Selenium	58 (66.7)	29 (33.3)	87(100)	47.4± 65.9	87(100)	0(0.0)	87(100)	4.2 ±9.3
Placebo	67 (77.0)	20 (23.0)	87(100)	30.9± 51.9	87(100)	0(0.0)	87(100)	2.4 ±7.3
Vit ACE	62 (72.1)	24 (27.9)	86(100)	37.8± 53.9	86(100)	0 (0.0)	86(100)	3.4 ±8.4
Total	187(71.9)	73 (28.1)	260(100	38.7± 57.8	260(100	0 (0.0)	260(100)	3.3 ±8.4

Figures in parentheses are percentile

This table 3 revealed that 33.3% in selenium group, 23% in placebo group and 27.9 % of Vitamin ACE group were drinking contaminated water before the beginning of the study. But after intervention it dropped down to none ($p<0.31$). Maximum concentration of arsenic found in their tube well before intervention was 243 microgram/L and mean concentration was 38.7± 57.8 microgram/L. It should be noted that among the study subjects 71.9% had already started taking the arsenic free drinking water.

Table 4: Inorganic arsenic intakes through food intake literature (N= 26)

SL	Food item	Intake(g/day)	Arsenic conc. (µg/kg)	Total As intake (µg/day)	inorganic Fraction	Inorganic As intake (µg/day)
1	Cereal	378.2	200.0	75.64	0.43	32.53
2	Potato	59.3	23.0	1.36	0.10	0.14
3	Vegetables	108.2	7.0	0.76	0.05	0.04
4	Pulses	22.5	200.0	4.50	0.43	1.94
5	Meat	29.3	22.0	.65	0.41	0.27
6	Fish	60.9	1662.0	101.22	0.10	10.12
7	Spices	37.2	7.0	0.26	0.05	0.01
8	Oils	10.6	19.0	0.20	1.00	0.2-
9	Fruits	2.4	6.4	0.02	0.10	0.002
10	Sugar	5.6	10.9	0.06	0.43	0.03
11	Milk	23.7	12.0	0.28	0.75	0.21
12	Misc	9.5	12.5	0.12	0.43	0.05
13	Total	747.4	2181.8	185.07	4.28	45.54

Tables 4 revealed that population in the study area were consuming on and average 45.54 microgram of inorganic arsenic through their daily dietary intake. This table also showed there was less fat and imbalance in protein intake .

Table 5 :Source and duration of water in the study area

Characteristics	Selenium (N = 87)	Placebo (N = 87)	Vit ACE (N = 86)	p-value
Source of drinking water, in %				0.28
a) Deep tube well	6.9	0.3	5.8	
b)Shallow tube well	86.2	87.4	93.0	
c) Pond	4.6	2.3	1.2	
d) filter/boil	2.3	0.0	0.0	
Duration of drinking water, in %				0.79
a) 1-3 years	11.5	6.9	9.3	
b) 4-6 years	23.0	23.0	23.3	
c) 7-9 years	29.9	37.9	39.5	
d) 10+ years	35.6	32.2	27.9	
Duration of drinking water in years, mean±SD	21.1±11.4	21.9±12.9	22.6±12.6	0.79
Source of cooking water, in %				0.45
a) Deep tube well	3.4	1.1	1.2	
b)Shallow tube well	2.3	2.3	0.0	
c) Pond	94.3	96.6	98.8	
Duration of cooking water, mean±SD	25.5±10.6	27.6±11.4	26.1±10.8	0.71
Arsenic conc in drinking water				0.31
a) before intervention	47.4±65.9	30.9±51.9	37.8±53.9	
b)during intervention	4.2±9.3	2.4±7.3	3.4±8.4	

In table 5 showed that 91.8% of study subjects were drinking contaminated water for more than 04 years and the mean duration of drinking water was 21.9±12.3 years (The mean were not mutually exclusive) ($p<0.79$). More than 94 % people cooked their meals with pond water which we have found free from arsenic. The mean

duration of cooking with water from this source was 26.4±10.9 years (p<0.71) (Appendix 7.14 & 7.15).

Table 6: Distribution of Body Mass Index (BMI) before and after intervention

Body Mass Index (BMI)	Group	Male				Female				Total			
		Before		After		Before		After		Before		After	
		N	%	N	%	N	%	N	%	N	%	N	%
<18.5	Selenium	13	46.4	10	45.5	17	28.8	12	30.8	30	34.5	22	31.9
	Placebo	9	26.5	5	17.9	18	34.0	7	15.9	27	31.0	12	16.7
	Vit ACE	11	35.5	7	28.0	18	32.7	12	24.5	29	33.7	19	25.7
18.5-24.9	Selenium	10	35.5	9	40.9	36	61.0	32	82.1	46	52.9	41	67.2
	Placebo	24	70.6	21	75.0	29	54.7	28	63.6	53	60.9	49	68.1
	Vit ACE	16	51.6	14	56.0	30	54.5	30	61.2	46	53.5	44	59.5
25 & above	Selenium	5	17.9	3	13.6	6	10.2	3	7.7	11	12.6	6	9.8
	Placebo	1	2.9	2	7.1	6	11.3	9	20.5	7	8.0	11	15.3
	Vit ACE	4	12.9	4	16.0	7	12.7	7	14.3	11	12.8	11	14.9
Total	Selenium	28	100.0	22	100.0	59	100.0	47	100.0	87	100.0	69	100.0
	Placebo	34	100.0	28	100.0	53	100.0	44	100.0	87	100.0	72	100.0
	Vit ACE	31	100.0	25	100.0	55	100.0	49	100.0	86	100.0	74	100.0
BMI Mean±SD	Selenium	20.2±4.3		20.6±4.3		20.3±3.1		21.3±2.8		20.3±3.5		21.0±3.4	
	Placebo	20.1±2.5		21.3±2.3		20.7±3.5		22.0±3.5		20.5±3.2		21.7±3.1	
	Vit ACE	20.3±3.6		20.4±3.3		20.8±3.8		21.4±3.3		20.4±3.4		21.2±3.5	

Mean BMI before intervention in were 20.3±3.5, 20.5±3.2 and 20.4±3.4 in selenium, placebo and Vitamin ACE groups respectively. After intervention it was 21.0±3.4, 21.7±3.1and 21.2±3.5 in selenium, placebo and Vitamin ACE group respectively (Table 6). There was 27% increase of BMI found in selenium group having BMI 18.5-25.

Figure 1: Shows distribution of duration of ailment of disease by sex in study population

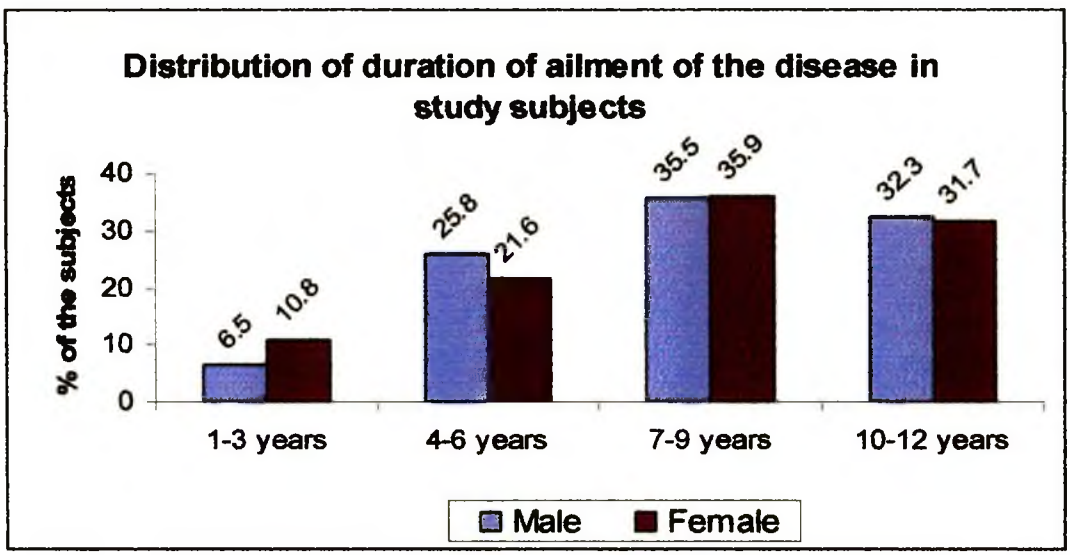


Figure 1 showed that majority of the study subjects suffered from the disease ailments for more than 07 years ($p < 0.81$)(Appendix 7.5).

Figure 2: Distribution of skin manifestations

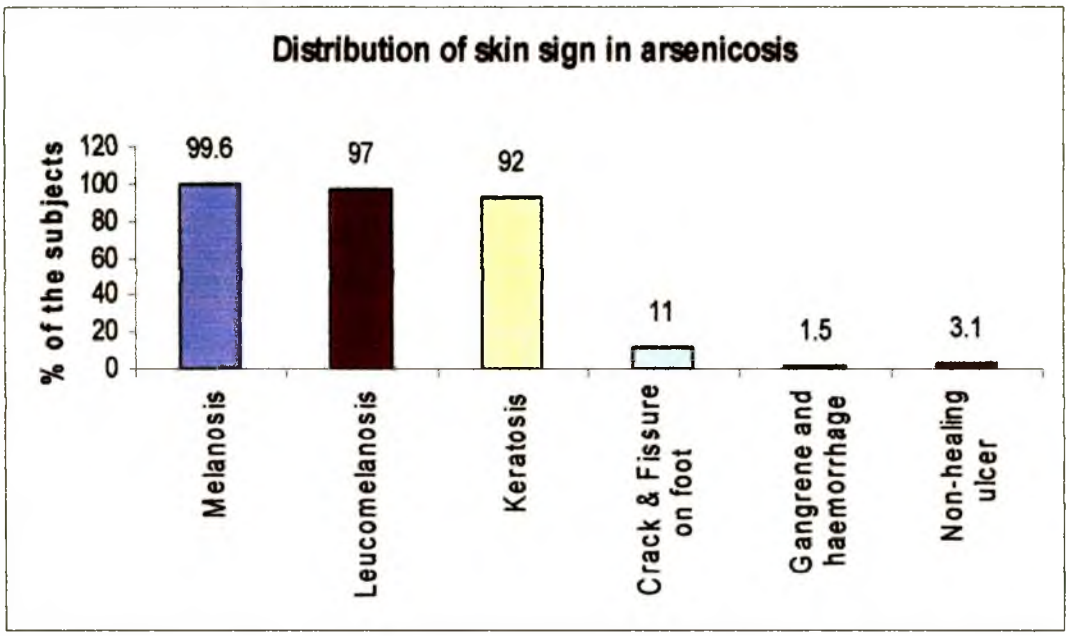
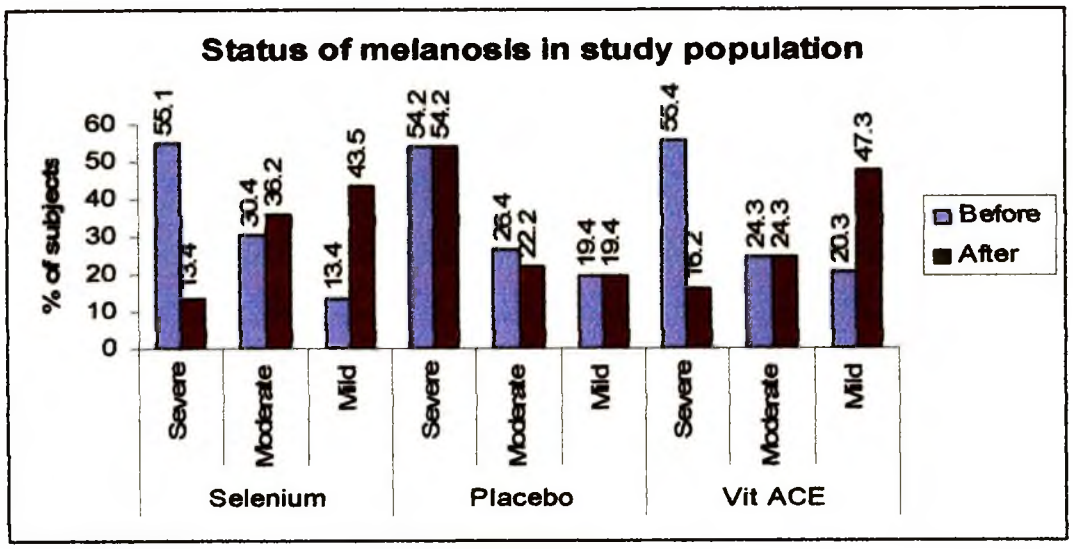


Figure 2 revealed that 99.6% cases had melanosis, 97 % had leucomelanosis and 92 % had palmo-planter hyperkeratosis. Multiple lesions were present in same subjects in many cases . Non-healing indolent ulcer were present in 3.1% cases which histopathologically revealed Bowen's disease 02 (0.8%) cases and squamous cell carcinoma 06 (2.3%) cases. (Appendix 7. 1 & 7.2)

Figure 3: Status of melanosis in study subjects



In figure 3 , Selenium treated group showed distinct reduction of severity of melanosis by 76% in the intervention period , no change in placebo group and little change in vit ACE group. (Appendix 7.2) .

Figure 4: Status of keratosis in study subjects

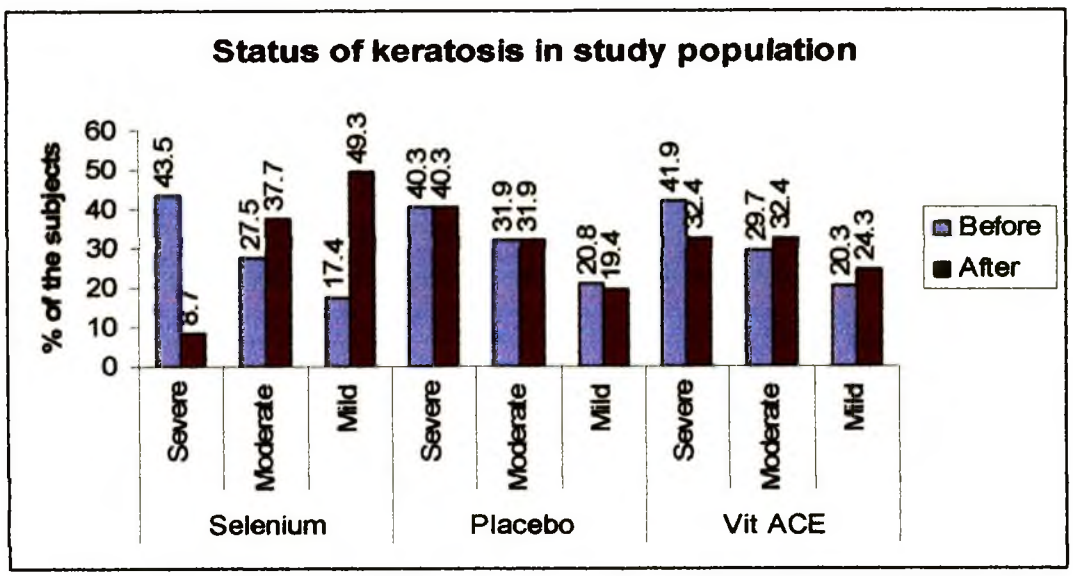


Figure 4 showed that keratosis in the palm and soles reduced markedly in selenium intervention group .The severity of keratosis was dropped from 43.5 % to 8.7% (80% reduced n=67) in selenium intervention groups and in Vitamin ACE groups it reduced 23% but no changes observed in placebo groups (Appendix 7.2).

Figure 5: Status of leucomelanosis in study population

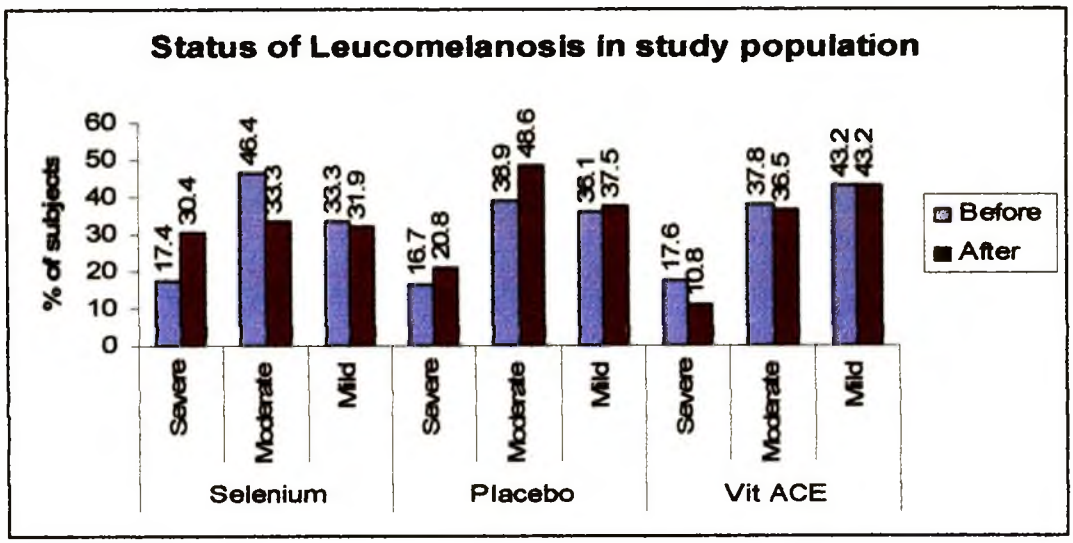
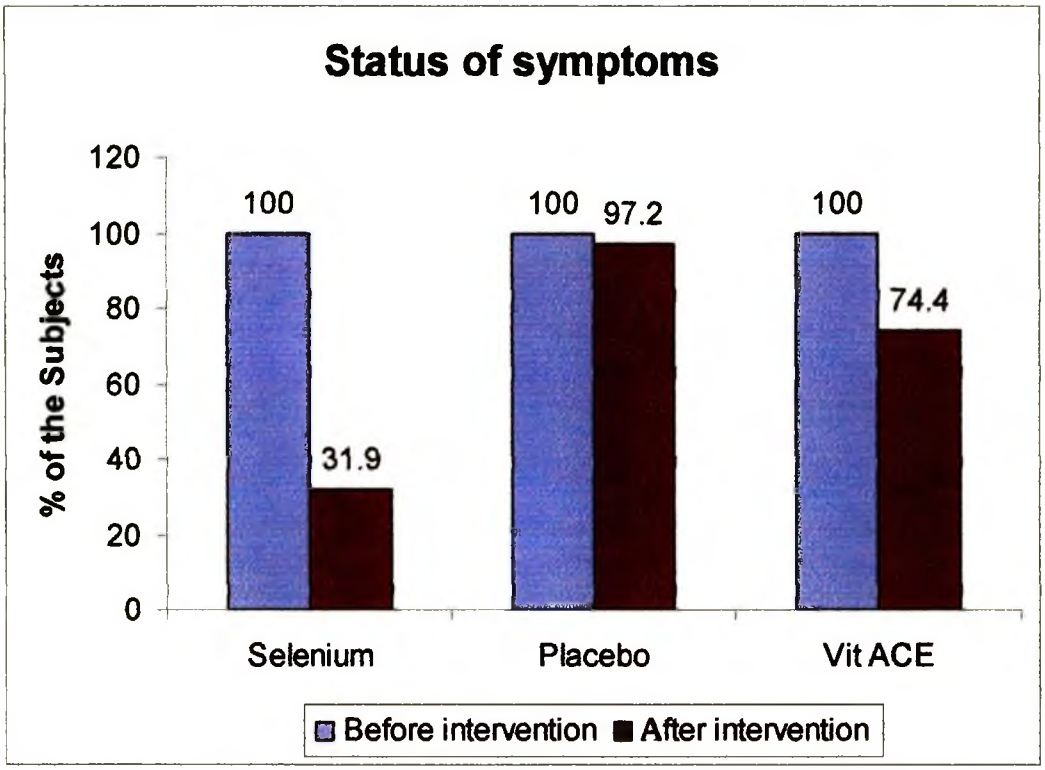


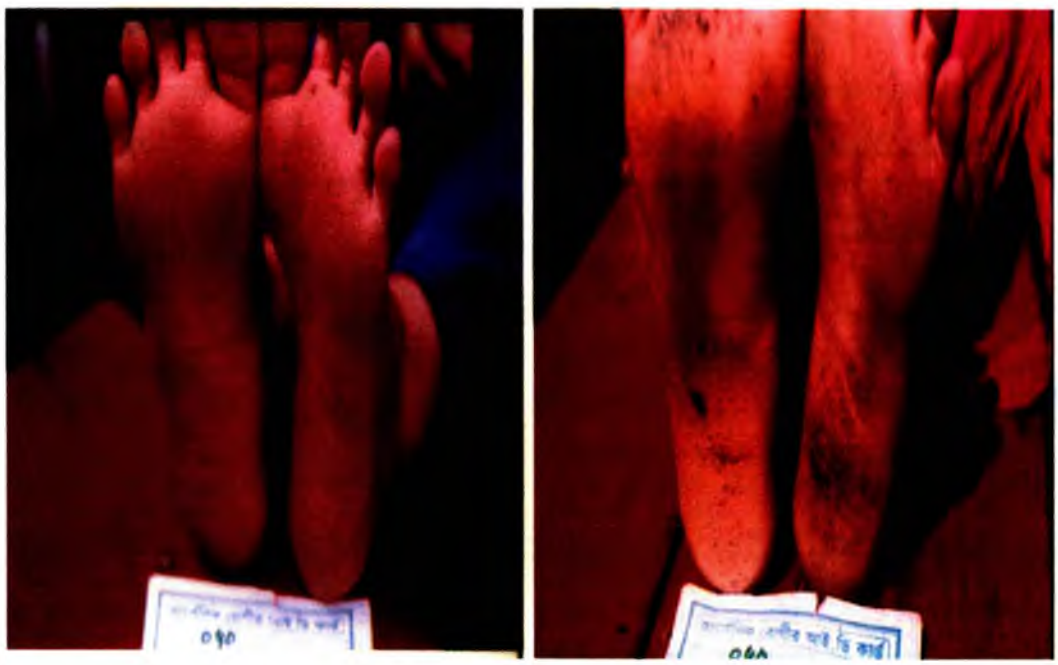
Figure 5 showed that more leucomelanosis was developed in selenium treated group after intervention. There was 75% increase of leucomelanosis in severely affected selenium intervention group and 25% increase in placebo group .But it dropped in severely affected Vitamin ACE group to 39% (Appendix 7.2).

Figure 6 : Status of symptoms in study subjects



In this figure 6 it was observed that in comparison to placebo and ACE group the selenium group showed 68% improvement in symptom ($p < 0.00$) (Appendix 7.3)

Photograph 1



After intervention **Before intervention**

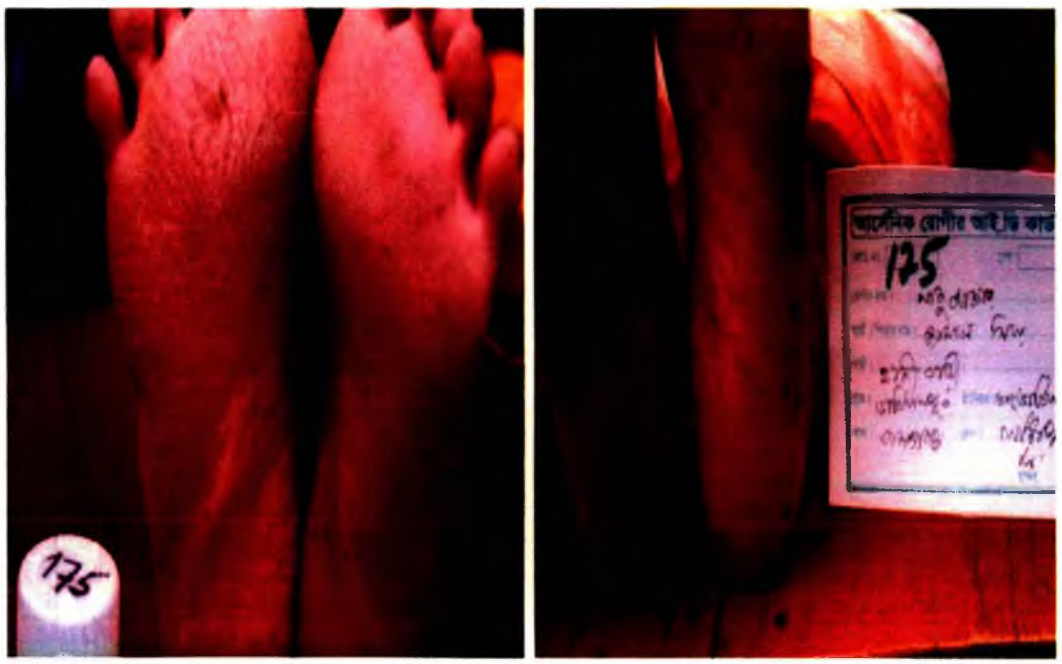
Photograph 1 showed hyper-keratosis with few keratotic horn present on the soles in a selenium treated subject .There was significant reduction particularly in size by 80% of the hyperkeratosis . Also there was much reduction in melanosis showing significant fairness in the after intervention photograph.

Photograph 2

After intervention **Before intervention**

Photograph 2 Showed melanosis in the trunk of a female subject in selenium treated group. There was 80% reduction of hyperpigmentation found in the after intervention photograph .

Photograph 3



After intervention **Before intervention**

Photograph 8 showed keratotic horn and papulo-nodules developed on the soles in a selenium treated subject .There was significant reduction of the hyperkeratosis particularly in size and in number but complete elimination did not happened . Also, there was much reduction of melanosis showing significant fairness in the photograph of after intervention .

Figure 7: Status of selenium in blood in the study subjects

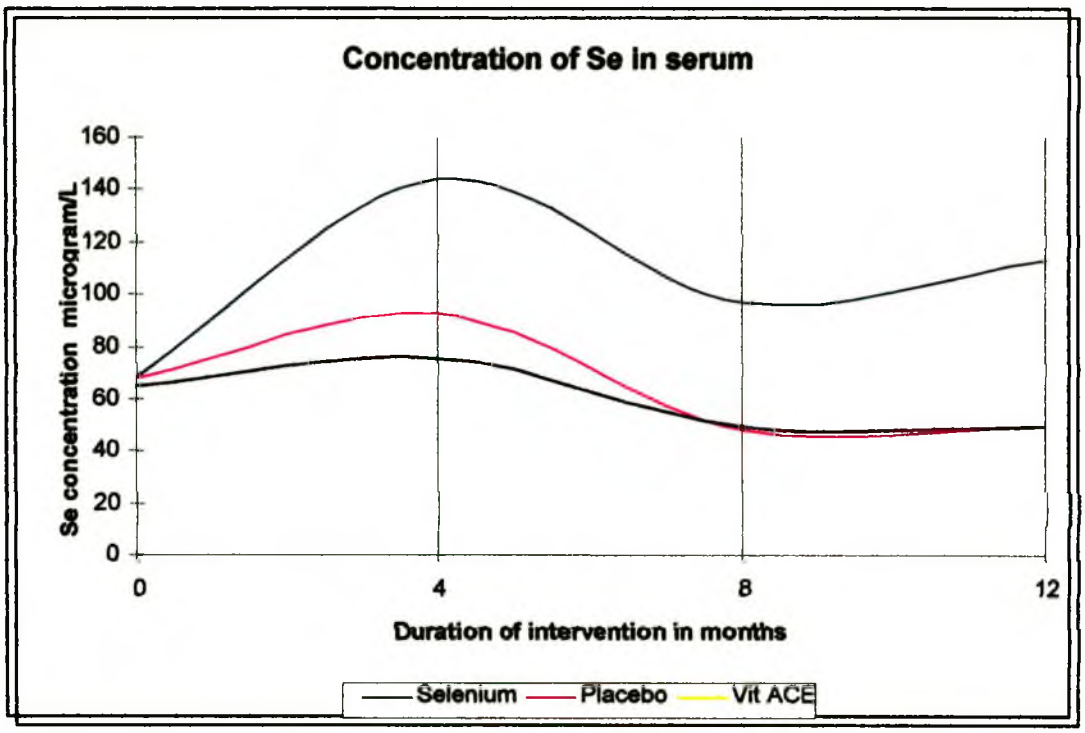


Figure 7 showed serum concentration of selenium in selenium, Placebo and Vitamin ACE group in µg/L level as mean±SD (Appendix 7.7). This figure showed that there were low concentrations of selenium in our study population . The concentration reached its peak in 4 month in the selenium treated groups.

Table 7: Mean &SD Arsenic concentrations of nail, Hair and Urine of study subjects

Parameters	Group	N	Before intervention	After intervention	P-value
Arsenic (As) Concentration in Nail (ppb)	Selenium Placebo Vit. ACE	55 61 64	3.41 ± 2.93 3.58 ± 4.26 3.80 ± 4.71	2.14 ± 1.90 3.11 ± 3.16 2.64 ± 2.40	0.00 (S) 0.48(NS) 0.01 (S)
Arsenic (As) Concentration in Hair (ppb)	Selenium Placebo Vit. ACE	60 67 71	1.31 ± 1.82 1.11 ± 1.30 1.37 ± 1.42	0.81 ± 1.79 0.80 ± 1.39 0.84 ± 0.84	0.01 (S) 0.06 (NS) 0.00(S)
Arsenic (As) Concentration in Urine (ppb)	Selenium Placebo Vit. ACE	60 64 66	67.73 ± 111.37 95.48 ± 146.97 122.25±187.96	31.75 ± 77.79 41.63 ± 78.82 44.11 ± 85.40	0.00 (S) 0.00 (S) 0.00 (S)
Selenium (Se) Concentration in Serum(ppb)	Selenium Placebo Vit. ACE	67 72 74	69.13 ± 24.48 69.92 ± 38.15 63.51 ± 28.61	97.49 ± 55.03 53.25 ± 68.50 48.31 ± 56.45	0.00 (S) 0.07(NS) 0.03(S)

S = Significant
NS = Not Significant

In table 7, it was observed that nail, hair and urine were loaded with arsenic initially. But finally after intervention significant changes occurred in selenium and Vitamin ACE group. Concentration in serum selenium showed a sharp rise in titer after intervention in selenium group.

Figure 8 : Cross tabulation of normal and abnormal arsenic level in hair

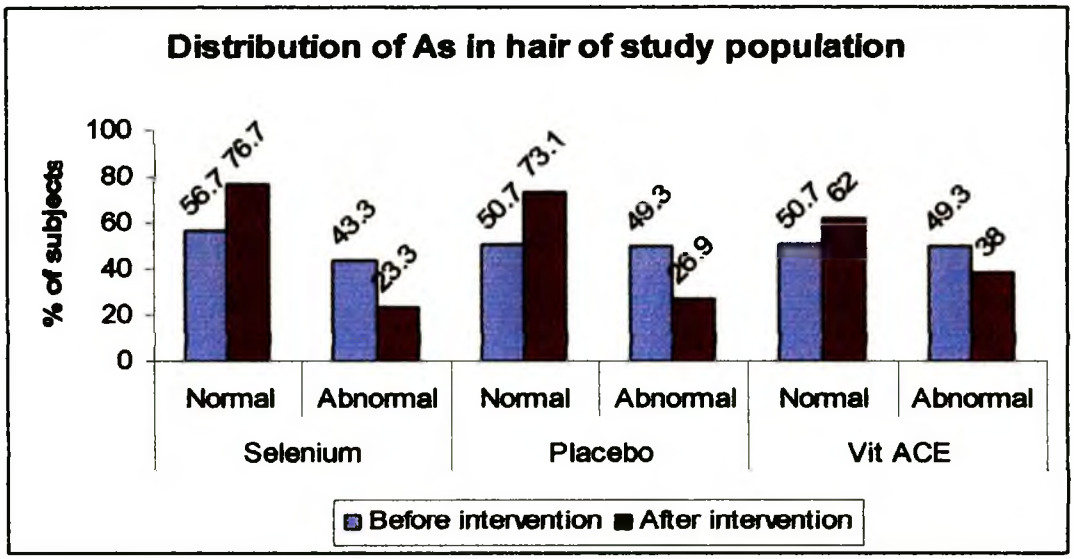


Figure 8 showed that there were significant changes in concentration of arsenic in hair of selenium group after intervention ($p<0.02$). The Arsenic load dropped after intervention from 43.3 % to 23.3 % (46% improved , n=60) in selenium treated group , from 49.3 % to 38% (23% improved , n=71) in Vitamin ACE group ($p<0.12$) and from 49.3 % to 26.9% (45% improved , n=67) in placebo ($p<0.01$) group respectively (Appendix 7. 11).

Figure 9 : Cross tabulation of normal and abnormal arsenic level in nail

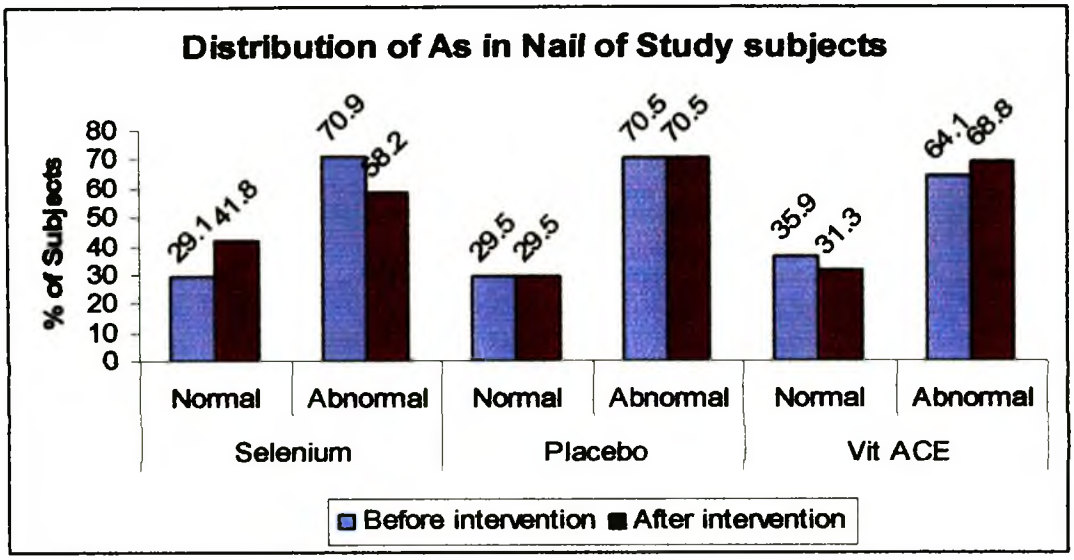


Figure 9 revealed that there were considerable reduction in concentration of arsenic load in the nail of selenium group after intervention ($p<0.12$). About 71% subjects of selenium group showed high concentration of arsenic in their nail before intervention but after intervention it become to 58.2% (18% improved, n=55). On the other hand, there were no changes found in placebo group and in Vitamin ACE group ($p < 0.56$ & $p < 0.35$ respectively) (Appendix 7. 12).

Figure 10: Status of arsenic in urine of study population

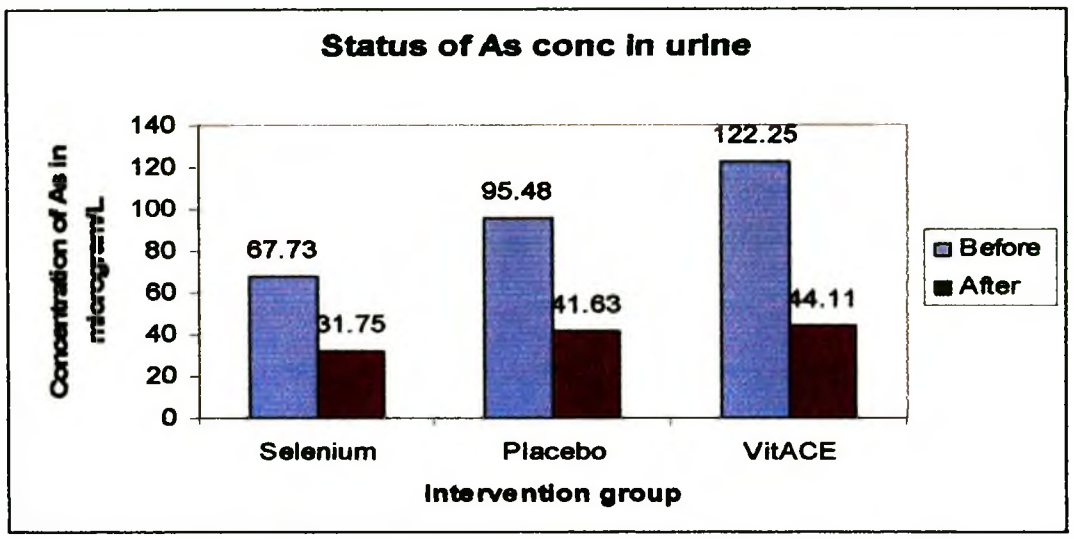


Figure 10 Showed concentration of Arsenic in Selenium, Placebo and Vitamin ACE group in µg/L as mean±SD level in urine .There were significant reduction of excretion in concentration of arsenic in all intervention group ($p<0.00$) but titer of concentration become lower in selenium treated group (Table 7).

Figure 11 : Trend of arsenic excretion in urine of study population

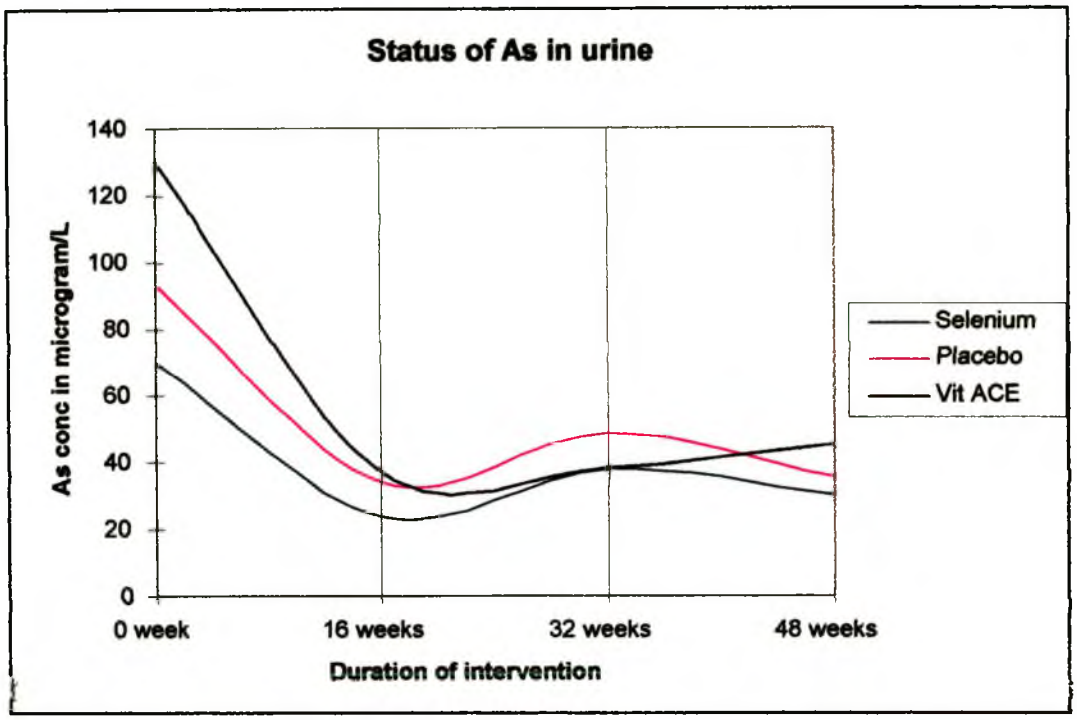


Figure 11 showed concentration of Arsenic in selenium, Placebo and Vitamin ACE group in microgram/L level as mean±SD in urine and their trend (Appendix 7.6). This figure revealed that there was sharp reduction in concentration of urinary arsenic by 16 weeks of intervention and this trend continued till the end of study .

Figure 12: Correlation of arsenic concentration in urine and water

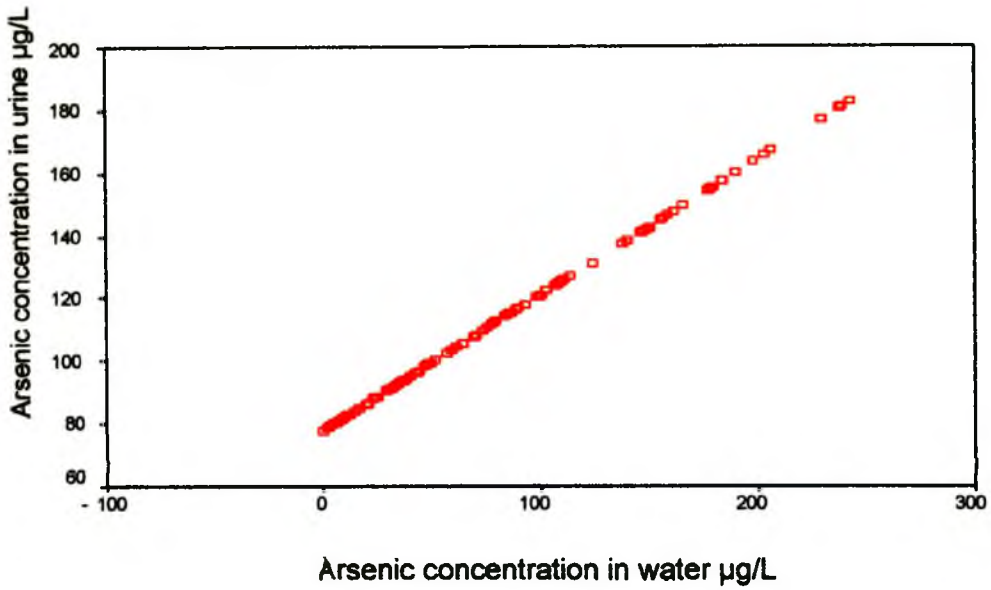


Figure 12 showed that there was a direct positive correlation between the amounts of arsenic consumed through drinking of contaminated tube well water and the concentration of arsenic in urine. The spearman coefficient of correlation, r = 0. 026 which indicates a strong and significant ($p<0.00$) relationship between arsenic contents in urine than that in tube well water. It was observed that higher the concentration in tube well water there was more excretion through urine.

Figure `13: Status of arsenic in nail of study subjects

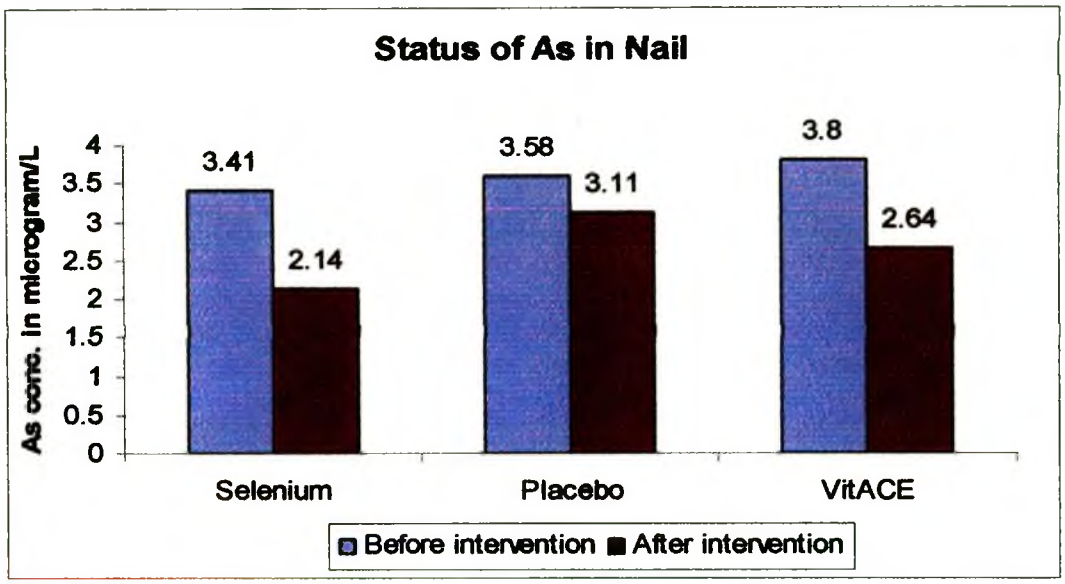


Figure 13 showed concentration of Arsenic in selenium, Placebo and Vitamin ACE group in µg/L level as mean±SD in nail. This figure revealed that there were significant reduction of arsenic in all groups of study subjects but more in selenium intervention group (37% reduced in selenium treated group, $p<0.00$, 30 % reduction in Vitamin ACE group, $p< 0.48$ and 13% reduction in placebo group, $p< 0.01$) (Table 7).

Figure 14: Correlation of arsenic concentration in nail and water

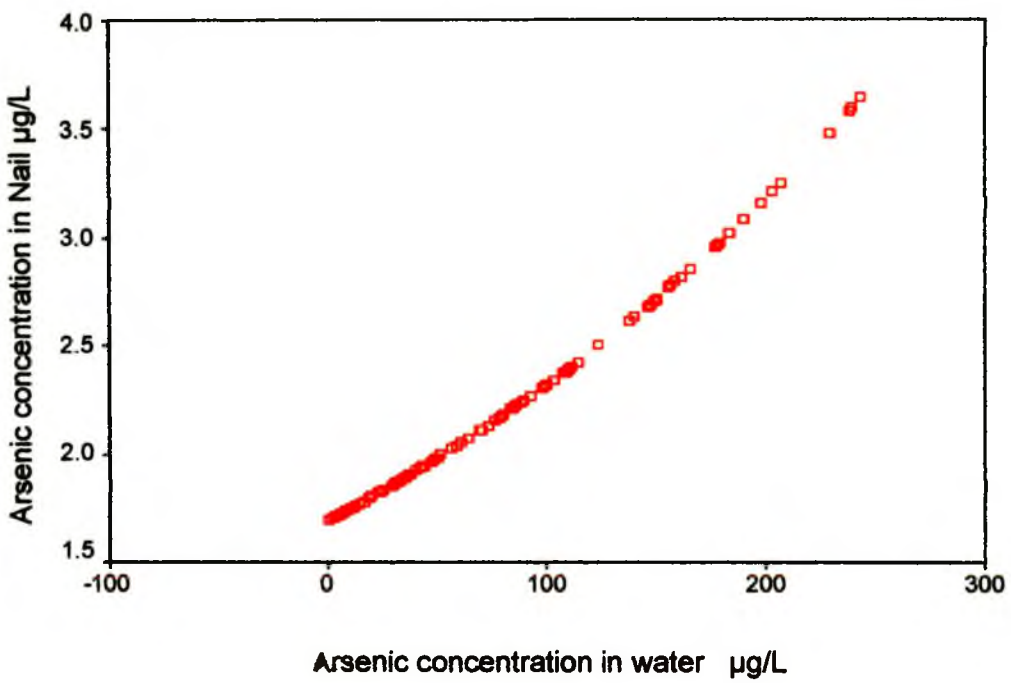
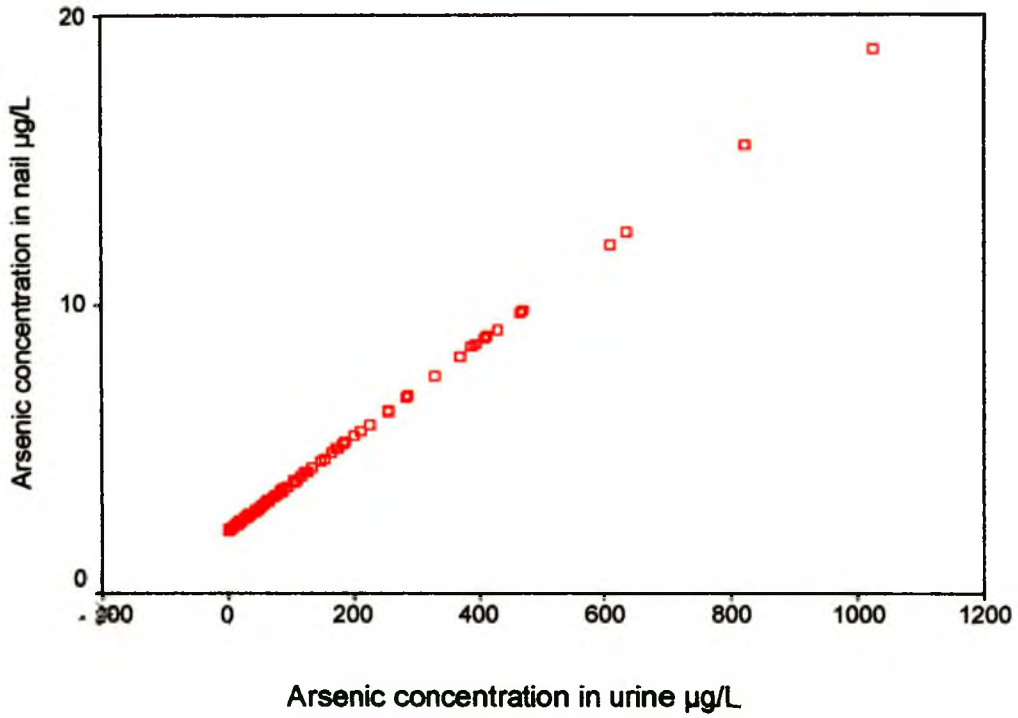


Figure 14 showed that there had a direct positive correlation between the amounts of arsenic consumed through drinking of contaminated tube well water and the concentration of arsenic in finger nail. The spearman coefficient of correlation, r = 0. 018 which indicates a strong and significant ($p<0.03$) relationship between arsenic contents in nail than that in tube well water. It was observed that higher the concentration in tube well water more will be deposited in nails.

Figure 15: Correlation of Arsenic concentration of Nail and urine



The nail concentration of total arsenic has a direct positive correlation with urine arsenic concentration and there was a very strong relationship (Figure 15). This was indicated by a significant ($p<0.00$) higher correlation coefficient for nail ($r= 0.402$). This linear relationship exists throughout the concentration ranges for nail with that of urine concentration.

Figure16: Status of arsenic in hair of study subjects

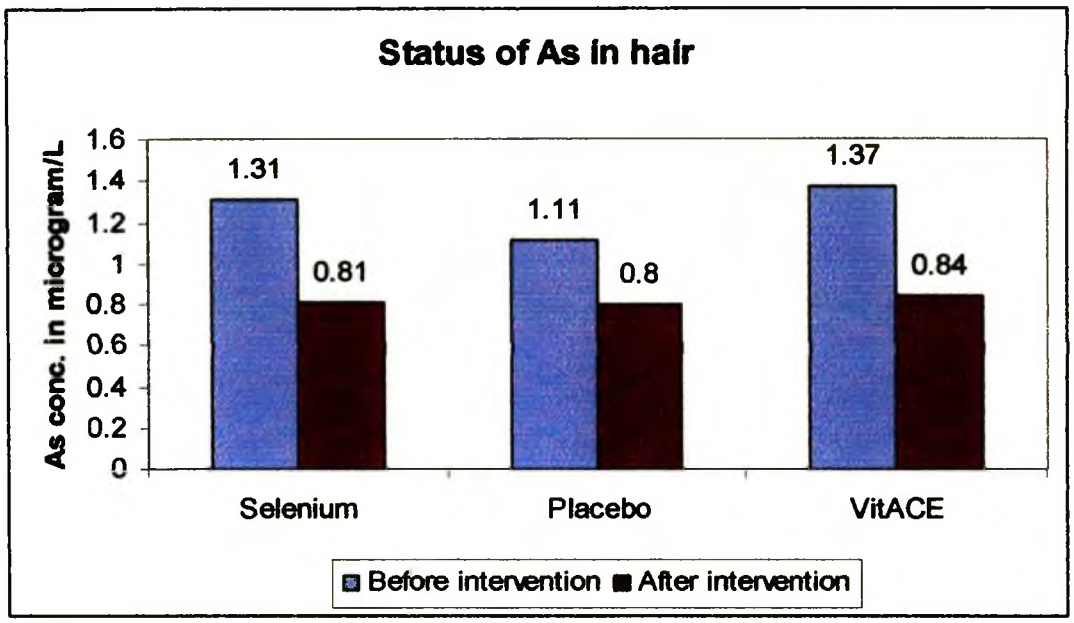


Figure 16 showed concentration of Arsenic in selenium, Placebo and Vitamin ACE group in µg/L level as mean±SD in hair. There were significant reduction of arsenic in all groups .38% ($p<0.00$) reduced in selenium, 28% ($p<0.05$) reduced in placebo and 39 % ($p<0.00$) reduced in Vitamin ACE groups (Table 7).

Figure 17: Correlation of hair and arsenic concentration in water

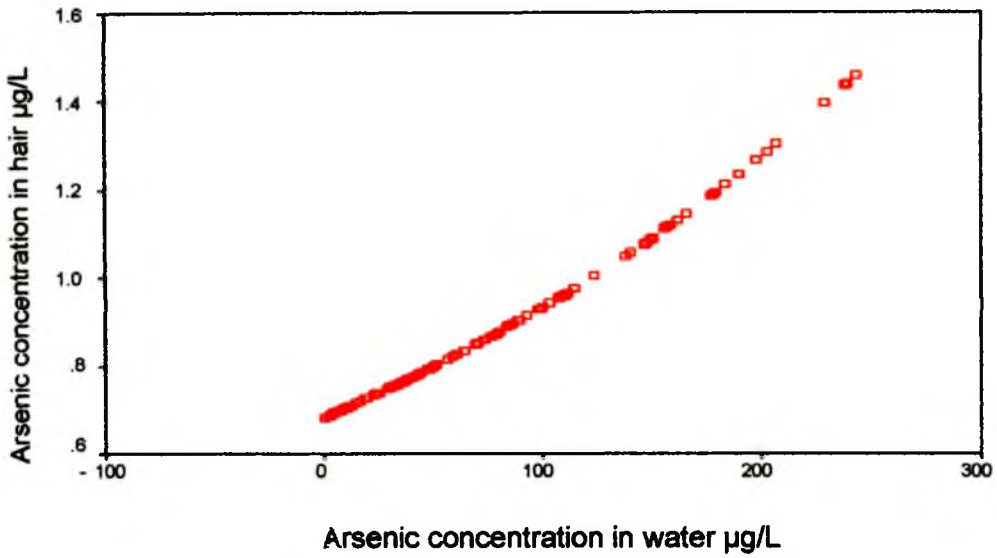
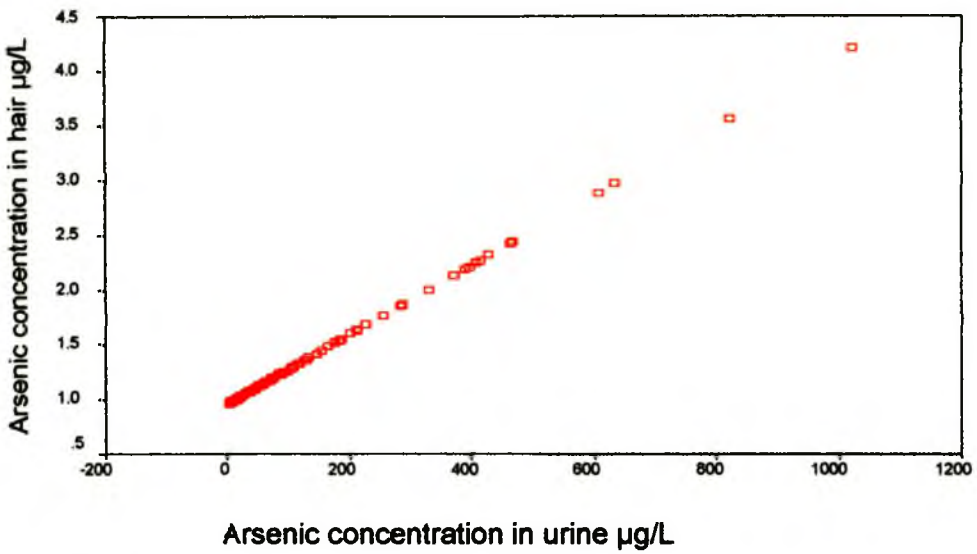


Figure 17 showed that there is a direct positive correlation between the amounts of arsenic consumed through drinking of contaminated tube well water and the concentration of arsenic in scalp hair. The spearman coefficient of correlation, r =0. 035 which indicates a strong and significant ($p<0.00$) relationship between arsenic contents in hair than that in tube well water.

Figure 18: correlation of arsenic concentration of hair and urine



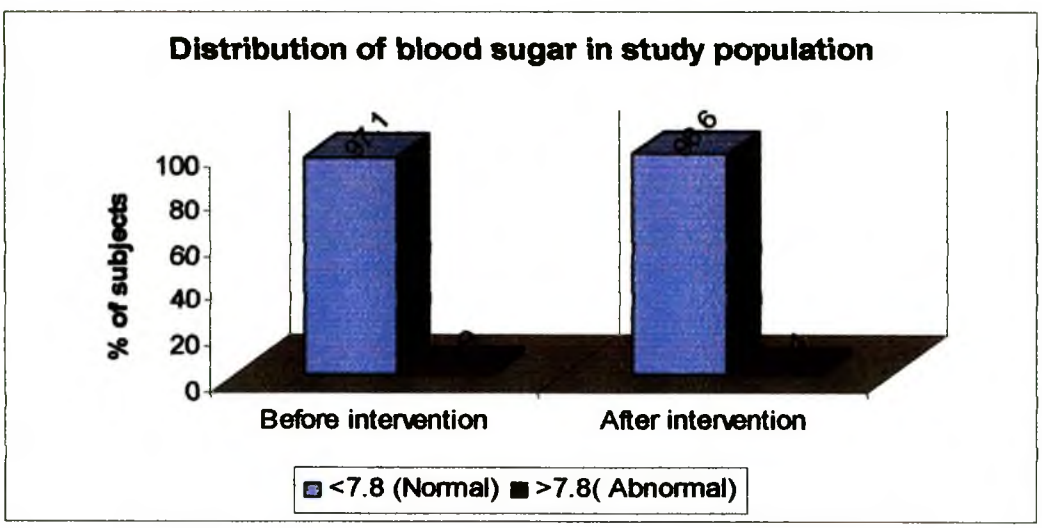
The hair concentrations of total arsenic have a direct positive correlation with urine arsenic concentration. Although hair concentration was little lower than in nails, there was a very strong relationship. This was indicated by a significant $p<0.00$ and correlation coefficient for hair $r=0.150$ .This linear relationship exists throughout the concentration ranges for hair with that of urine concentration (Figure 18).

Table 8 :Distribution of albumin in urine

Batch	Albumin	Selenium		Placebo		Vit ACE		Total	
		N	%	N	%	N	%	N	%
Before intervention	Normal	69	100	72	100	73	98.6	214	99.5
	Abnormal	0	00	0	00	1	1.4	1	0.5
	Total	69	100	72	100	74	100	215	100
After intervention	Normal	69	100	71	98.6	72	97.3	212	98.6
	Abnormal	0	00	1	1.4	2	2.7	3	1.4
	Total	69	100	72	100	74	100	215	100

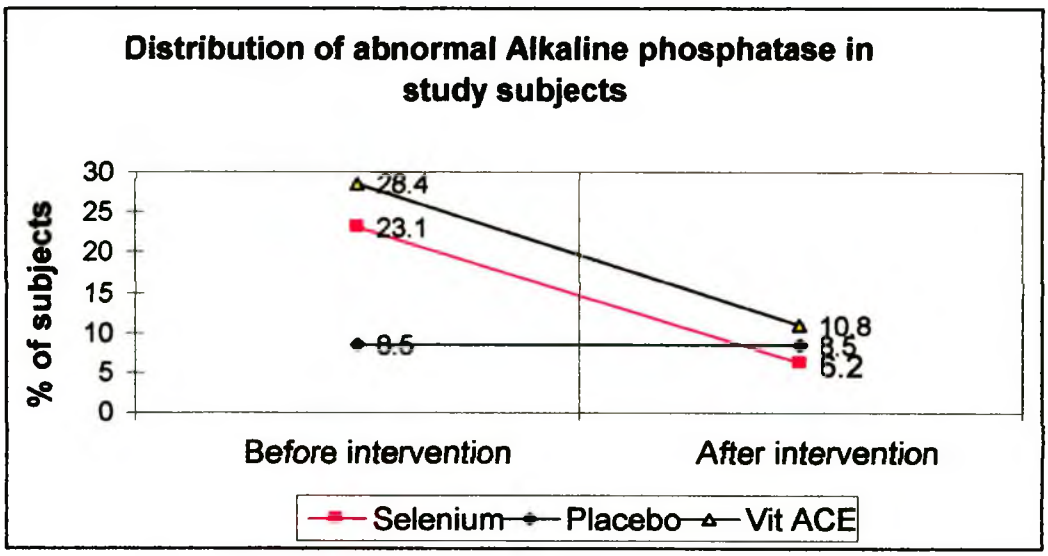
On urine examination, no albumin and cast were found in the study subjects before and after intervention (Table 8).

Figure 19 : Distribution of random blood sugar in the study subjects



There was no significant change found in the blood glucose level of the study population after intervention ($p<0.06$) (Figure 19, Appendix 7. 9)

Figure 20 : Distribution of Liver function test in study subjects



Liver function test showed 17.3% cases had high level of alkaline phosphatase but after intervention , there was considerable improvement found in selenium groups (66% reverses in selenium, 56% in Vitamin ACE group and 9% in placebo group respectively). But found no abnormality in SGPT level before intervention (Figure 20, Appendix 7. 10).

Table 9 : Summary of clinical and biochemical result of the study subjects

Parameters	Group	N	Before intervention	After intervention	Improvement
Melanosis	Selenium	67	55.1%	13.4%	75.7%
	Placebo	72	54.2%	54.2%	0.0%
	Vit. ACE	74	55.4%	16.2%	70.7%
Keratosis	Selenium	67	43.5%	8.7%	80%
	Placebo	72	40.3%	40.3%	0.0%
	Vit. ACE	74	41.9%	32.4%	22.7%
Symptoms	Selenium	67	100%	31.9%	68.1%
	Placebo	72	100%	97.2%	2.8%
	Vit. ACE	74	100%	74.4%	25.6%
Arsenic (As) Concentration in Nail (ppb)	Selenium	55	3.41 ± 2.93	2.14 ± 1.90	31.9%
	Placebo	61	3.58 ± 4.26	3.11 ± 3.16	13.1%
	Vit. ACE	64	3.80 ± 4.71	2.64 ± 2.40	30.5%
Arsenic (As) Concentration in Hair (ppb)	Selenium	60	1.31 ± 1.82	0.81 ± 1.79	38.2%
	Placebo	67	1.11 ± 1.30	0.80 ± 1.39	27.9%
	Vit. ACE	71	1.37 ± 1.42	0.84 ± 0.84	38.6%
Selenium (Se) Concentration in Serum(ppb)	Selenium	67	69.13 ± 24.48	97.49 ± 55.03	↑ 41.0%
	Placebo	72	69.92 ± 38.15	53.25 ± 68.50	↓ 23.8%
	Vit. ACE	74	63.51 ± 28.61	48.31 ± 56.45	↓ 23.9%

The result showed more improvement of arsenic signs and symptoms in selenium treated group than other control . The reduction of arsenic in hair and nail also showed greater improvement in selenium supplemented group , so this indicated that selenium is a choice to combat arsenicosis.

Chapter 4

DISCUSSION

4. Discussion

4.1 Clinical presentation and effect of treatment

In this study , it was found that there were gradual improvement of clinical sign and symptoms including skin changes in study subjects . A significant changes observed in the severity scores compared to their pre-treatment values . The severity of melanosis, leucomelanosis and keratosis were reduced to almost normal skin in many subjects. The severity of melanosis was dropped from 55.1 to 13.4 (76% reduced n=67) in selenium group after intervention and in Vitamin ACE group the severity dropped from 55.4% to 16.2% (reduction was 71% , n=74) but did not changed in Placebo group . The severity of keratosis was dropped from 43.5 % to 8.7% (80 % reduced n=67) in selenium intervention groups and in Vitamin ACE groups it dropped from 41.9 % to 32.4% (23 % reduction , N=74) but no changes in placebo groups. The symptoms like anorexia , weakness, nausea reduced from 100% to 31.9% (68% reduction) in selenium intervention groups , from 100% to 74.4% (26% reduction) in Vitamin ACE group but only 2.8 % reduced in placebo group (Figure 21,22,23,24) (Photograph 1-3). Our free discussions with patients and their own assessments are also consistent with these observations .

Our observation found similar to the study was done by Wang 2001, in China where they administered 100-200 μg selenium /day for 14 months and observed 75 % and 55 % reduction in clinical signs and symptoms in selenium treated group whereas in placebo group it was 25 % and 24.4 % respectively .

The most common presentation of our patients was skin pigmentation, predominantly in the form of cutaneous melanosis (99.5%), Leucomealnosis (97%) and palmo-planter hyperkeratosis (92%). General weakness, anorexia and nausea were present in 100% cases and 03 patients showed non-pitting pedal edema. About 32 (12.3%) cases, respiratory symptoms were the clinical manifestation in absence of definite pneumonia. There were no abnormalities noted in clinical vital sign e.g. body temperature, pulse rate or respiratory rate in all our study subjects .Only 02 cases had hepatomegly and 01 cases with ascitis. But 8(3.1%) cases were found non-healing ulcer in there hand or foot which we have done exicisional biopsy for histopathology. The histopathological findings revealed that 2 (0.8%) cases were Bowen's disease and 6 (2.3%) cases were squamous cell carcinoma in grade-1. The cases were observed closely and found no recurrence of the ulcerative lesions during the intervention period. Intermittent claudication with gangrene was observed in 4 cases (1.5%).

This observation found similar with our earlier study where we had studied 250 cases and found cutaneous manifestations like melanosis and keratosis in 100% cases but leucomelanosis was less. Conjunctivitis and hepatopathy were 6.3% and 2.2% respectively (Shikder, 1999; Momin, 2005). Again Khan 1997, found Melanosis 87.4%, Keratosis 67.7%, Hyperkeratosis 38.7%, Leukomelanosis 35.5% , Bronchitis 10.5%, Conjunctivitis 6.3% andHepatomegaly 2.2% (Khan, 1997).

In contrast , severe systemic involvement of the patients have been reported in West Bengal, India by Guha 1998.The authors studied 156 patients in West Bengal and observed 77% cases of hepatomegaly, 31% splenomegaly,53% lung disease, 1 case of skin cancer and 5 deaths . In Taiwan, only 7.1% showed keratosis and 18.4% had skin pigmentation (Tseng 1968, Tseng, 1977). It seems that the Indians were so severely affected than those in Bangladesh, although the socio-environmental background in these two adjacent but independent states of Bengal are similar. The possible explanation may be that the peoples in West Bengal might be exposed for longer period than those in Bangladesh. The other possibilities could be the difference in arsenic concentration in drinking water, pattern of water use, level of malnutrition and genetic predisposition between these two population groups.

In our study groups, it was found that young adult females were the most victims of arsenicosis. Illiterate subjects with preponderance to lower socio-economic group having large family members were suffered more. Peoples in the area, exposed to high level of arsenic (average 243 µg/L) through drinking contaminated water for more than 4 years were the usual victims of arsenicosis. As all our study subjects were from same homogenous environment, it was found that about 45.45 µg/L of total arsenic were also taken by each subject daily through their food chain (Table 6). So, during this chronic exposure to arsenic, clinical manifestations developed slowly and insidiously, which may take more than 5 to 10 years before the cases is clinically evident.

In this study , we have found no significant changes in the BMI of the study population in all groups . In an earlier study by Rabbani in Hajigonj area, which is adjacent to Chatkhil upazilla, observed that a considerable weight gained occurred in antioxidants treated group . Selenium treated patients were also feeling better after 48 weeks than placebo group . The present study found encouraging result in Arsenicosis after administration of selenium in this blind trial . Selenium administration improves chronic arsenic toxicity by 80% .

But in other intervention group it was also observed that there were considerable relief of clinical symptoms and mild improvement in their skin changes . The possible explanation might be the cessation of arsenic contaminated water has dramatically stop the arsenic load and by gradual excretion through urine , thereby reducing the arsenic –induced oxidative damage and enhancing body's oxidative defense mechanisms .

4.2 Environmental perspective of the study

Exposure to arsenic adversely affects multiple organ systems including liver, spleen, kidneys, heart, intestine, lungs, brain, muscle and thyroid gland .The toxicity of arsenic compounds depend on the amount, the chemical and physical form of arsenic and the duration of exposure to arsenic (Khan, 1997). The symptoms of acute toxicity include vomiting, diarrhea, muscle cramp, facial edema and cardiac abnormalities. But chronic toxicity develops insidiously after a long exposure.

There is no specific antidote yet been discovered. However, some specific antidotes including British Anti Lewisite (BAL), 2, 3-dimercaptosuccinic acid (DMSA), and d-penicillamine have been tried to treat arsenicosis but found ineffective (Guha, 1998). Besides these drug has toxic effects and some are costly .

Access to arsenic free water is thought to be an important step for mitigation of arsenicosis but the goal may be difficult to accomplish for a vast majority of the affected people. Avoidance of arsenic contaminated drinking water and high protein diet may help to improve the physical condition of the patients to some extent but can't prevent the late complications of arsenicosis (Sato Y2000) .Chelating agents, Vitamins, high nutritional diets , spirulina, all these have limitations in different grounds (Bates ,1992, Ahmad 1998, Huq ,2000) . Above all, people with clinical manifestations are not only the sufferer but millions of people who are exposed but clinical features are yet to develop, become easy prey to serious and delayed health effects of arsenicosis. Thousands of people may develop lung cancer, bladder cancer, skin cancer, liver cirrhosis etc if the silent accumulation of arsenic is not prevented (Cuzick 1992, Momin, 2002).

Many researchers found that selenium is a known antioxidant and has an ability to negate the toxic effects of several heavy metals including arsenic. The interaction between arsenic and selenium was reported by Levander in 1977 and concluded that arsenic has a protective effect against the toxicity of a variety of forms of selenium . He also documented the metabolic antagonism between arsenic and selenium as arsenite stimulated the excretion of

selenium in to the bile, so did selinite stimulate the excretion of arsenic (Levander, 1977). Antagonistic interactive effects were also observed between arsenic and selenium including complete to partial alleviation of the selenium toxicity (Hoffman, 1992). Moreover, both arsenic and selenium interact extensively with sulfhydryl (-SH) groups in tissues, it is possible that arsenic elimination is delayed in Se-deficiency state because there could be more target –SH groups for arsenic to react with because selenium is low (Kenyon, 1997) . On the contrary, if sufficient selenium is available to the tissues, the –SH group would not be left free for arsenic to react with and there would be abundance of antioxidant enzymes to counteract the per-oxidative stress by arsenic (Passwater, 2000). A Chinese study (Hu GG, 1989) done among the workers exposed to arsenic showed selenium found antagonize the toxic effect of arsenic.

It may justify to evaluate the therapeutic role of an antioxidant (selenium) to fight back against the curse of the arsenicosis in this country. We believe that a dietary supplement of selenium is feasible as well as inexpensive and may help to turn the heat off of a potentially massive health crisis in an already impoverished place. Thus, we found interest to evaluate the level of selenium of an arsenic endemic area of Bangladesh and accordingly we have designed a randomized, double-blind, placebo controlled

trial with Selenium and a second control antioxidant containing Vitamin ACE in 260 patients to observe the effect of selenium to relief clinical signs and symptoms and as effective remedies.

4.3 Characteristics of study of the patient

According to the user's recall, we have estimated that these subjects have been drinking arsenic contaminated water for 4 to 24 years and the mean was 21.9 ± 12.3 years (Appendix 7.14).

4.4 Effect of treatment on urinary excretion

A urinary concentration of total arsenic is a reliable indicator of arsenic consumption because urine is the primary route of elimination of most absorbed arsenicals (Boucher, 1983, Vahter, 1994). In this study , all the subjects were excreting arsenic in urine at an average concentration of 95 µg/L ,at the same time they were consuming inorganic arsenic at a average concentration of 243 µg/L through the drinking water and 45 µg/L their food daily . Amount of arsenic excreted in urine is likely to be related to that consumed in drinking water. After 4 months of treatment with selenium and antioxidants with consumption of arsenic free water, urinary concentration of total arsenic dropped to 50% of the pretreatment value in all groups. This drop was likely to be related to sudden

cessation of arsenic consumption accomplished by taking boiled or filtered pond water. The drop was unlikely to be related to drug action since there was no significant difference among the intervention group. That urinary reduction was reflecting the current consumption level that indicated by the observations in volunteers that 40%-60% of daily intake of inorganic arsenic were excreted each day in urine (Buchet, 1980, Farmer, 1990). This data are well fit to our observed arsenic concentrations in drinking water and urine of patients. Interestingly , however, during the next 4 months (i.e. end of 8 months), the urinary excretion of arsenic started increasing in all groups, but in the selenium treated group the excretion remains in lower trend. The initial lag period (4 months) before we observed any increase in urinary arsenic excretion probably reflects the time taken for mobilization and biotransformation of arsenic compounds into methylated forms for urinary excretion. However, we are not sure at what point in time, the urinary excretion actually began to increase, since arsenic concentrations were determined only at 4 months intervals. At 8 and 12 months after starting treatment, selenium treated patients were excreting half the amount of arsenic than that excreted by them before. However, the peak excretion levels did not reach a constant level or a plateau at the end of 12 months period; this indicates that treatment time might have to be prolonged to reach a maximum excretory threshold level. Since the total body

content of arsenic was not known in the subjects, it was difficult to establish a quantitative relationship among urinary excretion, total body load, rate of excretion, and time required for total elimination. The question may be raised whether patients would benefit from selenium or antioxidants treatment if they do not have an access to a source of arsenic-free water .Unfortunately any helpful clues can be provided from our observations since we did not study a group of subjects, obviously for ethical reasons, which were not given arsenic free water. Nevertheless, we believed that the beneficial effects of selenium in patients who continue to consume contaminated water would be less than those taking drugs and arsenic free water concurrently. The net effect, however, will depend on a balance between arsenic consumption and excretion; if the excretory effects of selenium are offset by substantial intake, arsenic will accumulate in the body. More study would require to conclude the excretory effects of selenium in arsenicosis .

4.5 Reduction of arsenic content in scalp hair

In this study , we have used arsenic contents in hair and nail as a biomarker of arsenic exposure . Although ,hair arsenic content is considered as a reliable indicator of exposure ,its value as an index of quantitative change may be limited .This is because , hair can accumulate arsenic from both endogenous and exogenous

sources . Arsenic concentrations at the root are in equilibrium with the concentrations in blood, the segmental distribution varies along the same strand of hair, being increasingly low at the terminal end. Hair is known to accumulate arsenic by adsorption due to direct contact with a variety of external sources including arsenic-rich water, air, soaps and shampoos. This has been shown in a study in Fairbanks, Alaska, where arsenic content of drinking water was 345 µg/L; people who drank essentially bottled water had low concentrations in urine (43 µg/L) but high concentrations in hair (5.7 µg/L) (Harrington ,1978) . Hair is considered an excretory pathway and once inorganic arsenicals are incorporated into sulfhydryl groups in hair keratin, these are not available for metabolic degradation .Thus it appears that arsenicals once incorporated into hair, can't be removed by any means other than destruction or cutting of the hair strand.

In our patients, who had been drinking average concentration of 243 µg/L of arsenic, the mean arsenic concentration in scalp hair as determined by HG-AAS was 0.82 µg/kg .This value is consistent with many other reports of hair arsenic concentrations found in different population with different level of exposure. For example Das, 1995, reported that hair arsenic concentrations varied from 3 to 10 µg/Kg in people of West Bengal of India exposed to similar

concentrations in drinking water . In California and Nevada , a concentration of arsenic 400 µg/L in drinking water corresponded to about 1.2 µg/L in hair and 100 µg/L in water to 0.5 mg/L in hair (Valentine, 1979). In Hajigonj, Rabbani, 2003, showed concentration of arsenic 619 mg/L in water corresponded to 2.8 µg/kg.

Some of the limitations of using hair arsenic as an indicator of changes in exposure levels may not be applicable to our study patients . Because hair deposition of arsenicals from external sources other than tube well water is highly unlikely in this population .These people in general are poor farm workers and rarely use soap or shampoos for their hair care. On the other hand , contamination of from atmospheric air is not possible because arsenicals in air is usually found in industrial neighborhood and mining areas, none of these conditions exist in and around our study villages .The only source of exposure to arsenic in our study populations is through drinking of contaminated underground water. Moreover, our study area is far away from seacoast, and these populations, by tradition, neither had an access to nor a had a habit of eating seafood. We believe that all their arsenic in the body (urine, nail and hair) had been coming exclusively from the contaminated drinking water .However, these people did use contaminated tube well water for washing and bathing which might have contributed, at

least in part, some arsenic deposition in their scalp hair. Nevertheless, arsenic contents in hair attributed to external sources would likely to be distributed among patients in all treatment groups similarly and thus would not confound the outcome variable.

In our study ,the effects of selenium in reducing arsenic contents in scalp hair should be reviewed in consideration of the above mentioned observations .We have found that selenium intervention significantly reduced arsenic concentration in scalp hair . The trend of decline continued more sharply in selenium and Vitamin ACE group than placebo group. The reduction in placebo treated groups is likely to be related to the introduction of arsenic – free water. The magnitude of reduction of hair arsenic in patients treated with arsenic free water alone was in the order 10-20% or less over a period of 12 months, this is significantly less than the reduction in patients given arsenic free water plus selenium and Vitamin ACE treatment. In contrast, Reduction of arsenic load in hair after intervention with selenium 200 microgram /day for 14 months in Mongolia found in Selenium treated group 73% and in Placebo group 52% (Yang, 2002). In another study Xia, 2000 in Inner Mongolia showed arsenic load was decreased, 54% from hair after 14 months oral intervention with organic selenium at dose of 200 mg/day (Xia, 2000). Thus it seems that access to safe water alone,

although useful in reducing arsenic load from the body , its quantative effect is smaller compared to selenium treatment .

So our observations support the observations by Tseng, 1968 in Taiwan, Guha, 1968, in India and Yang 2002 in Mongolia, Rabbani, 2003 in Bangladesh that supply of arsenic –free water alone is not sufficient enough to detoxify individuals exposed to arsenic for prolonged period.

4.6 Reduction of nail arsenic concentration

Since nail tissue is rich in sulphydryl (-SH) groups, there is a preferential uptake of inorganic arsenic by finger nails . However, the role of nail arsenic as an indicator of exposure to arsenic poisoning has not been well studied and data on nail arsenic are limited. In individuals, arsenicals binding with nail tissues seems to be fairly stable over a certain period of time since it has been shown that a single administration ,arsenic can be detected at the nail tip after 100 days (Pound ,1979,Pirl, 1983).

In our study, patients consuming arsenic in drinking water at an average concentration of 243 µg/L, the average concentrations were 3.59 µg/kg in nail of all groups before starting intervention. After treatment with selenium or antioxidants with

arsenic free water , nail arsenic concentration significantly reduced ($p<0.00$) in selenium and in Vitamin ACE ($p<0.02$) compared to patients with placebo treated group .The decline trend continued at 12 months, the concentration was significantly lower in selenium treated group . Reduction of nail arsenic concentration appears to be related to increased urinary excretion in absence of concurrent arsenic consumption due to drinking of safe water. It is likely that arsenic is deposited in nail root from the blood circulation and advances distally as the nail grows, usually at a rate of 0.12 mm per day. However, some of the nail arsenic concentrations may be attributed to external contact with arsenic contaminated water during washing, cooking, bathing and other household activities. Therefore the marginal reduction observed in the placebo treated patients given safe water only, may be offset by external deposition from contaminated water. In a study of 17 patients exposed to drinking water arsenic (500 µg/L in West Bengal, the nail arsenic concentration were 10-12 µg /kg. An intervention with arsenic free water for 2 years resulted in only marginal decrease in nail and hair arsenic concentrations which persistently fluctuated because of non-water sources of arsenic consumptions in those populations (Mondal, 1998).

The underlying mechanism of arsenic loss from the nail and hair tissues observed in this study may be due to selenium with its antioxidants effects and arsenic free water. Cessation of arsenic ingestion through drinking water may have reduced blood –borne deposition of arsenic at the hair and nail roots; at the same time, increased elimination of arsenic through urine is likely to reduce the metabolic pool in the body. In addition to periodic cutting of hair and nail from the tip provided an excretory pathway for arsenic removal from the body .Therefore, it is likely that continuous loss of arsenic through the nail and hair tissues together with reduction of blood-borne deposition might have resulted in reduced arsenic concentrations in hair and nails in our patients .

4.7 Co-relation among the hair, nail, urine and water arsenic level

Kinetic studies examining the interrelationship among arsenic exposure, metabolism, retention and excretion are limited and quantative aspects of exposure and its risk to human health are not completely known .In our study ,patients had been drinking arsenic in the contaminated water at a average concentration of 243 µg/L for more than 4 years before they were enrolled in the study .We observed good correlations between the amounts of arsenic exposure level and the concentration of arsenic in scalp hair ,

nail and urine . This relationship was shown in figure 31, 33 and 36. We found that the arsenic concentration in scalp hair, nail and urine were interrelated (Figure 34, 37). Both hair and nail concentration of total arsenic have a direct positive correlation with urine arsenic concentration. Although hair concentration was little lower than in nails, there was a very strong relationship. This was indicated by a significant ($p<0.00$) higher correlation coefficient for both hair ($r=0.150$) and nail ($r= 0.402$). This linear relationship exists throughout the concentration ranges for both hair and nail with that of urine concentration. These values are consistent with those observed in patients of Hajigonj by Rabbani 2003, in Bangladesh. In contrast, West Bengal, India, population exposed to drinking water containing 200-700 µg/L arsenic deposited in the hair 3.6-9.6 and nail 6.1-23.0 (Das 1996) .

In our patients, exposure to average 243µg/L of arsenic in drinking water resulted in mean 95.2 µg/L of total arsenic excreted in urine which consistent with the population exposed to 619 µg/L of arsenic in drinking water resulted in 110-115 µg/L of total arsenic in urine in Hajigonj, Bangladesh (Rabbani,2003). A water arsenic concentration of 400 µg/L corresponded to about 230 µg/L in urine and 100 µg/L in water to 75 µg/L in urine among different population groups in California and Nevada, USA (Valentine ,1979).

Alaskan population exposed to 400 µg/L of arsenic in drinking water had an average 180 µg/L in urine and those exposed to 50-100 µg/L had about 45 µg/L (Harrington, 1978). In Taiwan, where water contains 50- 300 µg/L arsenic and average urine concentration 140 µg/L was reported (Chiou, 1997). In comparison to all these observations, our observed value in urine excretion (95 µg/L) was lower relative to that consumed in drinking water 243 µg/L. The possible explanation of this lower urinary excretion (and increased body retention) of total arsenic might be due to low rates of methylation of inorganic arsenic in this population which could result from a variety of factors including nutritional deficiency, genetic polymorphism and environmental factors . Our study populations were malnourished and had lower than normal body mass index (BMI); the average BMI of our patients was 20 whereas the normal value is considered as 25. Poor nutritional status of our study population and relatively higher concentration of arsenic in drinking water may also contribute to inadequate methylation of inorganic arsenic in this population. It had been shown that clinical severity and poor methylation capacity of inorganic arsenic were common findings among population groups with poor nutritional status such as Taiwan, Chile, India and Bangladesh (Borgono, 1977, Tseng, 1977, Hsueh, 1995 Guha, 1998, Rabbani, 2003).

4.8 Clinical relevance of antioxidants to the antitoxic agent

Our study indicates that minerals like selenium is useful in reducing clinical severity and toxicity in patients with chronic arsenic poisoning in controlled doses without any serious side effects . Rabbani ,2003 shown in Hajigonj that antioxidants containing Vitamin A,C,E, selenium and zinc have significant effect in excreting arsenic through hair, nail and urine . There were much improvement in severity of keratosis and melanosis . It has been shown by Khan ,2001 that spirulina –a natural microalgae , is clinically useful in the treatment of arsenicosis .The active ingredients in Spirulina are believed to be its high contents of beta carotene, protein (65%) and mineral like selenium which possess antioxidant properties .Another uncontrolled study showed that treatment with vitamin ACE induced significant clinical improvement in a group of 43 Bangladeshi patients with arsenicosis (Ahmed ,1998). However, these results must be considered in relation to other clinical studies that have examined the effects of potential therapeutic agents in patients with chronic arsenic poisoning . Guha 1998, evaluated the effects of a chelating agent DMSA for 2-3 weeks, in the treatment of 21 adults with arsenicosis in India but did not find it useful for reducing clinical severity or increasing elimination of arsenic from the body . Whether ,it might have been useful to treat for longer period than 2-3 weeks given in that study and whether that would

have been justified clinically remain to be investigsted . Although, DMSA was reported to be useful in the treatment of acute and chronic arsenic poisoning in experimental animals (Kreppel, 1993, Flora, 1995). Earlier two other drugs , DMPS (DL-2,3-dimercaptopropane sulfone) and BAL (British anti-Lewisite) have been used as specific antidotes for arsenical poisoning, but later introduction of DMSA, a better tolerated agent , discouraged the use of DMPS and BAL. (Peters,1945, Aposhian,1995, Angel,1995) . Another drug, d-penicillamine, has been used in a small number of patients for a short course (2 weeks). The drug showed some clinical improvement but the drug is costly and has serious side effects like leucopenia, thrombocytopenia, hemolytic anemia and stevens-johnsons syndrome (Guha, 1996).

4.9 Effeots of arsenic free water alone on detoxification

We expected that cessation of arsenic contaminated water alone would reduce the severity in clinical sign and symptoms of arsenicosis in our patients. However we observed that patients in the placebo groups who were given arsenic free pond water alone reduced their total arsenic concentration in urine by 50% after the first 4 months of treatment. The same magnitude of reduction was also observed among patients given arsenic free surface water along with selenium or Vitamin ACE treatment. But after 8 months , there

were no further significant changes in urinary excretion of arsenic in patients given arsenic free water alone .However, in selenium and Vitamin ACE group ,urinary excretion were significantly increased in 8 and 12 months .Our study is supported by the study done by Rabbani, 2003 in Hajigonj in Bangladesh .

Our study do not support the belief , that introduction of arsenic free water alone would spontaneously detoxify patients within a reasonable period of time .There is little evidence in the literature in support of spontaneous cure hypothesis with arsenic free water . Risk assessment studies indicated that vast population affected in major arsenic polluted areas in the world including Taiwan, Chile, Argentina, Mongolia, India and Mexico continued to manifest clinical symptoms and develop cancers despite introduction of safe water (NRC 2000). Similar observations were reported by Guha, 1998, in India who assessed the effects of safe water containing less than 10 μg/L of arsenic was provided in 24 patients of arsenicosis for 2 years. The author reported that many of the clinical symptoms persist for long duration in spite of stopping ingestion of arsenic through drinking water and new symptoms appeared in some patients.

The study findings suggest that prolonged exposure to toxic levels of arsenic may induce changes in the body that are reversible by preventing further exposure to arsenic and supplementation with selenium with a therapeutic safe dose.

Chapter5

CONCLUSION

5 . Conclusion

The subjects of this study were drawn from an arsenic hyper endemic rural area of Noakhali district of Bangladesh and were divided into three groups called the selenium, placebo and the second control Vitamin ACE group. The mean age of the study population was 36.9±11.4 years. The females (64%) dominated over the males (36%). The literacy rate was poor, only thirty one percent were literate .The majority of the subjects have monthly income of about Taka 2782±3340. They were drinking water contaminated by arsenic with a concentration of average 243 microgram/liter of water for more than 4 years. The duration of drinking tube well water was about 21.9±12.3 years and majority was cooked food with pond water for more than 26±10.9 years. They were also consuming on an average 45.45 microgram of inorganic arsenic in 24 hours of their food. The study subjects showed the mean BMI was 20.3±3.5 kg/m^2 and a little improvement was noted after the trial.

The majority of the study population showed melanosis (99.6%), keratosis (92%), leukomelanosis (97%), skin cancer (3.1%) and gangrene (1.3%) but less systemic organopathy .Only 6.6 % showed hepatopathy, 12% cough and 3% pedal edema but there was 100% anorexia, nausea and weakness which improved about 68% in

selenium treated group .There was no substantial organopathy found in kidney function test, liver function test and random blood glucose in study population.

Our data showed that average arsenic concentration in nail was 3.59 microgram / kg and in hair was 1.26 microgram/kg which become significantly reduced in selenium treated group. Our data showed that peoples in our country excrete less arsenic in urine and retain more in the body .This is because of poor methylation capacity, may due to associated malnutrition in this population .Methylation of arsenical compounds in the body depends on availability of adequate methionine, an amino acid that must be provided through dietary intake . Our study subjects were marginally malnourished and likely to be associated with methionine deficiency which reduced their ability to arsenic methylation and excretion through urine. The urinary excretion of arsenic found lower threshold in selenium treated group .The serum selenium concentration was found about 69 microgram/liter in study subjects but after intervention the concentration raised up to 98 microgram/liter only in selenium treated group.

Thus, the present study found encouraging result with significant beneficial effect of selenium supplementation for 12

months in reducing the accumulation of arsenic after chronic exposure. Selenium treated arsenicosis patients showed rapid improvement of their skin clinical signs and symptoms, improves chronic arsenic toxicity by 80 %. There was no toxicity observed in study subjects. So, combination of 100 microgram of selenium as selenomethionine and arsenic free water per day is safe, effective and cheap remedy for arsenicosis.

5.1 Limitations

1. Food sources of arsenic of the study area were also high because, this area may have higher concentration of arsenic in shallow tube well water for a long time.
2. We could not ensure arsenic free water strictly
3. The substantial number of the subjects were frequently migrated out and in from and to the study area for economic reason.
4. Many of the sample population stated that they liked shallow tube-well water, while they went out of the study area.
5. For ethical reason, we did not refrain from advising arsenic free water to the placebo group.
6. Cognitive behavioral therapy is advocated but could not be done by clinical Psychologist.
7. We could not perform the speciation of arsenic in urine and selenium in blood due to lack of fund.
8. We could not assess the glutathione peroxidase activity in blood due to lack of fund.

Chapter 6

SUMMARY

6. Summary

6.1 Aim and Objectives

It is supported by literatures that Selenium has an effective positive role in the remedy of chronic arsenicosis. But evaluation of the therapeutic role of selenium in our country to fight back the curse of the arsenicosis of the millions sufferings from arsenicosis and its complications, are yet to be worked out. Our primary objectives are (1) to assess whether selenium supplement in the form of tablet significantly decrease the arsenic body load, particularly from hair, nail and urine and (2) to assess whether selenium supplement reverse the progression of clinical signs of arsenicosis, particularly a) melanosis and b) palmo-plantar keratosis without significant side effects. .

6.2 Methodology

A prospective randomized double blind phase 3 intervention trial was carried out during the period from November 2004 to November 2006. The study was approved by the ethical review board of the Bangladesh Medical Research Council (BMRC).

Arsenicosis patients were identified clinically from 11 villages of Shahpur Union, under Chatkhil Upazilla of Noakhali District

(about 170 km south west from Dhaka). Two hundred and sixty patients not receiving any drug were selected randomly from the sampling area. Suspected arsenicosis patients were identified by clinical examination and confirmed by urine tests.

After randomization, the attending patients were grouped in three groups, 87 patients were included in group 'A' and 87 patients in group 'B' and another 86 patients in group 'C' .Treatment of 'A', 'B' or 'C' were blindly coded and the code was kept confidential with Pharmacist .At the end of the trial the code was decoded and found 'A' for selenium alone, 'B' for placebo and 'C' for second control Vitamin ACE respectively. Neither the investigator nor the patients knew the intervention groups. The specific treatment was provided to the patients, supervision was strictly maintained at home level of patient, by the trained field workers. None of the patients were allowed to drink arsenic contaminated water throughout the study period.

Urine, and blood samples were collected at the beginning and at the end of 4, 8 and 12th months. Only hair and nail samples were collected at the beginning and at the end of study period. Sample of their drinking water was also collected at the beginning and at the end of first month. All the samples were transported to Dhaka within

next 24 hours for laboratory analysis in frozen containers and stored at -20° C until analysis was carried out.

The overall supervision both in clinic and in field was maintained by principal investigator, over 12 months follow-up. The assessment of the effectiveness of the intervention was assessed clinically and by biochemical examination.

6.3 Result

Out of 260 patients 215 (82.7%) completed follow-up throughout the whole period of the study. Among the study subjects, majority (60%) were young adult with an age range between 30 and 49 years ($p<0.83$). Female were 64% and the rest were male ($p<0.63$). About ninety one percent suffered from the disease for more than 04 years ($p <0.81$). Out of the arsenicosis subjects 99.6% had melanosis, 97 % leucomelanosis and 92 % palmo-planter hyperkeratosis. Non-healing indolent ulcer was present in 3.1% cases, histopathologically which revealed 2(1.5%) Bowen's disease and squamous cell carcinoma 6(8.5%) respectively. But after intervention, severity of the melanosis and keratosis decreased significantly in selenium group compared to other two groups.

The severity of melanosis was dropped from 55.1 to 13.4 with a

reduction of 76% in selenium treated group. The severity of keratosis was dropped from 43.5 % to 8.7%; the improvement rate was 80% in selenium intervention group. In Vitamin ACE group it dropped from 41.9 % to 32.4% (23% reduction), but no change was observed in placebo group. There were significant reduction of associated symptoms in selenium intervention group ($p<0.00$) .Symptoms reduced from 100% to 31.9% (68% reduction) in selenium intervention group , from 100% to 74.4% (26% reduction) in Vitamin ACE group but only 2.8 % reduced in placebo group .

On chemical analysis, it was revealed that significant changes observed in concentration of arsenic in hair of selenium treated group after intervention ($p<0.02$). The Arsenic load dropped from 43.3 % to 23.3 % (46% improved, n=60) after intervention in selenium treated group, whereas 23% improved in Vitamin ACE group ($p<0.12$) and 45% improved in placebo ($p<0.01$) group respectively. There were also considerable reduction in concentration of arsenic load in the nail of selenium group after intervention ($p<0.12$). Seventy one percent subjects of selenium treated group showed high concentration of arsenic in their nail before intervention but after intervention it become to 58.2% with 18% improvement. There were significant reduction of excretion in urinary concentration of arsenic in all intervention group ($p<0.00$)

but trend of concentration gradually lowered in selenium treated group. It was observed that there was low concentration of selenium in our study population. The concentration rises to 41% in selenium treated groups after intervention.

6.4 Conclusion

There is significant beneficial effect of supplementation of selenium for 12 months in reducing the accumulation of arsenic after chronic exposure. Selenium treated arsenicosis patients showed rapid and sustained improvement of their clinical signs and symptoms within 4 months of intervention. There were no toxicity in liver and kidney, observed in the subjects. So, a dose of 100 microgram of selenium as selenomethionine per day in combination with arsenic free water is safe, effective and cheap remedy for arsenicosis patients of Bangladesh.

Chapter 7

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Chapter 8

APPENDIX

8 . Appendix

Appendix 8.1: Table shows distribution of skin changes in study subjects before intervention

Skin Signs	Selenium				Placebo				Vit ACE				Total	
	Male		Female		Male		Female		Male		Female			
	N	%	N	%	N	%	N	%	N	%	N	%	N	%
Melanosis	28	100	59	100	34	100	52	98.1	31	100	55	100	259	99.6
Leucomelanosis	26	92.9	56	94.9	34	100	51	96.2	29	93.5	53	96.4	252	97.0
Keratosis	23	82.1	54	91.5	33	97.1	50	94.3	29	93.5	50	90.9	239	92.0
Crack & Fissure on foot	3	10.7	11	18.6	1	2.9	5	9.4	3	9.7	5	9.1	28	11.0
Gangrene and haemorrhage	1	3.6	1	1.7	0	0.0	0	00	2	6.5	0	0.0	4	1.5
Non-healing ulcer eg. Bowen's disease etc	2	7.1	0	00	1	2.9	1	1.9	1	3.2	3	5.5	8	3.1
Group Total	28	32.2	59	67.8	34	39.1	53	60.9	31	36.0	55	64.0	260	100

Appendix 8.2 :shows distribution of skin changes before and after intervention

Skin Sign	Selenium				Placebo				Vit ACE			
	Before		After		Before		After		Before		After	
	N	%	N	%	N	%	N	%	N	%	N	%
Grade 1 (Mild)												
Diffuse melanosis / Suspecious spotty pigmentation in trunk/limb	9	13.4	30	43.5	14	19.4	14	19.4	15	20.3	35	47.3
Mild thickening of palm/soles	12	17.4	34	49.3	15	20.8	14	19.4	15	20.3	18	24.3
Suspecious spotty depigmentation in trunk/limb	23	33.3	22	31.9	26	36.1	27	37.5	32	43.2	32	43.2
Grade 2 (moderate)												
Definite spotty pigmentation bilateral	21	30.4	25	36.2	19	26.4	16	22.2	18	24.3	18	24.3
Definite spotty depigmentation, bilateral	32	46.4	23	33.3	28	38.9	35	48.6	28	37.8	27	36.5
Severe diffuse thickening of palm/soles	19	27.5	26	37.7	23	31.9	23	31.9	22	29.7	24	32.4
Grade 111 (Severe)												
Definite spotty pigmentationon trunk&limb,bilateral	38	55.1	9	13.4	39	54.2	39	54.2	41	55.4	12	16.2
Definite spotty depigmentation over trunk& limb,bilateral	12	17.4	21	30.4	12	16.7	15	20.8	13	17.6	8	10.8
Mucosal Pigmentation on tounge or mucosa	0	00	0	00	0	00	0	00	2	2.7	0	00
Large nodules over thickened palm/soles or keratotic horn on palm & soles	30	43.5	6	8.7	29	40.3	29	40.3	31	41.9	24	32.4
Diffuse verrucous lesions with Crack and fissure on soles	11	15.9	7	10.1	5	6.9	5	6.9	4	5.4	3	4.1
Non-healing ulcer e.g Bowens disease etc	2	2.9	0	00	2	2.9	0	00	4	5.4	0	00
Gangrene of palm or sole	2	2.9	2	2.9	0	00	0	00	2	2.7	2	2.7
Group Total	69	100	69	100	72	100	72	100	74	100	74	100

Appendix 8.3 : Table shows Check list of Clinical Variables in study subjects before and after intervention

Clinical variables	Before intervention			After intervention		
	Selenium N (%)	Placebo N (%)	Vit ACE N (%)	Selenium N (%)	Placebo N (%)	Vit ACE N (%)
Anorexia	87(100)	87(100)	86(100)	22(31.9)	70(97.2)	68(91.9)
Nausea	87(100)	87(100)	86(100)	20(28.9)	70(97.2)	64(86.5)
Weakness	87(100)	87(100)	86(100)	20(28.9)	72(100)	48(64.9)
Dizziness	8(9.2)	11(12.6)	6(6.9)	1(1.4)	10(13.9)	1(1.4)
Vomitting	3(3.4)	5(5.7)	4(4.7)	0	3(4.2)	0
Abdominal Pain	6(6.9)	8(9.2)	8(9.3)	1(1.4)	7(9.7)	2(2.7)
Chest pain	1(1.1)	2(2.3)	1(1.1)	0	2(2.8)	1(1.4)
Cough	6(6.9)	14(16.9)	12(13.9)	6(8.7)	14(19.4)	6(8.1)
Paedal oedema	1(1.1)	3(3.4)	0	0	3(4.2)	0
Ascitis	0	0	1(1.1)	0	0	1(1.4)
Hepatomegaly	0	1(1.1)	1(1.1)	0	1(1.4)	0
Conjunctivities	1(1.1)	0	0	0	0	0
Tingling & numbness	1(1.1)	0	2(2.3)	0	1(1.4)	1(1.4)
Intermittent claudication	2(2.3)	13(14.9)	2(2.3)	1(1.4)	13	1(1.4)
Gangrene	1(1.1)	2(2.3)	1(1.1)	1(1.4)	2(2.8)	1(1.4)
ECG	1(1.1)	0	0	0	0	0
Blood pressure >100/90 Hg	10(11.5)	12(13.8)	10(11.6)	10(14.5)	9(12.5)	9(12.2)
Group Total	87 (100)	87 (100)	86 (100)	69 (100)	72 (100)	74 (100)

Figures in parentheses are percentile

Appendix 8.4 : Table shows Check list of Clinical Variables in study subjects during intervention

Systemic Check list

Variables	Beginning of study	1st follow-up visit	2st follow-up visit	final follow-up visit
Weakness				
Anorexia				
Nausea				
Vomiting				
Dizziness				
Diarrhoea				
Abdominal pain				
Chest pain				
Cough				
Conjunctivitis				
Hepatomegaly				
Cirrhosis				
Pedal oedema				
Tingling and numbness				
Intermittent claudication				
Blood pressure				

Skin Check list

Variables	Beginning of study	1st follow-up visit	2st follow-up visit	final follow-up visit
Grade 1				
Melanosis				
Leucomelanosis				
Kertatosis				
Grade 11				
Melanosis				
Leucomelanosis				
Kertatosis				
Grade 111				
Melanosis				
Leucomelanosis				
Kertatosis				
Crack and fissure				
Non healing ulcer				

Appendix 8.5 :Table Shows distribution of duration of ailment of disease by sex in study population

Study groups	Duration of ailment of the disease								Total	
	1-3 years		4-6 years		7-9 years		10-12 Years			
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
	N %	N %	N %	N %	N %	N %	N %	N %	N %	N %
Selenium	1(3.6)	9 (15.3)	8(28.6)	12(20.3)	8(28.6)	18(30.5)	11(39.3)	20(33.9)	28(100)	59(100)
Placebo	4(11.8)	2 (3.8)	7(20.6)	13(24.5)	15(44.1)	18(34.0)	8 (23.5)	20(37.7)	34(100)	53(100)
Vit ACE	1(3.2)	7 (12.7)	9(29.0)	11(20.0)	10(32.3)	24(43.6)	1(35.5)	13(23.6)	31(100)	55(100)
Total	6(6.5)	18(10.8)	24(25.8	36(21.6)	33(35.5)	60(35.9)	30(32.3)	53(31.7)	93(100)	167(100)

Figures in parentheses are percentile

Appendix 8.6 : Table shows distribution of Arsenic in urine in study population before and after intervention

	Specimen	Selenium	Placebo	Vit ACE
	Batch	Mean±SD (N = 56)	Mean±SD (N = 58)	Mean±SD (N = 61)
Arsenic(As) conc. in urine	Beforeintervention(1st)	70.0±114.5	93.2±147.6	130.3±193.3
	Duringintervention(2nd)	23.8±63.5	34.2±48.9	37.1±73.1
	During intervention(3rd)	37.6±80.1	48.8±73.9	38.2±63.0
	After intervention(4th)	30.1±76.9	35.8±73.8	45.2±88.3

Appendix 8.7 :Table shows distribution of Selenium in serum of study subjects before and after intervention

Specimen		Selenium	Placebo	Vit ACE
	Batch	Mean±SD (N=55)	Mean±SD (N=64)	Mean±SD (N =67)
Selenium (Se) concentration in Serum	Beforeintervention(1st)	68.5±24.6	67.4±30.5	65.1±28.7
	Duringintervention(2nd)	143.5±63.3	92.1±141.6	75.3±34.8
	Duringintervention(3rd)	96.4±54.2	47.8±37.3	49.3±58.4
	After intervention(4th)	113±75.9	49.1±39.7	49.4±34.7

Appendix 8.8 : Table shows distribution of Arsenic in nail,hair and water before and after intervention

Specimen		Selenium		Placebo		Vit ACE	
	Batch	Mean±SD	N	Mean±SD	N	Mean±SD	N
Nail for As	Beforeintervention	3.9±3.4	78	3.6±4.3	78	3.8±4.6	77
	After intervention	2.7±3.7	61	3.1±3.2	69	3.0±2.9	71
Hair for As	Beforeintervention	1.3±1.7	83	1.1±1.2	84	1.3±1.4	85
	After intervention	0.8±1.7	64	0.8±1.4	70	0.8±0.8	72
Water for As	Beforeintervention	47.4±66.0	87	30.9±52.0	87	37.8±54.0	86
	After intervention	4.2±9.3	87	2.4±7.3	87	3.4±8.4	86

Appendix 8.9 :Table Shows Distribution of random blood sugar in the study subjects

	Sugar	Selenium N		Placebo N		Vit ACE N	%	Total N	%
Before intervention	<7.8 (Normal)	64	98.5	69	97.2	71	95.9	204	97.1
	>7.8(Abnormal)	1	1.5	2	2.8	3	4.1	6	2.9
	Total	65	100	71	100	74	100	259	100
After intervention	<7.8 (Normal)	65	100	71	100	71	95.9	207	98.6
	>7.8(Abnormal)	0	00	0	00	3	4.1	3	1.4
	Total	65	100	71	100	74	100	210	100

Appendix 8. 10 : Table shows Distribution of Liver function test in study subjects

Study groups	Serum Glutamic pyruvate Trnsaminase				Serum Alkaline Phosphatase			
	Before intervention		After Intervention		Before intervention		After intervention	
	<45 iu Normal N (%)	>45 iu Abnorma N (%)	<45 iu Normal N (%)	>45 iu Abnom N (%)	<92 iu Normal N (%)	> 92 iu Abnorm N (%)	<92 iu Normal N (%)	> 92 iu Abnorml N (%)
Selenium	65 (100)	00 (0.0)	65 (100)	00 (0.0)	50(79.9)	15 (23.1)	61(93.8)	4 (6.2)
Placebo	71 (100)	00 (0.0)	71 (100)	0 (00)	65(91.5)	6 (8.5)	65 (91.5)	6 (8.5)
Vit ACE	74 (100)	00 (0.0)	73(98.6)	1 (1.4)	53(71.6)	21 (28.4)	66(89.2)	8 (10.8)
Total	210(100	00 (0.0)	209(995	1 (0.5)	168(80.0	42 (20.0)	192(91.4	18 (8.6)

Figures in parentheses are percentile

Appendix 8.11 : Table shows Cross tabulation of normal and abnormal arsenic level in hair

Intervention group	Status	Before intervention N	%	After intervention N	%	P - value
Selenium	Normal (<0.8µg/L)	34	56.7	46	76.7	0.02
	Abnormal(>0.8µg/L)	26	43.3	14	23.3	
Placebo	Normal (<0.8µg/L)	34	50.7	49	73.1	0.01
	Abnormal(>0.8µg/L)	33	49.3	18	26.9	
Vit ACE	Normal (<0.8µg/L)	36	50.7	44	62.0	0.12
	Abnormal(>0.8µg/L)	35	49.3	27	38.0	

Appendix 8.12 : Table shows Cross tabulation of normal and abnormal arsenic level in Nail

Intervention group	Status	Before intervention N	%	After intervention N	%	P - value
Selenium	Normal (<1.3 µg/L)	16	29.1	23	41.8	0.12
	Abnormal(>1.3µg/L)	39	70.9	32	58.2	
Placebo	Normal (<1.3 µg/L)	18	29.5	18	29.5	0.56
	Abnormal(>1.3µg/L)	43	70.5	43	70.5	
Vit ACE	Normal (<1.3 µg/L)	23	35.9	20	31.3	0.35
	Abnormal(>1.3µg/L)	41	64.1	44	68.8	

Appendix 8.13 : Table showed Distribution of Monthly Income among the study subjects by family size

Family size	Group	Household monthly income (Taka)							
		< 1500		1500-2999		3000 +		Total	
		N	%	N	%	N	%	N	%
1 - 4 (small)	Selenium	6	50.0	2	16.7	4	33.3	12	13.8
	Placebo	9	60.0	2	13.3	4	26.7	15	17.2
	Vit ACE	2	5.1	4	12.5	4	26.7	10	11.6
5 - 6 (medium)	Selenium	13	38.2	12	35.3	9	26.5	34	39.1
	Placebo	22	47.8	10	21.7	14	30.4	46	52.9
	Vit ACE	22	56.4	14	43.8	7	46.7	43	50.0
7 & above(large)	Selenium	17	41.5	9	22.0	15	36.6	41	47.1
	Placebo	10	38.5	9	34.6	7	26.9	26	29.9
	Vit ACE	15	38.5	14	43.8	4	26.7	33	38.4
Total	Selenium	36	41.4	23	26.4	28	32.2	87	100.0
	Placebo	41	47.1	21	24.1	25	28.7	87	100.0
	Vit ACE	39	45.3	32	37.2	15	17.4	86	100.0
Mean Income ±SD	Selenium	583 ± 189		2109 ± 248		6161 ± 4094		2782 ± 3340	
	Placebo	620 ± 237		2048 ± 218		5820 ± 3234		2459 ± 2810	
	Vit ACE	597 ±259		1969 ±177		4900 ±930		1858 ± 1597	

Appendix 8.14 : Table showed Distribution of patients according to their duration of drinking from contaminated source

Study groups	Duration of drinking from contaminated source									
	1-3 year N %	4-6 Year N %	7-9 Year N %	10+Yer N %	Male Mean ± SD	N	Female Mean ± SD	N	Total Mean ± SD	N
Selenium	1011.5	20 23	26 29.9	31 35.6	25.1±13.9	28	19.3±9.5	59	21.1±11.4	87
Placebo	6 6.9	20 23	33 37.9	28 32.2	24.0±15.5	34	20.6±11.0	53	21.9±12.9	87
Vit ACE	8 9.3	20 23.3	34 39.5	24 27.9	24.3±13.3	31	21.6±12.2	55	22.6±12.6	86
Total	24 9.2	60 23.1	93 35.8	83 32.9	24.4±14.1	93	20.5±10.9	167	21.9±12.3	260

Appendix 8.15 : Table showed Distribution of study subject according to drinking and cooking water sources

Study group	Source of cooking water			Duration of cooking water in years	Source of drinking water				Duration of drinking water in years	Total
	Deep TW N %	Shallow TW N %	Pond N %		Deep TW N %	Shallow TW N %	Pond N %	Filter/ Boil N %		N %
Seleniu	3(3.4)	2 (2.3)	82(94.3)	25.5±10.6	6(6.9)	75(86.2)	4 4.6)	2(2.3)	21.1±11.4	87 (100)
Placebo	1(1.1)	2 (2.3)	84(96.6)	27.6±11.4	9(10.3	76(87.4)	2 2.3)	0(0.0)	21.9±12.9	87 (100)
Vit ACE	1(1.2)	0 (0.0)	85(98.8)	26.1±10.8	5(5.8)	80 (93)	1(1.2)	0(0.0)	22.6±12.6	86 (100)
Total	5(1.9)	4 (1.5)	251(96.5	26.4±10.9	20(7.7	231(88.8	7 2.7)	2(0.8)	21.9±12.3	260(100)

TW = Tube well

Figures in parentheses are percentile

Appendix 8.16 shows Ethical clearance from BMRC

BMRC

বাংলাদেশ চিকিৎসা গবেষণা পরিষদ
Bangladesh Medical Research Council

Ref: BMRC/ERC/2001-2004/ 177 Date: 14/03/04

Ethical Review Committee

Dr. Abdul Momin
Associate Professor
Deptt. of Dermatology
Dhaka Medical College
Dhaka.

Subject: Ethical Clearance

With reference to your application on the above subject this is to inform you that your Research Proposal entitled "Selenium on Chronic Arsenicosis" has been reviewed and approved by the Ethical Review Committee of Bangladesh Medical Research Council (BMRC).

You are requested to please note the following ethical guidelines as mentioned at page 2 (overleaf) of this memo.

(Prof. Harun-Ar-Rashid)
MD, MSc, MPH, PhD, FRCP Edin
Director 14/3/04

Appendix 8.17 shows information leaflet

অবহিত পত্র

বর্তমানে বাংলাদেশের ৬১টি জিলার প্রায় সাড়ে সাত কোটি মানুষ আর্সেনিক বিষক্রিয়ায় আক্রান্ত। ভূগর্ভস্থ নলকূপের খাওয়ার পানিতে মাত্রাতিরিক্ত আর্সেনিক থাকার কারণে এই দূরারোগ্য আর্সেনিক বিষক্রিয়ায় আমাদের জনগণ আক্রান্ত হচেছ। আর্সেনিক দূষিত পানি বহুদিন যাবৎ পান করার ফলে শরীরের বিভিন্ন অঙ্গে নানাপ্রকার রোগের উপসর্গ দেখা দিতে পারে। অর্সেনিক বিষক্রিয়ার লক্ষণ হলো-শরীরে বৃষ্টির ফোটার মত ছড়ান সাদা কালো রং-এর দাগ, হাতের তালু ও পায়ের তালুতে শক্ত কড়া পড়া, কাশি, ডায়রিয়া, দুর্বলতা, খাওয়ার অরুচি, মাথা ঘুরানো, প্রশ্রাবে জ্বালাপোড়া ও চক্ষু জ্বালাপোড়া ইত্যাদি। এমনকি চর্ম, লিভার, কিডনী, ফুসফুস এবং মুত্রাশয়ের ক্যান্সার পর্যন্ত হতে পারে। কিন্তু অদ্যাবধি এ রকম মারাত্মক বিষক্রিয়াজনিত উপসর্গের কোন ফলপ্রসু চিকিৎসা ব্যবস্থা জানা যায় নাই। তাই আমরা এ রোগের চিকিৎসাকল্পে একটি নতুন পরীক্ষামূলক প্রকল্প হাতে নিয়েছি, যাতে আপনাদের স্বতঃস্ফুর্ত অংশগ্রহণ প্রয়োজন। ভক্ষণযোগ্য সেলিনিয়াম ইষ্ট (১০০ মাইক্রোগ্রাম) নিয়মিতভাবে ১২ মাস খেতে হবে। এ চিকিৎসা প্রকল্পে অংশগ্রহণের জন্য আপনার সম্মতি প্রয়োজন। এ প্রসঙ্গে বলা ভাল যে, এতে আপনার কোন ক্ষতি হবে না। যদি কিছু হয় তা বিনামূল্যে চিকিৎসা দেয়া হবে। আমরা আশা করছি যে, এ চিকিৎসায় আপনারা উপকৃত হবেন এবং আপনাদের অংশগ্রহণে প্রাপ্ত সাফল্য ভবিষ্যতে আর্সেনিক বিষক্রিয়াজনিত মহামারী আকারের এ বিপর্যয় রোধে সহায়ক হবে।

Appendix 8.18 shows consent form

সম্মতি পত্র

অবহিত পত্রে উল্লেখিত তথ্যাদি জানার পর এবং বুঝার পর আমি স্বতঃপ্রণোদিত এই পরীক্ষায় অংশগ্রহণে রাজী আছি।

রুগীনী

কর্তব্যরত চিকিৎসক

স্বাক্ষর

তারিখ ঃ-

নাম

ঠিকানা

Appendix 8.19 shows questionnaire form

"আরসিনোকোসিসের চিকিৎসায় সিলিনিয়ামের ভূমিকা" শীর্ষক গবেষণা
ঢাকা মেডিকেল কলেজ এবং পুষ্টি ও খাদ্যবিজ্ঞান ইনষ্টিটিউট, ঢাকা বিশ্ববিদ্যালয়।

প্রশ্নাবলী

ক্রমিক নং ☐☐☐

সাক্ষাতকারের তারিখ: ☐☐☐☐☐☐
দিন মাস বছর

ঃ আরসিনোকোসিসে আক্রান্ত রোগীর সাধারণ তথ্য ঃ

১। রোগীর (উত্তর দাতার) নাম ঃ ---------- পিতা/স্বামীর নাম----------

২। ঠিকানা ঃ বাড়ী----------গ্রামঃ----------

ইউনিয়ন---------- থানাঃ---------- জেলাঃ----------

৩। রোগীর বয়সঃ ----------বৎসর ; লিঙ্গঃ ☐1 পুরুষ ☐2 মহিলা

৪। রোগীর শিক্ষাগত যোগ্যতাঃ

☐1 নিরক্ষর ☐2 প্রাথমিক ☐3 মাধ্যমিক ☐4 এস এস সি
☐5 এইচ এস সি ☐6 স্নাতক ☐7 স্নাতকোত্তর ☐8 অন্যান্য

৫। ক) রোগীর পেশা ঃ ☐

খ) **পরিবারের মাসিক আয় ঃ** ☐☐☐☐☐

গ) **পরিবারের মোট লোকসংখ্যা ঃ** ☐☐ পুরুষ ☐ মহিলা ☐

৬। **রোগীর বসত বাড়ির ধরণ ঃ** ☐1 নিজস্ব বাড়ি ☐2 ভাড়া বাড়ি

৭। **ঘরের অবস্থা ঃ**

☐1 পাকা দালান ☐2 টিনসেড পাকা ☐3 সবটিন+পাকা মেঝে
☐4 সবটিন+কাঁচা মেঝে ☐5 টিনের চাল+বাঁশের বেড়া ☐6 খড়ের ঘর

ঃ পানির উৎস ও ব্যবহার ঃ

৮। **আপনারা পূর্বে কোন পানি পান করতেন ?**

☐1 গভীর নলকুপ ☐2 অগভীর নলকুপ ☐3 পুকুর ☐4 নদী ☐5 ফিল্টার/ফুটানো

৯। **কত বছর যাবৎ আপনারা এই পানি পান করছেন ?** ----------বছর ☐☐

১০। **আপনার পরিবারে রান্নার কাজে কোন ধরণের পানি ব্যবহার করতেন ?**

☐1 গভীর নলকুপ ☐2 অগভীর নলকুপ ☐3 পুকুর ☐4 নদী ☐5 ফিল্টার/ফুটানো

১১। কত বছর যাবৎ এই পানি রান্নায় ব্যবহার করছেন ? ----------বছর ☐☐

১২। বর্তমানে আপনারা কোন পানি পান করেন এবং রান্নায় ব্যবহার করেন ?

ক) রান্নায়: [1] গভীর নলকুপ [2] অগভীর নলকুপ [3] পুকুর [4] নদী [5] ফিল্টার/ফুটানো

খ) পান করায়: [1] গভীর নলকুপ [2] অগভীর নলকুপ [3] পুকুর [4] নদী [5] ফিল্টার/ফুটানো

১৩। আপনি কি মনে করেন আর্সেনিকে আক্রান্ত রোগীদের সামাজিক সমস্যা হয় ? [1] হ্যাঁ [2] না

উত্তর হ্যাঁ হলে, কি কি সমস্যা হয় (হ্যাঁ হলে 1, না হলে 2)

বিবাহের সমস্যা		অসুস্থ্য দেখায়	
লোকজন ঘৃণা করে		লেখাপড়া শিখতে সমস্যা হয়	
দেখতে কুৎসিত দেখায়		অন্যান্য	

ঃ আর্সেনিক আক্রান্ত বিস্তারিত তথ্য ঃ

১৪। আপনার আর্সেনিক রোগ কতদিন যাবৎ দেখা দিয়েছে ?

[1] ১-৩ বছর [2] ৩-৬ বছর [3] ৬-৯ বছর [4] ৯-১২ বছর

১৫। রোগীর শারিরীক লক্ষণ সমূহ ঃ (হ্যাঁ = 1, না = 2)

চামড়া রংয়ের পরিবর্তন		হাত-পায়ের তলায় ক্ষত	
চামড়ায় ক্ষত		আলসার	
চামড়ায় সাদা ছোপ		রক্ত ক্ষরণ	

১৬। রোগীর শারিরীক পরিমাপ ঃ

পরীক্ষার সময়কাল	উচ্চতা (সেঃমিঃ)	দৈহিক ওজন (কেজি)
১ম বার শুরুতে		
২য় বার ১২ মাস পর		

ঃ সিলিনিয়াম চিকিৎসার পর্যায়ক্রমিক ফলাফল ঃ

১৭। প্রয়োগকৃত ঔষধের প্রকার ঃ (A = 1, B = 2, C = 3) [ ]

১৮। ঔষধ প্রয়োগের ফলাফল ঃ

ক) বায়োকেমিক্যাল পরীক্ষা ঃ	১ম বার শুরুতেই	৪ মাস পর ২য় বার	৮ মাস পর ৩য় বার	১২ মাস পর ৪র্থ বার
রক্ত পরীক্ষা:				
গ্লুকোজ				
হিমোগ্লোবিন				
সিলিনিয়াম				
এস.জি.পি.টি				
এলকালাইন ফসফেট				
প্রসাব পরীক্ষা:				
আর্সেনিক				
নখ পরীক্ষা:				
আর্সেনিক				
চুল পরীক্ষা:				
আর্সেনিক				
ই.সি.জি পরীক্ষা:				