Prevalence of *Entamoeba histolytica* and *Giardia lamblia* infection among diabetic and non diabetic patients of Bangladesh.

A dissertation submitted to the University of Dhaka in fulfillment of the requirements for the degree of Doctor of Philosophy in Zoology (Parasitology)

By

TAHMINA SIDDIQUA

Registration No. 58

Session: 2011-2012 (Re)

Department of Zoology (Parasitology)

University of Dhaka

Dhaka-1000, Bangladesh

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DECLARATION

I hereby declare that this dissertation submitted to the University of Dhaka for the degree of Doctor of Philosophy is based on my own investigation, carried out under the supervision of Professor Dr. Hamida Khanum, Department of Zoology, University of Dhaka, and that this or any part of this work has not been submitted for my other degree anywhere.

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CERTIFICATE

This is to certify that the dissertation entitled "**Prevalence of *Entamoeba histolytica* and *Giardia lamblia* infection among diabetic and non diabetic patients of Bangladesh"** submitted by Tahmina Siddiqua for the degree of Doctor of Philosophy in Zoology (Parasitology), University of Dhaka, Bangladesh, embodies the record of original investigation carried out by her under my supervision.

Dr. Hamida Khanum
Professor
Department of Zoology
University of Dhaka
Dhaka 1000, Bangladesh

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ABSTRACT

The present study was conducted on 697 diabetic and 603 non-diabetic peoples (aged 25-75 years) in the Immunology Laboratory, Institute of Health Science (BIHS) Hospital, Mirpur, in Dhaka to investigate their association with protozoan infestation.
The use of appropriate technique plays an important role in the detection of the parasitic infections. ELISA with blood samples, formol-ether concentration technique (F-ECT) and zinc sulphate method (Z-SFM) were applied to faecal samples for the detection of prevalence of *Entamoeba histolytica* and *Giardia lamblia*. Out of 697 diabetic patients, by ELISA 15.93% and 17.65% were found infected by *E. histolytica* and *G. lamblia*; while out of 603 non-diabetic individuals, 27.53% and 28.03% were infected by *E. histolytica* and *G. lamblia*; respectively.

According to the results of F-ECT on diabetic patients, 26.83% and 28.41%; 34.66% and 34.99% were found among non-diabetic individuals infected by *E. histolytica* and *G. lamblia*; respectively.

According to the results of Z-SFM, on diabetic patients 12.05% and 13.34%; 15.09% and 16.09% were found among non-diabetic individuals infected by *E. histolytica* and *G. lamblia* respectively. A specimen was considered positive for *E. histolytica* and *G. lamblia* if either cysts or trophozoites or both stages were present.

Double parasitic infestation (8.03%) was found in diabetic patients and 13.10% was found in non-diabetic individuals. By these methods (ELISA, F-ECT and Z-SFM techniques), it reveals that, males were more infected than females. It was also observed that prevalence was higher among 25-45 years than old aged group (above 55 years).

By ELISA method, out of 697 diabetic blood samples, 18.68% of symptomatic types and 12.91% of asymptomatic types were positive for *E. histolytica* antibody. Similarly, 31.45% of non-diabetic symptomatic types and 21.21% of asymptomatic types were positive for *E. histolytica* antibody. Out of 697 diabetic blood samples, 21.15% was

positive in symptomatic types and 13.8% in asymptomatic types for *G. lamblia* antibody, 31.18% of non-diabetic symptomatic types and 22.94% of asymptomatic types were positive for *G. lamblia* antibody.

By F-EC technique, 31.87% of diabetic symptomatic types and 21.32% of asymptomatic types were positive for *E. histolytica*. Similarly, 37.63% of non-diabetic symptomatic types and 29.87% of asymptomatic types were positive for *E. histolytica*. Among diabetic 30.49% of symptomatic types and 26.13% of asymptomatic types were positive for *G. lamblia*. Among non-diabetic individuals, 38.71% of symptomatic types and 29% of asymptomatic types were positive for *G. lamblia*. By Z-SF method, 13.46% of symptomatic diabetic patients and 10.51% of asymptomatic types were positive for *E. histolytica*. Similarly, 19.35% of non-diabetic symptomatic types and 8.23% of asymptomatic types were positive for *E. histolytica*. Among diabetic patients, 14.56% of symptomatic and 12.01% of asymptomatic types was positive for *G. lamblia*. Similarly, 20.16% of non-diabetic symptomatic types and 9.12% of asymptomatic types were positive for *G. lamblia*.

Considering the age groups, in non-diabetic individuals, the highest prevalence of *E. histolytica* was 38.05% in the age group of 25-35 and in the case of *G. lamblia,* it was 40.13% found in the age group of 35-45 years detected by F-ECT.

In comparison of three techniques of blood and faecal samples, in single and double parasitic infections, the prevalence always found higher among the non-diabetes than the diabetic patients. The seasonal variation of parasitic infestation was higher during rainy season and lower in winter. It revealed that, F-ECT is most suitable method for detection of single and as well as double protozoan infections and is recommended as the best technique to get the concentration of the parasites present in faeces. Z-SFM used to diagnose infection with *E. histolytica* and *G. lamblia*, which is a quick and comparatively simpler examination and gives a precise and reliable diagnosis. The ELISA technique is the best of all techniques, which represents the present infection as well as the recent infection of amebiasis and giardiasis.

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INTRODUCTION

Bangladesh is a tropical country. Clinical amoebiasis is most prevalent in tropical and subtropical areas. It is a great public health problem in rural and urban areas with a wide spread endemicity. Low socio-economic conditions, poor hygienic habits and the most important is the lack of health educations are the main reasons behind this. In our country majority of the people are fighting with poverty. The density is 935 people per square kilometer, is the highest in the World; the humid temperature, squalid living condition favour parasitic infection (Shakur, 1993). The temperature in winter season is minimum, average temperature is 11ºc and maximum 29ºc in summer, the temperature remain maximum 34ºc and minimum 21ºc. The relative humidity is 99% in July-December and lowest humidity is 36%. Rainfall is on average 1194-3454 mm.

Congestion, soil character, source of water, socio-economic conditions, low living condition, unhygienic surroundings allow for transmission of the amoebic infection. The distribution is world wide, although the preponderance of morbidity and mortality is experienced in Central and South America, Africa and India (Yadav, *et al.,* 1995).

Entamoeba spp. is morphologically identical but biochemically and genetically different. This parasite colonized in the human gut, but only *E. histolytica* is thought to be capable of causing disease. Amoebiasis is defined as asymptomatic, invasive intestinal or extra intestinal diseases due to *E. histolytica* infection. It is one of the most common parasitic infections worldwide. Asymptomatic cyst is the most frequent manifestation of intestinal *Entamoeba* infection and 90% of *Entamoeba histolytica* infections are asymptomatic (WHO 1997).

The necessity to identify and treat asymptomatic carriers of *E. histolytica* is emphasized by the observation that 10% of invasive amoebiasis develops in due course (Gonzalez-Rulz, *et al.,* 1994). Additionally, asymptomatic carriers are more likely to spread the disease than

symptomatic persons with invasive disease, as the latter individuals seek medical attention (Mirelman, *et al.,* 1997).

Amoebic colitis and liver abscess are much more common in developing nations than in industrialized countries such as the United Status. *E. histolytica* infection is probably second only to malaria as a protozoan cause of death. The best estimate is that 4050 million cases of amoebic colitis and liver abscess occur annually in the world, resulting in 40,000-100,000 deaths (WHO, 2005). The prevalence of disease in the developing world is due to fecal-oral spread of infection via contaminated food and water.

Most amoebic infections occur in Central and South America, Africa and Asia. In 1987 and 1988 Mexican national serosurvey, for example, demonstrated 8.4% seropositivity for *E. histolytica*, there were an estimated 1 million cases of amoebiasis and 1216 deaths due to *E. histolytica* infection in Mexico (Caballero-Salcedo *et al*., 1994). The prevalence of disease in the developing world is due to fecal-oral spread of infection via contaminated food and water. In the United States, immigrants from and travelers to developing countries are those most likely to develop amoebiasis. A total of 2970 cases of amoebiasis in the United States were reported to the Centers for Disease Control and prevention in 1993; 33% 0f the patients were Hispanic immigrants and 17% immigrants from Asia or the Pacific Islands (Furness, 2000). Travelers to the tropics are at a low but definite risk for acquiring amoebic infection (Weinke *et al.,* 1989). The study on 2700 German citizens returning from tropical areas demonstrated 3% incidence of *E. histolytica* infection. Residents of institutions for the mentally retarded are also at increased risk for amebic colitis and liver abscess (Nagakura *et al.,* 1990).

Diarrhoeal diseases are one of the leading causes of mortality and morbidity worldwide especially in diabetic and non-diabetic individuals, children, travelers and immuno-compromised patients. Parasites can be protozoa having their choices of niche in human body. Most of protozoan parasites can be excreted with stool in both cyst and trophozoite stages (WHO, 1969).

Poor sanitation, illiteracy makes quite a good number of victims of diarrhea and other gastrointestinal discomforts. The first five year plan of Bangladesh (1973-78) reported that 645 million of the children of the country suffered from intestinal parasitic infection. Muttalib *et al.* (1976) showed that about 99% of non-diabetic children in rural areas of Bangladesh were infected by intestinal parasites. Intestinal protozoan parasite infections are amongst the most common ones in worldwide, particularly in tropical and subtropical developing countries. *Entamoeba histolytica,* which might cause the life threatening liver abscess, is estimated to cause sever disease in 48 million people and killing 70 thousand each year. Multiple infections with several different parasites like *Giardia lamblia* and amoebae are common and their harmful effects are often aggravated by coexistent malnutrition or micronutrient deficiencies (WHO, 2002).

Bangladesh is mostly a plain land and embedded with rivers and canals. The soil humidity and temperature contributes a lot towards parasitic infection. Several studies showed that intestinal parasitic infections are present all the time everywhere in this country (Muttalib *et al.,* 1975; Shakur *et al.,* 1993).

In Bangladesh, the protozoan parasites, *Entamoeba histolytica* and *Giardia lamblia* are common (Kuntz, 1960; Islam *et al.,* 1975; Saha and Chowdhury, 1981; Das, 1990; D'Silva *et al.,* 2003; Banu *et al.,* 2003 Uddin *et al.,* 2005 and Khanum *et al.,* 2008). The protozoan infections create different public health problems among the hosts directly or indirectly and can cause nutritional impairment, retard physical and mental development of diabetic and non-diabetic patients. Predispose of infection to enteric and some other diseases, such as diarrhea, dysentery, anaemia, appendicitis and other secondary infections. So, transmission of these parasites and prevention of infection are essential.

WHO (1994) reported iron deficiency anaemia during acute infections chronic infections with *Entamoeba histolytica* and *Giardia lamblia* with the hemoglobin status of diabetic and non-diabetic human hosts. Lee *et al.,* (2000) reported 87.5% infection among non-diabetic individuals in Philippines. In their study, infection rate was higher in rural population than in urban people. Parasites of diarrhoeal etiology are widespread, infecting a significant

proportion of the human population in third-world countries (Vignesh *et al.,* 2007), especially across the Asian subcontinent. With an ever-increasing population leading to overcrowding and unhygienic practices, these parasites pose a serious threat that is compounded by limited resources. Competency in the diagnosis and proficiency of laboratories in such peripheral setting remain questionable and may be attributed to these limited resources. Simple and cost-effective diagnostic methods may provide a solution to these difficulties.

Parasitic infestation in diabetic and non-diabetic peoples:

Protozoa are among the most important pathogens that can be cause infections in immuno-commprosied hosts. Over recent decades, parasitic protozoa have been recognized as having great potential to cause waterborne and food borne disease. The organisms of greatest concern in food production worldwide are *Entamoeba histolytica* and *Giardia lamblia*. Although other parasitic protozoa can be spread by food or water, current epidemiological evidence suggests that these two present the largest risk. Their occurrence depends on factors that include season, the age and other demographic characteristics of a population: among children age 1-5 years with diarrhea, *Giardia* may be the most frequently found as pathogen (Allison, 1988). Muttalib *et al.,* (1976) reported on the prevalence of intestinal parasites in rural children of Bangladesh. He found *E. histolytica* in 40.88% and *Giardia* 46.04%. Alaofe (2008) reported that parasitic diseases, especially in adolescent girls, lead to malnutrition and impairment of physical and mental development.

The principle protozoan parasites that present a risk to humans are: *E. histolytica, G. lamblia* and *Cryptospridium sp.* Three organisms have emerged as pathogens during recent decades, *G. lamblia* being the first to come to attention as a public health issue, followed by *Cryptospridium* and then *Cyclospora.* Recognition of the organisms as a health issue is partly due to improved disease surveillance, including better laboratory diagnosis. Kuntz (1960) examined intestinal protozoa in non-diabetic children of Dhaka city and found *E. histolytica* in 37% of the non-diabetic children examined. An investigation on the prevalence of intestinal parasites in pre-school children in Ghana has been done by

Khanum *et al.,* (1998) worked on the prevalence of intestinal parasitic infections in children aged between 0-5 years in Dhaka Shishu Hospital.

In developed countries the parasite is commonly found in persons living in closed communities in those who are immuno-compromised (Smith *et al.,* 1982), and in children attending day care centers (Carison, *et al.,* 1988). There are also documented outbreaks of amoebiasis and giardiasis due to contaminated water supplies (Green *et al*., 1988). Diarrhea is a major cause of diabetic and non-diabetic morbidity and mortality in developing countries (Islam *et al.,* 1983). Poor socio-economic condition, lack of knowledge of hygience and illiteracy is the common features of rural Bangladesh. As a result, intestinal diseases mainly diarrhea are highly prevalent in Bangladesh.

The crude soluble antigen (CSA) of *Giardia lamblia* trophozoites and their analytically purified fractions were characterized biochemically and immunologically to determine the most immunogenic fraction and is localization on the parasite (Chowdhury *et al.,* 1996). Serum sample from 20 Indian children with diarrhea were compared with those from 20 non-diabetic residents in the United Kingdom who had been diagnosed as having ulcerative colitis, or Crohn's disease, or indeterminate colitis using enzyme linked immunosorbent assays specific for *Entamoeba histolytia* and *Giardia lamblia.* More than 50% of the United Kingdom patients had high IgG responses in ELISAs for *G. lamblia* (Shetty *et al*., 1988a).

The epidemiology, clinical features, nutritional status, and causative agents of diarrhea were studied in 289 children (147 boys and 142 girls) of 2-5 years old. The use of improved diagnostic tests for amoebiasis enabled for the first time analysis of the contribution of *E. histolytica* to total diarrheal illness in this community setting (Haque *et al*., 2003).

Men who have sex with men were in the past predominantly infected with the nonpathogenic amoeba but recently invasive amoebiasis has been seen in this group (with and without HIV infection) (Black, *et al.,* 1980). *E. histolytica* has been found in as many

as 32% of homosexual men Northern America (David 1992). An increase severity of illness is seen in malnourished people, the very young and old, pregnant women, and patients receiving corticosteroids. According to Yavad *et al., (*1990) invasive amoebiasis in malnourished, hospitalized patients in Dhaka city, in adults older than 40 years age, there was an overall 29% case fatality rate despite hospitalization and anti-amoebic chemotherapy.

An increasing number of biochemical, immunological and genomic differences between the two species of amoeba recognized and this information finally led to the formal separation of the two species (Clark, 1993) with the name *E. histolytica* being retained for the pathogenic species and Brumpt's name *E. dispar* being revived for the non-pathogen. Under light microscopy, the cysts of the two species are indistinguishable.

The early literature of *E. histolytica* research has been reviewed by Kean, 1988 and by Clark *et al.,* 2000. Milestones in the study of *E. histolytica* were its description by Lesh, in 1875, the delineation amoebic liver abscess and colitis by Aristizabal and his colleagues in 1991, its axenic culture by Diamond in 1961, and differentiation of pathogenic from nonpathogenic in 1979. This patient of Lesh's was the first person known to have died from amoebiasis. Clinical disease results from the ability of *E. histolytica* to penetrate the wall of the large bowel and to spread extra- intestinally. In brief, penetration of the gut wall may lead to ulceration which, if extensive enough, produces the classical sings and symptoms of amoebic dysentery. Extra- intestinal spread most frequently involves the liver, causing hepatic amoebiasis or amoebic liver abscess; spread to other distant organs such as the brain is known but is very rare.

Attia *et. al.,* (1995), in a classic monograph, described the clinical and pathological evidence of the association of *E. histolytica* with dysentery and liver abscess. In 1954, the international Commission on Zoological nomenclature ruled that *E. coli* was to be the type species and that *E. coli* was to be used to be place of *Entamoeba* to describe *E. histolytica* (David and Brukner 1992).

In Bangladesh, a number of studies were carried out earlier on the prevalence of *E. histolytica.* Kuntz 1960) studied the intestinal parasite among different school children in Dhaka city. The total rates of *E. histolytica* infection were moderately high. He found that 37% of the children had *E. histolytica.* At the same time Dutt (1958) and Ghosh (1998) also reported an incidence of 11.1% *E. histolytica* in a study of hospital patients at Calcutta.

Muttalib (1970) reported the prevalence of *E. histolytica* to be 55% in those patients attending a private city clinic. Huq and Shaikh (1976) found the incidence of *E. histolytica* to be 42% when he studied the relationship between the socio-economic conditions of the people and their infestation by parasites. The children of slum area showed the higher incidence rate compared to the children living in Dhanmondi and Eskston, because they lived in crowded, slum and muddy areas and suffered from in environmental contamination by defection around their shelters.

A surveillance system (Haque, 1995) was set up at the International Center for Diarrhoeal Disease Research, Bangladesh, (ICDDR, B) hospital, to study about 100,000 non-diabetic patients with diarrhea. *E. histolytica* was isolated from 6% of the patients. All these studies showed that parasitic infestation is an important health problem in a large number of people in Bangladesh.

The genus *Entamoeba* contains many species, six of which (*E. histolytica, E. dispar, E. moshkovskii, E. polecki, Ecoli, E. hartmanni*) reside in the human intestinal lumen. *Entamoeba histolytica* is the causative agent of amoebiasis and is considered a leading parasitic cause of death worldwide in humans. The identification of *E. histolytica* is based on detection of *E.histolytica* –specific antigen and DNA in stool and other clinical samples (Fotedar *et al.,* 2007).

Waterborne parasitic infections are common worldwide due to the storage of drinking water, and unhygienic conditions of storage and manipulation of contaminated water in different daily purposes (WHO 1991). At least 325 reported water related outbreaks of

protozoan parasites worldwide where *Cryptospordium* and *Giardia* have been reported to account for a majority of these outbreaks affecting millions of people (Karanis *et al.* 2007).

Bangladesh is a country of third world and poverty is the common feature of most of the people. 70% of the people of this country live under the level of poverty. Poor socio-economic conditions, lack of knowledge of hygiene and illiteracy are the common features of rural Bangladesh. As a result, intestinal diseases mainly diarrhea are highly prevalent in Bangladesh.

Over 40 species have been described in the genus *Giardia* and there details have recently been complied by Thompson *et al.,* (1986) the majority of these species were based on host occurrence but most of transmission of *G. lamblia* is by the direct faecal-oral route from contaminated hands, food or water, which maintain widespread endemic areas of infection. About 200 million people have been symptomatic giardiasis worldwide, with prevalence of 2-5% in industrialized countries and 20-30% in developing regions of Asia, Africa and Latin America (Haque, 2007). Transmission of *Giardia* occurs by faecal-oral route of exposure and is sustained by zoonotic and anthroponotic cycles where several species and genotypes are enrolled (Rahim *et al*., 2009). Besides adults, children are highly susceptible whereas breast-fed infants (less than 6 months of age) are not likely to be infected (Stanton *et al.,* 1989).

The infected hosts shed a large number of infective cysts in the environment, contributing to an increase of environmental contamination, in particular water courses. The cysts remain infective for long periods in natural environment, resistant to the conventional water treatment processes and small infectious dose, representing a serious problem of public health (Klein *et al.,* 2011). *Entamoeba histolytica* and *Giardia lamblia* are the most frequently identified protozoan parasites causing waterborne disease outbreaks. The morbidity and mortality associated with these intestinal parasitic infections warrant the development of rapid and accurate detection and genotyping methods to aid public health efforts aimed at preventing and controlling outbreaks (Wang *et al*., 2004).

Amoebiasis in developed countries is most common in immigrants and travelers returning from the third world. Infection is acquired by ingestion of food or water containing the cyst form of the parasite *Endameba histolytic,* which is the cause of amoebic colitis and liver abscess. The trophozoite invades the intestinal epithelium and causes disease by destroying host tissues.

Amoebic colitis commonly has a sub acute onset with weight loss, and is characterized by diarrhea that usually contains occult or gross blood. In some developing countries, giardiasis is associated with a delay in growth and weight gain (failure to thrive) of non-diabetic young children. Symptoms effecting areas outside of the gastro intestinal tract are extremely rare. However, the development of swollen, reddish welts known as hives (urticaria); joint pain and inflammation (arthritis); and disease of the organs and ducts that remove bile from the body (biliary tract) and carry it to the intestines have been reported in the medical literature.

Immunity to *Entamoeba* species intestinal infection is associated with the presence of intestinal IgA antibodies against the parasite's galactose-inhibitable adherence lectin. These findings were correlated with the effects of epitoe-specific murine antilectin Immunoglobulin A (IgA) monoclonal antibodies (MAbs) on amebic in vitro galactose-specific adherence (Mohamed *et al.,* 2004).

Zaid *et. al.* (1999) reported that patients with diabetes had increased risk of mucocutaneous yeast infection. But not all types of yeast infection occur frequently in diabetes mellitus. Incidence and patterns of candida infection among diabetics according to their age and sex was debatable. Glycemic control has important role in yeast infection but such a relation is not absolute in all studies. To characterize the yeast infections among diabetics according to their anatomical sites, sex and duration of diabetes and glycaemic status, that have investigated 460 diabetic patients of candidiasis in Department at BIRDEM, Based on microscopy and culture, *Candida intertrigo* (47.8%), *C. paronychia* (23.5%), *C. nail* (9.6%), *C. vulvovaginitis* (6.7%), *Pityriasis versicolor* (6.7%), *C. balanitis* (2.6%), oral candidosis (2.2%) and perianal candidosis(0.7%) was diagnosed. *Pityriasis versicolor*

infection ($p<0.5$) and oral candidosis ($p<0.01$) were significantly higher ($p<0.001$) among females.

Agent of amoebiasis and giardiasis:

The trophozoite stage of *Entamoeba histolytica* is pathogenic to man and causes 'amoebiasis' or 'amoebic dysentery in primary stage. The trophozoite of *Giardia lamblia* is also pathogenic to man and causes 'giardiasis'.

Amoebiasis, causes by an enteric protozoa *Entamoeba histolytica* is known to occur in every part of world (Khanum *et al*, 2005). Infection by this parasite is about 500 million diabetic and non-diabetic people each year have amoebiasis of which only 10% experience symptomatic diseases (Walsh, 1986 and WHO, 1997). It is estimated to result in 50 million cases of colitis and liver abscess and 100,000 deaths worldwide each year, although its prevalence and severity might vary in different areas or increase in special circumstances (Wank *et al,* 1988).

In developing countries, colonization with *E. histolytica* has been observed in 5% or more of poor children. In Bangladesh one of the leading causes of the death of pre-school children (2-5 years) is diarrhea (khanum *et al,* 2005). The two major clinical syndromes of amebiasis are amebic colitis and amebic liver abscess. Patients with amebic colitis typically present with a several week history of gradual onset of abdominal pain and tenderness, diarrhea and bloody stools (dysentery). The principal site of extra-intestinal infection is the liver (Felnger, 1977).

The organisms invade the live tissue and form abscesses that increase in size, progressively damaging the liver, 85% of the patients with biopsy proven intestinal amoebiasis (Wank *et al,* 1988). The diagnosis of intestinal amebiasis is ideally made using an *E. histolytica*-specific stool antigen detection test (Haque *et al,* 1994). The pathological lesions in the colon include ulceration of the intestinal epithelium and invasion into the lamina propria by trophozoites. Amebic liver abscess is a rare form of the disease that is almost exclusively limited to adult males (Haque *et al.,* 2003).

E. histolytica infection (1.2 cases/100,000 USA population) results from ingestion of the quadrinucleate cyst of *E. histolytica* from fecally- contaminated food or water.
The pathological lesions in the colon include ulceration of the intestinal epithelium and invasion into the lamona propria by trophozoites. Infammation, with infiltrating neutrophils and mononuclear lymphocytes is pronounced, but inflammatory cells near the amabae are killed with pyknotic nuclei characteristic of apoptotic death. Amebic liver abscess is a rare form of the disease that is almost exclusively limited to adult males (Haque *et al.,* 2003).

Respiratory infections are the most common infection in the children after diarreal diseases. There is any interaction between the respiratory infection and enteric infections such as *E. histolytica* influence of co-infection with respiratory tract infections on the acquired immunity to *E. histolytica* is not known. In many people *E. histolytica* infection resolves without symptoms. For example in our studies in Dhaka Bangladesh, 40% of preschool children have been infected with *E. histolytica* annually, but diarrhea has occurred in only 10% of all children annually, and dysentery in 3% (Haque *et al.,* 2006).

Giardiasis, an illness that affects the digestive tract, is caused by a protozoan parasite called *Giardia lamblia. Giardia* is one of the chief causes of diarrhea in Bangladesh, and is transmitted through contaminated water (Stoll *et al.,* 1982). *Giardia* is considered to be the most common intestinal parasitic protozoa. Its major role in infecting diabetic and non-diabetic in under developing countries is recognized and it is believed to be an important cause of malnourishment and failure to thrive. It is an important cause of traveler's diarrhea. The symptoms include those of malabsorption as well as simple diarrhea and there may be nausea, vomiting and abdominal pain.

Young kids are three times more likely to have giardiasis than adults, which leads some experts to believe that our bodies gradually develop some form of immunity to the parasite as we grow older. But more than two thirds of people who are infected may have no sings or symptoms of illness, even though the parasite is living in their intestines (Joel Klein, 2011). The symptoms include those of malabsorption as well as simple diarrhea and there may be nausea, vomiting and abdominal pain (Isaac, 1991).

Giardia is endemic in Bangladesh and reported prevalence of infection ranges from 4% to 21% depending on the methods of examining stool samples and on whether the stools came from non-diabetic healthy individuals or from people with diarrhea (khanum, *et al,* 1998). In 1968 a study was done by Muazzem *et al.,* among the children and it was found that the prevalence of giardiasis was 3%. And in 1976, the prevalence rate was 2.93%-2.80% among 1-15 age group children (Haq and Sheikh, 1993).

Gilman *et al.,* (1985) worked in Bangladeshi children and said that the prevalence was higher in 5 to 10 year old village children (21%) and one to five year old malnourished children (51%). *G. lamblia* was uncommon in infants and found most frequently in urban hospital patients of Bangladesh aged 5 to 9 (21%) and this prevalence declined in relation with increasing age (Hossain *et al.,* 1983).

Justification of the study:

Amoebiasis and giardiasis have been diagnosed traditionally by detection of cysts or trophozoite in stool by microscopic examination which is a laborious, time consuming and expensive task and depends on the skill of the microscopes. Other diagnostic methods such as duodenal aspiration and biopsy to primarily identify trophozoites are also more expensive, uncomfortable and invasive. Serum antibody titers to *E. histolytica* and *G. lamblia* can be measured by using the indirect immunofluorescence technique. But serological tests have been proven to be a little value in *E. histolytica* and *G. lamblia* diagnosis because there is a little correlation between positive *Anti-Entamoeba* and *Anti-Guardia* antibody titers and the presence of active *Entamoeba* and *Giardia* infection. Further more, examination of a single stool specimen may miss 10 to 50% of *Entamoeba* and *Giardia* infection, because of the intermittent shedding of cyst in stool.

Amoebiasis and giardiasis may also be present in the absence of whole organisms and can be confused with other illness such as Crohn's disease and ulcerative colitis. When infection is present but parasites are not detected, sampling of duodenal fluid detects trophozites but this method is invasive and expensive. *Entamoeba* and *Giardia*

immunoflorescence, using *Entamoeba* and *Giardia* trophozoites as antigen, are insensitive method, although it is reasonably specific (Gally *et al.,* 1973).

The formal-ether technique had been recommended as the best over all technique for recording the abundance of parasite in faeces. The movement of *G. lamblia* was not seen in a formal-ether preparation because formalin killed living organism. The formalin fixes and preserves parasites. The required formal water was 10%v/v (prepared by mixing 50 ml of strong formal dehydrate solution with 450 ml of distilled or filtered water). In Mirzapur, 10% children were suffered from diarrhea where 16.7% children were infected by *G. intestinalis* (Khanum *et al.,* 1998).

Invasive disease caused by *Entamoeba histolytic* and *Giardia lamblia* are usually diagnosed on the basis of clinical symptoms and ultrasound or radiological examination WHO-pan American Health Organization. Expert panel recently recommended the development of improved methods, using technologies, appropriate for developing countries, for the specific diagnosis of *Entamoeba histolytica* and *Giardia lamblia* infection.

The faecal concentration techniques are commonly used to concentrate faecal parasites. Sedimentation techniques in which parasites are sediment by gravity or centrifugal force, e.g. formol ether concentration method which is the most frequently used technique because it concentrates a wide range of parasites with minimum damage to their morphology. Floatation techniques in which parasites are concentrated by being floated in solutions of high specific gravity, i.e. solutions that are denser than the parasites being concentrated; example includes the zinc sulphate method (Cheesbrough, 1987).

If the formol ether sedimentation technique was not used, a single floatation technique cannot be used to concentrate a wide range of parasites because of differences in the densities of parasites and the damage that can be caused by floatation fluids to some parasites. For certain parasites and situations, floatation techniques are recommended and can be easily performed in the field with the minimum of equipment, providing adequate health and safety measures were taken. Parasites can also be recovered from the surface of

the floatation fluid after centrifuging. If however, a centrifuge was available, the safer formol ether technique was recommended for concentrating and infective cysts from faecal specimens (Cheesbrough, 2005).

The most recommended Formol Ether Concentration Method (Cheesbrough, 1987) was applied which was suitable for concentration of parasite cysts in fresh or preserved faeces. The zinc sulphate technique (Cheesbrough, 1987) was also recommended for concentration the cysts of *Entamoeba histolytia* and *G. lamblia* species.

(**Note:** parasites can also be recovered from the surface of the floatation fluid after centrifuging. If however, a centrifuge was available; the safer formol ether technique was recommended for concentrating and infective cysts from faecal specimens, Cheesbrough, 1987; Noble and Noble, 1980; Chatterjee, 2004).

So, the need for the rapid, expensive methods to diagnose *Entamoeba* and *Giardia* infection have led to the recent development of enzyme-linked immunosorbent assy (**ELISA)** for the detection of *E. histolytica* and *G. lamblia* antigen in blood. These methods may permit large numbers of blood samples to be tested accurately within short time. Antigen detection of blood specimens by enzyme immunoassay has been found to be sensitive and specific. This sensitive and specific enzyme linked immunosorbent assay (ELISA) to detect small amount of antigen in blood has the potential to detect infections when neither cysts nor trophozoites are being excreted in the stools and may offer an efficient diagnostic technique for large scale epidemiological studies of infections with *Entamoeba* and *Giardia* as well as for clinical diagnosis of *Entamoeba histolytica* and *Giardia lamblia* infection.

So, the present study will provided an opportunity to assess the usefulness of an antigen capture ELISA as an epidemiological and diagnostic tool, and to evaluate its sensitivity and specificity.

Hypothesis:

Protozoan parasitic infestation may cause diarrhea and anaemia among diabetic patients and non-diabetic individuals, at the same time it might have less immune reaction of non-diabetic individuals and especially diabetic patients which may be influenced by

socioeconomic conditions/ factors. Diabetic patients also eventually involve immune system of the body.

Objectives of the study:

General objective:

To determine the prevalence of intestinal protozoan parasites (*E. histolytica* and *G. lamblia*) infection among adult diabetic patients and non diabetic individuals by three diagnostic techniques.

Specific objectives:

1. To determine the prevalence of the *E. histolytica* and *G. lamblia* among adult patients by antigen detection techniques from the blood specimen.

2. To determine the occurrence of *E. histolytica* and *G. lamblia* infection among males and female; diabetic and non diabetic patients of different age groups etc.

3. To investigate and determine the prevalence of *Entamoeba* and *Giardia* infection among asymptomatic and symptomatic patients.

4. Evaluation
   (a). by antigen detection with blood serum,
   (b). by formal ether concentration test with faecal samples,
   (c). by zinc sulphate floatation test with faecal samples,
   for diagnosis of *Entamoeba* and *Giardia* infection.

5. To compare the results of formal ether concentration technique with antigen detection test and zinc sulphate floatation method.

Identification of *Entamoeba histolytica* and *Giardia lamblia:*

Giardia is a binucleated flagellate protozoan parasite which exists in two forms: a non infectious, pear shaped trophozoite, 9-30 µm infecting the small intestine and the highly infectious cyst form which is elliptical in shape and ranges in size from 6-10 µm. survival outside its host varies greatly between the two forms, the trophozoite which is extremely labile lasting only a matter of hours outside the body, while the cyst from may survive for several days in an external environment.

A detailed examination of the use of species names in Zoological Nomenclature, the name of *G. duodenalis* is correct.

Historical background:

In 1969 WHO defined amoebiasis as "infection *Entamoeba histolytica, with* or without clinical manifestation," was implying that all strains were potentially pathogenic.

Several studies have implicated the intestinal protozoan *Giardia duodenalis* (=*G. intestinalis, G. lamblia*) as a cause of persistent diarrhea and growth retardation among children (WHO 1990). However the epidemiology of *Giardia* is poorly understood partly due to the perceived lack of a simple and reliable technique to diagnose infections. Diagnostic methods of giardiasis by microscopy are considered to be unreliable, because, the organism is known to be excreted intermittently (Garcia and Bruckner, 1997).

Morphology, Life cycle, Transmission Pathogenesis and Epidemiology of *Entamoeba histolytica:*

Morphology of different stages (Chattergee, 1984):

Entamoeba histolytic appears to be one of the most primitive Ekaryotes. There are three stages in the life cycle, the trophozoite which is a host tissue invasive stage, precystic stage, an intermediate stage and the cystic is host infective stage.

(a) Trophozoite stage: Shape and size: The shape is not fixed because of constantly changing position. The size ranges from 18-20µm average being 20-30µm under light microscope. The living parasite, in a warm stage is seen to exhibit slow gliding movement. Plasmalemma or surface membrane resembles eukaryotic cells. Amoeba surface is covered with glycocalyx attached to the outer leaflet.

(b) Precystic stage: It is smaller in size varying from 10-20µm. it is round or slightly ovoid with blunt pseudopodia projecting from the periphery. The endoplasm is free of red blood cells and other ingested food particles. The relatively larger nuclear structure retains the characteristics of the trophozoite.

(c) Cystic stage: A mature cyst is a quadrinucleate spherical body, ranging from 8-20µm in diameter, its cytoplasm is clear and hyaline and nuclear structure retaining the characters of trophozoite. During encystment, the parasite is surrounded by a highly refractive membrane called the cyst wall which is 2-3 nm diameters.

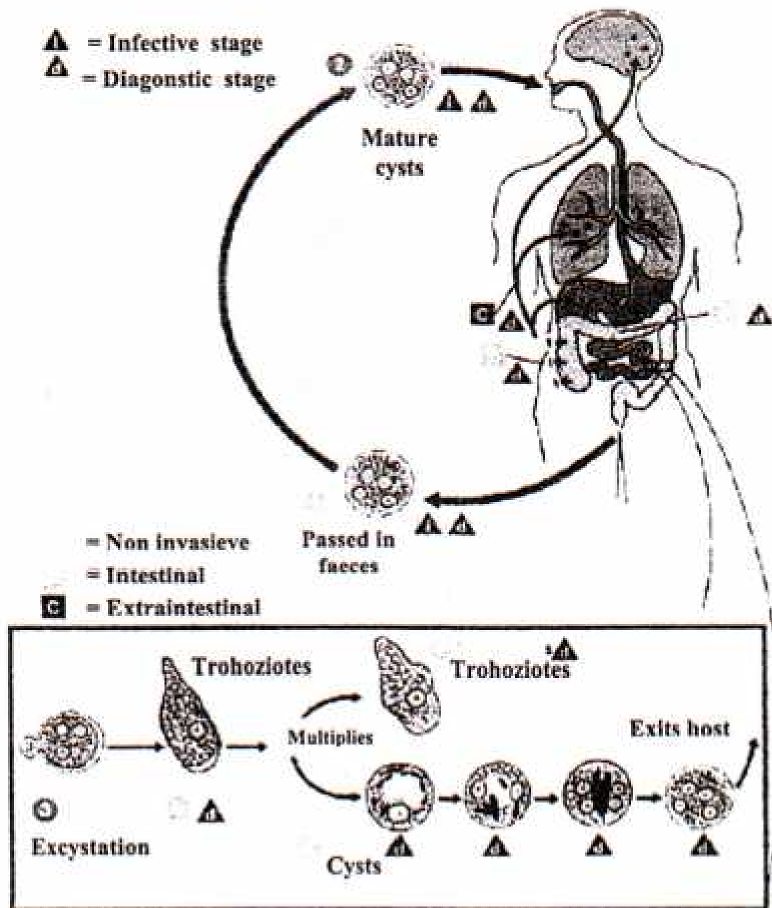
The cysts begin as a uninucleated body but soon divided by binary fission and develops into binucleated and quadrinucleate bodies. During the process of division, the nucleus undergoes gradual reduction in size becoming 2µm in diameter. The cytoplasm of the cyst shows in the stage of development the following characteristics:

Life Cycle of *Entamoeba histolytica:*

Entamoeba histolytica passes its life cycle only in one host-the man. The mature quadrinucleated cysts are the infective form of the parasite. Trophozoite if ingested is killed by stomach acids since they have no protective covering.

Encystation occurs in the lower ileum. The cyst wall is resistant to the action of gastric juice but is digested by the action of tripsin in the intestine. Each cyst liberates single amoebae with four nuclei which eventually forms eight amoebulae. The young amoebulae being actively motile invade the tissues and ultimately lodge in the sub mucous tissue of large gut and may destroy the tissue of the colon wall (by secreting an enzyme-cytolysine)

or my cause ulceration (acute dysentery) in which trophozoites are discharged along with the slough. Some trophozoites are carried away to the liver through the radicals of portal vein. Trophozoites may multiply in the lumen of the colon, the colon wall and other organ they invade. Trophozoites in the may either encysted or remain trophozoites and in either form pass in feces. Trophozoites are more common in dysentery stool. Most trophozoites disintegrate soon after passage. Cyst may be ingested and the life cycle continues.



Pathogenesis of Amoebiasis:

Clinical Features: The incubation period of intestinal amoebiasis can vary, ranging from a few days to months or years (Garcia and Bruckner, 1997), but is generally 1 to 4 weeks (Garcia, 1997). The wide spectrum of intestinal infection ranges from asymptomatic to transient intestinal inflammation to a fulminant colitis with an array of manifestations that may include toxic megacolon and peritonitis.

Asymptomatic Colonization: In up 90% of *E. histolytica* infections, the symptoms are absent or very mild (Gatti *et al.,* 2002; Jackson, 1985). These patients have normal rectosigmoidoscopic findings, without a history of blood in stool samples. Cysts and trophozoites lacking ingested RBCs may be visible on microscopy (Garcia and Bruckner, 1997). Interestingly, most individuals infected with *E. histolytica,* but not *E. dispar,* develop serum antibody responses to the parasite even in the absence of invasive disease (Abd-Alla *et al.,* 1998). It was thought that asymptomatic infection by *E. histolytica* is common; sings and symptoms of invasive amoebiasis develop in approximately 10% of the infected population (Jackson, 1990). Asymptomatic *E. dispar* infections do not show evidence of disease or a serum anti-amebic antibody response, while symptomatic *E. histolytica* intestinal infection does show a systemic immune response (Jackson, 1990).

Amebic Colitis and Dysentery: Although people can be asymptomatically colonized with *E. histolytica*, they should be treated. Otherwise, some of these subjects, called cyst carriers, may be dangerous environmentally or may develop colitis after a period of months (Jackson, 1990). Symptoms commonly attributed to *E. histolytica* colitis or dysentery with abdominal pain or tenderness and diarrhea (Restrepo *et al.,* 1996). A single stool examination has a low sensitivity of detecting the parasite (Markell *et al.,* 1999). The best diagnostic method is detection of *E. histolytica* antigen or DNA in stool (Haque *et al.,* 2006; Haque *et al.,* 1998).

Pathogenicity (Schmidt, 1981)

Entamoeba histolytica is unique among the amebas of humans in its ability to hydrolyze host tissue. Once in contact with the mucosa, the amebas secrete the photolytic enzymes, which enable them to penetrate the epithelium and begin moving deeper. The initial

lesion usually develops initially in the caecum appendix, or upper colon, then spreads the length of the colon. The number of parasites build up in the ulcer, increasing the speed of mucosa; destructions. The muscularies mucosae is somewhat of a barrier to further progress, and pockets of amebas form, communicating with the lumen of the intestine through a slender, duct-like ulcer. The lesion may stop at the basement membrane or at the muscular is mucosa and then begin eroding laterally, causing board, and swallowed areas of necrosis.

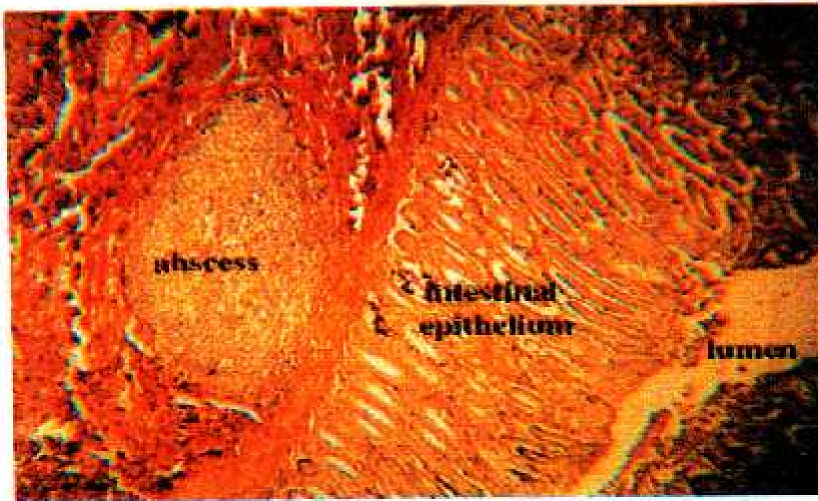
The tissues may heal nearly as fast as they are destroyed, or the entire mucosa may become poked. These early lesions usually are not complicated by bacterial invasion, and there is little cellular response by the host. In order lesion the amebas, assisted by bacteria, may break through the muscular is mucosa, infiltrate the sub mucosa, and even penetrate the muscle layers and serosa. This enables trophozoites to carried by blood and lymph to ectopic sites throughout the body where secondary lesion then form. A high percentage of deaths results from perforated colons with concomitant peritonitis. Surgical rapier of perforation is difficult because a heavily ulcerated colon becomes very delicate. Sometimes a granulomatous mass, called an ameboma, forms in the wall of the intestine and may obstruct the bowel. It is the result of cellular responses to a chronic ulcer and often still contains active trophozoites. The condition is rare except in Central and South America.

Secondary lesions have been found in nearly every organ of the body, but the liver is most commonly affected (about 50% of all cases). Regardless of the secondary site, the initial infection was an intestinal abscess, even through it may have gone undetected. Hepatic amebiasis results when trophozoites enter the mesenteric venules and travel to the liver through the hepatoportal system. They digest their way through the portal capillaries and enter the sinusoids, where they begin to form abscesses. The lesions thus produced may remain pinpoint size or they may is continue to grow, sometimes reaching the size of a grapefruit. The center of the abscesses filled with necrotic fluid, a median zone consists of liver tissue being attacked by amebas, although it is bacteriologically

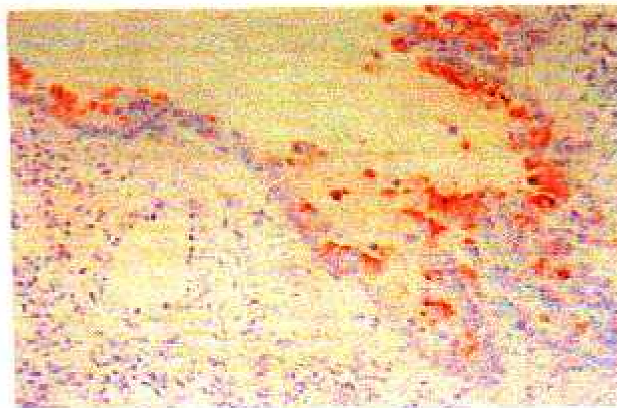
sterile. The abscess may rupture, pouring debris and organisms into the body cavity, where they attack other organs.

Pulmonary amebiasis is the next most common secondary lesion. It usually develops by metastasis from a hepatic lesion but may originate indecently. Most cases originate when a liver abscess ruptures through the diaphragm. Other ectopic sites occasionally encountered in the brain, skin and pains possibly acquired venereally). Rare ectopic sites are kidneys, adrenals spleen, male and female ganitalia, pericardium and others. As a role, all ectopic abscesses are bacterially sterile. Symptoms of infections vary greatly between cases. The strain of *Entamoeba histolytica* present the host's natural or acquired resistance to that strain, and the host's physical condition when challenged all affect the course of the disease in any individual. When conditions are appropriate, a highly pathogenic strain can cause a sudden onset of severe disease. This usually is the cases with water-borne epidemics.

More commonly, the disease develops slowly, with intermittent diarrhea, cramps, vomiting and general malaise. Infection in the cecal area may mimic the symptoms of appendities. Some patients tolerate intestinal amebiasis for years with no sign of colitis (but are passing cysts) then suddenly succumb to an ectopic lesion. Depending on the number and distribution of intestinal lesions, the patient might develop pain in the entire abdomen, fulminating diarrhea, dehydration, and loss of blood. Amebic diarrhea is marked by bouts of abdominal discomfort with four to six loose stools per day but little fever.



Photograph- : Intestinal amoebiasis.



Photograph- : liver abscess

Acute amebic dysentery is a less common conditional, but the sufferer from this affliction can best be described as miserable. The on set may be sudden after an incubation period of 8 to 10 days after a long period as an asymptotic cyst passer. In acute onset there may be headache, fever, severe abdominal cramps, and sometimes prolonged infective straining at stool, average of 15 to 20 stools are passed per day, consisting of liquid feces flecked with bloody mucus. Death may occur from peritonitis, resulting from gut perforation, of from cardiac failure and exhaustion. Bacterial involvement may lead to

extensive scarring loss of peristalsis. Symptomatic arising from ectopic lesion is typical for any lesion of the effected organ.

Epidemiology:

Entamoeba histolytica comprises an unknown number of pathogenic and non pathogenic strains. Infection by *Entamoeba histolytica* is ubiquitous and approximately ten present of the world's population carry the parasite (Walsh,1986), but prevalence of invasive amebiasis is a major health problem in western and south eastern Africa, southeast Asia, Chaina and Latin America specially in Mexico. At present on a global scale this infection represents one of the mint common causes of death from intestinal parasitic diseases (WHO 1987).

In many regions amebiasis is an important cause of diarrhea and dysentery. In Mexico City up to 15% of acute diarrhea and dysentery in children were found to be associated with *Entamoeba histolytica* (WOH 1987). Endemic intestinal infection is usually found in communities with inadequate sanitation and poor socio-economic condition. The prevalence of *Entamoeba histolytica* has been found to be 0.5% to 38% in Asia, 0.6% to 37% in Africa and 0-49.4% in America (Walsh 1988).

Transmission of Parasites:

Transmission of *E. histolytica* from man to man is done through its encysted stage and infection occurs through the ingestion of cysts. Faecal contamination of drinking water food and vegetables may cause this infection. Eating of uncooked or poorly cooked vegetables and fruits, which have been fertilized with infected human faeces, has often led to the occurrence of the disease. There are two types of carriers, **Contact carrier** and **Convalescent carrier**. The former are supposed to be so-called "healthy" carriers who have never suffered from amoebic dysentery and whose health appears to remain impaired. The latter are those who have recovered from a clinical attack of acute amoebic dysentery. Housefly transmit cyst while passes from faeces to unprotected foodstuff. The cysts of *E. histolytica* have been found in the dropping of cockroaches, which also sever as a source of infection.

Morphology, Life cycle, Transmission Pathogenesis and Epidemiology of *Giardia lamblia*

Morphology of Trophozoite:

The trophozoite is usually described as being tear-drop shaped from the front with the posterior end being pointed. If one examines the trophozoite from the side it resembles the curved portion of a spoon. The concaved portion is the area of the sucking disc. There are four pairs of flagella, 2 nuclei, 2 axonemes, and two slightly curved bodies which are called the median bodies. The trophozoites usually measures from 10-20 µm in length and from 5-15 µm in width.

Morphology of cyst:

The cyst may be either round or oval and contain 4 nuclei, axonemes, and median bodies. Often some cysts appear to be shrunk or distorted and one may see 2 holes, one around the cyst wall itself and one inside the cyst wall around the shrunken organism. The holes effect around the outside of the cyst is particularly visible on the permanent stained smear. Cysts normally measure from 11 to 14 µm in length and from 7 to 10 µm in width.

Life cycle of *G. lamblia*:

G. lamblia is a flagellated protozoan parasite that colonizes and reproduces in the small intestine, causing giardiasis. Both the trophozoite and cyst are included in the life cycle of *G. lamblia*. In the trophozoite stage the parasite multiply in the intestine of man by means of longitudinal binary fission, thus producing 2 daughter trophozoites. The most common location of the organism is in the crypts within the duodenum, when conditions in the duodenum are unfavourable, encystment occurs, usually in the large intestine. During encystment, a thick resistant wall is secreted by the parasite and the cell then divides into two within the cyst. Infection of man is brought about by ingestion of cysts, within 30 minutes of ingestion, the cyst hatches out two trophozoites which then multiply in enormous number and colonies in the duodenum. To avoid the high acidity of duodenum

Giardia often localizes in the biliary tract (gall bladder). Excystation would normally occur in the duodenum or appropriate culture media (Boreham, 1981).

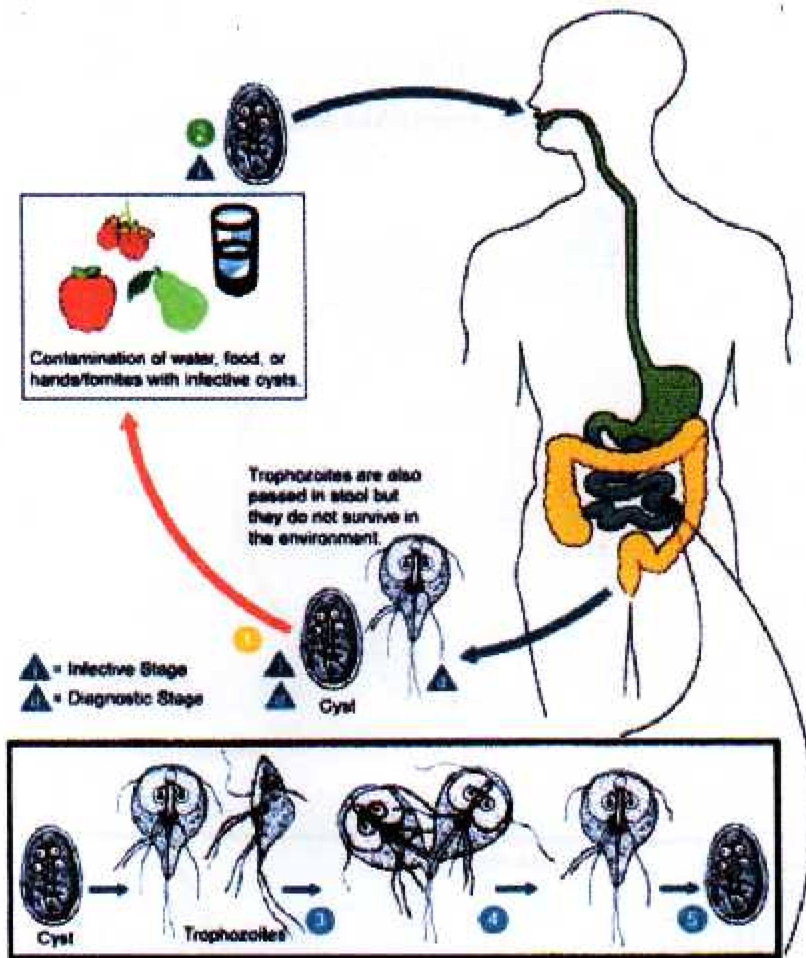


Fig: Life cycle of *Giardia lamblia.*

Transmission of *G. lamblia*:

Transmission of *G. lamblia* is predominantly by faecal-oral contamination. Levels of infection are therefore highest under condition of poor hygienic and sanitation, particularly in tropical and subtropical environments. Numerous cases of *G. lamblia*

infection have been associated with contaminated water. There is sufficient evidence to show that at least some isolates of *G. lamblia* are not host-specific and that human and a variety of other animals naturally share the parasite. Most authorities therefore regarded giardiasis as a zoonosis (Bapta; 1990).

Epidemiology and Prevention:

Transmission is by ingestion of viable cysts. Although contaminated food or drink may be the source, intimate contact with an infected individual may also provided the infection mechanism. This organism tends to be found more frequently in children or in groups that live in close quarters (Danciger and Lopez 1975).

Treatment:

In the majority of cases, giardiasis can be eliminated with the use of quinacrine (Atabrine). Metronidazole is also very effective, but is listed as the second drug of choice due to potential carcinogenicity in rats and mutagenic changes in bacteria. Although these changes have never been demonstrated in humans, it is not recommended for pregnant women.

Immunity

Immunity is the state of protection of an individual against foreign organism or substances (Kuby 1997a). Immunity can be either innate or acquired (adaptive), active or passive and either natural or artificial.

Active Natural- It develops slowly after contact with infection, is long term and antigen specific.

Active Artificial- It develops slowly after immunization, lasts for several years and is specific to the antigen for which the immunization is given.

Passive Natural- It can cross the placental barrier to the fetus and develops immediately and is responsible for the 3 to 6 months immune protection of newborns.

Passive Artificial- Conferred by injection of gamma globulin, develops immediately (Choudhury 1999).

The immune system is composed of many interdependent cell types that collectively protect the body from bacterial, parasitic, fungal, viral infections and from the growth of tumor cells. When an antigen is initially encountered, the cells of the immune system recognize the antigen as non-self and either elicit an immune response or become tolerant to it, depending on the circumstances. An immune reaction can take the form of cell mediated immunity (Immunity dependent on $CD4^{+}$TH1 and TH2 cells and CD8 T cytotoxic lymphocytes or CTLs, macrophages, neutrophils, eosinophils and NK cells.) or humoral immunity (involve the production of antibodies) directed against antigen (Turgeon 1996). Most of these cells can engulf bacteria; kill parasites, tumor cells or virus-infected cells. Often these cells depend on the T-helper subset for activation signals in the form of secretions formally known as cytokines, lymphokines or more specifically interleukins (Roitt 2001).

Antibodies (Immunoglobulins):

Antibodies are specific glycoproteins referred to as immunoglobulins (Ig). These are symmetrical, four-chain molecules and consist of two identical glycosylated heavy chains and two identical non-glycosylated light chains (Gally 1973). The B-lymphocyte cells of the immune system produce antibodies in plasma and in many body fluids such as tears, saliva and colostrums (Turgeon, 1996). Antibodies react against microorganisms (viruses, yeast, parasites, and bacteria) and allow' them to be killed by the white blood cells. Since antibodies are proteins, they can themselves function as potent immunogens to induce an antibody response. There are five major classes (IgA, IgG, 1gM, IgD and IgE) of antibody. Each class has a unique chemical structure and a specific function. IgG stands for immunoglobulin G or antibody G and so forth.

Immunoglobulin G (lgG):

The major immunoglobulin in normal serum is IgG. It is a 7S molecule with a molecular weight of approximately 150,000 (Turgeon, 1996). IgG accounts for 70-75%, of the total immunogiobulin pool. There are four IgG subclasses in humans, numbered in accordance with their decreasing average serum concentrations: IgGl (9 mg /mI), IgG2 (3 mg/mi), IgG 3 (1 mg/mI) and IgG 4 (0.5 mg/mi). The structural characteristics that distinguish

these subclasses from one another are the size of the hinge region and the number and position of the interchain disulfide bonds between the heavy chains. The subtle amino acid differences between subclasses of IgG affect the biological activity of the molecule.

IgGI, IgG3 and IgG4 are readily cross the placenta and play an important role in protecting the developing fetus.

IgG3 is the most effective complement activator, followed by IgGI; IgG2 is relatively inefficient at complement activation and IgG4 is not able to activate complement at all. IgGi and IgG3 bind with a high affinity to Fc receptors on phagocytic cells; IgG4 has n intermediate affinity and IgG2 has an extremely low affinity. Fc binding permits IgG to function as an opsonin (Kuby, 1997b).

During the fetal and newborn age, almost all IgG antibodies, irrespective of subclasses, in the circulation are of maternal origin. This immunoglobulin diffuses more readily than other immunoglobulins into the extra vascular spaces and it neutralizes toxins or binds to microorganism in extra vascular spaces.

GENERATION OF B. CEIL AND CELL RESPONSES

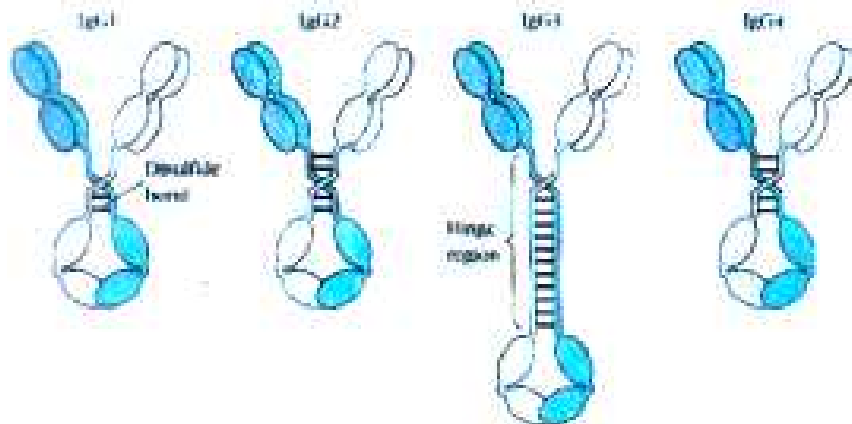


Figure 1: General structure of the four subclasses of human IgS (Kuby 1997)

Natural antibodies

When a fetus born, the mother provides the fetus with a wide repertoire of antibody developed by her immune system against antigen to which she has become immune. The fetus is able to synthesize all IgG subclasses, early in fetal life; however, the contribution is minimal and accounts for only about I % of the IgG in cord blood (Morell *et. al.* 1976).

Cells bearing surface immunoglobulin are present in fetal spleen, blood and lymph nodes at relative isotype frequencies similar to those in normal adults. 1gM synthesis has been demonstrated in the fetus by 10th weeks of gestation; IgG synthesis occurs later, and IgA synthesis occurs at 30th weeks. The transfer of maternal immunoglobulins (IgG) to infants starts about the 6th month of gestation and increases sharply and fetal level of IgG attains the maternal level during the 8th month of gestation. IgG levels then gradually rises with age to become indistinguishable from adult levels by 5-Il yrs of age (Lee *et. al.* 1986).

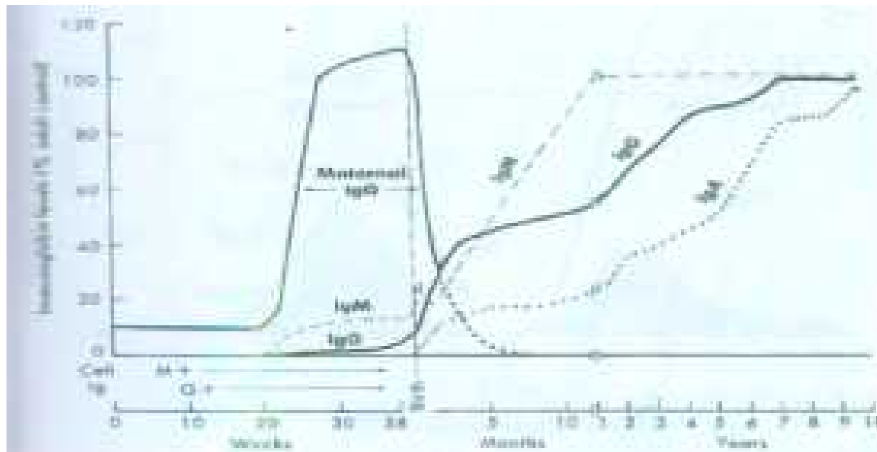


Figure 2: Immunoglobulin concentration in newborn, infant and children

Antibody synthesis and humoral effector response

Production of antibody is induced when the host's immune system comes into contact with a foreign antigenic substance and reacts to this antigenic stimulation. Humoral effector response involves interaction of B cells with antigen and their subsequent proliferation and differentiation into antibody-secreting plasma cells. Antibody functions as the effectors of the humoral response by binding to antigen and neutralizing it or facilitating its elimination. Antibody can cross-link the antigen, forming clusters that are more readily ingested by phagocytic cells (NK=natural killer cells, Macrophages etc).

Binding of antibody to antigen on a microorganism also can activate the complement system, resulting in lysis of the foreign organism. The kinetics and other characteristics of the humoral response differ considerably depending on whether they result from activation of naive lymphocytes (primary response) or memory lymphocytes (secondary response). A primary response is characterized by a lag phase, during which naive B cells undergo clonal selection in response to the antigen and differentiate into plasma cells and memory cells.

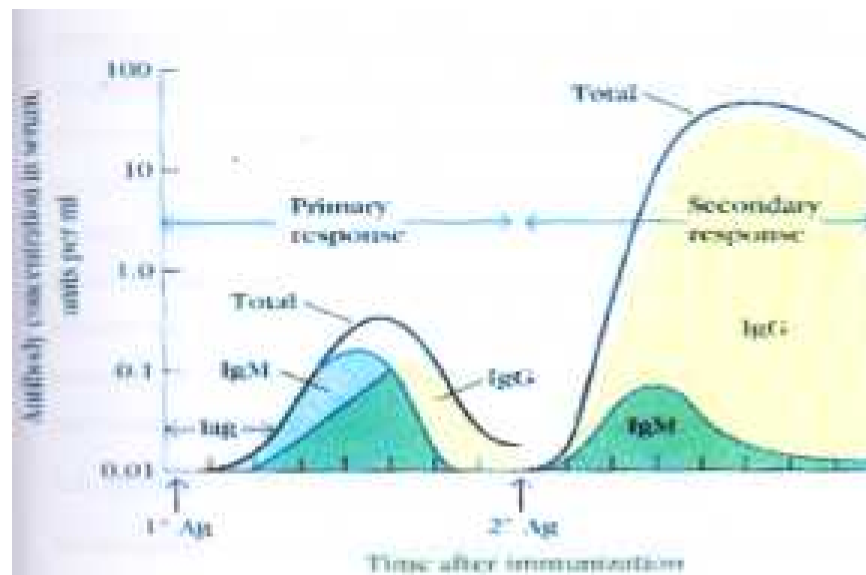


Figure 3: Concentration and isotype of serum antibody after primary and secondary

Immunization (Kuby, 1997)

The lag phase is followed by a logarithmic increase in serum antibody level, which reaches a peak, plateaus for a variable time and then declines. The lag phase lasts 3-4 days when eight or nine successive cell division occur, peak plasma cell levels are attained within *4-5* days and peak serum antibody levels are attained by *5-7* days. In primary humoral response 1gM is secreted initially, often followed by IgG. The memory B cells formed during a primary response stop dividing and enter the Go phase of the cell cycle. These cells have variable life spans and some persist for the life of the individual. The kinetics of the primary response, as measured by serum antibody level, vary depending on the nature of the antigen, the route of antigen administration, the presence or absence of adjuvants and the species or strain being immunized.

The capacity to develop a secondary humoral response depends on the existence of a population of memory B cells and memory T cells. Antigen activation of this memory cells result in a secondary antibody response that is faster, of higher affinity and causes secretion of IgG isotype of 1g. It has been demonstrated that memory B cells express antigen specific receptors with higher affinity (Gibb *et. al.* 1995). The population of memory B cells specific for a given antigen is considerably larger than the population of corresponding naive B cells, accounting in part for some of the difference between the primary and secondary response. Moreover, memory B cells are more effective than naive B cells in capturing antigen and processing it rapidly.

The higher level of antibody coupled with the overall higher affinity provide an effective host defense against re infection. The change in isotype that occurs in the secondary response provides antibodies whose biological effector functions are particularly suited to eliminate a given pathogen (Kuby, 1997c).

Immune mechanisms involved in antiviral immunity

The immune system has mechanisms which can attack the virus in both extra and intra cellular phases of its life cycle and which involve both non-specific and specific effector mechanisms.

Non-Specific Mechanisms

Interferons:

Viral infection of cells directly stimulates the production of interferons. “Type 1” interferons which are produced non-specifically by many cell types in response to viral infection are quite distinct from the T cell cytokine gamma interferon which is produced by CD4+ and CD8+ T cells in response to antigenic stimulation. Type 1 interferons lead to the induction of an “antiviral state” in the cells, which is characterized by inhibition of both viral replication and cell proliferation, and also enhancement to the ability of natural killer cells to lyse virally infected cells.

Natural Killer Cells

Natural killer (NK) cells are a subset of lymphocytes found in the blood and tissues, which lack antigen specific surface receptors (TCR or immunoglobulin receptors). Phenotypically, NK cells do not express the characteristic cell surface markers that define T cells and B cells, and so NK cells represent a distinct lineage of lymphocytes. NK cells possess the ability to recognize and lyse virally infected cells and certain tumor cells. The main advantage that NK cells have over antigen-specific lymphocytes in antiviral immunity is that there is no “lag” phase of clonal expansion for NK cells to be active as effectors, as there is with antigen-specific T and B lymphocytes. Thus NK cells may be effective early in the course of viral infection, and may limit the spread of infection during this early stage, while antigen-specific lymphocytes are being recruited and clonally expanded.

Specific Mechanisms:

Both humoral and cell mediated arms of the immune response play a role as specific effector mechanisms in antiviral immunity.

Antibody

Specific antibodies are important in and may protect against viral infections. During the course of a viral infection, antibody is most effective at an early stage. The most effective type of antiviral antibody is ‘neutralizing’ antibody this is antibody which binds to the

viral usually to the viral envelope or capsid proteins, and which blocks the virus from binding and gaining entry to the host cell. Virus specific antibodies may also act as opsonins in enhancing phagocytosis of virus particles - this effect may be further enhanced by complement activation by antibody-coated virus particles. In addition, in the case of some viral infections, viral proteins are expressed on the surface of the infected cell (Roitt 2001). These may act as targets for virus-specific antibodies, and may lead to complement-mediated lysis of the infected cell, or may direct a subset of natural killer cells to lyse the infected cell through a process known as Atibody-Directed Cellular Cytotoxicity (ADCC).(Cates, 1973).

Cytotoxic T Cells:

The principal effector cells, which are involved in clearing established viral infections, are the virus specific CD8+ cytotoxic T lymphocytes **(CTL).** These cells recognize (viral) antigens that are presented at the cell's surface as short peptides associated with self-class I MHC molecules. MHC class I molecules are expressed on almost all somatic cells, so virtually any cell, on infection with virus, can act as a "target" cell for antigen specific CTL.

Immunopathology of *E. histolytica* infection (Denis and Chadee, 1988):

About 10% of the world's population is infected with *E. histolytica* and most infections are asymptomatic — ie. Infected patients show no clinical signs of the disease. However, about 10% of infected individuals suffer from one form or another of amoebiasis. Of these, 80—90% show symptoms of dysentery with amoebic invasion presumably limited to the intestinal mucosa. In advanced forms of the disease, the amoebae disseminate to extra-intestinal sites, most notably the liver, causing amoebic liver abscesses. Therefore, in symptomless carriers, which account for about 90% of infected individuals, the parasite is believed to exist as a harmless commensal. At present, it is not known what host factors or virulent traits in amoebae trigger pathogenicity, and there is disagreement about parasite stability in terms of isoenzyme electrophoresis patterns (zymodeme pro-files) as a marker for virulent strains.

Infections with *E. histolytica* that are confined to the intestinal lumen in the absence of invasion do elicit an antibody response but peripheral blood lymphocytes are unresponsive to amoebic antigens. In asymptomatic individuals, amoebic antigens or soluble amoebic products can pass out of the intestinal lumen and elicit a systemic immune response. It may be that specific amoebic proteins cause a local transient immunosuppressant in the gut facilitating amoebic invasion.

While there is no evidence of defective cell-mediated immune responses in asymptomatic individuals, patients with invasive disease show a spectrum of immune dysfunction mechanisms. It is not clear whether intestinal invasion (amoebic colitis), in the absence of abscesses in extra-intestinal sites, elicits a differential immune response and, there- fore, this review will deal exclusively with invasive amoebiasis (regardless of the organ involved) and its immunopathological consequences.

Mechanisms of cytotoxicity

The pathogen city of virulent *E. histolytica* seems to act via two mechanisms — one dependent on contact, and another acting from a distance via the release of soluble components (amoebapore or cytotoxins).

The contact required for contact- dependent killing by trophozoites is mediated by a surface adhesin molecule that can be inhibited by galactose (Gal), N-acetylgalactosamine (Ga1NAc) or galactose-terminal oligosaccharides.

This adherence process is an absolute prerequisite for in vitro amoebic cytolytic activity.

Pathogenic amoebae are incredibly strong chemoattractants for neutrophils. However, these cells possess little or no cytotoxic potential towards virulent *E. histolytica* trophozoites — instead, neutrophils are rapidly killed upon contact with amoebae. Neutrophil lysis may contribute considerably to tissue damage, as suggested by the observation that amoeba—neutrophil interaction in vitro leads to a significant destruction of Chinese ovary cells, apparently independent of reactive oxygen intermediates (ROIs).

Immune complexes:

Trophozoites of *E. histolytica* have been found to release a small molecular weight factor that can inhibit the locomotion of human monocytes, but not polymorphonuclear leucocytes (PMNLs). Keeping the monocytes at bay by directly affecting their locomotion not only disturbs the afferent limb of cell-mediated immunity, but also prevents the harmful effects of macrophages upon amoebae, should they come close to the parasite. This constitutes a likely escape mechanism of *E. histolytica* from the host defences. As such, the lack of macrophages in hepatic and intestinal amoebic lesions may explain, in part, the remarkable absence of scar tissue observed upon recovery from these lesions, since macrophages play an important role in stimulating collagen deposition, a crucial part of the wound healing process. Intestinal lesions show a spectrum of findings. Immunosuppression accompanies invasive amoebiasis, but unlike leprosy, tuberculosis and malaria, the immunosuppression appears specific in its expression.

Meanwhile, splenic and peritoneal macrophages were not significantly down-regulated in these various functions, even in the late stages of the disease. Like abscess-derived macrophages, uninfected murine and gerbil peritoneal liver and splenic macrophages treated with a crude *E. histolytica* extract *in vitro* showed gross down-regulation in their responsiveness to lymphokines, their release and in their oxidative metabolism and immune phagocytosis (M. Denis and K. Chadee, 1988). This defect was titrated with different doses of the crude amoebic extract, showing that nanogram amounts of the extract, or proteins released in amoebic-conditioned medium, were efficient at producing these dysfunctions. These results strongly suggest that secreted and/or intrinsic amoebic proteins can exert profound dysfunction at the afferent and efferent levels of an immune response. (Bartholomew, 1987).

The contention that virulent amoebae induce cellular immunosuppression which may allow tissue invasion is sup- ported by the findings of Bruchard *et al.,* (1998) who showed the suppressive potential of *E. histolytica* extracts on the immune response of hamsters. An exhausting restricted or polyclonal T-cell activation by amoebic antigens or by a lipid-free aqueous extract from *E. histolytica* could result from over stimulation to

depression of the host cellular immune response. Amoebic invasion may then mimic the situation found in tumour bearing animals— amoebic antigens may transiently act as potent suppressors of cell-mediated immunity, allowing the parasite to invade the tissues. In addition, local intestinal anaphylactic reactions, in which histamine and other mediators are released, may have suppressive effects upon T-cells leading to the degree of immuno-suppression required for invasion.

Amoebic killing by macrophages and lymphocytes:

Indirect evidence for a role for macro- phages in the killing of amoebae has been obtained from several studies. *In vitro,* macrophages can be activated by lymphokines to kill *E. histolytica* trophozoites. This killing mechanism is contact-dependent and is mediated by both oxygen- and non-oxygen-dependent pathways.

The cytotoxic properties of lymphocytes from recovered patients may depend on lymphocytotoxins, since direct contact of lymphocytes and amoeba does not always appear to be essential for the lytic effect. Supernatant fluid from such lymphocytes stimulated with amoebic antigen has been claimed to be equally effective. Collectively, these observations suggest that (1) invasive amoebiasis triggers a substantial immunosuppressive response in the host, affecting both the afferent and accessory functions of macrophages, that is seemingly triggered by amoebic proteins secreted in the environment, and (2) killing of virulent amoebae by macrophages is dependent upon sequential activation of these cells by different soluble factors, similar to the activation pattern for the acquisition of tumoricidal activity. Furthermore, it appears that resident tissue macrophages, mainly Kupffer nd spleen cells, are refractory to activation for the acquisition of cytotoxic activity against *E.histolytica.*

Diabetes

The disease was called diabetes by Greek and Roman physicians because the word diabetes means a siphon, referring to the large urine volume. Rather courageously, they distinguished diabetes mellitus from diabetes insipidus by the sweet taste of the urine; mellitus= sweet, insipidus= tasteless.

Diabetes Mellitus:

Diabetes mellitus is characterized by hyperglycaemia due to an absolute or relative deficiency of insulin. The disease can present with asymptomatic glycosuria detected on routine physical examination or with symptoms of diabetes, ranging from polyuria to coma as a result of diabetic ketoacidosis.

Diabetes mellitus (DM) is a metabolic syndrome which is usually characterized by a permanent or relative lack of pancreatic hormone insulin. Prevalence of non insulin dependent diabetes mellitus are higher in urban (7-9.7%) than in rural subjects (3.84%). Diabetes is associated with alteration in central monoaminergic system, changes which are also linked to depression. Therefore, a functional link between diabetes and depression may exist at the level of brain monoamine system. (Alteras, 1979).

Major Symptoms:

Appetite and weight changes, disturbed defaecation, hair distribution, lethargy, polyuria and lump in the neck (goiter).

Syndromes: 1. Thyrotoxicosis: Preference for cooler weather, weight loss, increased appetite (polyphagia), palpitation, increased sweeting, nervousness, irritability, diarrhea, amenorrhoea, muscle weakness, exertional dyspnoea.(Bagan, 1988).

Pothyroidism (myxoedema): Preference for warmer weather, lethargy, swetting of eyelids (oedema), hoarse voice, constipation, coarse skin, hypercarotenaemia

Diabetes mellitus: Polyuria, polydipsia, thirst, blurred vision, weakness, infections, groin itch, rash (pruritus vulvae, balanitis), weight loss, tiredness, lethargy and disturbance of conscious state. Diabetes mellitus is also characterized by polyuria, polydipsia, thirst, blurred vision, weakness, infections, groin itch, rash (pruritus vulvae, balanitis), weight loss, tiredness, lethargy and disturbance of conscious state (Sayeed, 1997).

1. **Hypoglycaemia:** Morning headaches, weight gain, seizures, sweatin
2. **Primary adrenal insufficiency:** Pigmentation, tiredness, loss of weight, anorexia, nausea, diarrhea, nocturia, mental changes, seizures (hypotension, hypoglycaemia).

3. **Acromegaly:** Fatigue, weakness, increased sweating, heat intolerance, weight gain, enlarging hands and feet, enlarged and coarsened facial features, headaches, decreased vision, voice change, decreased libido, impotence.

General Inspection: assess for evidence of dehyhration because the osmotic diuresis caused by a glucose load in the urine can cause massive fluid loss. Note obesity (non-insulin-dependent diabetics are usually obese) or sings of recent weight loss (this can be evidence of uncontrolled glycosuria). The abnormal endocrine facies (e.g., Cushing's syndrome or acromegaly) and for pigmentation (e.g., haemochomatosis-bronze diabetes) as these may cause secondary diabetes. The patient may be comatose due to dehydration, acidosis or plasma hyperosmolality. Kussmaul's breathing ('air hunger') is present in diabetic ketoacidosis due to the acidosis (this occurs because fat metabolism is increased to compensate for the lack of availability of glucose; excess acetyl-CoA is produced which is converted in the liver to ketone bodies, and two of these are organic acids).
The causes of diabetes are listed below: Criteria for diagnosis of diabetes mellitus: fasting blood suger level of 7.8 m. mol/L or more, or a two-hour postprandial blood suger level of 11.1 mmol/L or more, on more, on more than one occasion.

Primary Type 1**:** Insulin-dependent diabetes mellitus (juvenile onset); Type 2**:** Non-insulin-dependent diabetes mellitus: Obese: Maturity-onset diabetes of the young (MODY).
Secondary: Hormone-induced states (rare), acromegaly and glucagonoma.
The Abdomen history: Palpate for hepatomegaly (fatty infiltration, or due to haemochromatosis).
Change in bowel habit: Diarrhoea is associated with hyperthyroidism and hypercalcaemia and with gastrointestinal disease.
Anaemia, connective tissue disease, chronic infection (e.g., HIV, infective endocarditis), chronic liver disease, renal failure and occult malignancy may result in lethargy. Importantly, depression is a common cause of this very common symptom. And diabetes mellitus can present with this problem. (Wats, 1979).

The Endocrine history: Hormones control so many aspects of body function that the manifestations of endocrine disease are protein. Symptoms can include changes in body weight, appetite, bowel habit, hair distribution, pigmentation, sweating, height and menstruation, as well as polydipsia, polyuria, lethargy, headaches and impotence. Many of these symptoms have other causes as well and must be carefully evaluated. In particular, there may be a history of a diabetes mellitus. (Muller, 1966).

Blood:

Blood in the urine (haematuria) is abnormal and can be seen with the naked eye if 0.5mL is present per liter of urine. A positive dipstic testis abnormal and suggest haematuria, haemolobinuria (uncommon) or myglobinuria (also uncommon). The presence of more than a trace of protein in the urine in addition suggests that the blood is of renal origin. False-positive or negative results can occur if vitamin C is being taken.

There are four types of haemorrhages: Streaky haemorrhages near the vessels (linear or flame-shaped), large ecchymoses that obliterate the vessels, petechiae which may be confused with microaneurysms, and subhyaloid haemorrhages (large effusions of blood which have a crescentic shape and well-marked borders and a fluid level may be seen). The first two types of haemorrage occur in hypertensive and diabetic retinopathy. They may also result from any cause of raised intracranial pressure or venous engorgement, or a bleeding disorder. The third type occurs in diabetes mellitus, and the fourth is characteristic of subarachnoid haemorrage. (Vazquez, 1995).

The Urine sediment:

Every patient with suspected renal disease should have the urine sediment from the first morning urine sample (to avoid dilute urine) examined. Centrifuge 10 mL of the urine at 2000 rpm for four minutes. Remove the supernatant, leaving 0.5 ml- shake well to re-suspend, then place one drop on a slide with a cover slip. Look at the slid using a low-power microscope, and at specific formed elements under the high-power field for identification. And diabetes mellitus can present with this problem. (Liotta, 1987).

Immuno pathogenesis in diabetic patients

Humans are the only natural host for measles virus. The virus gains access to the body via the respiratory tract, where it multiplies locally; the virus then spreads to the regional lymphoid tissue where further multiplication occurs. Primary viremia disseminates the virus, which then replicates in the reticuloendothelial system. Finally, a secondary viremia seeds the epithelial surfaces of the body, including the skin, respiratory tract and conjunctiva, where focal replication occurs (Brooks *et. al.,* 1998). Viral antigens, which can be found by immunofluorescent techniques in and on the surface of the cells and lymphocytes, induce the immune response. First, natural killer cells and cytotoxic T cells mount a cell-mediated reaction that contains the virus and limits its spread within cells. Later, B cells are primed to produce antibody. Defects in the cellulaiç immune system, as in severe malnutrition, cancer or primary immuno deficiencies allow widespread multiplication of the virus to cause fatal giant cell pneumonia. Around day 8, the blood carries measles viruses, either free or in mononuclear cells, to the target tissue, which are the epithelia of the eye, lung and gut. The first antibody to appear is to the nucleoprotein antigen. The second, who is largely responsible for neutralization of the virus, is to the haemagglutinin. Finally, the antibody to the fusion glycoprotein appears in a low titer.

This antibody stops cell-to-cell spread of the virus. At this stage, the child is markedly immunosuppressed and thus susceptible to secondary infection of eye, mouth, gut and lung. The mechanism of immunosuppression is complex: T lymphocytes specially the T-helper subset, are infected with the virus and depleted, antigen processing is defective, as is the production of lymphokines and a suppressor factor, possibly an immune complex, circulates in the serum. By the third week, around day 21 as the diabetic patient recovers, antibody is in full production. Levels remain elevated for the rest of the diabetic patients life, either because of repeated sub clinical infections or because the virus persists in latent from in the spleen and other organs, so stimulating antibody (Whittle and Aaby 1996).

REVIEW OF LITERATURES

The purposes of review of literatures were to collect the information regarding the background, history, and previous relevant studies. A remarkable number of national and international literatures were cited and reviewed which were closely related to the present study. The summarized information's / literatures were given below:

1. Parasitic infestation among diabetic and non-diabetic patients in Bangladesh and other countries:

Of the principal four, the important point is that the cysts of *E. coli* and *E. hartmanni* may be distinguished by light microscopy applying well-understood criteria from those of *E. histolytica* and *E. dispar* but the latter two are indistinguishable from each other. After much research and argument, it is generally accepted that, what was earlier known as *E. histolytica* actually comprises two genetically distinct but morphologically indistinguishable species the invasive disease causing parasite *E. histolytica* (formerly known as the pathogenic zymogene of *E. histolytica*), and the non-pathogenic parasite *E. dispar* (formerly known as the non-pathogenic zymogene of *E. histolytica*). *E. dispar* has never been documented to cause colitis or liver abscess, but is responsible for many case of asymptomatic infection (Kappus, 1934).

In 1934, a study at intestinal parasites in the United States was done by Kappus. The analyzed results of 216,275 stool specimens examined by the state diagnostic laboratories, parasites were found in 20%, percentage were highest for protozoan: *Giardia lamblia* (7.2) and *Entamoeba histolytica* (0.9%). Identifications of *G. intestinalis* increased. Krogstad from the 4% average found in 1978, with 40 states reporting increase and seven reporting decrease. Seasonally, *Giardia* identifications increased in the summer and fall, especially in the Data for 1991: *Giardia* was found in 5.6%. States

reporting percentages of *Giardia* identification in the highest quartile for both 1987 and 1991 were located in the Midwest or in Northwest.

Muazzem and Ali (1968) in a study on children of Dhaka noted the following prevalence of parasites- *Entamoeba histolytica* (12.15%) and *Giardia lamblia* (14.4%).

Nuruzzaman (1974) and Huda (1976) carried out a study with hospitalized patients. The incidence rate was over 70%. They reported the prevalence rate of parasites separately where 15.3 % for *Entamoeba histolytica* and 13.8% for *Giardia lamblia.*

In 1975, Islam *et al.,* examined 933 students of Dhaka University. Mean hemoglobin levels were 80.5% for men and 70% for women. Among the student, 57.33% had single or multiple intestinal parasitic infections. Of this *Entamoeba histolytica* and *Giardia lamblia* were commonest. It was suggested that chronic nutritional deficiencies compounded by these two parasites, leading to low weight and low hemoglobin's count.
Muttalib *et al.* (1975) in a study on Dhaka University student's who came from different villages of Bangladesh, found that the total prevalence of intestinal parasites was 56.98%.

Haque and Sheikh (1976) noted that overall incidence of intestinal parasites in Dhaka city was 65.80%. The male children were slightly more infected than the female children. They observed that both housing and economic status played vital role in the occurrence of intestinal parasites where the housing and environmental conditions were found to be more influential than economic status.

Muttalib *et al.,* (1976) studied on the prevalence of intestinal parasites in rural children of Bangladesh and he found that about 92.9% children in rural areas of Bangladesh were infected by intestinal parasites; *Entamoeba histolytica* in 40.88% and *Giardia lamblia* in 46.04%.

Antigen detection test for *E. histolytica* and *G. lamblia* infection have been used by several authors in several countries. *E. histolytic* and *G. lamblia* are common human intestinal

protozoan parasites found in both industrialized and less developed countries (Boreham *et al.,* 1981). In both settings, a large proportion of individuals are asymptomatic (Lopez *et al.,* 1980). At present there is no single, simple marker which can differentiate between asymptomatic intestinal infection and symptomatic disease. *E. histolytica* and *G. lamblia* infection may also be difficult to diagnose by stool examination alone because the organism is exerted episodically (Danciger and Lopez, 1975).

Gardner *et al.,* (1980) studied on comparison of direct wet mount and trachoma staining techniques for detection *Entamoeba* species trophozoites in stools and he find the results of the percentage of detection by direct mount was 4.8%. The percentage of detection of the trophozoites by direct mount plus permanent stain was 58.5%.

Hossain *et al.,* (1983) a studied on campylobacter - associated diarrhoea in Egyptian infants: epidemiology and clinical manifestations of disease and high frequency of concomitant infections was done. In this case, the study population was 880 children (mean age 9.8 months) presenting with diarrhoea at one of two hospitals. The prevalence of *G. intestinalis* trophozoite 21.3% and *E. histolytica* was 3.9%.

Dr. Jahan Ara *et al.* (1987) studied was carried out among the employees of three different class of restaurants of Dhaka city Stool examination revealed that out of 153 respondents 91 59 8%) had one or more type of parasitic infestation and *E histolytica* positive cases were 7 (4.59%).

Ali-Shtayen *et al.,* (1989) examined stool samples for intestinal parasites investigation. A total of 22970 stool specimens collected from patients attending the Central Medical Laboratory in the city of Nablus in the period of 1981-1986. Of these 7412 (32.3%) were positive where, *E. histolytica* (22.9%), *G. lamblia* (27.3%) were the more prevalence parasites. Seasonal variation of intestinal parasites in the West Bank of Jordan was also studied in the period January 1981-August 1987. Lower prevalence rates of intestinal parasites generally occurred during winter and early spring. Peak incidence occurred during summer and early autumn.

Janoff *et al.,* (1989) a studied on the effect of *Giardia* intestinalis ophozoite on trypsin was done and showed tryptic activity was decreased after incubation with *Giardia* trophozoite. This reduction was time dependent and linear over the incubation period of 2 h. At a trypsin concentration at 18 BAEE units/tm there was a 35 5 + /1- 40% reduction in enzyme activity attar 2 h compared to controls.

Entamoeba histolytica is one of the parasitic amoebae of the genus *Entamoeba* that are known to infect humans *E. coli, E. gingivalis, E. moshkovskii and E. hartmani* are not associated with pathologic squeal. There are four species of *Entamoeba (E. histolytica, E. dispar, E. coli, E. hartmanni)* may regularly be found in the human large bowel, only one of which is a pathogen. There are also a few rare species: "atypical," "low temperature" or "Laredo" strains of *E. histolytica* now known to be the normally free-living species *E. moshkovskii* (Clark and Diamond 1991). *E. polecki, E. chattoni and E. gingivalis*.

Chowdhury *et al.,* (1991) noted that in urban slums of Bangladesh more than 40% children aged 1-12 years were moderately to heavily infest.

In 1993, Lascurain *et al* studied on the prevalence of *Giardia* and other intestinal parasites in children, dogs and cats from aboriginal communities in the Kimberly was done. Duodenalis was the most prevalent parasite in children and adults (32.1% in children, no of children 361, 12.5% in adults, no. of adult 24). The high prevalence acuities of *Giardia* and other enteric parasites in this survey are indicative of poor living condition and low levels of hygiene.

Al-Tukhi *et al.,* (1993) examined a total of 16,592 stool specimens were randomly collected from different age groups in Central Northern and Southern Iran in both urban and rural areas. The sample was examined by direct and formalin- ether concentration method. A total of 226 samples were positive for *E. histolytic* cyst. The study showed that

79% of isolates were *E. histolytic.* The ratio of *E. histolytica* was higher in Southern region than in the other two regions.

Shakur and Ehsan (1993) observed that the land areas of Bangladesh are mostly plain and embedded with rivers and canals. The soil, humidity and temperature contribute a lot towards parasites. In their study, they found that prevalence of *G. lamblia* was 21%and *E. histolytica* was 19% in the children of the up to 5 years in Dhaka Shishu Hospital.

Singh *et al.,* (1994) noted higher percentage of parasites in males (76.6%) than females (63.6%). The difference was statistically significant in rural community of Varanas, India. He also determined age-specific prevalence, and recorded a maximum of 92.5% from 5 to 9 years of age and a minimum of 65% from 50 years older.

Haque (1994) carried out a study with 593 stools from Mirpur and Mohakhali in Institute of public Health, Mohakhali, Dhaka and found total parasitizes of 63% among the under 20 years of age of people.

Chowdhury (1996) examined 710 faucal specimens at the clinical pathology laboratory Unit of the International Center for Diarrheal Research, Mohakhali, and Dhaka, Bangladesh. The overall prevalence of diarrhoeal protozoan parasites was 8.o2% (570 where the prevalence of *E. histolytica* was 0.56%.

UNICEF (1997) worked in rural areas of Bangladesh, and showed prevalence of intestinal parasites among adult healthy rural population was 33.3% of which 35.8% in females and 27.9% in males. Highest prevalence (40%) was found in the age group of 18-35 years. Significant association was found between hand washing with soap and prevalence of intestinal parasite infestation.

Haque *et al.,* (1998) examined 98 faucal specimens from with diarrhea Parasitological laboratory (LSD) of the International Centre for Diarrhoeal Research, Bangladesh

(ICDDR, B). Analyzed were single stool samples from 88 patients diagnosed with *E. histolytica* by microscopy.

In 1998 Haque *et al*., used PCR, isoenzyme analysis and antigen detection for diagnosis of *Entamoeba histolytica* infection. They examined stool samples from 98 patients from Dhaka and concluded that all three techniques for specific identification of *E. histolytica* in fresh stool showed excellent correlation. Only the tech lab *E. histolytica* antigen detection test was both rapid and technically simple.

In 1999, M.A. Chowdhury *et al.,* studied on dissecting microscopic and light microscopic of the intestinal mucosa of Bangladeshi population was done. In their study they incidently found that 44 subjects out of 67 were parasitic infested. Among them two was infected by *G. intestinalis.*

Taylor *et al.,* (1999) observed that people living in rural areas and in urban slums of Bangladesh would be particularly at risk to intestinal parasites because of poor sanitation, unclear water and lack of personal hygiene.

Hall *et al*. (1999) in a study on parasitic infestation in lower socioeconomic group in Chandigarh, North India reported similar observation, as mentioned in Kang (1998), regarding the association of parasites, prevalence and factors like living condition, climatic, soil environment, etc. In India overall prevalence rates ranged from 12.5% to 66% with varying prevalence rates for individual parasites about 50% of the urban and 68% of the rural population. They suggested that colonization does not necessarily produce immunity to reinfection.

Khatun (2000) conducted a community based prospective study where among randomly selected 300 children's aged less than 5 years and the incidence of *E. histolytica* was 0.7%.

Dhaka University Institutional Repository

Amoebiasis is a significant world wide health problem, especially in developing countries. *Entamoeba histolytica* is the etiological agent of amoebiasis. It is presently one of the third most common causes of death by parasitic diseases. It has been also estimated that approximately 500 million individuals are infected with *Entamoeba histolytica* each year and only about 10% experience symptomatic diseases (D'Silva *et al*., 2003). An estimated 40,000- 100,000 people die of invasive amoebiasis annually (Stanley, 2003).

Hussain (2003) worked in Chongra Charimukh area of hill tracts district of Khagrachari, found the following prevalence- *G. lamblia* (11.3%) and *E. histolytica* / *E. dispar* (8.5%).

Bordley (2003) observed an intestinal parasitosis of 59.5% in non-diabetic patients in Gond tribal community in Kundam block of Jabalpur district, Madhya Pradesh, India. Prevalence of intestinal parasites among boys was 57.0% and girls were 61.7%. Most of the population was chronically affected with intestinal parasites.

Haque *et al.,* (2003) examined the epidemiology, clinical feature, nutritional status, and causative agent of diarrhea were studied in 289 Bangladesh children (147 boys and 142 girls) 2-5 years old. The use of improved diagnostic tests for amebiasis enabled for the first time analysis of the contribution of *Entamoeba histolytica* to total diarrheal illness in this community setting. The average incidence rate of diarrhea was 1.8% child-year, and the average number of diarrheal days was 3.7 days/ child-year over an average observation period of 2.8 years/child. Seventy-five percent of the diarrheal episodes were 32 days in duration. Persistent diarrhea was relatively uncommon (0.2% of the children) and chronic diarrhea was observed in only one episode. Compared with malnourished and/or stunted children, better-nourished children experienced significantly fewer diarrheal episodes.

Kebede *et al.,* (2004 a, b) examined 108 stool specimens from different hospital and health centers from patients in Ethiopia. He found only one specimen out of 108 stool sample.

Dryden. *et al.,* (2005) studied on occurrence *Giardia* sp. cysts and cryptosporidium sp. oocysts in faeces from public parks in the west of Scotland was done by Dryden. *et al.* one hundred faecal specimens, randomly collected from various locations within seven public parks were examined, 11% of samples contained *Giardia* sp. cysts. Occurrence of data from individual parks varied from 0 to 40% for *Giardia* sp.

John and David (2006) examined *Entamoeba histolytica* is a protozoan parasite of humans that causes 40,000-100,000 death annually. Clinical amoebiasis results from the spread of the normally luminal parasite into the colon wall and beyond; the key development in understanding this complex multistage process has been the publication of the *E. histolytica* genome, from which has come an explosion in the use of micro arrays to examine changes in gene expression that result from changes in growth conditions.

Adamu *et al.,* (2006) in a study in Ethiopia, noted that low socio-economic status, poor living conditions, over crowded areas, poor environmental sanitation, improper garbage disposal, unsafe water supply and unhygienic personal habits were the causes of major proportion of burden of the diseases and death in developing countries. Opportunistic parasites detected by them in diarrhoeal children were *Cryptosporidium parvum* (8.1%), *G. lamblia* (6.3%) and *E. histolytica* (1.4%). Sixty one percent of pediatric diarrhoeal patients were infected with parasites.

Fotedar *et al.,* (2007) examined the genus *Entamoeba* contains many species, six of which (*Entamoba histolytica, Entamoeba dispar, Entamoeba moshkovskii, Entamoeba polecki, Entamoeba coli,* and *Entamoeba hartmanni)* reside in the human intestinal luman. *Entamoeba histolytic* is the causative agent of amebiasis and is considered a leading parasitic cause of death worldwide in humans. The identification of *E. histolytic* is based on detection of *E. histolytic*-specific antigen and DNA in stool and other clinical samples.

Jacobsen *et al.,* (2007) carried out a study on Ecuador's rural areas and found that the prevalence of *E. histolytica* 57.1% and *G. lamblia* was 51.5%. They also found that older children aged 3-4 years had higher prevalence of parasite than younger children aged 1-2 years. Water related risk factors such as consistent latrine use, participation in a community based clear water project and storage and treatment of water were not associated with significant differences in prevalence of intestinal parasites.

Uddin (2007), worked in Chittagong city found the overall prevalence of infestation by intestinal parasites was 16.58%. *G. lamblia* was 9.6% and *E. histolytica* was 7.52%. The prevalence in the female was 18.34% and the male was 15.24%. The prevalence was highest in May (20.45%) and lowest in April (10.82%). Among the subjects of different age groups, the highest prevalence was observed in 31-40 years old groups (23.47%) and lowest in 61-70 years old group (10.34%).

2. Purification of trophozoites and cyst:

The patient young peasant with bloody dysentery was passing large numbers of amebas in stool many of these, Craig observed, contained erythrocytes in their food vacuoles. Although Craig found the trophozoites of this ameba in colobic ulcers and was able to induce dysentery in a dog (which he inoculated with the patients bloody mucus stools containing the organisms), but he failed to recognize the casual relationship between the amoeba and the acute colitis. He thought that ulcers were caused by some other agents and the amebas merely interfered with their healing. Craig named these amebic trophozoites as *Ameba coli* (Schimdt, 1981; Craig, 1970).

Avron *et al.,* 1983 described a method for the separation and purification of cyst, precyst and trophozoite of *E. histolvtica* and *E. invadens* by centrifugation on discontinuous density gradients of Percoll. They regarded that different cell forms in the life cycle of *E. invadens* were rapidly and quantitatively separated on density step gradients of polyvinylpyrolidone-coated colloidal silica particles (Percoll). This method was also efficient in separation different forms of *E. histolytica* and bacteria. They concluded that

after purification on Percoll, trophozoites display no evidence of damage when examined by light microscopy and no loss in viability as judged by their ability to multiply.

1988 Wanke *et al.,* described the epidemiological and clinical features associated with invasive amebiasis in Bangladesh. 85 hospitalized diarrhoea patients with hematophagous trophozoites of *Entamoeba histolytica* is their stools were compared to a control group of 84 hospitalized diarrhoeal patients without amoebiasis. Post-mortem examinations were carried out in 22 deaths due to amoebiasis. Then results indicated that invasive amebiasis in this population differs from other diarrhoeal diseases, affecting mainly children >2 years and adults and causing severe and fatal illness characterized by extensive colitis with diverse systemic consequences.

Walderich *et al.,* 1998 established a new method for the purification of protozoan cysts from feces, allowing for isolation of native cysts. The procedure consists of two sucrose-density gradients and enzymatic digestion of cellulose particles by cellulase and can be accomplished in a few hours. The cyst fractions were differentiated into *E. histolvtica* and *E. dispar* using the DNA probes P145 and 8133 and a dot-blot test.

3. Immunological detection:

Muttalib *et al.,* 1975 found 12.2% of newly admitted Dhaka University student positive for *Entamoeba histolytica.* A study among 1,820 slum dwellers of Dhaka city showed 7.7% positively rate for *Entamoeba histolytica* (Annual report ICDDR, B. 1989).

Hautaner *et al,* 1978 studied on the use of ELISA for measuring immunoconglutinins (IKS). IKS are allowed to bind to solid - Phase 3 and are then assayed using alkaline phosphatase coupled to anti IgG or anti IgM. IK activity was detected in some patient's sera. They found the significant correlation was obtained between the levels of IgM — IKS measured by ELISA and by conventional hemagglutination assay.

Yang and Kennedy (1979) evaluated an enzyme linked immunosorbant assay (ELISA) for the detection of antibodies in *Entamoeba histolytica.* Comparison of the ELISA with indirect fluorescent antibody and indirect hemagglutination techniques showed that the former was slightly more sensitive then the two latter methods. The assay was positive in 26% of 461 patients with suspected amoebiasis and in all of 53 patients with amebic liver absences. The ELISA was found to be a specific, highly sensitive and reliable procedure for detecting anti *Entamoeba histolytica* antibodies in human.

Randall *et al,* (1984) used commercial immunozyme kit reported results of microscopy and ELISA on paired stool specimens from 107 patients in San Francisco. In 93% of the cases, the results of the two tests were similar indicating that light microscopy and ELISA were equally good tests. Five specimens were Positive by both tests, 95 negative by both. In contradistinction to Mexico studies, Randall *et al.* reported seven specimens positive by microcopy, which were negative by ELISA. There were no ELISA positive, microscope negative findings.

Speelman *et al,* (1986) sampled 251 expatriates in Bangladesh and reported that 8.6% of them were infected with *E. histolytica* during the study period of one year, but 90% of the subject infected with *Entamoeba histolytica* was asymptomatic.

Bangan *et al,* (1988) worked on the efficacy of enzyme-linked immunosorbent assay (ELISA) in the diagnosis of amoebiasis in comparison with indirect florescent antibody test (IFT) and found that ELISA test with as sensitive as IFT for a serodiagnosis of amoebiasis.

Knisley *et al.* (1989) used enzyme-linked immunosorbent assay for the detection of *G. lamblia* in faecal samples. They found that stool specimens were positive by ELISA in (36.92%) of 39 patients with giardiasis. They concluded that, the ELISA was a simple, sensitive and specific diagnostic test for *G. lamblia* that would be useful in diagnosis, in follow up treatment, and in large scale studied direct at defining the epidemiology and pathophysiology of *G. lamblia* infection.

Ravdin *et al,* (1990) studied on the recognition of *Entamoeba histolytica* galactose inhibitable adherence lectin by antibodies. All sere by ELISA for antibody to lectin 99% from liver abscess patients, and all 4 from individual asymptotically infected with pathogenic *Entamoeba_histolytica* were positive, all from the 40 healthy American controls and the 29 infected with other parasites were negative. The prevalence of serum anti-lectin antibodies was identical (25%) in asymptomatic. Thus the presence of serum antibodies to lectin seems to indicate current or prior invasive amebiasis or asymptomatic intestinal infection with pathogenic *Entamoeba histolytica.*

Yadav *et al.,* (1990) examined stools of 634 individuals from Varanasi for *Entamoeba histolytica.* Serology was done in these subjects by enzyme linked immunosorbent assay (ELISA) employing filter paper technique. Stools were positive for *E. histolytica* in 16.9%, and serology in 15.9%. Both the tests were positive.

Bruchard *et al,* 1991 in Mexico reported the first extensive trail of the ELISA developed by Root *et al.,* (1978) and marketed commercially as the immunozyme test (Millipore Corporation, Bedfored, Mass). The authors tested 717 stool specimens by ELISA and microscopy. They reported 155 specimens positive and 307 specimens negative, by ELISA and microscopy. In 255 specimens, there was no correlation between the methods, the ELISA was positive while microscopy was negative.

Bruckner, (1992) regarded pathogenicity of *E. histolytica* as a genotypic trait or could be changed by environmental influences which not been resolved. Exchange of genetic material between strains of amoebae could influence zyrnodeme patterns. Development of monoclonal antibodies and DNA probes specific for pathogenic zyrnodemes may be beneficial for clinical laboratory testing and therapeutic decisions.

Gonzalez *et al.,* (1994) worked on diagnosis of amoebic dysentery by infection of *E. histolytica* faecal antigen by an invasive strain - specific, monoclonal antibody-based

enzyme-linked immunosorbent ay (ELISA) was done in Bangladesh. The limit at detection of the assay for invasive E. *histolytica* crude antigen diluted in phosphate buffered saline or in stools was 0.58 and 3.9 mg/ml, respectively, which is the equivalent of approximately 72 and 487 E. *histolytica* trophozoite per well, respectively. The sensitivity, specificity and efficiency of the FAC—ELISA were 87, 100 and 98% respectively, for the detection of invasive E. *histolytica* antigens and 100, 100 and 100% respectively tor the diagnosis at amoebic dysentery.

Hassan *et. al.,* (1995) studied on detection of *Giardia* antigen in stool samples before and after treatment. A double antibody sandwich ELISA technique, using a chromatography purified *Giardia* antiserum, was applied to detect faecal antigen in patients infected with *Giardia lamblia* before and atter treatment the assay could detected antigens in 98% of infected cases with E. *histolytica*. There was a significant direct relation between the antigen level in stool samples and the number of *Giardia* cysts. The mean level of copra-antigen was slightly lower in children, below 10 years, than older patients, without significant difference. On the other hand, the lowest cyst count was noticed in elder patients, over 20 years. The level of faecal antigens decreased significantly successful treatment in patients with giardiasis.

Walderich *et al.,* (1998) established a new method for the purification of protozoan cysts from feces, allowing for isolation of native cysts. The procedure consists of two sucrose-density gradients and enzymatic digestion of cellulose particles by cellulase and can be accomplished in a few hours. The cyst fractions were differentiated into *E. histolvtica* and *E. dispar* using the DNA probes P145 and 8133 and a dot-blot test.

Braga *et al.,* (1998) worked on a slum community in northeastern Brazil and found that 20% of a sample population was colonized with *Entamoeba histolytica* or *Entamoeba dispar* and 10.6% was colonized with *E. histolytica* alone. No correlation between seropositivity for anti-Ga1NAc lectin antibody and colonization was found. They suggested that colonization does not necessarily produce immunity to reinjection.

Parija *et al*., (1999) a study of detection of *Giardia lamblia* cysts in stool samples by immuno-fluorescence using monoclonal antibody. one hundred fifty specimens from clinically suspected *Giardia* infections and 50 control samples from microscopically proved *Giardia* infections were tested *Giardia* cysts found in 15 of 150 (10%) samples tested by light microscopy. whereas immuno-florescence microcopy detected 35 of 150 (23%) positive samples. Forty six of the 50 reference samples previously shown to contain *Giardia* cysts were positive apparently, the four samples contained very low numbers of parasites, as none would be detected by conventional microscopy. The results show that *Giardia lamblia* cysts are detected significantly more frequently using the antibody marker.

Pillai and Kain, (1999) showed that BIOSITE Triage Immunochromatographic Strip was 68.3% sensitive and 100% specific for the detection of *Entamoeba histolytica-E. dispar* compared to Alexon-Trend's ProSpecT test (reference standard) using fresh-frozen stool. Neither test is able to distinguish *E. histolytica* from *E. dispar.* Triage was 83.3% sensitive and 100% specific compared to microscopy (formalrn-ether concentrates and permanent stains) for the detection of *Giardia lamblia.*

Haque *et al.,* (2001) reputed a method of detection in serum of circulating E. *histolytica* Gal/GalNAc lectin to diagnose amebic liver abscess, which was used in patients from Dhaka, Bangladesh. The TechLab *E. histolytica* II test (which differentiates the true pathogen *E. histolytica* from *Entamoeba dispar)* detected Gal/Ga1NAc lectin in the sera of 22 of 23 (96%) amebic liver abscess patients tested prior to treatment with the antiamebic drug metronidazole and 3 of 7 (70%) controls. After 1 week of treatment with metronidazole, 9 of 11 (82%) patients became serum lectin antigen negative. Antigen detection was a more sensitive test for infection than antilectin antibodies, which were detected in only 76 of 98(78%) amebic liver abscess patients and in 26 of 50 (52%) patients with intestinal infection. They concluded TechLab *E. histolytica* II kit as a sensitive means to diagnose hepatic and intestinal amoebiasis prior to the institution of metronidazole treatment.

Ali *et al.,* (2003) examined faecal specimens collected from 106 preschool child 2-5 years from Murmur, an urban slum in Dhaka, Bangladesh. Results based on PCR in stool DNA samples, where positive for *E. histolytic* (15.6%).

Delialioglu, *et al.,* (2004) collected stool specimens from patients with blood or mucus diarrhoea at the Mersin University. Eight-eight stool specimens collected from 40 child and 48 adults. 88 stool was positive (20.4%) in trichrom staning. ELISA was positive in 29.5% (26). The positive rate in children and adults were 27.5% and 14.5% with trichrom staning and 32.5% (13) and 27% with ELISA.

Goncalvesa *et al.,* (2004) showed that it is possible to detect copro-antigen of *E. histolytica* in historic and prehistoric human faecal remains, using a commercially available enzyme immunoassay (ELISA) kit. The kit used monoclonal antibody-peroxidase conjugate specific for *E. histolytica* adhesion. A total of 90 specimens of desiccated faeces found in mummies and ancient organic sediment from South America, North America, Africa and Europe were examined. The ELISA detected 20 positive samples, dated to about 5300 years before present to the 19th Century. The positive samples are from archaeological sites in Argentina, USA, France, Belgium and Switzerland. They regarded that detection of Protozoan antigen using immunoassays is a reliable tool for the studies of intestinal parasites in the past.

Mohamed *et al.,* (2004) found that immunity to *Entamoeba* species intestinal infection is associated with the presence intestinal IgA antibodies against the parasite's galactose-inhibitable adherence lectin. These finding were correlated with the effects of epitope-specific murine antilectin immunoglobulin A(IgA) monoclonal antibodies (mAbs) on abebic in vitro galactose-specific Africa were recently cured of amebic liver abscess(ALA) with or without concurrent *Entamoeba histolytica* intestinal infection or were infection free 1 year after cure.

Haque *et al.,* (2006) studied on the contribution of amoebiasis of the burden of diarrheal disease in children and the degree to which immunity is acquired from natural infection

were assessed in a 4-years prospective observational study of 289 preschool children in an urban slum in Dhaka, Bangladesh. *Entamoeba histolytica* infection was detected at least once in 80%, and repeat infection in 53%, of the children who completed 4-years of observation. Annually there were 0.09 episodes/child of *E. histolytica*-associated with diarrhea and 0.03 episodes/child *E. histolytica*-associated dysentery. Feacal immunoglobulin A (IgA) anti-parasite Gal/GalNAc lection carbohydrate recognition domain (anti-CRD) was detected in 91% (183/202) of the children at least once and was associated with a lower incidence of infection and disease.

Vreden *et al.,* (2007) studied on host parasites relationship in amoebiasis. *E. histolytica* has been described as the separate sp. *E. histolytica* and *E. dispar*. At the same year Azian *et al.,* observed another study of socio-economic factors associated with intestinal parasites among children living in Gambak, Malaysia was done. A total of 458 faecal specimens collected from school children, the most common parasite found *E. histolytica* (9.9%). The prevalence of infection was found to be associated with socio-economic status, water supply, sanitary disposal of faeces and family size.

Haque *et al.*, (2007) used ELISA. The killing of host cells by extra cellular amebic trophozoites requires contact via the parasite Gal/ GalNAc lectin. The overall seropositivity was 14.6% (171 of 11640 and 4.6% (54 of 1164) for antilectin and anti delta antibodies.

4. Prevalence of *Entamoeba histolytica* and *Giardia lamblia*:

Wanke *et al.,* (1988) described the epidemiological and clinical features associated with invasive amoebiasis in Bangladesh, 85 hospitalized diarrheal patients with hematophagous trophozoites of *Entamoeba histolytica* in their stools were compared to a control group of 84 hospitalized diarrheal patients without amoebiasis. Postmortem

examinations were carried out in 22 deaths due to amoebiasis. For the patients with amoebiasis, there was a bimodal age distribution with peaks at 2-3 years and greater than 40 years, whereas the control patients had a unimodal distribution with the peak at 0-1 year. The sex distribution was equal in childhood but young adults were predominantly female and older adults predominantly male.

Crompton (1993) studied on intestinal parasitic infections and showed the prevalence options caused by *E histolytica* and *G intestinalis* may Pease among the rural Populations who are migrating to these Ian and suburban settings owing to the favorable conditions for transmission.

Sultana (1994) examined 450 faecal specimens, collected from three rural areas where the prevalence of *E. histolytica* was 0.67%.

Gatti *et at,* (1995) screened 77 mentally retarded male inpatients residing in a psychiatric institution in northern Italy for the presence of stool parasites, *Entamoeba histolytica* particularly. Parasitological stool examination showed *Entamoeba* spp. *(E. histolytica* and/or *E. dispar)* in 26 cases (33.7%). In vitro culture on Robinson's medium was positive in (6 cases (61. %): in II cases we could stabilize and clone the isolates and proceed to electrophoretic assays. In all cases, patterns of pathogenic zymodemes were found (zymodeme II, 3 isolates; zymodeme XII, 4 isolates; zymodeme XIV 4 isolates). All isolates were therefore identified as *E. histoitica.*

Gatti *et at,* (1998) conducted an epidemiological survey to characterize *Entamoeba hictolytica/E dixpar* isolates from 123 human subjects was carried out in the Wonji area of Central Ethiopia, where an increased incidence of amoebic infection has been reported. In a randomized, coproparasitological studied, 93 (75.6%) of the subjects were found positive for at least one species of intestinal parasite: 14 (15.1%) harboured only one species and 79 (84.9%) were infected with at least two species. In-vitro culture in Robinson's medium revealed amoebic parasites in 52 (82.5%) of the 63 case tested. Of the 29 amoebic isolates successfully stabilized, cloned and characterized by Sargeaunt's

electrophoretic technique, 27(93.1%) were of it. *dispar* zymodemes (19 of zymoderne 1, two each of zymodemes ill, V and Xl, and one each of zymodemes X and XV) and two (6,9°/o) were of *E. histolytica* (zymodeme XIII).

Tachibana *et al.,* (2000) isolated *Entamoeba histolytica/Entamoeba dispar* from 50 asyrnptomatic amebic cyst passers in three institutions for the mentally retarded in Kanagawa Prefecture, Japan. To distinguish between *E. histolytica* and *E. dispar,* the isolates were analyzed by PCR, reactivity to monoclonal antibodies, and zymodemes. All isolates were identified as *E. histolytica.* They conceived that, in Japan, *E. histolytica* is predominant even in asymptomatic cyst passers.
Vreden *et at,* (2000) described an outbreak of amoebiasis in a family in The Netherlands. They demonstrated that even with Western standards of hygiene, persistent cyst passage might result in the transmission *of E. histolytica* to household contacts.

Amin, (2002) tested fecal specimens of 2,896 patients in 48 states and the District of Columbia for intestinal parasites during the year 2000. Multiple infections with 2-4 parasitic species constituted 10% of 916 infected cases. *Blastocystis hominis* infected 662 patients (23% or 72% of the 916 cases) and the prevalence appeared to be increasing in recent years. Eighteen other species of intestinal parasites were identified. *Cyptosporidium parvurn* and *Entamoeba histolytica/E. dispar* ranked second and third in prevalence, respectively. Prevalence of infection was lowest (22- 27%) in winter, gradually increased during the spring, reached peaks of 36-43% between July and October, and gradually decreased to 32% in December. The author also described a new superior method of parasite detection using the Proto-fix CONSED system for fixing, transport, and processing of fecal specimens.

Gatti *et at.,* (2002) conducted an epidemiological field study in the village of Borbon in Esmeraldas province in northern Ecuador to compare different parasitological methods in the diagnosis of infection with the *Entamoeba histolytica/Entamoeba dispar* complex. The results of two stool antigen detection assays (the Prospect *Entamoeba histolytica* microplate assay and the *E. histolytica* 11 assays) were compared with isoenzyme

characterization of the amebic isolates and showed that immunochromatographic tests have different degrees of sensitivity and specificity when compared with isoenzyme characterization as the reference technique.

Haque *et al.* (2003) detained the prevalence of infection by the invasive parasite *Entamoeba histolytica* and the noninvasive parasite *Entamoeba dispar* in 2000 children in Bangladesh. Antigen detection identified more cases of it. *histolytica-E. dispcir* infection than did culture or microscopy. They suggested that microscopic identification of *E. histolytica-E. dispar* complex infection in stool did not equate *with* the diagnosis of amebic dysentery because most amebic infections in this population were due to *E. dispar.* They found 4.2°/s prevalence of *E. histolytica* infection and 6.5% prevalence of *E. disper* infection in urban children with diarrhea. Rural asymptomatic children had a 1.0% prevalence of *E. histolytica* infection and *a* 7.0% prevalence of it. *dispar* infection.

Gonin and Trudel, (2003) reported a reference differentiation technique for *Entamoeba histolytica* and *Entamoeba dispar.* Stool samples were tested by microscopy, TechLab enzyme-linked immunosorbent assays (ELISAs), and an in- house PCR. The target for the PCR amplification was a small region (135 bp) of the SSU rRNA selected to increase the sensitivity of the test. They suggested that for differentiation, PCR performed well on simulated samples, while ELISA gave a discordant result for one of the two samples PCR positive for *E. histolytica* during the study. They also confirmed that *E. dispar* infection is significantly higher among travelers and underlines the possibility of acquiring *E. histolytica* infection in regions that are not areas of endemicity.

Blessmann *et al.,* (2003) performed a longitudinal study over an observation period of 15 months with a group of 383 randomly selected adult individuals (mean age, 38.5 years) living in an area of amoebiasis endemicity in central Vietnam. Ameba infection was diagnosed by using species-specific PCR and DNA extracted directly from fcal samples. They indicated an *E. histolytica* prevalence of 11.2% and an annual new infection rate of 4.1% in the study population. Follow-up of the 43 individuals who were *E. histolytica* positive at enrollment suggested a regular exponential decline in infection of about 3%

per month. They calculated the mean half-life of *E. histolytica* infection that was 12.9 months (95% confidence interval, 10.2 to 15.6 months).

Nesbitt *et al.,* (2004) found higher rate of amoebal infection among the children of the age of five years compared to less than that of a five-year-old population; however. To investigate misdiagnosis of amoebiasis, 226 patients (passive cases) in three hospitals and 616 individuals (active cases) from three different localities in Kilimanjaro were examined. In passive cases, the prevalence of *Entamoeba histolytica* and *Entamoeba dispar* were 1% and 7.3%, respectively. Among active cases, 1% was infected with *E. histolytica,* and 15% were infected with *E. dispar.* There were no significant differences in amoebal infection between the male and female populations. A pool of 842 stool samples was used for diagnosis of *E. histolytica* and *E. dispar* by microscopic examination or ELISA kits. The microscopic examination indicated 8.7% amoebal infection; however, using ELISA as the gold standard, the prevalence of *histolytica/dispar* was 0.8% and 7.4%, respectively. They indicated that *E. dispar* infection was 14.5 times more prevalent than *E. histolytica* infection.

5. Comparison of diagnostic technique:

However, in 1925, Emile Brumpt suggested an alternative explanation, that there were in fact two species. One capable of causing invasive disease and one that never cause disease, which he called *Entamoeba dispar* Brumpt's hypothesis was dismissed by other workers. In the 1970's data started to accumulate that gave support to Brumpt's hypothesis of the existence of two distinct organisms within what was being called *Entamoeba hisolytica* Biochemical immunological and genetic data continued to accumulate and in 1993 a formal redecoration of *Entamoeba histolytica* was published separating it from *Entamoeba dispar.*

Haque *et al.,* 1993 reported a field trial of commercial antigen detection kits designed to rapidly detect and differentiate *E. histolytica* from *E. dispar* in stool specimens, compared with culture and microscopic identification of the *E. histolytica-E. dispar*

complex was 60% sensitive and 79% specific, while the screening antigen detection test for the *E. histolytica-E. dispar* complex was 80% sensitive and 99% specific. Differentiation of *E. dispar* from *E. histolytica* by the *E. histolytica-specific* test was *95%* sensitive and 93% specific compared with zymodeme analysis. They concluded that the antigen detection test for the *E. histolytica-E. dispar* complex is more sensitive and specific than microscopy and that the *E. histolytica-specific* antigen detection test is as reliable as and much more rapid than zymodeme analysis for the differentiation of *E. histolytica* from *E. dispar*

Hassan *et al*. (1995) studied on detection of *E. histolytica* antigen in stool samples, the assy detected antigen in 98% of infection cases with *E. histolytica*.

Talukdar *et al.,* (2002) collected faecal samples from 500 healthy army recruits aged 17 to 20 years and examined by direct method as well as by concentration method. He found 62% men were suffering from enteric parasitic infection. Out of them 52.80% had single infections and 9.20% had multiple parasitic infections. About 60% of men in the study group were anaemic.

Oguma *et al.,* (2007) studied on comparisons of direct smear and formol-ether concentration techniques in diagnosing intestinal parasites. The study compared results of one direct smear and one formol-ether concentration examination executed on each of 103 stool samples from patients attending a hospital and a medical diagnostic laboratory in Owerri. Formol-ether concentration technique detected 65.26% of positive specimens for one or more intestinal parasites. Direct smear was 34.74% effective. A significant number of the infected population was missed by direct smear method. This accounts for the prevalence of intestinal parasites in the studied population, part of which is ensuing from misdiagnosis. This study furthermore showed that the age group (9-13) has the highest occurrence (15.79%). Formol-ether detected 23.16% prevalence in this age group while direct smear found 14.74%.

Feleke *et al.*, (2010) studied on the prevalence of intestinal parasites in children of primary school age. in Anambra State, Nigeria. Stool samples were taken from children belonging to a 8 primary school in the area of the 1,536 samples examined using light microscopy after fomol ether centrifugation, 922 (60%) harbored one or more parasites. The prevalence of *E. histolytica* was 6.8%. The overall infection was 31.9% for male and 27.5% for female. Infection was more prevalent in male and female children (10-12) years. Mixed infections were observed. *E. histolytica* combinations with other disease agent were more frequently encountered. After these, various investigations carried out among these the following works have been done of which mostly based on ELISA techniques to detect amoebiasis.

Khanum *et al.,* (2010) conducted a study on intestinal parasitic infestation among the outdoor patients of Dhaka University medical centre and reported the prevalence of parasitic infestation was 24.73%. The prevalence of *E. histolytica* was 3.95%, *G. intestinalis* 6.31%. The prevalence was higher in case of staff (32.31%) and lower in case of teacher (13.16%). Highest prevalence observed during the rainy season (29.3%) and lowest in winter (19.4%). Female patient showed higher prevalence (31.25%) compared to male (24.14%). The rate of infection was higher (28.3%) among the patients, who used to drink unboiled water.

Khanum *et al.,* (2013) studied the seasonal pattern showed that highest (30%) prevalence occurred in rainy season and lowest (17.19%) in winter season.

Barua *et. al.,* (2014) reported the prevalence *Blastocystis hominis* was 14.76% in asymptomatic samples and 6.985 in diarrheal samples by in- vitro culture.

6. Contamination from water, food, vegetables and sanitation:

Roche (1999) studied on water borne *Giardia* cyst and cryptosporidium oocysts in the HMSO, Canada was done. The objective of this study was to collect information relevant to waterborne giardiasis and cryptosporidiosis in the Yukon including epidemiological data and analysis of water, sewage and animal faecal samples. Pristine water samples

were found to be contaminated with *Giardia* cysts (7 of 22 or 32%) *Girdia* cysts were found in 21% 13 of 61) of animal scats, Whitehorse's drinking water was episodically contaminated With *Giardia* cysts (7 of 42 or 17%).

Orlandi. Faecal (2002) studied on socio-economic factors associated with intestinal parasites among children living in Gambak, Malaysia, specimens collected from 456 school children. The most common parasite found was *G. intestinalis* (14.7%) and *E. histolytica* (9.9%). The prevalence at infection was to be associated with the soda-economic status, water supply, sanitary disposal of faeces and family size.

Khanum *et al*., (2012) worked a study on food borne giardiasis in a corporate office setting was done showed food borne in November 2007, such an outbreak among insurance company employees resulted laboratory confirmed and 9 suspected cases of giardiasis. A case-control study of 26 ill and 162 well employees implicated raw sliced vegetables served in the employee cafeteria and prepared by a food handler infected with *Gardia lamblia* as the probable vehicle (Odds ratio, 5.1; 95% confidence interval, 1.4 - 22.7). This outbreak illustrates the potential for transmission of *Giardia* organism to occur in commercial establishments through a frequently served food item.

MATERIALS AND METHODS

The present study was undertaken to find out the prevalence of *Entamoeba histolytica* and *Giardia lamblia* among the people of diabetic and non diabetic patients (25-70 years) in Bangladesh.

Sample size and duration of the study:

The present investigation was a cross sectional study with a sample size of 1300 diabetic patients and non-diabetic individuals (697 samples were from diabetic patients and 603 were from non-diabetic individuals) and conducted during the period of June 2011 to July 2013.

Study location:

The entire study was carried out in the Department of Immunology (Immunology Laboratory) Bangladesh, Institute of Health Sciences (BIHS) Hospital, Mirpur, Dhaka.

Detection of Diabetes:

Diabetes was detected with Glukotest strips (Boehringer Mannheim) sensitive to glycosuria on day 5 giving STZ. Fasting blood glucose determinations were made (mg/100ml) using a glucometer 11 reflectance photometer. *(STZ= Steptozotocin-Diabetic Rats).

The following techniques were used to diagnose the infection with *Entamoeba histolytica* and *Giardia lamblia*.

(1). ELISA test,

(2). Formol- Ether concentration method and

(3). Zinc sulphate floatation method.

Laboratory Diagnostic Techniques:

For several years, researchers have been searching for methods that will allow an accurate and reliable assessment of amoebiasis and giardiasis. Laboratory diagnosis of amoebiasis and giardiasis are usually based on microscopy (Direct smear, Formol-ether concentration method and Zinc sulphate floatation methods) and serological methods including enzyme-linked immunosorbent assay (ELISA), indirect hemagglutination assay (IHA), and latex agglutination. During the last decade, there has been remarkable development in molecular biology-based diagnostic procedures to detect *E. histolytica* and *G. lamblia,* to the point where today they are the preferred approach. Accurate diagnosis is important not just for patients with dysentery but also for the 90% of *E. histolytica* and *G. lamblia* infection that are asymptomatic, because infection may easily be transmitted from person to person, especially in developing countries which have poor hygienic conditions and inadequate water treatment (Jackson, 2000).

Trophozoites are more frequently observed in fresh stool specimens that contain mucus, pus and trace amounts of blood. In wet mounts, the trophozoite nuclei cannot easily be seen (Proctor, 1991). Charcot-Leyden crystals (products of degenerated eosinophils) and clumped RBCs can be seen in a wet mount preparation (Garcia and Bruckner, *et al,* 1997, Markell *et al.,* 1999). Motility of *E. histolytica* and *G. lamblia* in fresh preparations usually occurs in a linear (not random) fashion, with the clear hyaline ectoplasm flowing to form blunt-ended pseudopodia, which guide the endoplasm containing the nucleus (Proctor, 1991).

Occasionally motile trophozoites are seen even after 4 h. at this temperature (Ravdin, 1995). Iodine stains, including Giemsa, methylene blue, Chorazole black E, Wright's and iodine-trichrome, may be used successfully, Wheatly's trichrome staining or one of the modified iron hematoxylin stains for permanent smears has been suggested for routine use in the diagnosis of *E. histolytica/e. dispar* (Garcia and Bruckner, 1997, Ravdin, 2000). Shetty and Prabhu found that D'Antoni's iodine was much better than saline or buffered

methylene blue for detection of *E. histolytica* and *G. lamblia* cysts while saline and buffered methylene blue were equally good for detection of *E. histolytica* and *G. lamblia* (Shetty and Prabhu, 1988a,b). There are several factors that adversely affect the results of microscopy.

Collection of blood specimen:

Blood samples were collected by sterile needles and 10-ml syringes and were placed into 10 ml blood separator tubes.

Preparation of Serum:

The serum was separated from blood by clotting and then by centrifuge mechanism. Then serum samples were preserved at 2^0C. Blood samples were centrifuged for 5 minutes and were used for ELISA for the detection of anti-amoebic antibodies.

ELISA:

ELISA is among the most popular methods used in the diagnostic laboratories throughout the world. ELISA method depends on the conjugation of an antigen (Ag) or antibody (Ab). The sensitivity of detection of specific antibodies to *E. histolytica* in serum is reported to be near 100% which is promising for diagnosis of ALA (Ravdin *et al.,* 1990). Serum antilectin immunoglobulin G (IgG) antibodies could be present within 1 week after the onset of symptoms of patients with amebic colitis and ALA, with a value over 95% (Abd-Alla *et al.,* 1992). Antigen diction test for *G. lamblia* infections has been used by several authors in several countries. *G. lamblia* is common human intestinal protozoan parasites found in both industrialized and less developed countries (Haque *et. al.,* 1997). In both settings, a large proportion of individuals are asymptomatic (Lopez *et al.,* 1980). At present there is no single, simple marker which can differentiate between asymptomatic intestinal infection and symptomatic disease. *G. lamblia* infections may also be difficult to diagnose by blood examination alone because the organism is exerted episodically (Danciger and Lopez, 1975).

Antigen Detection:

Antigen-based ELISA has several significant advantages over other methods currently used for diagnosis of amoebiasis and giardiasis: (a) some of the assays differentiate *E. histolytica* from *E. dispar*, (b) They have excellent sensitivity and specificity; (c) They are readily usable by even nonexperienced laboratory personnel; and (d) the use of a 96-well plate format enhances their potential as large-scale screening tools in epidemiological studies, such as waterborne outbreak situations (Gonzalez-Ruiz *et al.,* 1994). Today, antigen-based ELISA Kits that are reported to be specific for *E. histolytica* use monoclonal antibodies against the Gal/GalN Ac-specific lection of *E. histolytica* (*E. histolytica* test 11; Tec lab, Blacksburg, Va.) or monoclonal antibodies against the serine-rich antigen of *E. histolytica.* In addition to these clinical assays, research-based detection has included the use of a monoclonal antibody against a lysine-rich surface antigen, a lipophosphoglycan, a salivary 170-KDa adherence lectin antigen and an uncharacterized antigen (Abd-Alla *et al.,* 2000).

Immunological Detection: Antibody Detection:

Amoebiasis and giardiasis are endemic in developing countries and particularly affects children. Symptoms commonly attributed to *E. histolytica* and *G. lamblia* may be absent in the majority of cases. Serological tests are more helpful for the identification of *E. histolytica* and *G. lamblia* infection in industrialized nations, where *E. histolytica* and *G. lamblia* infection are not common (Ohnishi and Murata, 1997; Walderich *et al.,* 1998). Serum antibody to *E. histolytica* and *G. lamblia* can be detected in 75 to 85% of patients with symptomatic *E. histolytica* and *G. lamblia* infection. Assays that have been used so far involve IHA (Hung *et al.,* 1999), counterimmunoelectrophoresis (CIE) (Haque, 2001), amoebic gel diffusion test (Jackson, 2000), complement fixation (CF) (Lotter *et al.,* 1993), indirect fluorescence assay (IFA) (Tachibana *et al.,* 2000;), latex agglutination (Cummins *et al.,* 1994; Lotter *et al.,* 1995), and ELISA (Agundis *et al.,* 1996; Kelsall *et al.,* 1994; Pal *et al.,* 1996).

A genetic study of the human-pathogen-environment relationship proposed that underlies susceptibility to amebiasis. 8 years ago, a prospective cohort study of *E. histolytica* infection in 300 two to five year old children living in the Mirpur urban slum of Dhaka, Bangladesh. The study discovered that amebiasis is common (Haque *et al.,* 2006) and associated with malnutrition (Mondal *et al.,* 2006), that parasite genotypes differ in propensity to cause disease, and that mucosal IgA (Haque *et al.,* 2001) and systemic IFN- are markers of protective immunity. Despite a substantial burden of disease, noted that children differ markedly in their susceptibility to amebiasis, with only 25% of infections resulting in disease (Haque *et al.,* 2006).

A study of a cohort of Bangladeshi mother and their children from birth revealed that 42% of children were infected at least once in their first year of life (Islam *et al.,* 1983), while 2 serological surveys in Bangladesh have shown that specific serum antibodies are not a reliable marker of current infection (Islam *et al.,* 1983).

In many people *E. histolytica* and *G. lamblia* infections resolve without symptoms. For example in previous studies in Dhaka, Bangladesh, 40% of pre-school children annually, and dysentery in 3% (Haque *et al.,* 2006). A major emphasis of the current proposal is to study the host, environment and parasite factors controlling susceptibility to protozoan parasites infection and disease, to begin to answer the question of why all infections do not cause disease.

Identification of the parasites and determination of the prevalence were done by following technique:

A. Immunodiagnostic technique for blood: Antigen detection test: Enzyme-Linked Immunosorbent Assay (ELISA):

The ELISA method depends on the conjugation of an antigen (Ag) or antibody (Abs). A rapid and simple approach to diagnosis of *Entamoeba histolytica* and *Giardia lamblia* infections based on antigen detection by enzyme-linked immunosorbent assay (ELISA).

Principle of the procedure:

The micro test wells were coated with *E. histolytica* and *G. lamblia* antigen. During the first incubation with the diluted patients' sera, any antibodies which were reactive with the antigen will bind to the coated wells. After washing, removed the rest of the sample, the Enzyme Conjugate was added. If antibodies have been bound to the wells, the Enzyme Conjugate will then bind to these antibodies. After another series of washes, a chromogen (tetramethlybenzidine or TMB) was added. If the Enzyme Conjugate was present, the peroxidase will catalyze a reaction that consumes the peroxide and turns the chromate from clear to blue. By adding of the Stop Solution, ends the reaction and turns the blue color to a bright yellow color. The reaction may then be read visually or with an ELISA reader.

Reagents:

(a). **Test strips:** micro wells containing *E. histolytic* and *G. lamblia* stains NIH-200 antigens - 96 or 48 test wells in a test wells in a test strip holder.
(b). **Enzyme conjugate**: one (1) bottle containing 11 or 6 ml of protein A conjugated to peroxides.
(c**). Positive control serum**: one (1) vial containing 1 ml of diluted positive rabbit serum.
(d**). Negative control serum**: One (1) vial containing 1 ml of diluted negative human serum.
(e).**TMB Substrate**: One (1) bottle containing 11 ml of the chromate tetramethylbenzidine (TMB).
(f). **Wash concentrate solution (20X):** one (1) bottle containing 25 ml of concentrated buffer and surfactant.
(g). **Dilution buffer**: Two (2) 0r one (1) bottle(s) containing 30 ml of buffered protein solution.
(h). **Stop solution**: one (1) bottle containing 11 ml of 1 M phosphoric acid.

Storage Conditions:

Reagents, strips and bottled components:
(a). Stored between 2-8^{0}C
(b). Squeezed bottle containing diluted wash buffer may be stored at room temperature.

Preparation:

Wash Buffer- Removed cap and added contents of bottle to 475 ml of reagent grade water. Place diluted wash buffer into a squeeze bottle with narrow tip opening.

Note: Washings consist of filling to the top of each well, shaking out the contents and refilling. Avoid generating bubbles in the wells during the washing steps.

Collection and Preparation of Serum:

Coagulated blood and removed serum. Freeze sample at -20^{0}C or lower if not used immediately. Heated was not inactivate serum and avoid repeated freezing and thawing of samples.

Test samples: 1:64 dilutions of patient sera were made by using the dilution buffer (e.g. 5 µl sera and 315 µl dilution buffer).

Procedure: Materials Provided: Amebiasis and Giardiasis serology Micro well ELISA Kit.

Materials Required:

1. Pipettes
2. Squeeze bottle for washing strips (narrow tip is recommended)
3. Reagent grade water and graduated cylinder
4. Tubes for sample dilution
5. Absorbent

Micro titer plates were used as ELISA plate reader with a 450 nm and a 650 to 620 nm filter. That was "Nune-ELISA Plate" DRG International Inc., USA

Performance of Test:

1. Break off number of wells needed (two for controls plus number of samples) and placed in strip holder.
2. Added 100 µl (or two drops) of the negative control to well #1, 100 µl of the positive control to well #2 and 100 µl of the diluted (1.64) test samples to the remaining wells.

Note: Negative and positive controls were supplied prediluted.

3. Incubated at room temperature (15 to 25^0C) for 10 minutes.
4. Shaked out contents and washed 3 times with the diluted wash buffer.
5. Added 2 drops of Enzyme Conjugate to each well.
6. Incubated at room temperature for 5 minutes.
7. Shaked out contents and washed 3 times by using wash buffer. Slap wells against paper towels to remove excess moisture.
8. Added 2 drops of the chromogen to every well.
9. Incubated at room temperature for 5 minutes.

10 Added 2 drops of the Stop Solution and mixed by tapping strip holder.

Quality Control:

The used of controls allows validation of kit stability. The kit was not used if any of the controls are out of range. Expected values for the controls were:

Negative- 0.0 to o.3 OD units

Positive- 0.5 OD units and above.

Interpretation of Results – ELISA Reader:

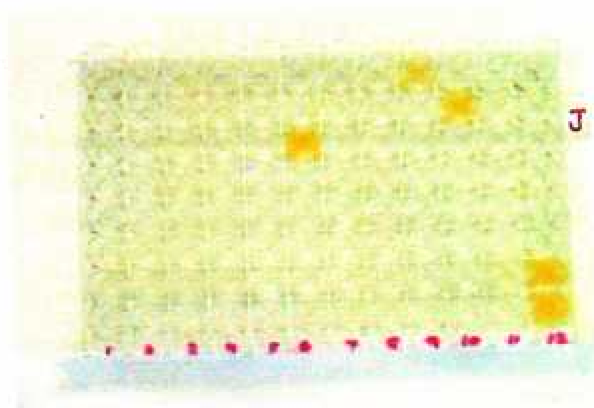
Zero ELISA reader on air. Read all wells at 450/650-620 nm.

Positive- Absorbance reading greater than 0.4 OD units

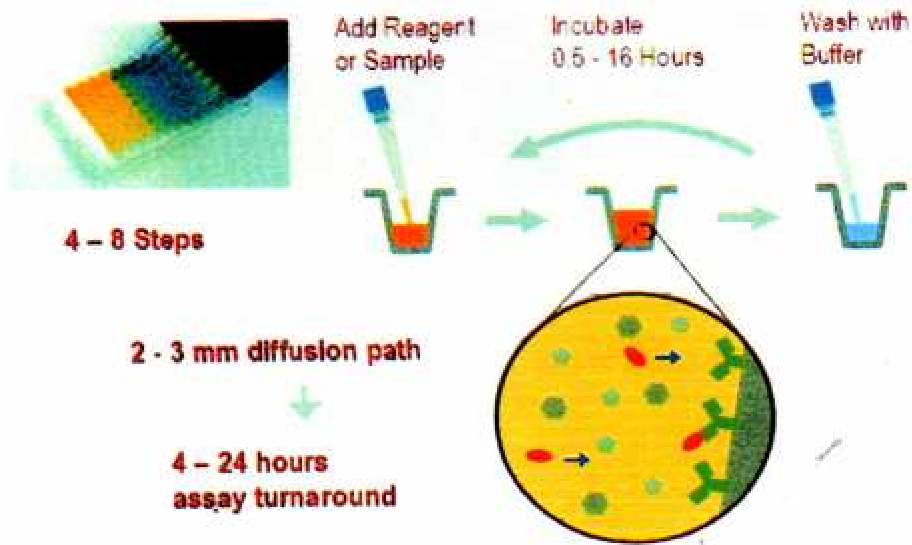
Negative- Absorbance reading less than 0.4 OD units.

A positive OD reading indicated that the patient may be infected by *E. histolytica* and *G. lamblia*

A negative OD reading indicated that the patient had no detectable level of antibodies. This may be due to lack of infection or poor immune response by the patient.



Photograph : A multiwell ELISA plate



Interpretation of Results- (Visual): Compared the results to the controls. A sample was interpreted as positive if the degree of color was significant and obvious.

Faecal concentration technique and Zinc sulphate floatation method:

Stool examination for intestinal protozoan parasites is one of the most frequently performed examinations in parasitological laboratories. Most of protozoan parasites can be excreted in stool in both cyst and trophozoite stages. Cysts of *Giardia* occur in the aquatic environment through out the world. They have been found in moist surface water, where their concentration is related to the level of faecal pollution or human use of water (Hassan, *et al.,* 1995). *E. histolytica* and *G. lamblia* trophozoites are usually identified in dysenteric specimens by their motility and ingested red cells (Cheesbrough, 2005).

The formal-ether technique is recommended as the best overall technique for the concentration of the parasites in faeces that have been preserved in Beyer's solution, Merthiolate iodine formaldehyde (MIF), and other faucal fixatives (Khanum, *et al.,* 2000).

Collection of stool specimen:

Fresh stool specimens were collected in a physically and chemically clean and dry container. The following procedure was maintained while collecting the stool samples:

(a). The stool samples were collected in a clean and leak proof, transparent container. No antiseptic was used. Stool samples were not contaminated with urine.

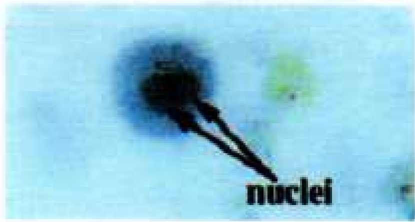
(b). No medication was used before taking these samples.

(c). Identification label was put on the container (patient's number, laboratory number and date of collection)

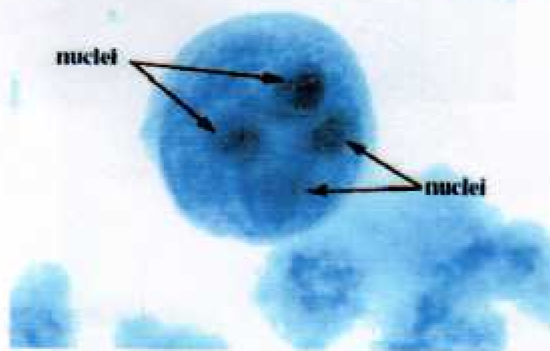
Processing of stool samples:

Examination of specimen was performed as soon as possible, within one hour of collection. In most instances liquid stool or those specimens mucus and or blood was examined on a priority basis.

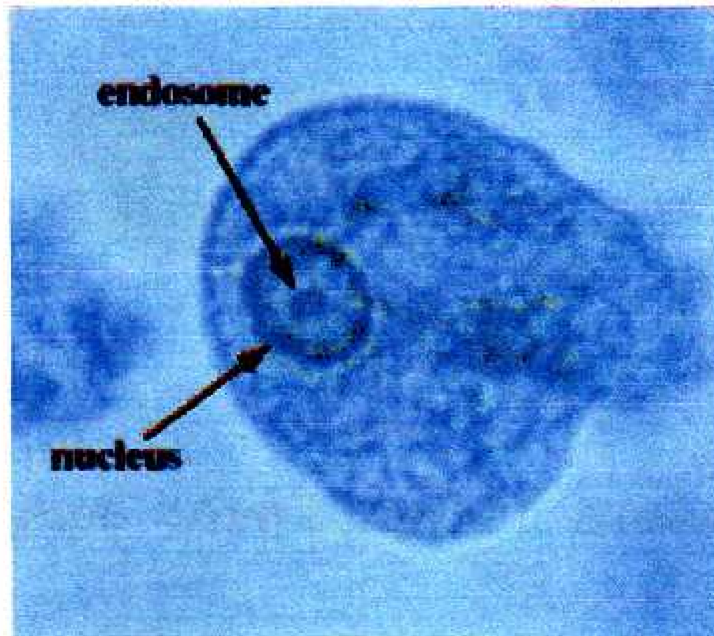
Stool samples were preserved in 10% formal saline and also stored specimens freeze (-20^0c) which could not be performed within 72 hours of collection.



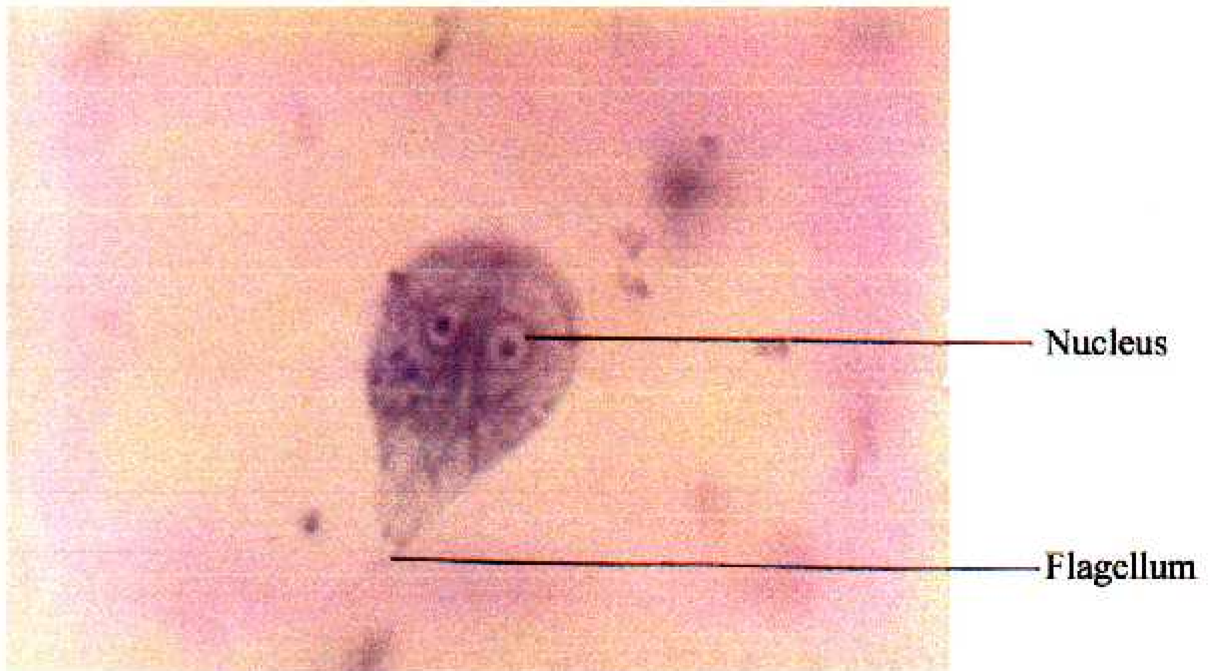
Photograph- : Cystic stage of *E. histolytica* .



Photograph- : Precystic stage *E. histolytica* .



Photograph- : Trophozoite or invasive or vegetative stage of *E. histolytica*



Photograph : Trophozoites of G. intestinalis [From Ash and Orihel, 1990. Atlas of human parasitology, 3rd edition. American Society of Clinical Pathologists, Chicago.]

B. Microscopic stool examination by Formal-ether concentration technique (Cheesbrough, 1987):

The formal-ether technique is recommended as the best over all technique for the concentration of parasites in faces. The movement of these parasites will not be seen in a formal-ether preparation because formalin kills living materials. The formalin fixes the parasite and therefore their morphology is preserved. Cysts are fixed and sediment and the faucal debris are separated in a layer between the ether and formal water. Faecal fat is dissolved in ether.

The formal-ether technique is a suitable method for concentrating parasites in faces that have been preserved in Beyer's solutions, Merthiolate iodine formaldehyde MIF, and other faucal fixatives.

Requirement for formal-ether concentration method:

(1). Formal water, 10% v/v (Prepare by mixing 50 ml of strong formaldehyde solution with 450 ml of (distilled water).

(2). Ether (diethyl ether or ethyl acetate).

(3). Sieve (strainer) with small holes, preferably 400-450 µm in size.

Formal-ether concentration method:

The stool were preserved in 10% formalin and weighed and that was the total weight of the stool sample. The weight of the sample was determined by subtracting the total weight from the weight of formalin. The stool samples were mixed in a vortex machine. The stool samples were then strained through two layers of gauze in to centrifuge tubes. Saline water (0.9) was added to the samples in the tube, to bring the total to 10 ml. the samples were centrifuged at 1000 to 2000 rpm for 5 minutes, to partially sediment the parasites. The supernatant was discarded after which a little amount saline and 3 ml of ether were added to bring up the volume to 10 ml. stopper the tubes and shook vigorously. The samples were

centrifuged at 1000-2000 rpm for 5 minutes. After this centrifugation, the stool samples became separated into 4 layers.

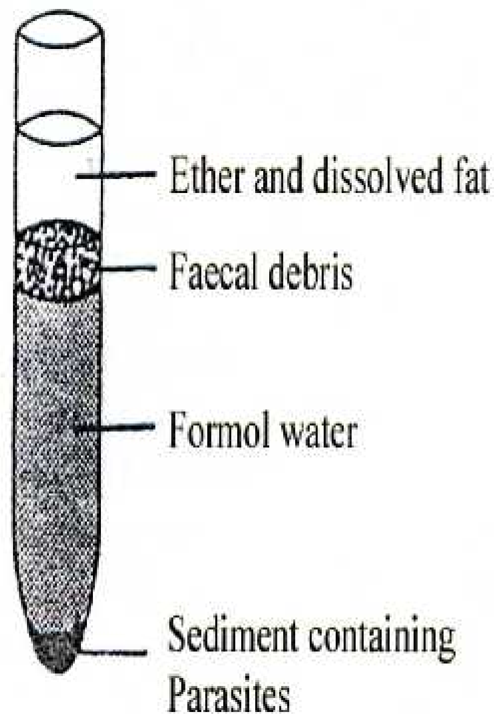


Fig. Formal ether sedimentation concentration technique, after centrifugation.

(a). ether at the top,

(b). plug of debris,

(c). formalin solution and

(d). sediment.

The top three layers were decanted. Then added 0.9% normal saline with a disposable glass pipette. Mixed well and counted the drops. A small portion of the specimen was taken with a drop of solution on a slide; covered with a cover slip and examined under the microscope. The parasites were identified and counted. The number of parasites was calculated as follows:

$$\frac{\text{Number of parasites} \times \text{Drops counted}}{\text{Wt. of stool samples in grams}}$$

C. Zinc sulphate floatation technique (Cheesbrough, 1987):

The zinc sulphate technique was recommended for concentrating the cysts of *E. histolytica* and *G. lamblia.*

Principle:

A zinc sulphate solution was used which had a specific gravity, 1.180-1.200. Faeces were emulsified in the solution and the suspension was left undisturbed for the cysts to float to the surface. They were collected on a cover glass.

Requirement for the zinc sulphate floatation method:

Zinc suleplate solution, 33% w/v. Reagent No 69, specific gravity, 1.180-1.200.

Used a hydrometer to check that specific gravity of the solution correct. Adjusted with distilled water or more chemical if required.

(a). Test tube (without a lip) of about 15 ml capacity which had a completely smooth rim.

(b). Strainer

Fig. Zinc Sulphate Floatation method

Zinc sulphate floatation method:

1. Filled the tube about one quarter full with the zinc solution. Add estimated1 gm of faces (or 2 ml if a fluid specimen). By using a rod or stick, emulsify the specimen in the solution.
2. Filled the tube with the zinc specimen in the solution and mix up well. Strain the faucal suspension to remove large faucal particles.
3. By using a plastic bulb pipette or Pasteur pipette added further solution to ensure the tube is filled to the brim.
4. Carefully placed a completely clean (grease-fee) cover glass on top of the tube. Avoid trap any air bubbles.
5. Left undisturbed for 30-45 minutes to time for the cysts to float.
6. Microscopically examined the entire preparation using the 10x objective with the condenser iris closed sufficiently to give good contrast. Used a drop of iodine under the cover glass to identify the cysts.
7. Number of *E. histolytica* and *G. lamblia* cysts counted per gram of faces.

The presence of parasite cysts was detected. The findings were confirmed by the help of experts and renowned books (Neva and Brown, 1994; Chatterjee, 2004). All the findings of stool samples were recorded in the respective questionnaire.

Data management:

Individual questionnaire was checked for completeness and consistency. Separate code number was given to each of the questionnaires. The code number was used in subsequent handling of respective questionnaire. Then data were entered into computer in SPSS 12.

DATA ANALYSIS

Data processing and Analysis:

$$\text{Specificity} = \frac{\text{Number of patients without diseases with negative test results}}{\text{Number of patients without disease}} \times 100$$

$$\text{Sensitivity} = \frac{\text{Number of diseased patients with positive test results}}{\text{Number of diseased patients}} \times 100$$

(Abd-Alla *et al.,* 1992)

Prevalence was calculated by using following formula:

$$\text{Prevalence} = \frac{\text{Number of infected hosts}}{\text{Total number of hosts}} \times 100$$

(Margolis *et al.,* 1982)

Formula for statistical analysis:

The Statistical analysis of the present study was done by using personal computer (PC) and for solving the equation following formula and software was used:

Correlation:

$$r = \frac{\sum (X-\bar{X})(Y-\bar{Y})}{\sum (X-\bar{X})^2 \sum (Y-\bar{Y})^2} \times 100$$

Here,

X= The value of 1st variable. $\bar{X}$= Mean of X value

Y= The value of 2nd variable. $\bar{Y}$= Mean of Y value

P value:

$P<0.05$; is significant, highly significant.

$P>0.05$; is not significant at 5% level.

Software's: Following software were utilized during the present study –

1. **Windows SPSS 10.1**
2. **Kc Junior, USA**
3. **EPI info 2000**
4. **iCycler 2.031**
5. **Beacon Designer 2.1**
6. **Quantity one 2.1**
7. **Diversity data base 2.1.1**

Photographs:

Required microphotographs of worms were taken using Olympus PM-6 compact photomicrograph equipment and with digital handy camera.

OBSERVATION AND RESULTS

The study was undertaken to find out the prevalence of *Entamoeba histolytica* and *Giardia lamblia* among the people of diabetic and non diabetic peoples (25-70 years) in Bangladesh. This investigation was a cross sectional study with a sample size of 1300 diabetic patients and non-diabetic individuals and conducted during the period of June 2011 to July 2013. The entire study was carried out in the Department of Immunology (Immunology Laboratory) Bangladesh, Institute of Health Science (BIHS) Hospital, Mirpur, Dhaka. The collected blood and stool samples were detected by ELISA, Formol Ether Concentration technique and Zinc Sulphate method.

In the present investigation, out of 1300 blood and stool samples, 697 from diabetic and 603 from non-diabetic samples were collected for the identification of different protozoan parasite species.

5.1 Parasitic infestation among diabetic and non diabetic peoples:

According to ELISA, out of 1300 (diabetic and non-diabetic peoples) blood serum samples, 15.93% diabetic patients showed positive and 27.53% non-diabetic showed positive for *Entamoeba histolytica* antibody; similarly 17.65% diabetic patients showed positive and 28.03% non-diabetic were positive for *Giardia lamblia* antibody (Table-1).

According to the result of formol-ether concentration technique (F-ECT), out of 1300 stool samples (diabetic and non-diabetic individuals), 26.83% diabetic patients showed positive and 34.66% non-diabetic individuals were positive for *E. histolytica*; 28.41% diabetic patients and 34.99% non-diabetic were positive for *G. lamblia* (Table-2).

Table-1: Comparative analysis of the prevalence of *E. histolytica* and *G. lamblia* infection among diabetic and non-diabetic patients detected by ELISA.

Name of parasites	Patient groups	Total samples examined	Blood serum		Diabetic and non-diabetic positive cases
			ELISA technique Positive cases		P-value of proportion test
			n	%	
E. histolytica	Diabetic	697	111	15.93	0.025**
	Non-diabetic	603	166	27.53	
G. lamblia	Diabetic	697	123	17.65	0.040*
	Non-diabetic	603	169	28.03	
Total	Total diabetic positive cases	697	234	33.5	0.0000**
	Total non-diabetic positive cases	603	335	55.56	
Overall	Total diabetic and non-diabetic samples	1300	569	43.77	

*Significant, ** highly significant, $P<0.05$; ns= not significant at 5% level, $P>0.05$;

Table-2: Comparative analysis of the prevalence of *E. histolytica* and *G. lamblia* infection among diabetic and non-diabetic patients detected by formol-ether concentration technique.

Name of parasites	Patient groups	Total samples examined	Stool		Diabetic and non-diabetic positive cases
			Formol-ether concentration technique (Positive cases)		P-value of proportion test
			n	%	
E. histolytica	Diabetic	697	187	26.83	0.093 ns
	Non-diabetic	603	209	34.66	
G. lamblia	Diabetic	697	198	28.41	0.154 ns
	Non-diabetic	603	211	34.99	
Total	Total diabetic positive cases	697	385	55.24	0.0000**
	Total non-diabetic positive cases	603	420	69.65	
Overall	Total diabetic and non-diabetic samples	1300	805	61.93	

* Significant, ** highly significant, $P<0.05$; ns= not significant at 5% level, $P>0.05$;

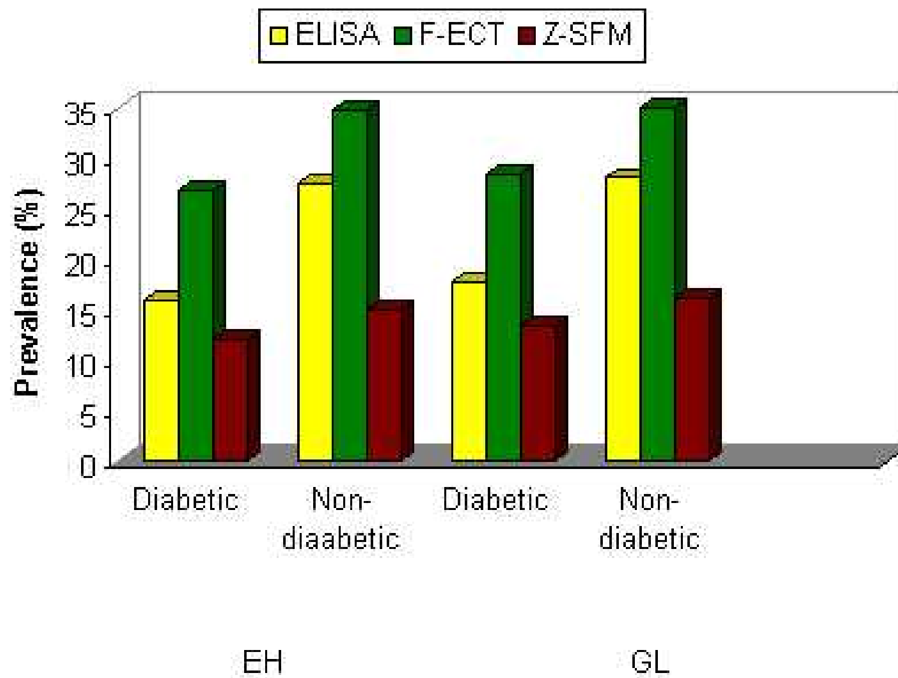
According to zinc sulphate floatation method (on stool samples of diabetic and non-diabetic individuals), 12.05% of diabetic patients and 15.09% of non-diabetic individuals were positive for *E. histolytica*; 13.34% diabetic patients and 16.09% non-diabetic were positive for *G. lamblia* (Table-3).

Table-3: Comparative analysis of the prevalence of *E. histolytica* and *G. lamblia* infection among diabetic and non-diabetic patients detected by zinc sulphate floatation method.

Name of parasites	Patient groups	Total samples examined	Stool		Diabetic and non-diabetic positive cases
			Zinc sulphate floatation method (Positive cases)		P-value of proportion test
			n	%	
E. histolytica	Diabetic	697	84	12.05	0.558 ns
	Non-diabetic	603	91	15.09	
G. lamblia	Diabetic	697	93	13.34	0.593 ns
	Non-diabetic	603	97	16.09	
Total	Total diabetic positive cases	697	177	25.39	0.220 ns
	Total non-diabetic positive cases	603	188	31.18	
Overall	Total diabetic and non-diabetic samples	1300	365	28.08	

* Significant, ** highly significant, P<0.05; ns= not significant at 5% level, P>0.05;

(Prevalence= % of infestation).



EH= *Entamoeba histolytica* GL= *Giardia lambia*

Fig.-1: The prevalence of *E. histolytica* and *G. lamblia* infection among diabetic and non-diabetic by different techniques.

Interpretation: (a). In overall observation, it is evident from the present study that, formol-ether concentration technique is most suitable and sensitive method for detection of protozoan infections (Table-1, 2, 3).

(b). According to the blood and stool samples were examined by different techniques, the prevalence of *E. histolytica* and *G. lamblia* were found higher among the peoples without diabetic than the diabetic patients (Table-1, 2, 3); (Fig.-1).

5.2 Double parasitic infestation:

The presence of two intestinal parasites species at a time in a single host was considered as double infection.

Out of 697 diabetic blood serum samples by ELISA, the prevalence of single infection of *E. histolytica* and *G. lamblia* antibody were 15.93% and 17.65%, respectively; and the prevalence of double infections were 4.45%. Out of 603 non-diabetic blood serum samples, the prevalence of single infection of *E. histolytica* and *G. lamblia* antibody were 27.53% and 28.03%; and the prevalence of double infections were 8.79% (Table-4).

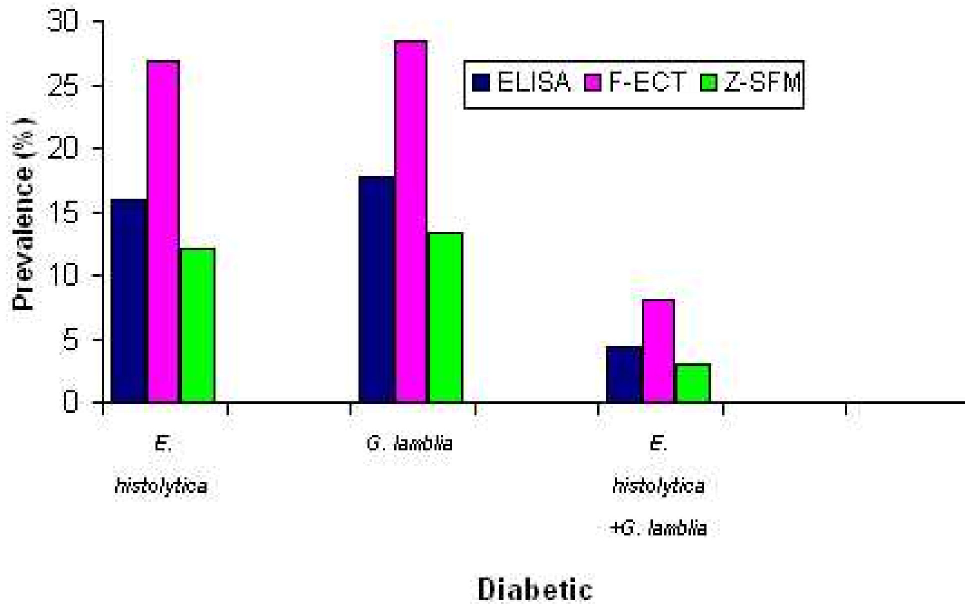


Fig.-2(A): The prevalence of single and double infection among diabetes by three techniques.

According to the result of formol-ether concentration technique, the prevalence of single infection of *E. histolytica* and *G. lamblia* were 26.83% and 28.41%, respectively; and the prevalence of double infections were 8.03%. Out of 603 non-diabetic stool samples, the prevalence of single infection of *E. histolytica* and *G. lamblia* were 34.66% and 34.99%, respectively; and the prevalence of double infections were 13.10% (Table-4).

Table-4: Comparative analysis of the prevalence of single and double infection of *E. histolytica* and *G. lamblia* among diabetic and non-diabetic patients detected by different techniques.

Name of techniques	Patient groups	Total no. of stool and blood samples examined	Single infestation				Double infestation of *E. histolytica* and *G. lamblia* (positive cases)		P-value of proportion test	
			E. histolytica (positive cases)		*G. lamblia* (positive cases)					
			n	%	n	%	n	%	P-value	Parasitic infection
ELISA (Blood serum)	Diabetic	697	111	15.93	123	17.65	31	4.45	0.025**	*E. histolytica*
	Non-diabetic	603	166	27.53	169	28.03	53	8.79	0.040*	*G. lamblia*
									0.459 ns	*E. histolytica* +*G. lamblia*
F-ECT (Stool)	Diabetic	697	187	26.83	198	28.41	56	8.03	0.093 ns	*E. histolytica*
	Non-diabetic	603	209	34.66	211	34.99	79	13.10	0.154 ns	*G. lamblia*
									0.355 ns	*E. histolytica* +*G. lamblia*
Z-SFM (Stool)	Diabetic	697	84	12.05	93	13.34	21	3.01	0.558 ns	*E. histolytica*
	Non-diabetic	603	91	15.09	97	16.09	34	5.64	0.593 ns	*G. lamblia*
									0.654 ns	*E. histolytica* +*G. lamblia*

* Significant, ** highly significant, P<0.05; ns= not significant at 5% level, P>0.05; (F-ECT= Formol-ether concentration technique), (Z-SFM=Zinc sulphate floatation method).

By zinc sulphate floatation method, the prevalence of single infection of *E. histolytica* and *G. lamblia* were 12.05% and 13.34%, respectively; and the prevalence of double infections was 3.01%. On the other hand, non-diabetic stool samples, the prevalence of single infection of *E. histolytica* and *G. lamblia* were 15.09% and 16.09%, respectively; and the prevalence of double infections was 5.64% (Table-4).

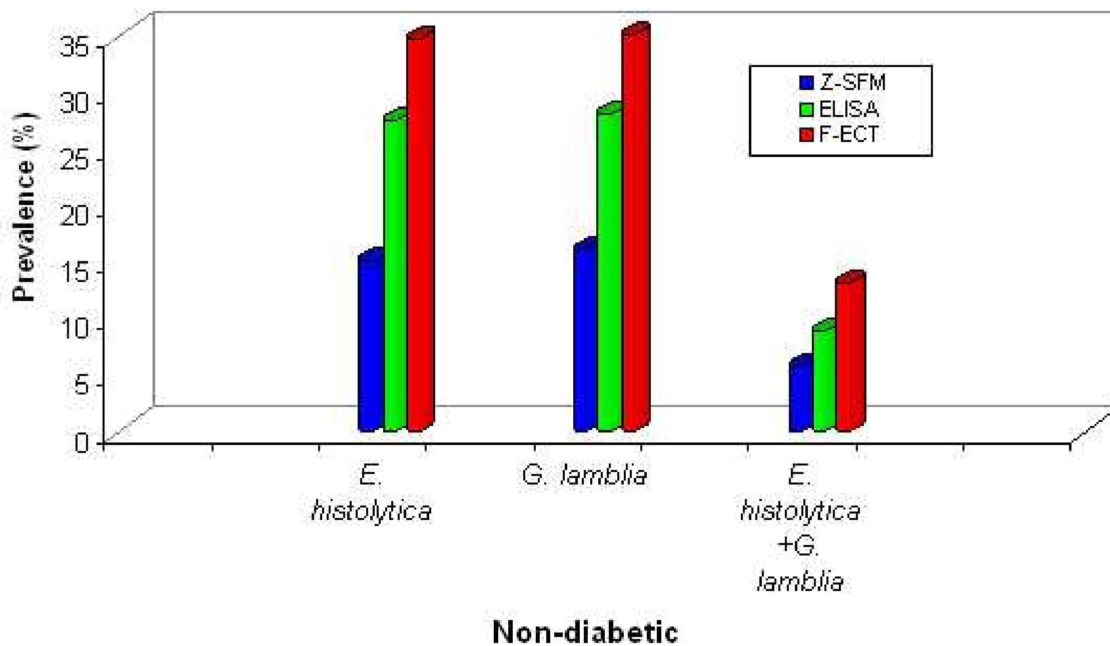


Fig.-2(B): The prevalence of single and double infection among non-diabetes by three techniques.

Interpretation:

(a). Single parasitic infections were found highly prevalent compared to the double parasitic infections among diabetic and non-diabetic patients. In comparison of the three techniques of blood and faecal samples, for single and double infections, the prevalence found always higher among the non-diabetes than the diabetic patients (Table-4); (Fig.-2A).

(b). By ELISA in diabetic patients and non-diabetic individuals showed significant result; and other two techniques (F-ECT and Z-SFM) in single and double infestation of (*E. histolytica* + *G. lamblia*) protozoan parasites showed non significant result (Table-4); (Fig.-2B).

(c). As diabetes is a chronic non-communicable disease, if uncontrolled it became gradually effect all the vital organs of the body. It also eventually involves immune system of the body. The less immune reactivity against *E. histolytica* and *G. lamblia* in diabetic patient was consistent with the present findings (Table-4).

5.3 Sex wise prevalence of *Entamoeba histolytica* and *Girdia lamblia* in blood and stool samples among diabetic and non-diabetic peoples based on different diagnostic techniques:

Total 697 diabetic blood serum samples were examined by ELISA, 359 females and 338 were males. The prevalence of *E. histolytica* was 14.48% in female and 17.46% in male. In case of *G. lamblia*, the prevalence was 15.88% in female and 19.53% in male. On the other hand, by F-ECT out of 697 diabetic stool samples, the prevalence of *E. histolytica* was 25.07% in female and 28.70% in male; and *G. lamblia* was 27.02% in female and 29.88% in male. *E. histolytica* was found 11.14% in female and 13.02% in male; and *G. lamblia* 11.70% in female and 15.09% in male by Z-SFM (Table-5); (Fig. 3A).

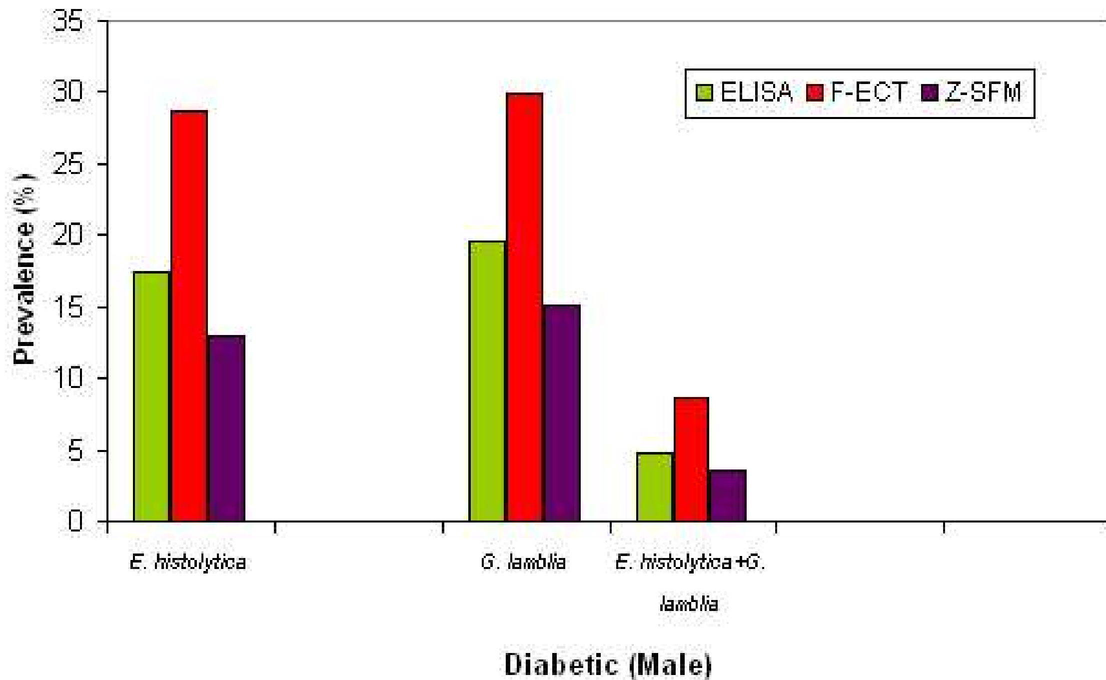


Fig.-3(A): The prevalence of single and double infection of male among diabetic by three techniques.

In diabetic patients, the prevalence of double infections (*E. histolytica*+ *G. lamblia*) was 4.18% in female and 4.73% in male were found by ELISA; and 7.52% in female and 8.58% in male were found by F-ECT and 2.51% in female and 3.55% in male by Z-SFM. In case of non-diabetic individuals, the prevalence of double infections (*E. histolytica*+ *G. lamblia*) was 7.57% in female and 10.14% in male was found by ELISA; and 11.67% in female and 14.69% in male were found by F-ECT and 4.73% in female and 6.64% in male by Z-SFM (Table-5); (Fig. 3B).

Table-5: Comparative analysis of the prevalence of *E. histolytica* and *G. lamblia* infection among diabetic and non-diabetic patients detected by different techniques by sex.

Name of techniques	Patients group	sex	Single infestation (positive cases) *E. histolytica*		*G. lamblia*		Double infestation *E. histolytica* and *G. lamblia*		P-value of proportion test	
			N	%	n	%	n	%	P-value	Parasitic infection (male+female)
ELISA (Blood serum)	Diabetic	female (n=359)	52	14.48	57	15.88	15	4.18	0.670ns	*E. histolytica*
		male (n=338)	59	17.46	66	19.53	16	4.73	0.599ns	*G. lamblia*
		(female+ male)							0.94ns	*E. histolytica* +*G. lamblia*
F-ECT (Stool)	Diabetic	female (n=359)	90	25.07	97	27.02	27	7.52	0.576ns	*E. histolytica*
		male (n=338)	97	28.70	101	29.88	29	8.58	0.645ns	*G. lamblia*
		(female+ male)							0.884ns	*E. histolytica* +*G. lamblia*
Z-SFM (Stool)	Diabetic	female (n=359)	40	11.14	42	11.70	9	2.51	0.792ns	*E. histolytica*
		male (n=338)	44	13.02	51	15.09	12	3.55	0.635ns	*G. lamblia*
		(female+male)							0.893ns	*E. histolytica* +*G. lamblia*
ELISA (Blood serum)	Non-diabetic	female (n=317)	81	25.55	84	26.50	24	7.57	0.549ns	*E. histolytica*
		male (n=286)	85	29.72	85	29.72	29	10.14	0.642ns	*G. lamblia*
		(female+ male)							0.745ns	*E. histolytica* +*G. lamblia*
F-ECT (Stool)	Non-diabetic	female (n=317)	104	32.81	104	32.81	37	11.67	0.554ns	*E. histolytica*
		male (n=286)	105	36.71	107	37.41	42	14.69	0.484ns	*G. lamblia*
		(female+ male)							0.695ns	*E. histolytica* +*G. lamblia*
Z-SFM (Stool)	Non-diabetic	female (n=317)	45	14.20	46	14.51	15	4.73	0.803ns	*E. histolytica*
		male (n=286)	46	16.08	51	17.83	19	6.64	0.659ns	*G. lamblia*
		(female+ male)							0.532ns	*E. histolytica* +*G. lamblia*

* Significant, ** highly significant, P<0.05; ns= not significant at 5% level, P>0.05;(F-ECT= Formol-ether concentration technique; Z-SFM=Zinc sulphate floatation method).

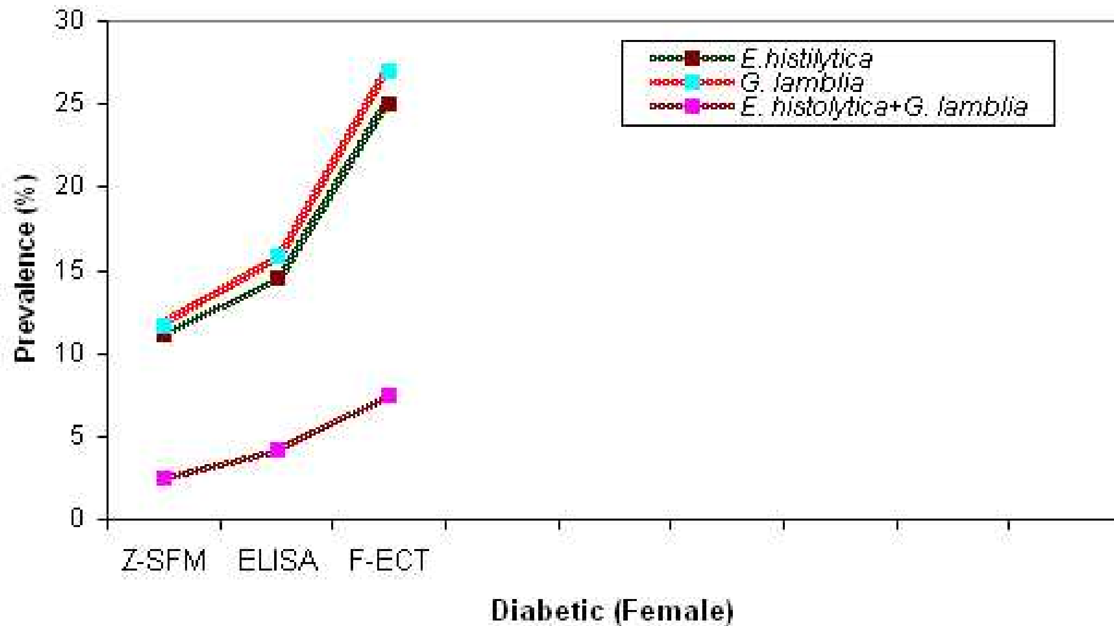


Fig.: 3(B): The prevalence of single and double infection of female among diabetic by three techniques.

According to ELISA, total 603 non-diabetic blood serum samples were examined; the prevalence of *E. histolytica* was 25.55% of female and 29.72% of male. In case of *G. lamblia*, the prevalence was 26.50% of female and 29.72% of male. On the other hand, out of 603 non-diabetic stool samples, the prevalence of *E. histolytica* was 32.81% of female and 36.71% of male; and *G. lamblia* was 32.81% of female and 37.41% of male were found by F-ECT *E. histolytica* was found 14.20% of female and 16.08% of male; and *G. lamblia* 14.81% of female and 17.83% of male by Z-SFM (Table-5).

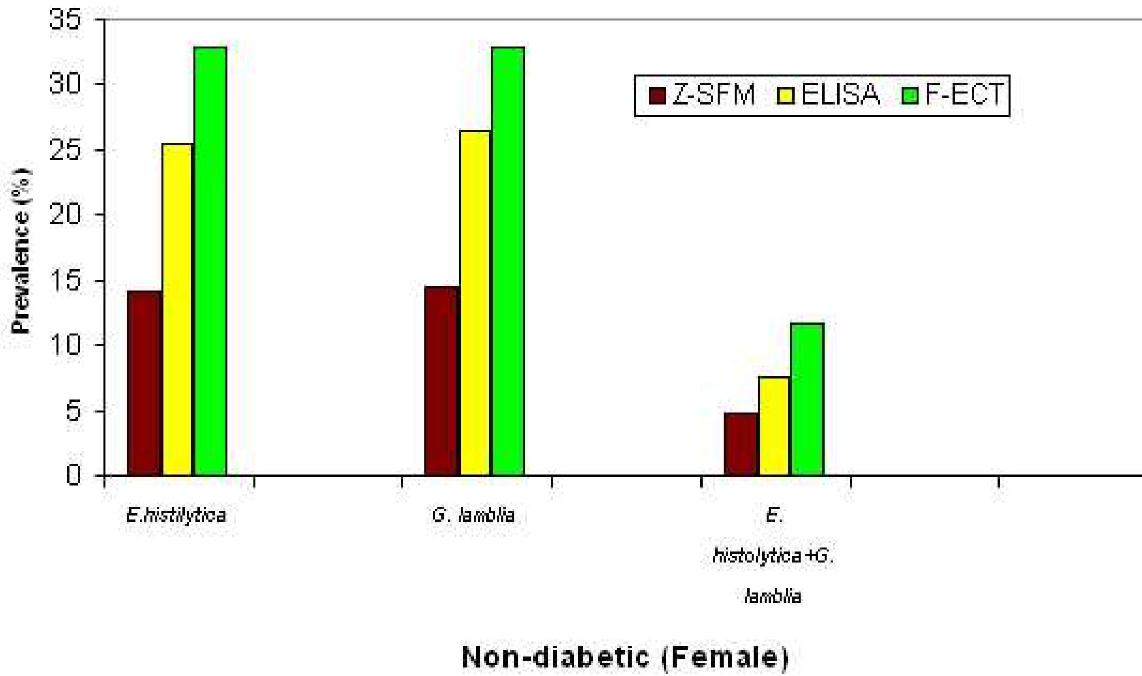


Fig.-4(A)): The prevalence of single and double infection of female among non-diabetic by three techniques.

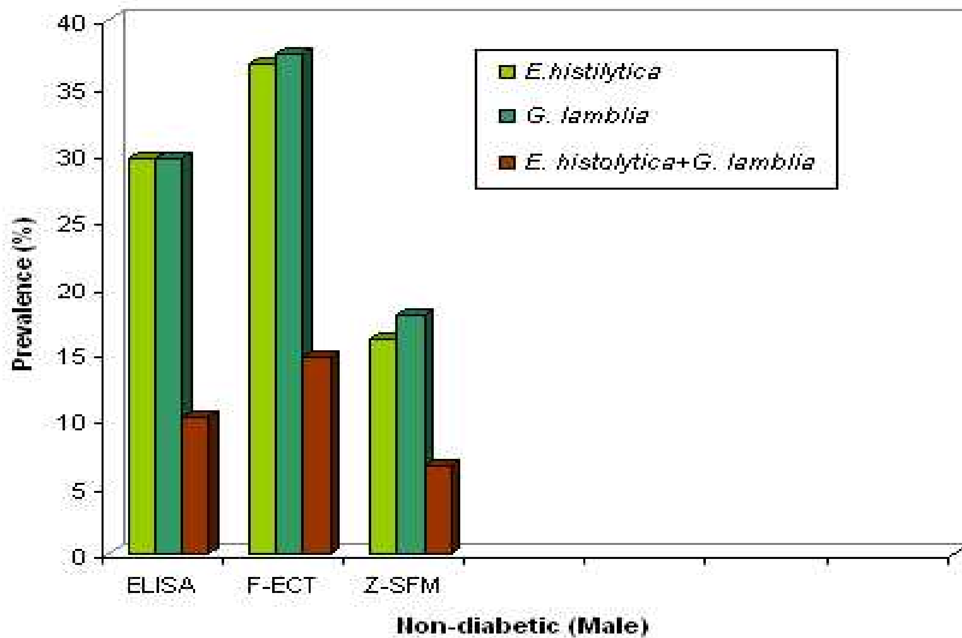
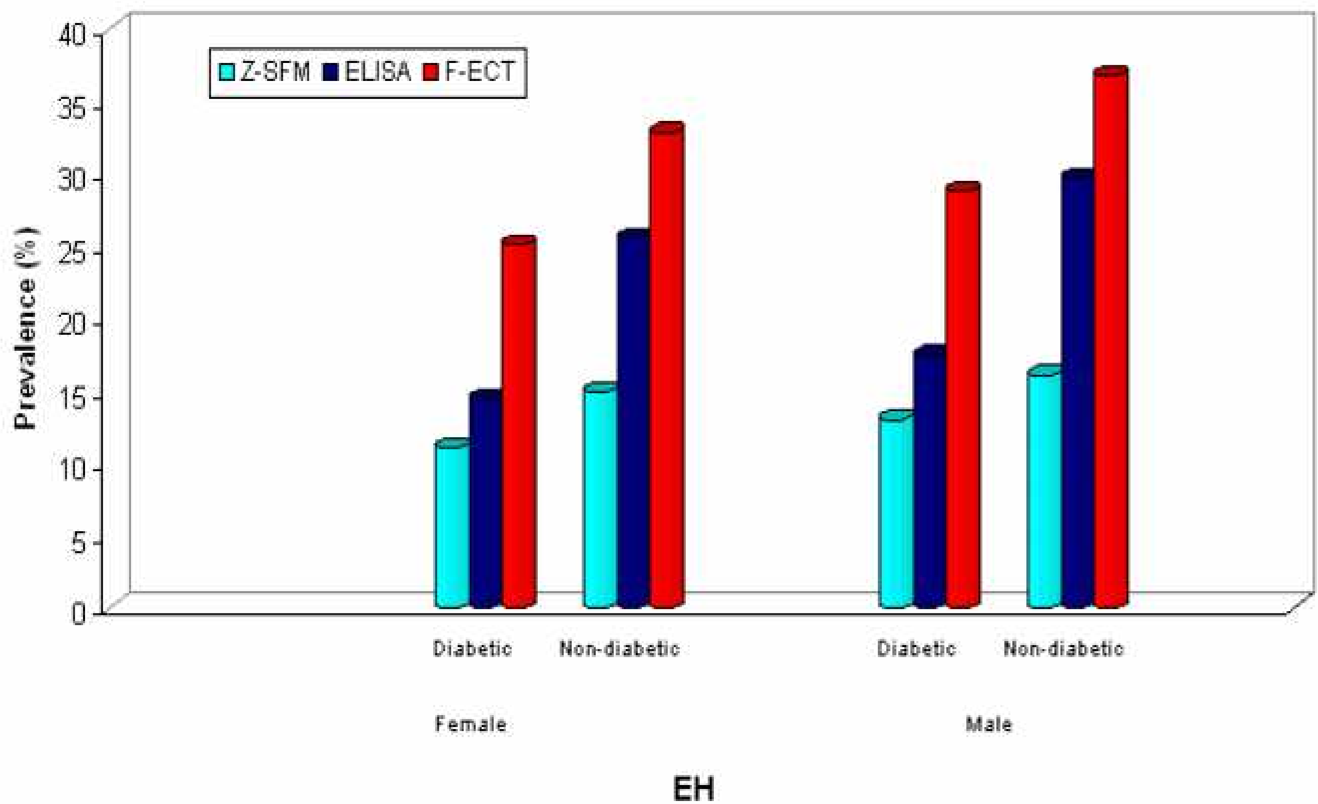


Fig.-4(B): The prevalence of single and double infection of male among non-diabetic by three techniques.

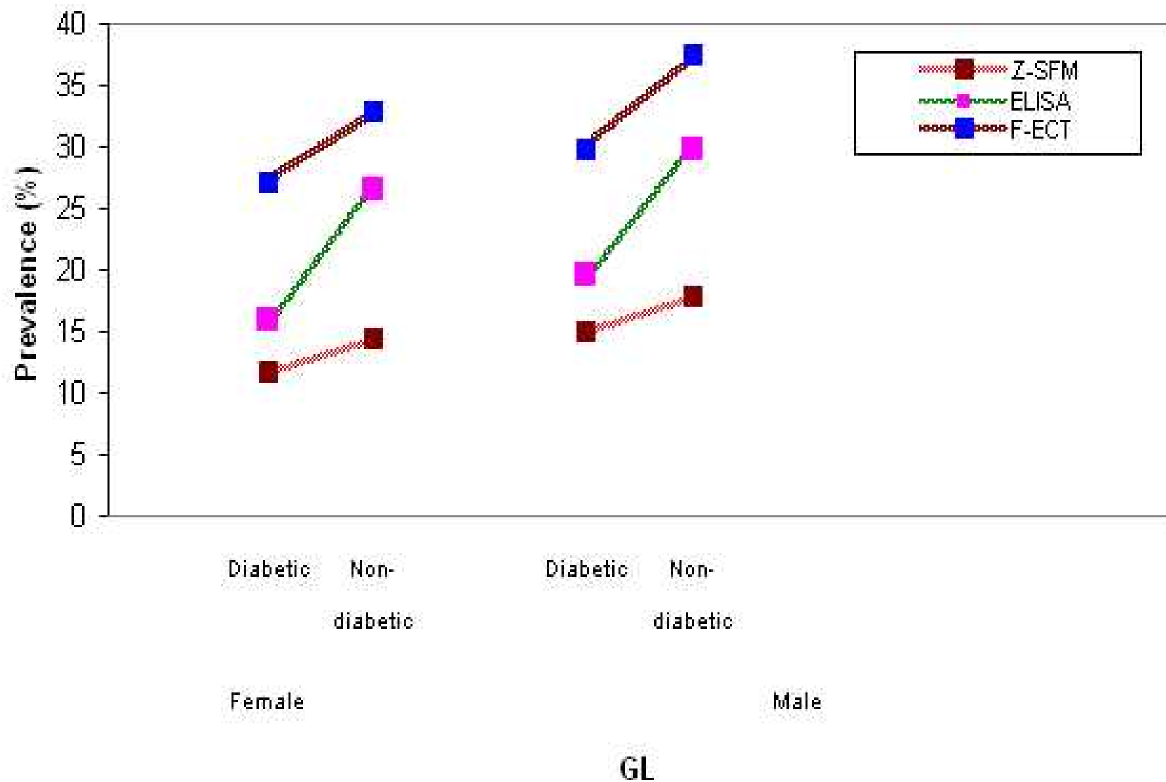
Interpretation:

Males were (slightly) more infected than females. But by three techniques result showed that both male and female were non significant. By this comparative study it can be said that in almost each cases *G. lamblia* (both female and male) had slightly greater and higher response than *E. histolytica* (both female and male) (Table-5); (Fig. 4A, B).



ET= ***Entamoeba histolytica***

Fig.-5(A): The prevalence of *E. histolytica* infections in female and male among diabetic and non-diabetic by three techniques.



GL = *Giardia lamblia*

Fig.- 5(B): The prevalence of *G. lamlia* infections of female and male among diabetic and non-diabetic by three techniques.

Interpretation:

The infection rate of *E. histolytica* and *G. lamlia* are considered to be associated with the socioeconomic status of their host; because, these organism are being spread through ingested food, contaminated water or by venereal transmission. The transmission rate of *E. histolytica* and *G. lamlia* cyst also associated with their host's sewage disposal, source of drinking water and latrine structure (Table-5-6); (Fig. 4A).

Table-6: Comparative analysis of the prevalence of *E. histolytica* and *G. lamblia* infection of male and female among diabetic and non-diabetic patients detected by different techniques.

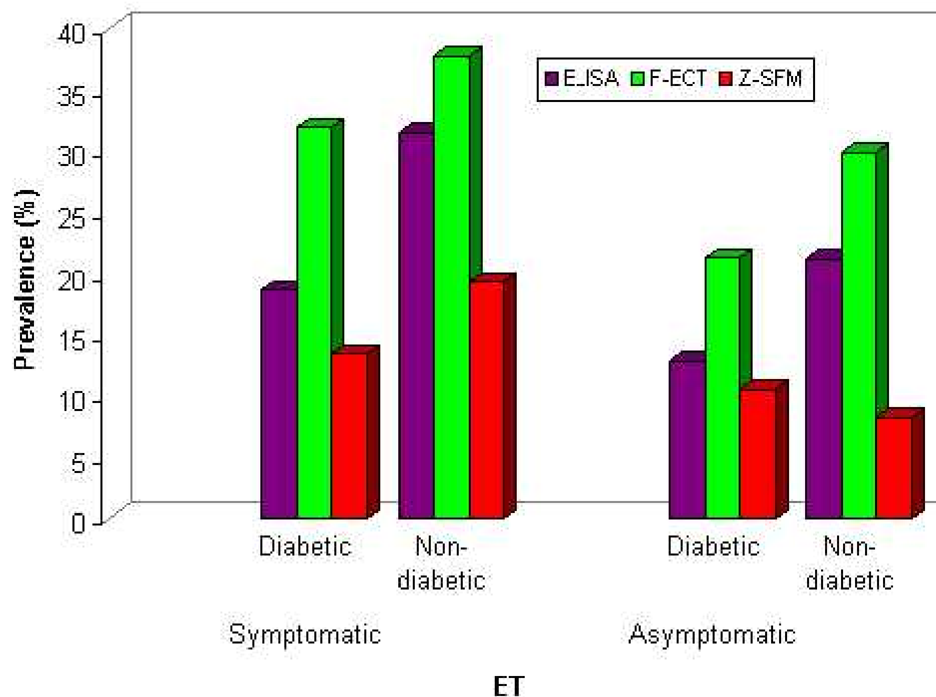
Name of parasites	Sex	Patient groups	Total samples examined	Blood serum ELISA (Positive cases)		Stool F-ECT (Positive cases)		Stool Z-SFM (Positive cases)		P-value of proportion test	
				n	%	n	%	n	%	P-value	Technique
E. histolytica	Female	Diabetic	359	52	14.4	90	25.07	40	11.14	0.130 ns	ELISA
		Non-diabetic	317	81	25.55	104	32.81	45	14.96	0.238 ns	F-ECT
										0.604 ns	Z-SFM
	Male	Diabetic	338	59	17.46	97	28.70	44	13.02	0.095 ns	ELISA
		Non-diabetic	286	85	29.72	105	36.71	46	16.08	0.227 ns	F-ECT
										0.682ns	Z-SFM
G. lamblia	Female	Diabetic	359	57	15.88	97	27.02	42	11.70	0.138 ns	ELISA
		Non-diabetic	317	84	26.50	104	32.81	46	14.51	0.367 ns	F-ECT
				0.138 ns		0.367 ns		0.698 ns		0.698 ns	Z-SFM
	Male	Diabetic	338	66	19.53	101	29.88	51	15.09	0.155 ns	ELISA
		Non-diabetic	286	85	29.72	107	37.41	51	17.83	0.155 ns	F-ECT
										0.709 ns	Z-SFM

* Significant, ** highly significant, P<0.05; ns= not significant at 5% level, P>0.05;

Interpretation: The prevalence of amoebiasis and giardiasis among male was higher, than that of female, as the male population are more exposed to external environment, they use public toilet, take food and water from outside and cannot maintain proper personal hygiene when they are outside of their residence (Table-5-6); (Fig. 4B).

5.4 Parasitic infestation among the diabetic and non diabetic peoples (symptomatic and asymptomatic):

Out of 697 diabetic blood serum samples were examined by ELISA. In total blood samples (from symptomatic), 18.68% was positive, and (from asymptomatic) blood samples, 12.91% was for *E. histolytica* antibody. Out of 603 non-diabetic blood serum samples, 372 samples (from symptomatic) and 231 (from asymptomatic) were positive. In total blood samples of (from symptomatic) 31.45% was positive, and on the other hand, blood samples (from asymptomatic), 21.21% was for *E. histolytica* antibody. The serological diagnosis by ELISA, (from symptomatic and asymptomatic) both diabetic patients and non-diabetic individuals samples showed highly significant result (Table-7); (Fig 6A).



ET= *Entamoeba histolytica*

Fig-6(A): Prevalence of *E. hitolytica* among diabetic and non-diabetic patients on the basis of symptomatic and asymptomatic samples by three techniques.

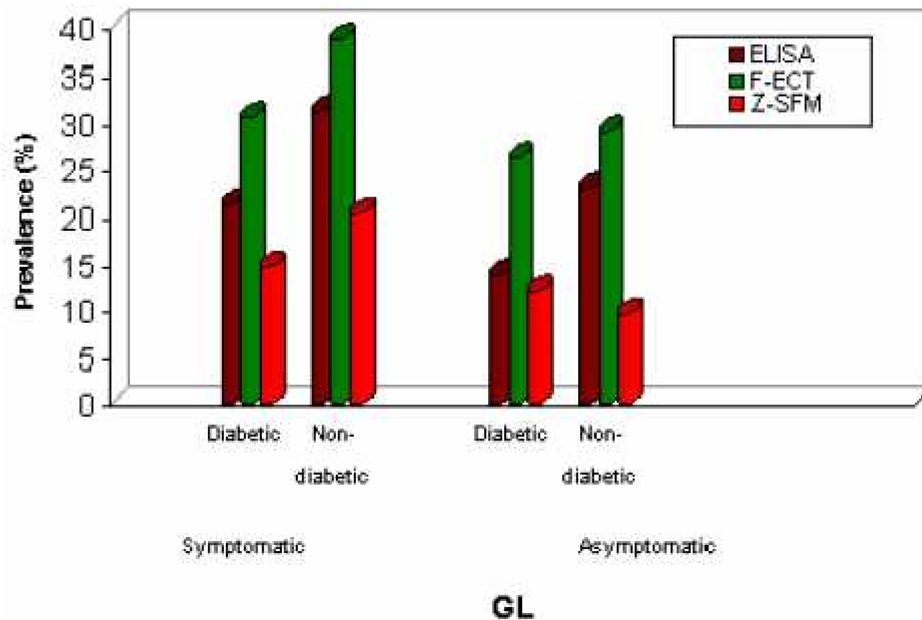
Table-7: The prevalence of *E. histolytica* among diabetic and non-diabetic patients on the basis of symptomatic and asymptomatic samples detected by three techniques.

Type of samples	Patient groups	Total number of samples examined	Prevalence of *E. histolytica*						
			Blood serum		Stool				
			ELISA (Positive cases)		F-ECT (Positive cases)			Z-SFM (Positive cases)	
			n	%	n		%	n	%
Symptomatic	Diabetic	364	68	18.68	116		31.87	49	13.46
	Non-diabetic	372	117	31.45	140		37.63	72	19.35
P-value of proportion test			0.059*		0.337 ns			0.398 ns	
Asymptomatic	Diabetic	333	43	12.91	71		21.32	35	10.51
	Non-diabetic	231	49	21.21	69		29.87	19	8.23
P-value of proportion test			0.277 ns		0.248 ns			0.788 ns	
Total of symptomatic and asymptomatic samples	Total diabetic positive cases		697	111	15.93	187	26.83	84	12.05
	Total non-diabetic positive cases		603	166	27.53	209	34.66	91	15.09
	P-value of proportion test			0.025 **	0.093 ns		0.558 ns		
P-value of proportion test between symptomatic and asymptomatic sample of diabetic patients				0.426 ns	0.120 ns		0.685 ns		
P-value of proportion test between symptomatic and asymptomatic sample of non-diabetic patients				0.184 ns	0.270 ns		0.254 ns		
Over all	Total diabetic and non-diabetic samples	1300		277	21.31	396	30.46	175	13.46

* Significant; ** highly significant; ns= not significant at 5% level.

According to F-ECT, out of 697 diabetic stool samples, among symptomatic (diarrhea) types, 31.87% was positive and 21.32% was asymptomatic types for *E. histolytica.* Out of 603 non-diabetic stool samples, 37.63% was positive in symptomatic (diarrhea) types and 29.87% asymptomatic types, was for *E. histolytica* (Table-7); (Fig 6A).

On the other hand, from Z-SFM, diabetic stool samples, 13.46% was positive among symptomatic (diarrhea) types and 10.51% asymptomatic types were for *E. histolytica*. Out of 603 non-diabetic stool samples, 19.35% was positive from symptomatic types and 15.09% was for *E. histolytica*; asymptomatic types, respectively. Other two techniques (F-ECT, Z-SFM) techniques showed non significant result (Table-7).



GL= *Giardia lamblia*

Fig-6(B): Prevalence of *G. lamlia* among diabetic and non-diabetic patients on the basis of symptomatic and asymptomatic samples by three techniques.

According to ELISA, out of 364 diabetic blood serum samples (from symptomatic) and 333 samples (from asymptomatic) were examined. Among blood (from symptomatic) samples, 21.15% was positive, and in (from asymptomatic) blood samples, 13.8% for *G. lamlia* antibody. Out of 603 non-diabetic blood samples, 372 samples (from symptomatic) and 231 samples (from asymptomatic) were examined. Among blood (from symptomatic) samples 31.18% was positive, and in (from asymptomatic) samples, 22.94% was for *G. lamlia* antibody (Table-8); (Fig 6B).

According to F-ECT, among symptomatic (diarrhea) types, 30.49% was positive and 26.13% in asymptomatic types was for *G. lamlia*. In samples of symptomatic types, 38.71% was positive and in asymptomatic types, 29% was for *G. lamlia* (Table-8).

On the other hand, by Z-SFM, among diabetic symptomatic types 14.56% was positive and in asymptomatic types 12.01% was for *G. lamlia*. Non-diabetic symptomatic types 20.16% was positive and in asymptomatic types 9.52% was for *G. lamlia* (Table-8).

Interpretation:

Comparatively higher prevalence of *E. histolytica* and *G. lamlia* were found in symptomatic stool samples; ELISA also detected high prevalence in (from symptomatic) blood samples than (from asymptomatic) blood samples. A major part of the infected patients are found asymptomatic in both diabetic and non-diabetic patients (Table-7, 8).

Table-8: The prevalence of *G. lamlia* among diabetic and non-diabetic patients on the basis of symptomatic and asymptomatic stool samples detected by different techniques.

Type of samples	Patient groups	Total number of samples examined	Prevalence of *G. lamlia*					
			Blood serum		Stool			
			ELISA (Positive cases)		F-ECT (Positive cases)		Z-SFM (Positive cases)	
			n	%	n	%	N	%
Symptomatic	Diabetic	364	77	21.15	111	30.49	53	14.56
	Non-diabetic	372	116	31.18	144	38.71	75	20.16
P-value of proportion test			0.126 ns		0.174 ns		0.416	
Asymptomatic	Diabetic	333	46	13.81	87	26.13	40	12.01
	Non-diabetic	231	53	22.94	67	29.00	22	9.52
P-value of proportion test			0.248 ns		0.692 ns		0.934	
Total of symptomatic and asymptomatic samples	Total diabetic positive cases	697	123	17.65	198	28.41	93	13.34
	Total non-diabetic positive cases	603	169	28.03	211	34.99	97	16.09
	P-value of proportion test		0.042*		0.154 ns		0.593 ns	
P-value of proportion test between symptomatic and asymptomatic sample of diabetic patients			0.311 ns		0.501 ns		0.722 ns	
P-value of proportion test between symptomatic and asymptomatic sample of non-diabetic patients			0.273 ns		0.171 ns		0.253 ns	
Overall	Total diabetic and non-diabetic samples	1300	292	22.46	409	31.46	190	14.62

* Significant; ** highly significant; ns= not significant at 5% level;

Interpretation:

By ELISA, (from symptomatic and asymptomatic) both diabetic patients and non-diabetic individual samples showed significant result; and other two techniques (F-ECT, Z-SFM) techniques showed non significant result (Table-7, 8).

According to ELISA, out of 364 diabetic (from symptomatic) blood samples were examined, 17.46% of female and 20% of male were positive for *E. histolytica* antibody. From 333 diabetic (from asymptomatic) individuals, 11.18% of female and 14.72% in male was positive. On the other hand, out of 372 non-diabetic (from symptomatic) individuals, 31.25% of female and 31.67% of male were positive. From 231 non-diabetic asymptomatic individuals, 16.8% of female and 26.42% of male were positive for *E. histolytica* antibody (Table-9).

According to F-EC, out of 364 diabetic symptomatic (diarrhea) samples, 30.16% of female and 33.71% of male were positive for *E. histolytica*. From 333 diabetic asymptomatic individuals, 19.41% of female and 23.31% of male were positive. On the other hand, out of 372 non-diabetic symptomatic (diarrhea) individuals, 34.38% of female and 41.11%; of male were positive. From 231 non-diabetic asymptomatic individuals, 30.4% of female and 29.25% of male were positive for *E. histolytica* (Table-9).

Table-9: Comparative observation of the prevalence of *E. histolytica* among diabetic and non-diabetic patients on the basis of symptomatic and asymptomatic samples (female and male) detected by three techniques.

<table>
<tr><th rowspan="3">Patients group</th><th rowspan="3">Type of samples</th><th rowspan="3">Sex groups</th><th rowspan="3">Total number of samples examined</th><th colspan="3">Prevalence of E. histolytica</th></tr>
<tr><th>Blood serum</th><th colspan="2">Stool</th></tr>
<tr><th>ELISA (%)</th><th>F-ECT (%)</th><th>Z-SFM (%)</th></tr>
<tr><td rowspan="10">Diabetic</td><td rowspan="3">Symptomatic</td><td>Female</td><td>189</td><td>33(17.46%)</td><td>57(30.16%)</td><td>24(12.69%)</td></tr>
<tr><td>Male</td><td>175</td><td>35(20%)</td><td>59(33.71%)</td><td>25(14.29%)</td></tr>
<tr><td>Total</td><td>364</td><td>68(18.68%)</td><td>116(31.87%)</td><td>49(13.46%)</td></tr>
<tr><td colspan="3">P-value of proportion test between male and female</td><td>0.789 ns</td><td>0.682 ns</td><td>0.870 ns</td></tr>
<tr><td rowspan="3">Asymptomatic</td><td>Female</td><td>170</td><td>19(11.18%)</td><td>33(19.41%)</td><td>16(9.41%)</td></tr>
<tr><td>Male</td><td>163</td><td>24(14.72%)</td><td>38(23.31%)</td><td>22(13.50%)</td></tr>
<tr><td>Total</td><td>333</td><td>43(12.91%)</td><td>71(21.32%)</td><td>38(11.41%)</td></tr>
<tr><td colspan="3">P-value of proportion test between male and female</td><td>0.734 ns</td><td>0.691 ns</td><td>0.637 ns</td></tr>
<tr><td rowspan="8">Non- diabetic</td><td rowspan="3">Symptomatic</td><td>Female</td><td>192</td><td>60(31.25%)</td><td>66(34.38%)</td><td>34(17.71%)</td></tr>
<tr><td>Male</td><td>180</td><td>57(31.67%)</td><td>74(41.11%)</td><td>38(21.11%)</td></tr>
<tr><td>Total</td><td>372</td><td>117(31.45%)</td><td>140(37.63%)</td><td>72(19.35%)</td></tr>
<tr><td colspan="3">P-value of proportion test between male and female</td><td>0.961 ns</td><td>0.414 ns</td><td>0.717 ns</td></tr>
<tr><td rowspan="3">Asymptomatic</td><td>Female</td><td>125</td><td>21(16.8%)</td><td>38(30.4%)</td><td>11(8.8%)</td></tr>
<tr><td>Male</td><td>106</td><td>28(26.42%)</td><td>31(29.25%)</td><td>8(7.54%)</td></tr>
<tr><td>Total</td><td>231</td><td>49(21.21%)</td><td>69(29.87%)</td><td>19(8.23%)</td></tr>
<tr><td colspan="3">P-value of proportion test between female and male</td><td>0.427 ns</td><td>0.917 ns</td><td>0.922 ns</td></tr>
<tr><td rowspan="3">Total of symptomatic and asymptomatic samples</td><td colspan="2">Total diabetic samples</td><td>697</td><td>111(15.92%)</td><td>187(26.83%)</td><td>84(12.05%)</td></tr>
<tr><td colspan="2">Total non-diabetic samples</td><td>603</td><td>166(27.53%)</td><td>209(34.66%)</td><td>91(15.09%)</td></tr>
<tr><td colspan="3">P-value of proportion test between diabetic and non diabetic samples</td><td>0.025 **</td><td>0.093 ns</td><td>0.499 ns</td></tr>
<tr><td colspan="4">Total diabetic and non-diabetic samples 1300</td><td>277(21.31%)</td><td>396(30.46%)</td><td>175(13.46%)</td></tr>
</table>

* Significant; ** highly significant; ns= not significant at 5% level.

(F-ECT= Formol-ether concentration technique; Z-SFM=Zinc sulphate floatation method)

On the other hand, from Z-SF method, diabetic symptomatic (diarrhea) individuals, 12.69% of female and 14.29% of male were positive for *E. histolytica*; asymptomatic types, 9.41% of female and 13.50% of male were positive. In the case of non-diabetic symptomatic (diarrhea) individuals, 17.71% of female and 21.11% of male; non-diabetic asymptomatic individuals, 8.8% of female and 7.54% of male were found for *E. histolytica* (Table-9).

According to ELISA, out of 364 diabetic (from symptomatic) blood samples were examined, 19.58% of female and 22.86% of male were positive for *G. lamlia* antibody. From 333 diabetic (from asymptomatic) individuals, 11.77% of female and 15.95% of male was positive. On the other hand, out of 372 non-diabetic (from symptomatic) individuals, 30.73% of female and 31.67% of male were positive. From 231 non-diabetic (from asymptomatic) individuals, 20% of female and 26.42% of male were positive for *G. lamlia* antibody (Table-10).

According to the result of F-EC technique, diabetic symptomatic (diarrhea) stool samples, 28.57% of female and 32.57% of male were found for *G. lamlia.* Diabetic asymptomatic individuals, 25.29% of female and 26.99% of male were positive. In the case of non-diabetic symptomatic (diarrhea) individuals, 36.46% of female and 41.11% of male were positive. Non-diabetic asymptomatic type, 27.2% of female and 31.13% of male were positive (Table-10).

Table-10: The prevalence of *G. lamlia* among diabetic and non-diabetic patients on the basis of symptomatic and asymptomatic samples (female and male) detected by different techniques.

Patients group	Type of samples	Sex groups	Total number of samples examined	Prevalence of *G. lamlia*		
				Blood serum	Stool	
				ELISA (%)	F-ECT (%)	Z-SFM (%)
Diabetic	Symptomatic	Female	189	37(19.58%)	54(28.57%)	23(12.17%)
		Male	175	40(22.86%)	57(32.57%)	30(17.14%)
		Total	364	77(21.15%)	111(30.49%)	53(14.56%)
	P-value of proportion test between male and female			0.726 ns	0.648 ns	0.588 ns
	Asymptomatic	Female	170	20(11.77%)	43(25.29%)	19(11.18%)
		Male	163	26(15.95%)	44(26.99%)	21(12.88%)
		Total	333	46(13.81%)	87(26.13%)	40(12.01%)
	P-value of proportion test between male and female			0.688 ns	0.857 ns	0.870 ns
Non- diabetic	Symptomatic	Female	192	59(30.73%)	70(36.46%)	38(19.79%)
		Male	180	57(31.67%)	74(41.11%)	37(20.56%)
		Total	372	116(31.18%)	144(38.71%)	75(20.16%)
	P-value of proportion test between male and female			0.913 ns	0.568 ns	0.934 ns
	Asymptomatic	Female	125	25(20%)	34(27.2%)	8(6.4%)
		Male	106	28(26.42%)	33(31.13%)	14(13.21%)
		Total	231	53(22.94%)	67(29%)	22(9.52%)
	P-value of proportion test between female and male			0.583 ns	0.724 ns	0.625 ns
Total of symptomatic and asymptomatic samples	Total diabetic samples		697	123(17.65%)	198(28.41%)	93(13.34%)
	Total non-diabetic samples		603	169(28.03%)	211(34.99%)	97(16.08%)
	P-value of proportion test between diabetic and non diabetic samples			0.040**	0.154 ns	0.594 ns
Total diabetic and non-diabetic samples			1300	292(22.46%)	409(31.46%)	190(14.62%)

* Significant; ** highly significant; ns= not significant at 5% level

(F-ECT= Formol-ether concentration technique; Z-SFM=Zinc sulphate floatation method).

On the other hand, from Z-SF method, diabetic symptomatic (diarrhea) individuals, 12.17% of female and17.14% of male were positive for *G. lamlia.* Asymptomatic types, 11.18% of female and 12.88% of male were positive. In the case of non-diabetic symptomatic (diarrhea) individuals, 19.79% of female and 20.56% of male were positive. Non-diabetic asymptomatic types, 6.4% of female and 13.21% of male were positive for *G. lamlia* (Table-10).

Interpretation:

(a). Males were more frequently infected by *E. histolytica* and *G. lamblia* than females. A major part of the infected patients were found asymptomatic in both diabetic and non-diabetic patients (Table-9, 10). .

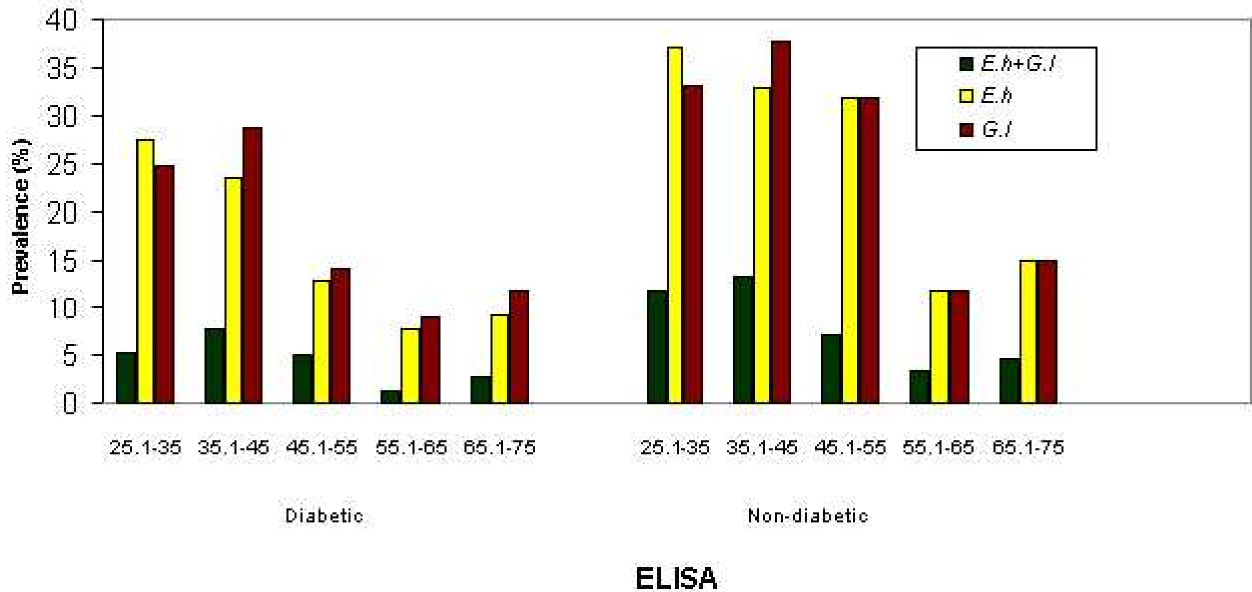
(b). By ELISA, (from symptomatic and asymptomatic) both diabetic patients and non-diabetic individuals samples showed significant result; and other two techniques (F-ECT, Z-SFM) techniques showed non significant result (Table-9, 10).

5.5 Age wise prevalence of *E. histolytica* and *G. lamblia* in stool and blood samples among diabetic and non-diabetic peoples based on different diagnostic techniques:

Age may be an important factor for parasitic infection. Ages of diabetic and non-diabetic individuals were categorized in five groups in the present study. Age of the diabetic and non-diabetic individuals, appear to be an important variable for the infestation of intestinal parasites; nevertheless, the infestation of double parasite was not highly remarkable in the present study.

Among all five age groups, among diabetic patients by ELISA, the highest prevalence of *E. histolytica* was 27.43% in the age group of 25-35 years and the lowest 7.74% in 55-65 years. In the case of *G. lamblia,* the highest prevalence was 28.66% found in the age group of 35-45 years and the lowest 9.03% in 55-65 years. In case of double infection

(diabetic) of *E. histolytica* and *G. lamblia,* the highest prevalence was 7.64% in the age of 35-45 years and the lowest prevalence was 1.29% in 55-65 years (Table 11); (Fig 7A).



E.h= E. histolytica* ; *G.l = G. lamblia;

E.h + G.l = E. histolytica + G. lamblia.

Fig-7(A): The prevalence of single and double infection in age groups among diabetic and non-diabetic by ELISA.

Considering all of the five age groups, non-diabetic individuals of age group 25-35 years was more infected (37.09%) as compared with other age groups and lowest 10.75% was found in 55-65 year group for *E. histolytica*. Similarly the highest prevalence was 37.76% found in the age group of 25-35 years and the lowest 11.83% in 55-65 years for *G. lamblia*. In case of non-diabetic the double infection of *E. histolytica* and *G. lamblia,* highest (13.29%) in the age of 35-45 years and the lowest prevalence was 3.23% in 55-65 years (Table 11).

Table-11: The prevalence of single and double infection of *E. histolytica* and *G. lamblia* in different age groups among diabetic and non-diabetic patients detected by ELISA.

Patient Groups	Age groups (in year)	Total samples examined	Single infestation				Double infestation	Prevalence (%)
			E. histolytica (positive cases)	*E. histolytica* (%)	*G. lamblia* (positive cases)	*G. lamblia* (%)	*E. histolytica* and *G. lamblia* (positive cases)	*E. histolytica and G. lamblia* (%)
Diabetic	(25.1-35)	113	31	27.43	28	24.78	6	5.31
	(35.1-45)	157	37	23.57	45	28.66	12	7.64
	(45.1-55)	163	21	12.88	23	14.11	8	4.91
	(55.1-65)	155	12	7.74	14	9.03	2	1.29
	(65.1-75)	109	10	9.17	13	11.93	3	2.75
Non-diabetic	(25.1-35)	151	56	37.09	50	33.11	18	11.92
	(35.1-45)	143	47	32.87	54	37.76	19	13.29
	(45.1-55)	129	40	31.01	41	31.78	9	6.98
	(55.1-65)	93	10	10.75	11	11.83	3	3.23
	(65.1-75)	87	13	14.94	13	14.94	4	4.59
Total diabetic samples across the age group		697	111	15.93	123	17.65	31	4.45
Total non-diabetic samples across the age group		603	166	27.53	169	28.03	53	8.79

* Significant; ** highly significant, P<0.05; ns= not significant at 5% level, P>0.05

In diabetic patients, correlation coefficient (r) between age and prevalence of infection were given below:

Single infestation cases, (i). *E. histolytica*: r = – 0.93*, p=0.019, n=5; (ii). *G. lamblia*: r = – 0.83, p=0.075, n=5; and

Double infestation cases, (iii) *E. histolytica and G. lamblia:* r = – 0.74, p=0.152, n=5

In non-diabetic patients, correlation coefficient (r) between age and prevalence of infection were

Single infestation cases, (i). *E. histolytica*: r = – 0.963**, p=0.008, n=5, (ii). *G. lamblia*: r = – 0.843, p=0.073, n=5

Double infestation cases, (iii) *E. histolytica and G. lamblia*: r = – 0.90, p=0.097, n=5

P-value of proportion test between diabetic and non diabetic samples were (i). *E. histolytica*: P=0.025**; (ii). *G. lamblia*: P=0.040* (iii) *E. histolytica and G. lamblia:* P=0.459 ns.

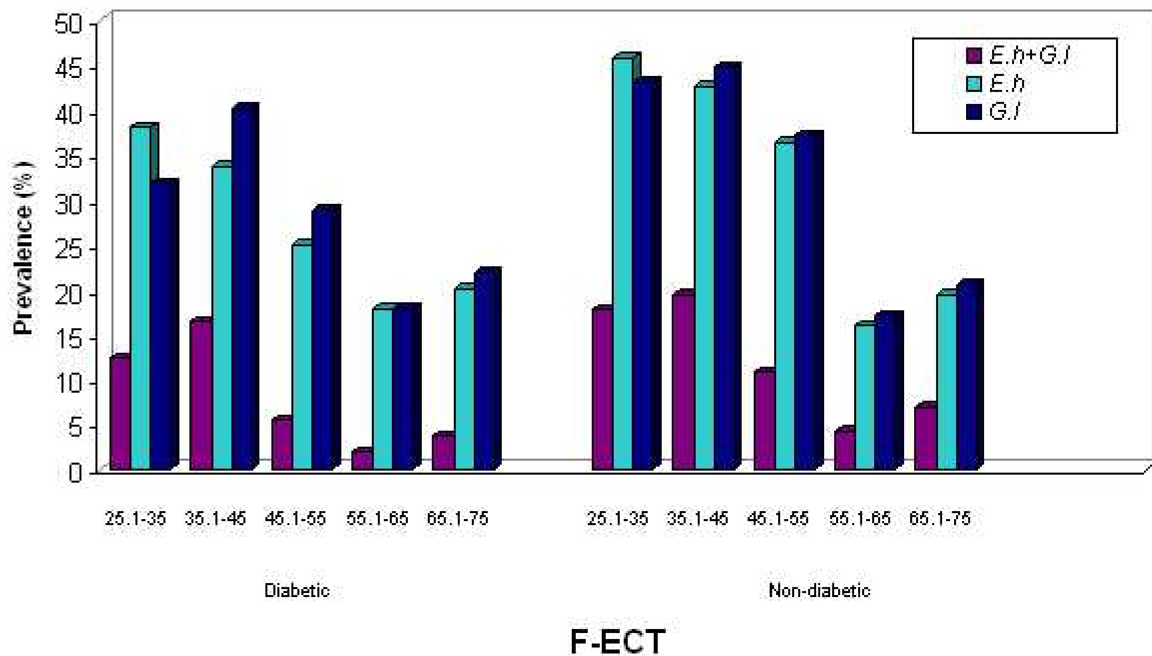
Interpretation: Strength of relationship or linear of relationship between age and prevalence of infection was found to be significantly co-related.

Among diabetic patients, the prevalence of *E. histolytica* was negatively correlated with ages (r = – 0.93**, p<0.019) which implied that as the age increased, protozoan parasite infection tends to decrease.

Among diabetic patients, the prevalence of *G. lamblia* was found negatively correlated with ages (r = – 0.83, p>0.075). Age group was not significantly associated (p>0.075) with infection by *G. lamblia* parasite. On the other hand, infection by *E. histolytica* parasite was found to be highly significant and associated (p<0.019) with age groups. Age of the diabetic patients, the infestation of double parasite was not significant results: (r = – 0.74, p=0.152) (Table-11).

In *E. histolytica* cases (non-diabetic patients), the prevalence was negatively correlated with age (r = -0.963**, p<0.008). In *G. lamblia* cases (non-diabetic patients), the prevalence was found negatively correlated with age groups (r = - 0.843). Age group was not significantly associated (p>0.073) with infection by *G. lamblia* parasite. On the other hand, infected by *E. histolytica* parasite was found to be significantly associated (p<0.008) with age groups (Table-11).

According to F-ECT, among all five age groups, of diabetic patients, the highest prevalence of *E. histolytica* was 38.05% in the age group of 25-35 years and the lowest 38.05% in 55-65 years. *G. lamblia*, infection found highest (40.13%) in the age group of 35-45 years and the lowest (18.06%) in 55-65 years. In case of double infection (diabetic), the highest prevalence was 16.56% in the age of 35-45 years and the lowest 1.94% in 55-65 years (Table 12).



E.h= E. histolytica* ; *G.l = G. lamblia;

E.h + G.l = E. histolytica + G. lamblia.

Fig-7(B): The prevalence of single and double infection in age groups among diabetic and non-diabetic by F-ECT.

Considering the five age groups, among non-diabetic individuals, 25-35 years was more infected (45.69%) as compared with other age groups and lowest infection (16.13%) was found in 55-65 year group for *E. histolytica.* Similarly the highest (non-diabetic) prevalence (44.76%) was found in the age group of 35-45 years and the lowest (17.20%) in 55-65 years for *G. lamblia*. In case of double infection (non-diabetic) the highest was 19.58% in the age of 35-45 years and the lowest prevalence was 4.30% in 55-65 years (Table 12); (Fig 7B).

Table-12: The prevalence of single and double infection of *E. histolytica* and *G. lamblia* in different age groups among diabetic and non-diabetic patients detected by F-ECT.

Patient groups	Age groups (in year)	Total examined samples	Single infestation				Double infestation	Prevalence (%)
			E. histolytica (positive cases)	*E. histolytica* (%)	*G. lamblia* (positive cases)	*G. lamblia* (%)	*E. histolytica and G. lamblia* (positive cases)	*E. histolytica and G. lamblia*
Diabetic	(25.1-35)	113	43	38.05	36	31.86	14	12.39
	(35.1-45)	157	53	33.76	63	40.13	26	16.56
	(45.1-55)	163	41	25.15	47	28.83	9	5.52
	(55.1-65)	155	28	18.06	28	18.06	3	1.94
	(65.1-75)	109	22	20.18	24	22.02	4	3.67
Non-diabetic	(25.1-35)	151	69	45.69	65	43.05	27	17.88
	(35.1-45)	143	61	42.66	64	44.76	28	19.58
	(45.1-55)	129	47	36.43	48	37.21	14	10.85
	(55.1-65)	93	15	16.13	16	17.20	4	4.30
	(65.1-75)	87	17	19.54	18	20.69	6	6.89
Total diabetic samples across the age group		697	187	26.83	198	39.84	56	8.03
Total non-diabetic samples across the age group		603	209	34.66	211	34.99	79	13.10

* Significant; ** highly significant; ns= not significant at 5% level

Single infestation cases,

(i). *E. histolytica*: r = – 0.943, p=0.016, n=5; (ii). *G. lamblia*: r = – 0.766, p=0.13, n=5; and

Double infestation cases, (iii) *E. histolytica and G. lamblia:* r = – 0.817, p=0.092, n=5

In **non-diabetic** patients, correlation coefficient (r) between age and prevalence of infection were given below:

Single infestation cases,

(i). *E. histolytica*: r = – 0.979, p=0.004, n=5, (ii). *G. lamblia*: r = – 0.892, p=0.04, n=5

Double infestation cases,

(iii) *E. histolytica and G. lamblia*: r = – 0.881, p=0.048, n=5

P-value of proportion test between diabetic and non diabetic samples were

(i). *E. histolytica*: P=0.093 ns**;** (ii). *G. lamblia*: P=0.311ns; (iii) *E. histolytica and G. lamblia:* P=0.354 ns.

According to Z-SF, among the diabetic patient, the highest prevalence of *E. histolytica* was 16.81% in the age group of 25-35 years and the lowest 8.39% in 55-65 years. *G. lamblia* infection was highest (19.75%) in the age group of 35-45 years and the lowest (7.74%) in 55-65 years for *G. lamblia*. In case of double infection (diabetic), the highest prevalence was 5.73% in the age of 35-45 years and the lowest was 0.65% in 55-65 years (Table 13).

Table-13: The prevalence of single and double infection of *E. histolytica* and *G. lamblia* in different age groups among diabetic and non-diabetic patients detected by Z-SFM.

Patient groups	Age groups (in year)	Total examined samples	Single infestation				Double infestation	Prevalence (%)
			E. histolytica (positive cases)	*E. histolytica* (%)	*G. lamblia* (positive cases)	*G. lamblia* (%)	*E. histolytica and G. lamblia* (positive cases)	*E. histolytica and G. lamblia*
Diabetic	(25.1-35)	113	19	16.81	19	16.81	5	4.42
	(35.1-45)	157	23	14.65	31	19.75	9	5.73
	(45.1-55)	163	19	11.66	20	12.27	4	2.45
	(55.1-65)	155	13	8.39	12	7.74	1	0.65
	(65.1-75)	109	10	9.17	11	10.09	2	1.83
Non- diabetic	(25.1-35)	151	35	23.18	31	20.53	11	7.28
	(35.1-45)	143	27	18.88	32	22.38	13	9.09
	(45.1-55)	129	18	13.95	19	14.73	7	5.43
	(55.1-65)	93	5	5.38	7	7.53	1	1.08
	(65.1-75)	87	6	6.70	8		2	2.30
Total diabetic samples across the age group		697	84	12.05	93	13.34	21	3.01
Total non-diabetic samples across the age group		603	91	15.09	97	16.09	34	5.64

* Significant; ** highly significant; ns not significant at 5% level

Single infestation cases,

(a). *E. histolytica*: r = – 0.962, p=0.012, n=5;

(b). *G. lamblia*: r = – 0.821, p=0.088, n=5;

Double infestation cases, (c) *E. histolytica and G. lamblia:* r = – 0.795; p=0.108, n=5

In **non-diabetic** patients, correlation coefficient (r) between age and prevalence of infection were given below:

Single infestation cases,

(a). *E. histolytica*: r = – 0.996, p=0.000, n=5,

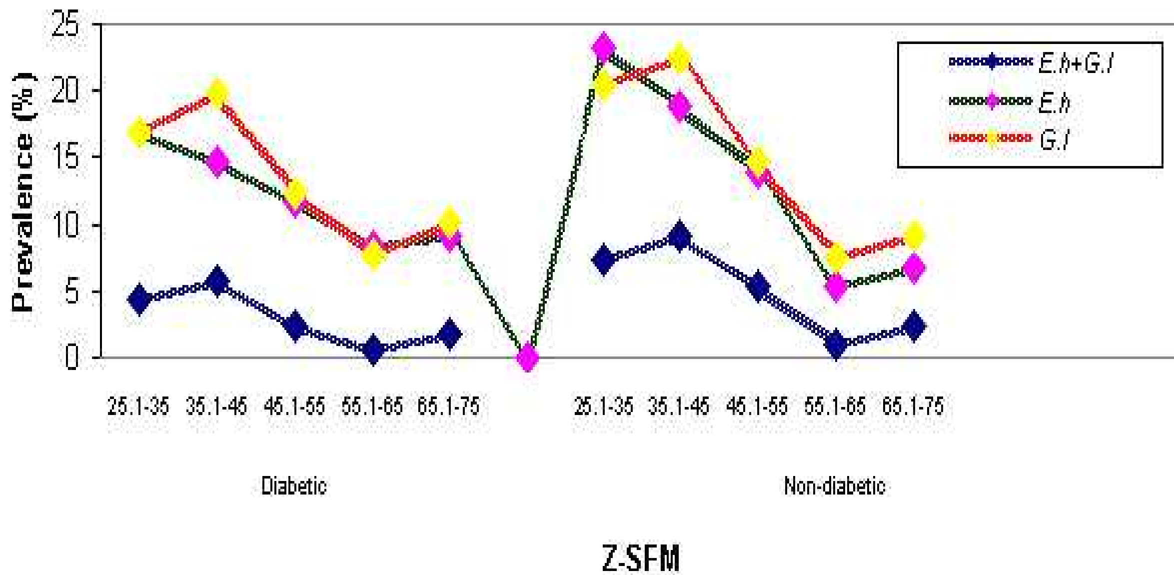
(b). *G. lamblia*: r = – 0.898, p=0.038, n=5

Double infestation cases, (c) *E. histolytica and G. lamblia*: r = – 0.849, p=0.069, n=5

P-value of proportion test between diabetic and non diabetic samples were

(a). *E. histolytica*: P=0.560ns**;** (b). *G. lamblia*: P=0.593ns

(c) *E. histolytica and G. lamblia:* P=0.654ns.



E.h= E. histolytica* ; *G.l = G. lamblia;

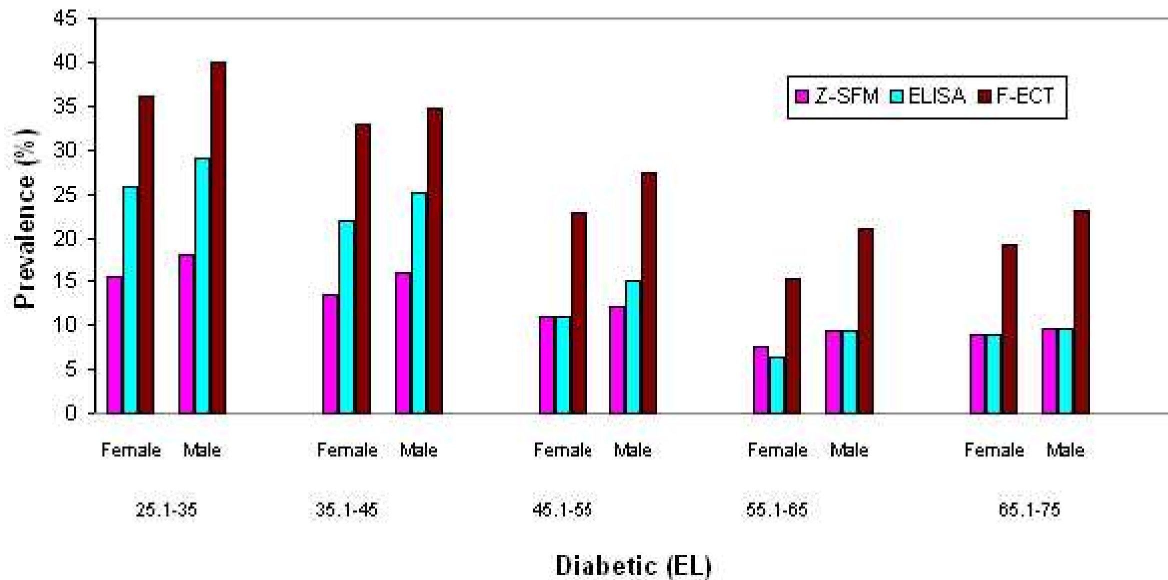
E.h + G.l = E. histolytica + G. lamblia.

Fig-7(C): The prevalence of single and double infection in age groups among diabetic and non-diabetic by Z-SFM.

Considering the five age groups, among non-diabetic individuals of age group 25-35 years was more infected (23.18%) as compared with other age groups and lowest infection (5.38%) was found in 55-65 year group for *E. histolytica* . Similarly the highest (non-diabetic) prevalence (22.38%) was found in the age group of 35-45 years and the lowest (7.53%) in 55-65 years for *G. lamblia*. In case of double infection (non-diabetic), the highest prevalence was 9.09% in the age of 35-45 years and the lowest prevalence was 1.08% in 55-65 years (Table 13); (Fig 7C).

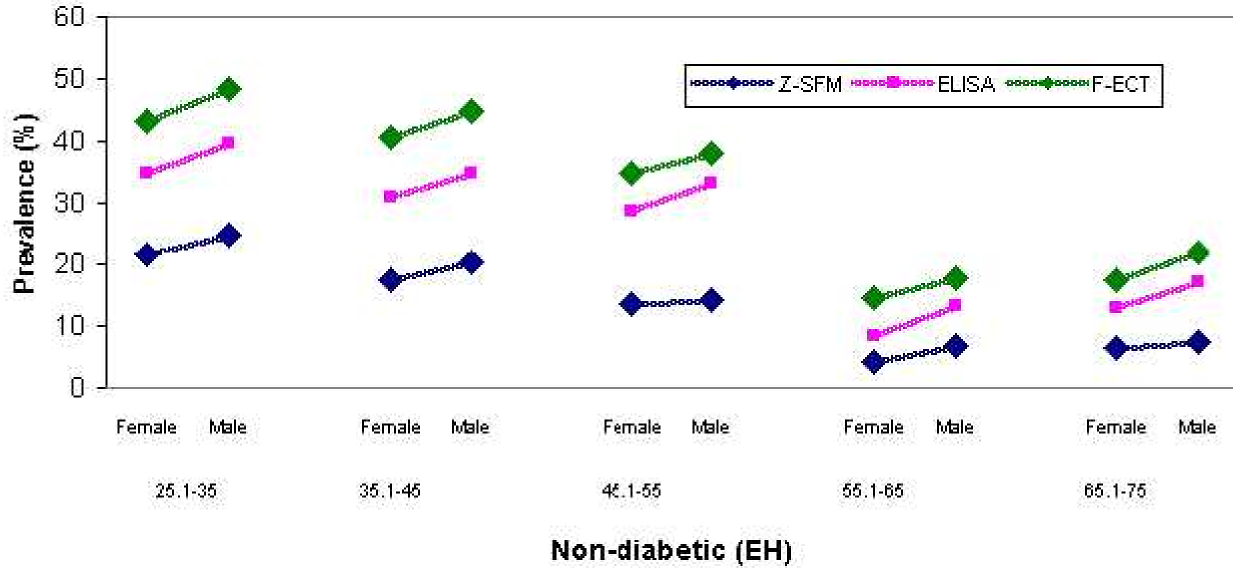
5.6 Age groups and sex prevalence of *E. histolytica* and *G. lamblia* :

According to ELISA, in diabetic female, the highest prevalence was 25.86 % and in male, 29.09% at the age group of 25-35 years and the lowest 6.33% in female and 9.21% in male, at 55-65 years for *E. histolytica* (Table-14).



EL = *Entamoeba histolytica*

Fig- 8(A): The prevalence of *E. histolytica* infections in different age groups, in sexes among diabetic patients by different techniques.



EL = *Entamoeba histolytica*

Fig- 8(B): The prevalence of *E. histolytica* infections in different age groups, in sexes among the non-diabetes patients by different techniques.

Table-14: The prevalence of *E. histolytica* infections in different age groups on the basis of sex among diabetic patients detected by three techniques.

Age group (years)	Patients group	Total number of samples	Prevalence of *E. histolytica*		
			ELISA (%)	F-ECT (%)	Z-SFM (%)
(25.1-35)	Female	58	15(25.86%)	21(36.21%)	9(15.52%)
	Male	55	16(29.09%)	22(40%)	10(18.18%)
	Total	113	31(27.43%)	43(38.05%)	19(16.81%)
P-value of proportion test between male and female			0.841 ns	0.799 ns	0.878 ns
(35.1-45)	Female	82	18(21.95%)	27(32.93%)	11(13.41%)
	Male	75	19(25.33%)	26(34.67%)	12(16%)
	Total	157	37(23.57%)	53(33.76%)	23(14.65%)
P-value of proportion test between male and female			0.810 ns	0.894 ns	0.862 ns
(45.1-55)	Female	83	9(10.84%)	19(22.89%)	9(10.84%)
	Male	80	12(15%)	22(27.5%)	10(12.5%)
	Total	163	21(12.88%)	41(25.15%)	19(11.66%)
P-value of proportion test between female and male			0.783 ns	0.736 ns	0.911 ns
(55.1-65)	Female	79	5(6.33%)	12(15.19%)	6(7.59%)
	Male	76	7(9.21%)	16(21.05%)	7(9.21%)
	Total	155	12(7.74%)	28(18.06%)	13(8.39%)
P-value of proportion test between female and male			0.860 ns	0.748 ns	0.918 ns
(65.1-75)	Female	57	5(8.77%)	11(19.30%)	5(8.77%)
	Male	52	5(9.62%)	11(23.08%)	5(9.62%)
	Total	109	10(9.17%)	22(20.18%)	10(9.17%)
P-value of proportion test between female and male			0.964 ns	0.830 ns	0.964 ns
Total diabetic samples		697	111(15.93%)	187(26.83%)	84(12.05%)

ns= not significant at 5% level

According to F-EC technique, in female, the highest prevalence was 36.21% and in male, 40% at the age group of 25-35 years. From Z-SF method, in female, the highest prevalence was 15.52% and in male, 18.18% at the age group of 25-35 years (Table-14).

According to ELISA, in non-diabetic female, the highest prevalence was 34.94% and in male, 39.71% at the age group of 25-35 years for *E. histolytica.* According to F-EC technique, in non-diabetic female, the highest prevalence was 43.37% and in male, 48.53% at the age group of 25-35 years. From Z-SF method, in non-diabetic female, the highest prevalence was 21.69% and in male, 25% at the age group of 25-35 years (Table-15).

Table-15: The prevalence of *E. histolytica* infections in different age groups on the basis of sex among non-diabetic individuals detected by different technique.

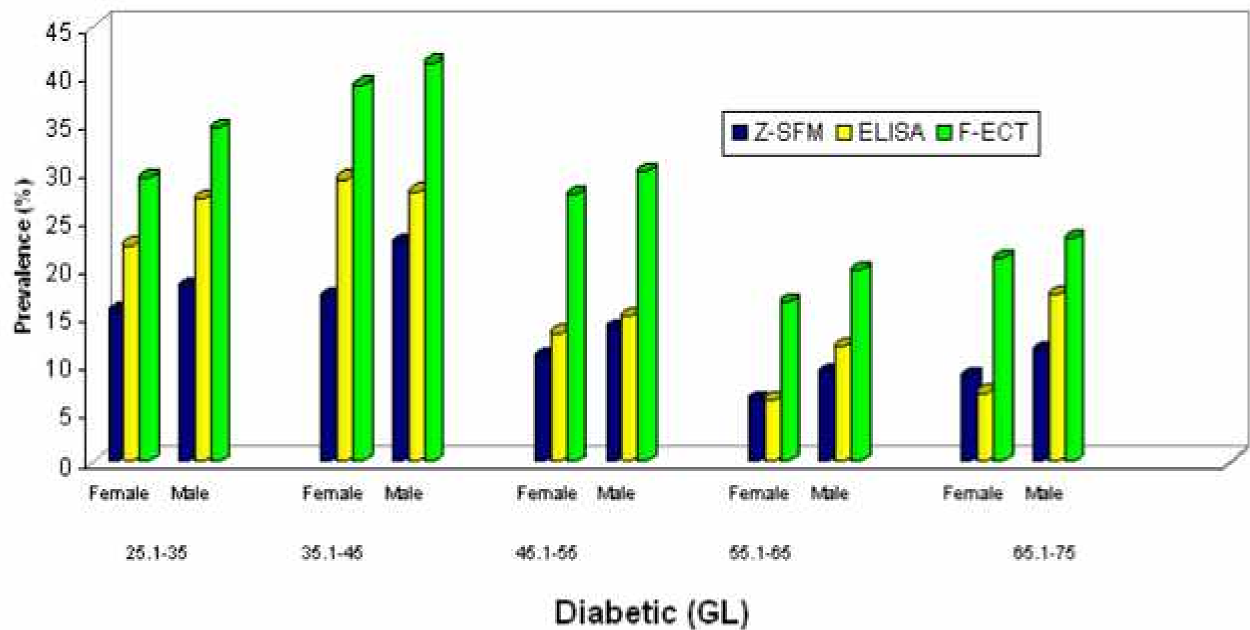
Age group (years)	Patients group	Total number of samples	Prevalence of *E. histolytica*		
			ELISA (%)	F-ECT (%)	Z-SFM (%)
(25.1-35)	Female	83	29(34.94%)	36(43.37%)	18(21.69%)
	Male	68	27(39.71%)	33(48.53%)	17(25%)
	Total	151	56(37.09%)	69(46.36%)	35(23.17%)
Proportion test between female and male			Ns	Ns	Ns
(35.1-45)	Female	74	23(31.08%)	30(40.54%)	13(17.57%)
	Male	69	24(34.78%)	31(44.93%)	14(20.29%)
	Total	143	47(32.87%)	61(42.65%)	27(18.88%)
Proportion test between female and male			Ns	Ns	Ns
(45.1-55)	Female	66	19(28.79%)	23(34.85%)	9(13.64%)
	Male	63	21(33.33%)	24(38.10%)	9(14.29%)
	Total	129	40(31.01%)	47(36.43%)	18(13.95%)
P-value of proportion test between female and male			Ns	Ns	Ns
(55.1-65)	Female	48	4(8.33%)	7(14.58%)	2(4.17%)
	Male	45	6(13.33%)	8(17.78%)	3(6.67%)
	Total	93	10(10.75%)	15(16.13%)	5(5.38%)
Proportion test between female and male			Ns	Ns	Ns
(65.1-75)	Female	46	6(13.04%)	8(17.39%)	3(6.52%)
	Male	41	7(17.07%)	9(21.95%)	3(7.32%)
	Total	87	13(14.94%)	17(19.54%)	6(6.89%)
Proportion test between female and male			Ns	Ns	Ns
Total non-diabetic samples		603	166(27.52%)	209(34.66%)	91(15.09%)

ns= not significant at 5% level

Interpretation: Males were more infected than females. But in three techniques showed that both female and male were non significant (result). In the diabetic and non-diabetic

patients, 25-35 years of age-group represents the high risk group and prevalence was very low in 55-65 years of age-group for *E. histolytica*. So, the above results revealed that, among all of these three techniques, the prevalence declined according the increase of age, usually after 35 years old (Table-14-15).

According to ELISA, in diabetic female, the highest prevalence was 24.78% and in male, 29.27% at the age group of 25-35 years and the lowest 6.33% in female and 11.84% in male, at 55-65 years for *G. lamblia* (Table-16).



GL= *G. lamblia.*

Fig- 9(A): The prevalence of *G. lamblia* infections in different age groups, in sexes among diabetic patients by different techniques.

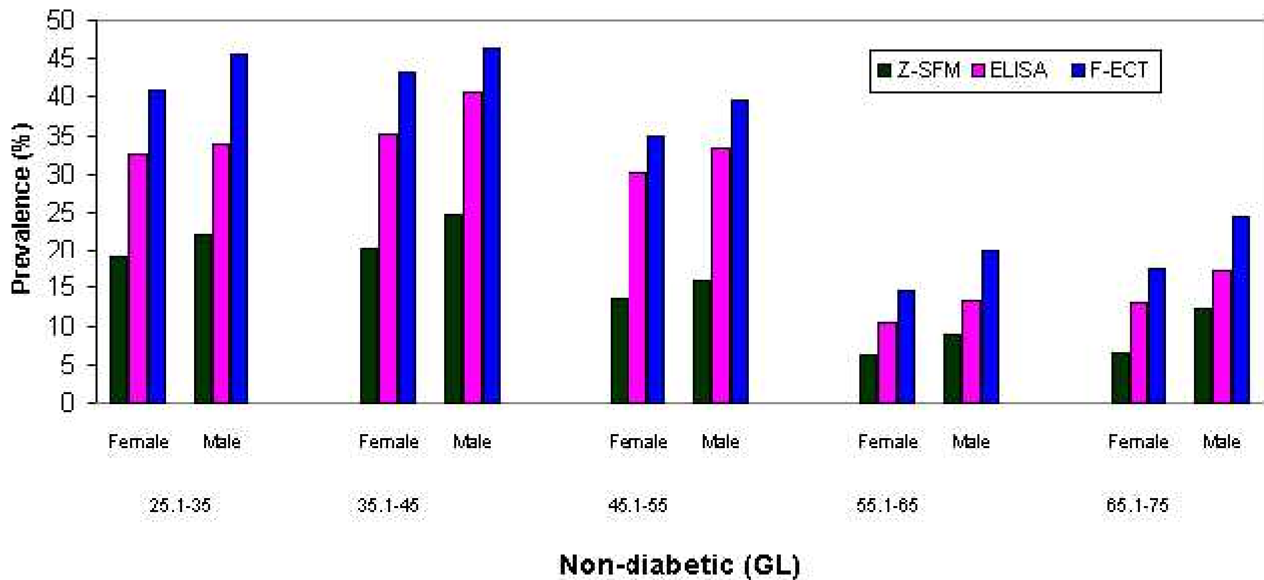
Table-16: The prevalence of *G. lamblia* infections in different age groups on the basis of sex among diabetic patients detected by different technique.

Age group (years)	Patients group	Total number of samples	Prevalence of *G. lamblia*		
			ELISA (%)	F-ECT (%)	Z-SFM (%)
(25.1-35)	Female	58	13 (22.41%)	17(29.31%)	9(15.52%)
	Male	55	15(27.27%)	19(34.55%)	10(18.18%)
	Total	113	28(24.78%)	36(31.86%)	19(15.29%)
Proportion test between female and male			ns	ns	Ns
(35.1-45)	Female	82	24(29.27%)	32(39.02%)	14(17.07%)
	Male	75	21(28%)	31(41.33%)	17(22.67%)
	Total	157	45(28.66%)	63(40.13%)	31(20.38%)
Proportion test between female and male			ns	ns	Ns
(45.1-55)	Female	83	11(13.25%)	23(27.71%)	9(10.84%)
	Male	80	12(15%)	24(30%)	11(13.75%)
	Total	163	23(14.11%)	47(28.83%)	20(12.27%)
Proportion test between female and male			ns	ns	Ns
(55.1-65)	Female	79	5(6.33%)	13(16.46%)	5(6.32%)
	Male	76	9(11.84%)	15(19.74%)	7(9.21%)
	Total	155	14(9.03%)	28(18.06%)	12(7.74%)
(65.1-75)	Female	57	4(7.02%)	12(21.05%)	5(8.77%)
	Male	52	9(17.31%)	12(23.08%)	6(11.54%)
	Total	109	13(11.93%)	24(22.02%)	11(10.09%)
Proportion test between female and male			ns	ns	Ns
Total diabetic samples	697		123(17.65%)	198(28.41%)	93(13.34%)

* Significant, ** highly significant; ns= not significant at 5% level

According to F-EC technique, in diabetic female, the highest prevalence was 31.86% and in male, 39.02% at the age group of 25-35 years. From Z-SF method, in diabetic female, the highest prevalence was 15.29% and in male, 17.07% at the age group of 25-35 years (Table-16).

According to ELISA, in diabetic female, the highest prevalence was 35.14% and in male, 40.60% at the age group of 25-35 years for *G. lamblia*. According to F-EC technique, in diabetic female, the highest prevalence was 43.24% and in male, was 46.38% at the age group of 25-35 years. From Z-SF method, in diabetic female, the highest prevalence was 20.27% and in male, 24.64% at the age group of 25-35 years (Table-17).



GL = *Giardia lamblia*

Fig-9(B): The prevalence of *G. lamblia* infections in different age groups and in females and males among non-diabetes patients by different techniques.

Table-17: The Prevalence of *G. lamblia* infections in different age groups on the basis of sex among non-diabetic patients detected by different technique.

Age group (years)	Patients group	Total number of samples	Prevalence of *G. lamblia*		
			ELISA (%)	F-ECT (%)	Z-SFM (%)
(25.1-35)	Female	83	27(32.53%)	34(40.96%)	16(19.28%)
	Male	68	23(33.82%)	31(45.59%)	15(22.06%)
	Total	151	50(33.11%)	65(43.05%)	31(20.72%)
Proportion test between female and male			ns	ns	Ns
(35.1-45)	Female	74	26(35.14%)	32(43.24%)	15(20.27%)
	Male	69	28(40.60%)	32(46.38%)	17(24.64%)
	Total	143	54(37.76%)	64(44.76%)	32(22.38%)
Proportion test between female and male			ns	ns	Ns
(45.1-55)	Female	66	20(30.30%)	23(34.85%)	9(13.64%)
	Male	63	21(33.33%)	25(39.68%)	10(15.87%)
	Total	129	41(31.78%)	48(37.21%)	19(14.73%)
(55.1-65)	Female	48	5(10.42%)	7(14.58%)	3(6.25%)
	Male	45	6(13.33%)	9(20%)	4(8.89%)
	Total	93	11(11.82%)	16(17.20%)	7(7.53%)
Proportion test between female and male			ns	ns	Ns
(65.1-75)	Female	46	6(13.04%)	8(17.39%)	3(6.52%)
	Male	41	7(17.07%)	10(24.39%)	5(12.20%)
	Total	87	13(14.94%)	18(20.69%)	8(9.20%)
Proportion test between female and male			ns	ns	Ns
Total non- diabetic samples		603	169(28.03%)	211(34.99%)	97(16.09%)

* Significant, ** highly significant, ns not significant at 5% level

Interpretation: Males were more susceptible to the disease than females and 35-45 years of age-group were more susceptible to the disease. So, the above results revealed that, among all these three techniques, the prevalence declined with the increase of age, usually after 45 years old (Table-16-17).

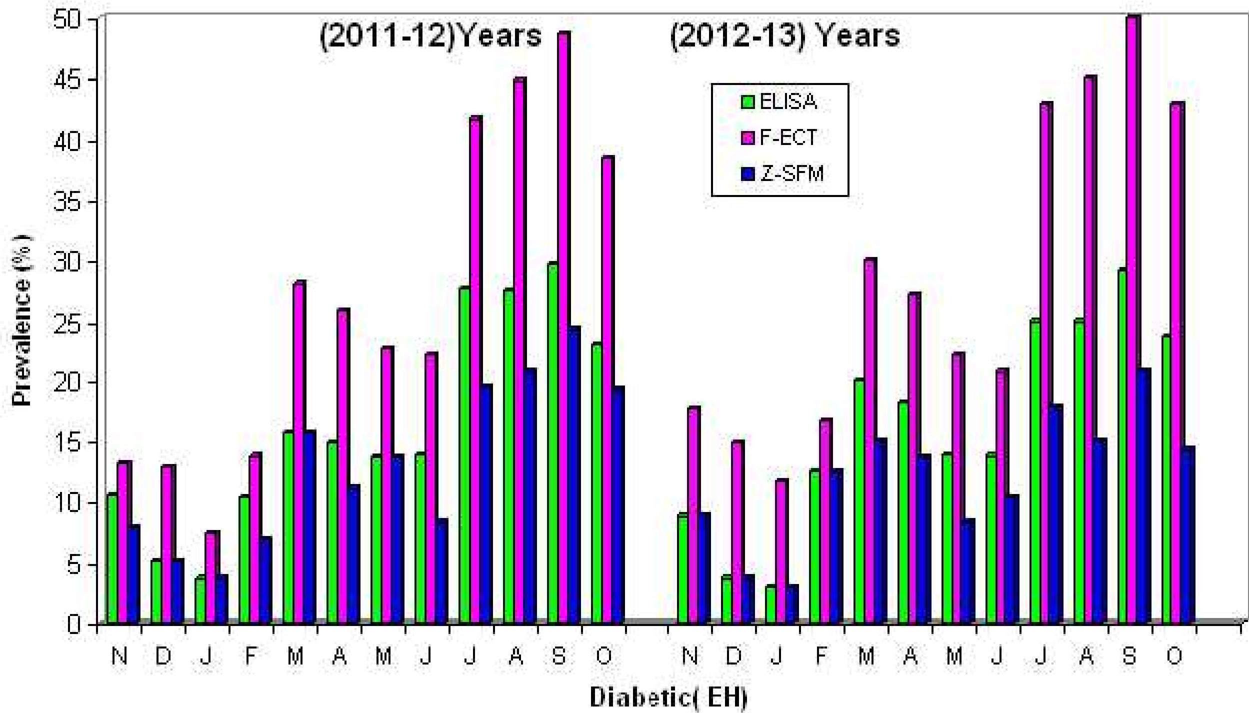
5.7 Monthly variation of parasitic infection among diabetic and non-diabetic individuals:

During November 2011 to October 2012, out of 378 diabetic blood serum examined by ELISA, 16.40% were infected with *E. histolytica*. On the other hand, out of 378 diabetic stool samples, 26.72% was found positive by F-ECT and 12.70% by Z-SFM (Table-18A).

During November 2012 to October 2013, out of 319 diabetic blood serum examined by ELISA, 15.36% were infected with *E. histolytica*. On the other hand, out of 319 diabetic stool samples, 26.96% was found positive by F-ECT and 11.29% by Z-SFM (Table-18B).

Table-18(A): The monthly variation in prevalence of *E. histolytica* infestation among the diabetic patients detected by three techniques.

Months (2011-12)	Name of parasite	Patients group	Total samples examined	ELISA Positive cases (%)	F-ECT Positive cases (%)	Z-SFM Positive cases (%)
November	*E. histolytica*	Diabetic	38	4(10.53%)	5(13.16%)	3(7.89%)
December			39	2(5.13%)	5(12.82%)	2(5.13%)
January			27	1(3.70%)	2(7.41%)	1(3.70%)
February			29	3(10.34%)	4(13.79%)	2(6.90%)
March			32	5(15.63%)	9(28.13%)	5(15.63%)
April			27	4(14.81%)	7(25.93%)	3(11.11%)
May			22	3(13.64%)	5(22.73%)	3(13.64%)
June			36	5(13.89%)	8(22.22%)	3(8.33%)
July			36	10(27.78%)	15(41.67%)	7(19.44%)
August			29	8(27.59%)	13(44.83%)	6(20.69%)
September			37	11(29.73%)	18(48.65%)	8(24.32%)
October			26	6(23.08%)	10(38.46%)	5(19.23%)
Total			378	62(16.40%)	101(26.72%)	48(12.70%)



EH= ***Entamoeba histolytica***

Fig.:- 10(A): The monthly variation of *E. histolytica* infections among diabetic patients by different techniques.

During the first year of the present study (2011-12), the infection recorded highest in the month of September and lowest in January. In 2012, higher prevalence was found in August, September and October while infection remained low in March, April, May and June (Table-18A); (Fig. 10A).

Table-18(B): The monthly variation in prevalence of *E. histolytica* infestation among the diabetic patients detected by three techniques.

Months (2012-13)	Name of parasite	Patients group	Total samples examined	ELISA Positive cases (%)	F-ECT Positive cases (%)	Z-SFM Positive cases (%)
November	*E. histolytica*	Diabetic	34	3(8.82%)	6(17.65%)	3(8.82%)
December			27	1(3.70%)	4(14.81%)	1(3.70%)
January			34	1(2.94%)	4(11.76%)	1(2.94%)
February			24	3(12.5%)	4(16.67%)	3(12.5%)
March			20	4(20%)	6(30%)	3(15%)
April			22	4(18.18%)	6(27.27%)	3(13.63%)
May			36	5(13.89%)	8(22.22%)	3(8.33%)
June			29	4(13.79%)	6(20.70%)	3(10.34%)
July			28	7(25%)	12(42.86%)	5(17.86%)
August			20	5(25%)	9(45%)	3(15%)
September			24	7(29.17%)	12(50%)	5(20.83%)
October			21	5(23.81%)	9(42.86%)	3(14.28%)
Total			319	49(15.36%)	86(26.96%)	36(11.29%)

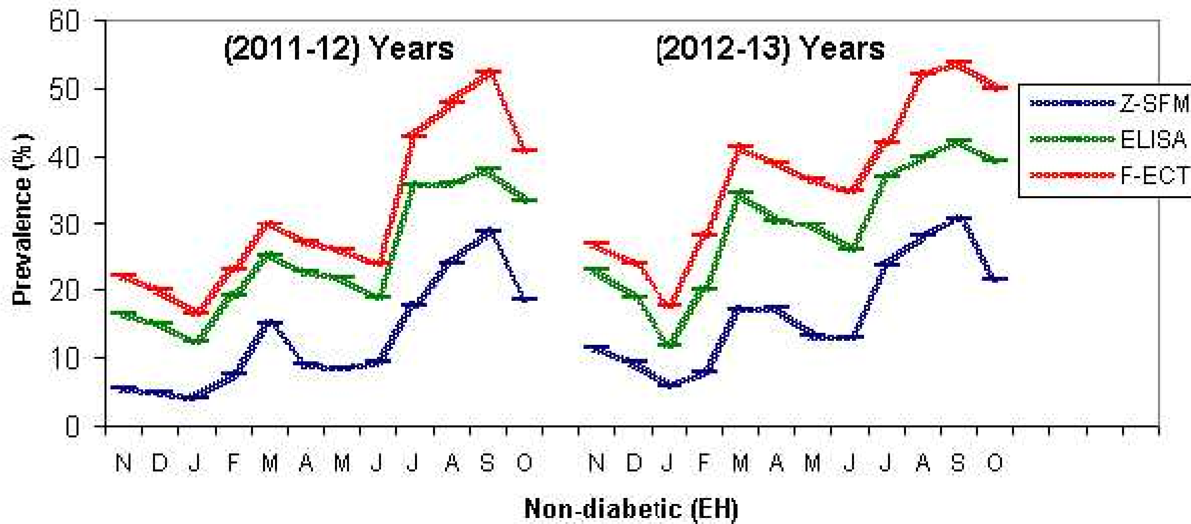
In (2012-13), the observation was quite similar to that of the previous year. The maximum infection of *E. histolytica* was in the month of September, 2013 and lowest in the month of January, 2012. During 2013, higher prevalence was found in August, September and October while, in March, April, May and June infection remained low (Table-18B); (Fig. 10A).

During November 2011 to October 2012, out of 275 non-diabetic blood serum 25.09% were infected with *E. histolytica*, 31.64% was found positive by F-ECT and 13.09% by Z-SFM (Table-19A).

During November 2012 to October 2013, out of 328 non-diabetic blood serum (2012-13), 29.57% found infected with *E. histolytica*, 37.20% were positive by F-ECT and 16.79% by Z-SFM for *E. histolytica* infection (Table-19B).

Table-19(A): The monthly variation in prevalence of *E. histolytica* infestation among the non-diabetic patients detected by three techniques.

Months (2011-12)	Name of parasite	Patients group	Total samples examined	ELISA Positive cases (%)	F-ECT Positive cases (%)	Z-SFM Positive cases (%)
November	*E. histolytica*	Non-diabetic	18	3(16.67%)	4(22.22%)	1(5.56%)
December			20	3(15%)	4(20%)	1(5%)
January			24	3(12.5%)	4(16.67%)	1(4.17%)
February			26	5(19.23%)	6(23.08%)	2(7.69%)
March			20	5(25%)	6(30%)	3(15%)
April			22	5(22.73%)	6(27.27%)	2(9.09%)
May			23	5(21.74%)	6(26.09%)	2(8.70%)
June			21	4(19.05%)	5(23.81%)	2(9.52%)
July			28	10(35.71%)	12(42.86%)	5(17.86%)
August			25	9(36%)	12(48%)	6(24%)
September			21	8(38.10%)	11(52.38%)	6(28.57%)
October			27	9(33.33%)	11(40.74%)	5(18.52%)
Total			275	69(25.09%)	87(31.64%)	36(13.09%)



EH = *E. histolytica*

Fig.:- 10(B): The monthly variation of *E. histolytica* infections among non-diabetes patients by different techniques.

Table-19(B): The monthly variation in prevalence of *E. histolytica* infestation among the non-diabetic patients detected by three techniques.

Months (2012-13).	Name of parasite	Patients group	Total samples examined	ELISA Positive cases (%)	F-ECT Positive cases (%)	Z-SFM Positive cases (%)
November	*E. histolytica*	Non - diabetic	26	6(23.08%)	7(26.92%)	3(11.54%)
December			21	4(19.05%)	5(23.81%)	2(9.52%)
January			34	4(11.76%)	6(17.65%)	2(5.88%)
February			25	5(20%)	7(28%)	2(8%)
March			29	10(34.48%)	12(41.38%)	5(17.24%)
April			23	7(30.43%)	9(39.13%)	4(17.39%)
May			30	9(30%)	11(36.67%)	4(13.33%)
June			23	6(26.09%)	8(34.78%)	3(13.04%)
July			38	14(36.84%)	16(42.11%)	9(23.68%)
August			25	10(40%)	13(52%)	7(28%)
September			26	11(42.31%)	14(53.85%)	8(30.77%)
October			28	11(39.29%)	14(50%)	6(21.43%)
Total			328	97(29.57%)	122(37.20%)	55(16.79%)

The first year of the study (2011-12), it was noticed that the infection was higher in September 2011 and the lowest in January 2012 (Table-19A); (Fig 10B).

In the present study (2012-13), the infection was higher in August, September and October and lower in January (Table-19B); (Fig 10B).

Out of 378 diabetic blood serum examined by ELISA (2011-2012), 17.99% found infected with *G. lamblia*. 28.31% by F-ECT and 12.96% by Z-SFM found positive (Table-20A).

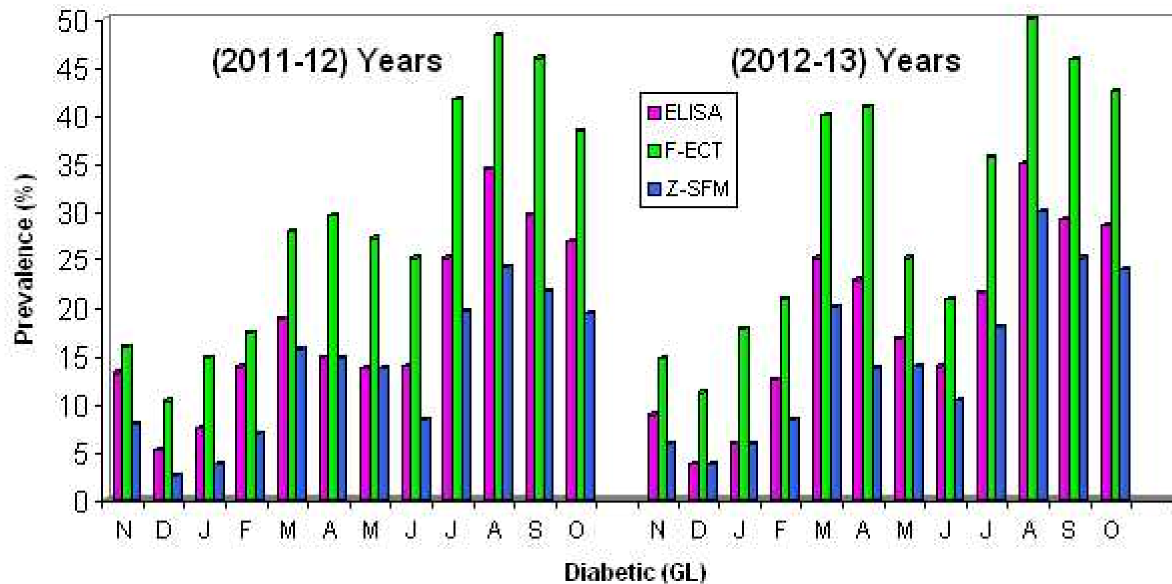
Out of 319 diabetic blood samples (2012-2013), 17.24% were infected with *G. lamblia* by ELISA; 28.53% by F–ECT and 13.79% by Z-SFM positive for *G. lamblia* infection (Table-20B); (Fig 11A).

Table-20(A): The monthly variation of prevalence of *G. lamblia* infestation among the diabetic patients detected by three techniques.

Months (2011-12)	Name of parasite	Patients group	Total samples examined	ELISA Positive cases (%)	F-ECT Positive cases (%)	Z-SFM Positive cases (%)
November	*G. lamblia*	Diabetic	38	5(13.16%)	6(15.79%)	3(7.89%)
December			39	2(5.13%)	4(10.26%)	1(2.56%)
January			27	2(7.41%)	4(14.81%)	1(3.70%)
February			29	4(13.79%)	5(17.24%)	2(6.90%)
March			32	6(18.75%)	9(28.13%)	5(15.63%)
April			27	4(14.81%)	8(29.63%)	4(14.81%)
May			22	3(13.64%)	6(27.27%)	3(13.64%)
June			36	5(13.89%)	9(25%)	3(8.33%)
July			36	9(25%)	15(41.67%)	7(19.44%)
August			29	10(34.48%)	14(48.28%)	7(24.14%)
September			37	11(29.73%)	17 (45.95%)	8(21.62%)
October			26	7(26.92%)	10(38.46%)	5(19.23%)
Total			378	68(17.99%)	107(28.31%)	49(12.96%)

Table-20(B): The monthly variation of prevalence of *G. lamblia* infestation among the diabetic patients detected by three techniques.

Months (2012-13)	Name of parasite	Patients group	Total samples examined	ELISA Positive cases (%)	F-ECT Positive cases (%)	Z-SFM Positive cases (%)
November	*G. lamblia*	Diabetic	34	3(8.82%)	5(14.71%)	2(5.88%)
December			27	1(3.70%)	3(11.11%)	1(3.70%)
January			34	2(5.88%)	6(17.65%)	2(5.88%)
February			24	3(12.5%)	5(20.83%)	2(8.33%)
March			20	5(25%)	8(40%)	4(20%)
April			22	5(22.73%)	9(40.91%)	3(13.64%)
May			36	6(16.67%)	9(25%)	5(13.89%)
June			29	4(13.79%)	6(20.69%)	3(10.34%)
July			28	6(21.43%)	10(35.71%)	5(17.86%)
August			20	7(35%)	10(50%)	6(30%)
September			24	7(29.17%)	11(45.83%)	6(25%)
October			21	6(28.57%)	9(42.86%)	5(23.81%)
Total			319	55(17.24%)	91(28.53%)	44(13.79%)



GL = *G. lamblia*

Fig.:- 11(A): The monthly variation of *G. lamblia* infections among diabetic patients by different techniques.

During the first year of the study (2011-12), the infection recorded highest in the month of August and lowest in December. In 2012, higher prevalence was found in August, September and October while in March, April, May and June infection remained low (Table-20A).

In (2012-13), the observation was quite similar to that of the previous year. The maximum infection of *G. lamblia* was in the month of August, 2013 and lowest in the month of December, 2012. During 2013, higher prevalence was found in August, September and October while, in March, April, May and June infection remained low (Table-20B).

Out of 275 non-diabetic blood serum were examined by ELISA (2011-12) 25.45% were infected with *G. lamblia*, and 31.64% by F-ECT and 14.55% by Z-SFM for *G. lamblia* (Table-21A).

During November 2012 to October 2013, out of 328 non-diabetic blood serum were examined by ELISA, 30.18% were infected with *G. lamblia*, on the other hand, from 328 non-diabetic stool samples, 37.80% were found positive by F-ECT and 17.38% by Z-SFM (Table-21B).

Table-21A: The monthly variation of prevalence of *G. lamblia* infestation among the non-diabetic patients detected by three techniques.

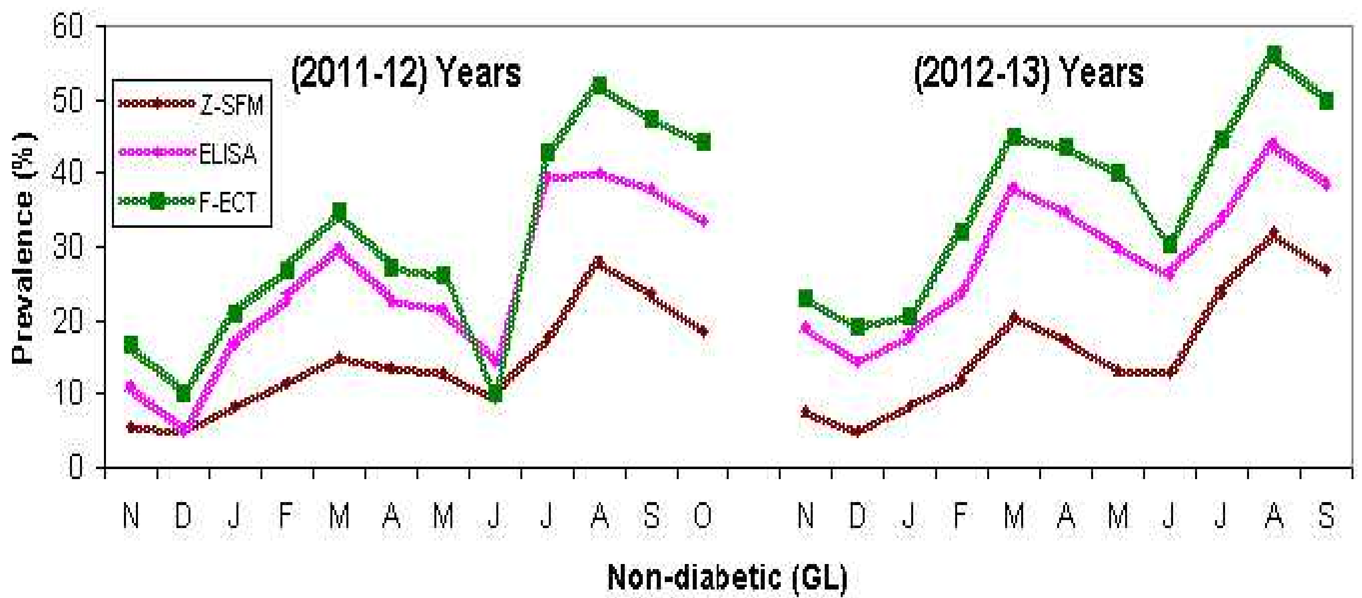
Months (2011-12).	Name of parasite	Patients group	Total samples examined	ELISA Positive cases (%)	F-ECT Positive cases (%)	Z-SFM Positive cases (%)
November	*G. lamblia*	Non - diabetic	18	2(11.11%)	3(16.67%)	1(5.56%)
December			20	1(5%)	2(10%)	1(5%)
January			24	4(16.67%)	5(20.83%)	2(8.33%)
February			26	6(23.07%)	7(26.92%)	3(11.54%)
March			20	6(30%)	7(35%)	3(15%)
April			22	5(22.73%)	6(27.27%)	3(13.64%)
May			23	5(21.74%)	6(26.09%)	3(13.04%)
June			21	3(14.29%)	4(19.05%)	2(9.52%)
July			28	11(39.29%)	12(42.86%)	5(17.86%)
August			25	10(40%)	13(52%)	7(28%)
September			21	8(38.10%)	10(47.61%)	5(23.81%)
October			27	9(33.33%)	12(44.44%)	5(18.52%)
Total			275	70(25.45%)	87(31.64%)	40(14.55%)

Table-21B: The monthly variation of prevalence of *G. lamblia* infestation among the non-diabetic patients detected by three techniques.

Months (2012-13).	Name of parasite	Patients group	Total samples examined	ELISA Positive cases (%)	F-ECT Positive cases (%)	Z-SFM Positive cases (%)
November	*G. lamblia*	Non-diabetic	26	5(19.23%)	6(23.08%)	2(7.69%)
December			21	3(14.29%)	4(19.05%)	1(4.76%)
January			34	6(17.65%)	7(20.59%)	3(8.82%)
February			25	6(24%)	8(32%)	3(12%)
March			29	11(37.93%)	13(44.83%)	6(20.69%)
April			23	8(34.78%)	10(43.48%)	4(17.39%)
May			30	9(30%)	12(40%)	4(13.33%)
June			23	6(26.09%)	7(30.43%)	3(13.04%)
July			38	13(34.21%)	17(44.74%)	9(23.68%)
August			25	11(44%)	14(56%)	8(32%)
September			26	10(38.46%)	13(50%)	7(26.92%)
October			28	11(39.29%)	13(46.43%)	7(25%)
Total			328	99(30.18%)	124(37.80%)	57(17.38%)

In the first year (2011-12), the infection of *G. lamblia* was highest during the month of August (2012). The infestation of *G. lamblia* lowered down from December (2012). In 2012, highest prevalence was found in July to September, while, during April to November (2011) the infection remained low. The infection again started to increase after October 2012 (Table-21A).

During the final year of the study (2012-13), the infection of *G. lamblia* was highest in of August 2013 and lowest in December, 2012. The infection started to increase after July 2013 (Table-21B); (Fig 11B).



GL =*G. lamblia*

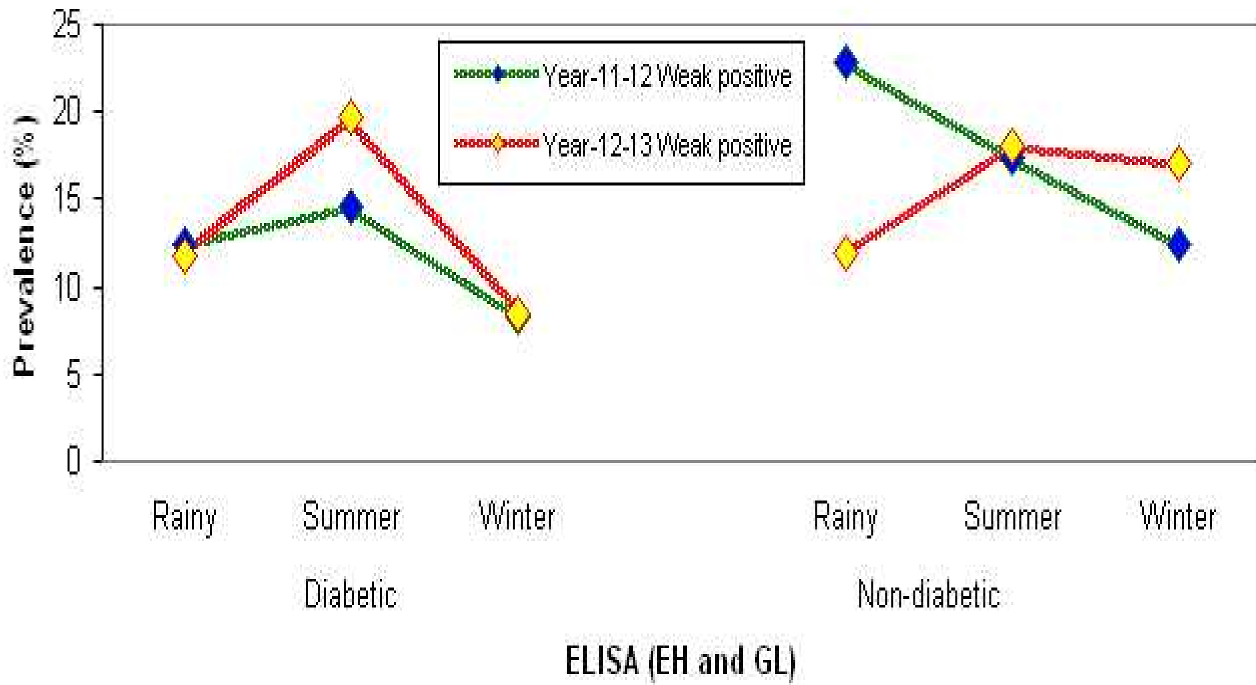
Fig. 11(B): The monthly variation of *G. lamblia* infections among non-diabetic patients by different techniques.

Interpretation: The present epidemiological study showed that amoebiasis and giardiasis were present throughout the year but seasonal peak of transmission occurred in September during monsoon season and minimum incidence was observed in March in summer season (Table-18-21).

5.8 Seasonal variation of parasitic infection among diabetic and non-diabetic individuals detected by ELISA:

In rainy season (2010-11), by ELISA, total 128 (diabetic) blood serum samples were examined, strong positive was 21.09% and 6.25% was weak positive for *E. histolytica* antibody. In the case of *G. lamblia* antibody, strong positive was 22.66% and 6.25% was

weak positive. On the other hand, out of 101 (non diabetic) blood serum samples, the strong prevalence was 24.75%; and 10.89% was weak for *E. histolytica* antibody. *G. lamblia* antibody was strong positive (25.74%) and 11.88% was weak positive (Table-22A).



EH = *E. histolytica* and GL = *G. lamblia*.

Fig-12(A): Comparison of weak positive infections in three seasons by ELISA.

Table-22(A): The prevalence of *E. histolytica* and *G. lamblia* infection in rainy season among the diabetic and non-diabetic patients detected by ELISA (2011-12).

Name of technique	Patient Group	Name of parasites	Total samples (blood)	Rainy season (July, August, September, October)						P-value of proportion test between strong and weak infection
				Strong positive	(%)	Weak positive	(%)	Total positive	(%)	
ELISA	Diabetic	*E. histolytica*	128	27	21.09	8	6.25	35	27.34	0.341 ns
		G. lamblia		29	22.66	8	6.25	37	28.91	0.345 ns
	Proportion test between *E.histolytica* and *G.lamblia*			ns		ns		ns		
	Non-diabetic	*E. histolytica*	101	25	24.75	11	10.89	36	35.64	0.349 ns
		G. lamblia		26	25.74	12	11.88	38	37.62	0.339 ns
	Proportion test between *E.histolytica* and *G.lamblia*			ns		ns		ns		
	Total diabetic patient		128	56	43.75	16	12.5	72	56.25	0.025**
	Total non-diabetic patient		101	51	50.50	23	22.77	74	73.27	0.028**
	p-value of proportion test between diabetic and non-diabetic			0.486 ns		0.422 ns		O.032**		
	Total diabetic and non-diabetic samples		229	107	46.72	39	17.03	146	63.76	0.001**

* Significant; ** highly significant; ns= not significant at 5% level

Positive control=2.773; Strong positive=2.4
Negative control=0.461; Weak positive=0.525

In rainy season (2011-12) by ELISA, total 93 (diabetic) blood serum samples were examined, strong positive was 20.43% and 5.38% was weak positive for *E. histolytica* antibody. In the case of *G. lamblia* antibody, strong positive was 21.51% and 6.45% was weak positive. On the other hand, 117 (non diabetic) blood serum samples, the strong

prevalence was 32.47%; and 6.83% was weak for *E. histolytica* antibody. For *G. lamblia* antibody, 33.33% was strong and 5.13% was weak (Table-22B).

Table-22(B): The prevalence of *E. histolytica* and *G. lamblia* infection in rainy season among the diabetic and non-diabetic patients detected by ELISA (2011-12).

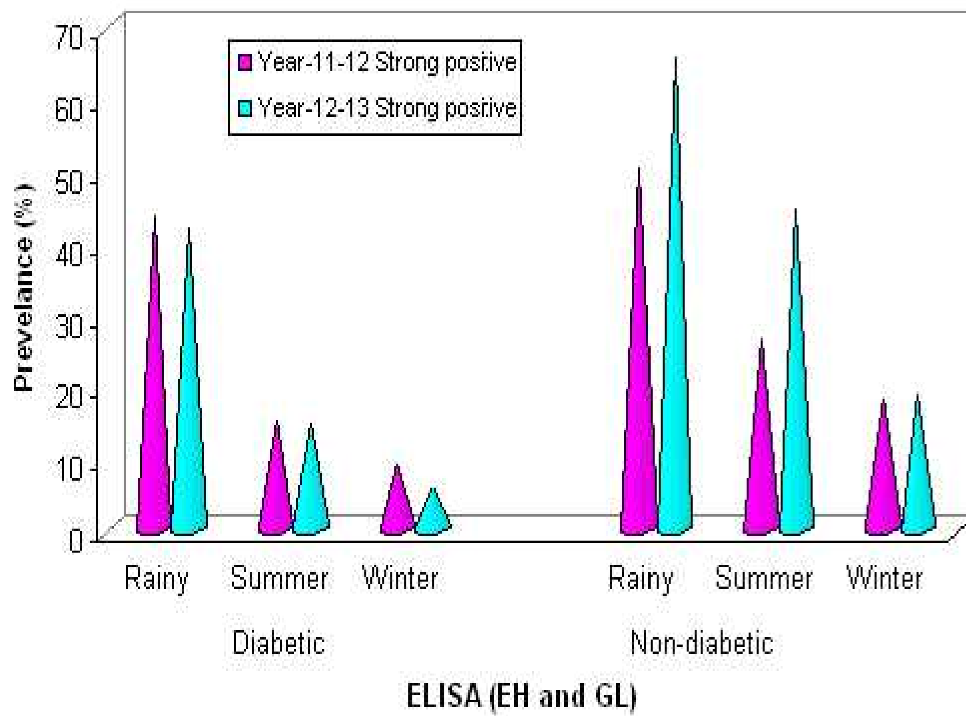
Name of	Patient groups	Name of parasites	Total samples (blood)	Rainy season (July, August, September, October)						P-value of proportion test between strong and weak infection
				Strong positive	(%)	Weak positive	(%)	Total positive	(%)	
ELISA	Diabetic	*E. histolytica*	93	19	20.43	5	5.38	24	5.81	0.437 ns
		G. lamblia		20	21.51	6	6.45	26	7.96	0.408 ns
	Proportion test between *E. histolytica* and *G. lamblia*			ns		ns		Ns		
	Non-diabetic	*E. histolytica*	117	38	32.47	8	6.83	46	39.31	0.149 ns
		G. lamblia		39	33.33	6	5.13	45	38.46	0.166 ns
	Proportion test between *E. histolytica* and *G. lamblia*			ns		ns		Ns		
	Total diabetic patient		93	39	41.94	11	11.83	50	53.76	0.074 ns
	Total non-diabetic patient		117	77	65.81	14	11.97	91	77.78	0.0003**
	p-value of proportion test between diabetic and non-diabetic			0.015**		0.991 ns		0.003**		
	Total diabetic and non-diabetic samples		210	106	50.48	23	10.95	141	67.14	0.0007**

* Significant; ** highly significant; ns= not significant at 5% level

Positive control=2.529; Strong positive=2.5
Negative control=0.004; Weak positive=0.445

In the rainy season, the present study showed that, infestation in diabetic patients and non-diabetic individuals with *E. histolytica* antibody and *G. lamblia* antibody were highly significant. During the year of the study (2011-12), the prevalence was more positive than that of the previous year (2010-11) (Table-22A-22B) (Fig 12A).

In summer season (2011-12), by ELISA, 117 (diabetic) blood serum samples were examined, strong positive was 7.69% and 6.84% was weak for *E. histolytica* antibody; and 7.69% and 7.69% were for *G. lamblia* antibody respectively. On the other hand, out of 86 (non diabetic) blood serum samples, 12.79%; and 9.30% were *E. histolytica* antibody; and 13.95% and 8.14% were for *G. lamblia* antibody respectively (Table-23A).



EH = *E. histolytica* and GL = *G. lamblia*.

Fig-12(B): Comparison of strong positive infections in three seasons by ELISA.

Table-23(A): The prevalence of *E. histolytica* and *G. lamblia* infection in summer season among the diabetic and non-diabetic patients detected by ELISA (2011-12).

Name of technique	Patient groups	Name of parasites	Total samples (blood)	Summer season (March, April, May and June)						Proportion test between strong and weak infection
				Strong positive	(%)	Weak positive	(%)	Total positive	(%)	
ELISA	Diabetic	*E. histolytica*	117	9	7.69	8	6.84	17	4.53	Ns
		G. lamblia		9	7.69	9	7.69	18	15.38	Ns
	Proportion test between *E.histolytica* and *G.lamblia*			ns		ns		ns		Ns
	Non-diabetic	*E. histolytica*	86	11	12.79	8	9.30	19	22.09	Ns
		G. lamblia		12	13.95	7	8.14	19	22.09	Ns
	Proportion test between *E.histolytica* and *G.lamblia*			ns		ns		ns		Ns
	Total diabetic patient		117	18	15.38	17	14.53	35	29.91	Ns
	Total non-diabetic patient		86	23	26.74	15	17.44	38	44.19	Ns
	Proportion test between diabetic and non-diabetic			ns		ns		ns		
	Total diabetic and non-diabetic samples		203	41	20.20	32	15.76	73	35.96	Ns

* Significant ** highly significant, P<0.05; ns= not significant at 5% level, P>0.05;

Positive control=2.773; Strong positive=2.4
Negative control=0.461 Weak positive=0.525

The results on diabetic patients and non-diabetic individuals with *E. histolytica* antibody and *G. lamblia* antibody were non significant (Table-23A).

Table-23(B): The prevalence of *E. histolytica* and *G. lamblia* infection in summer season among the diabetic and non-diabetic patients detected by ELISA (2012-13).

Name of technique	Patient groups	Name of parasites	Total samples (blood)	Summer season (March, April, May and June)						Proportion test between strong and weak infection
				Strong positive	(%)	Weak positive	(%)	Total positive	(%)	
ELISA	Diabetic	*E. histolytica*	107	7	6.54	10	9.35	17	15.89	Ns
		G. lamblia		9	8.41	11	10.28	20	18.69	Ns
	Proportion test between *E. histolytica* and *G. lamblia*			ns		ns		Ns		
	Non-diabetic	*E. histolytica*	105	22	20.95	10	9.52	32	30.48	Ns
		G. lamblia		25	23.81	9	8.57	34	32.38	Ns
	Proportion test between *E. histolytica* and *G. lamblia*			ns		ns		Ns		
	Total diabetic patient		107	16	14.95	21	19.63	37	34.58	Ns
	Total non-diabetic patient		105	47	44.76	19	18.10	66	62.86	P= 0.046*
	Proportion test between diabetic and non-diabetic			ns		ns		Ns		
	Total diabetic and non-diabetic samples		212	63	29.72	29	13.68	103	48.48	Ns

* Significant ** highly significant, P<0.05; ns= not significant at 5% level, P>0.05;

Positive control=2.529; Strong positive=2.5
Negative control=0.004; Weak positive=0.445

In summer season, (2012-13) by ELISA, 107 diabetic blood serum samples, strong positive was 6.54% and 9.35% was weak for *E. histolytica* antibody. In the case of *G. lamblia* antibody, strong positive was 8.41% and 10.28% was weak. On the other hand, 105 non diabetic blood serum samples, the strong positive was 20.95%; and 9.52% was weak for *E. histolytica* antibody for *G. lamblia* (Fig. 12B).

The results on diabetic patients with *E. histolytica* and *G. lamblia* were non significant; and non-diabetic patients with *E. histolytica* and *G. lamblia* were significant (Table-23B). In winter season, (2011-12) by ELISA, out of 133 (diabetic) blood serum samples were examined, strong positive 4.51% and 3.01% were weak for *E. histolytica* antibody; while *G. lamblia* antibody strong positive were 4.51% and 5.26% was weak. On the other hand, 88 (non diabetic) blood serum samples, 9.09%; and 6.82% for *E. histolytica* antibody; and 9.09%; and 15.68% for *G. lamblia*; respectively (Table-24A).

Table-24(A): The prevalence of *E. histolytica* and *G. lamblia* infection in winter season among the diabetic and non-diabetic patients detected by ELISA (2011-12).

Name of technique	Patient group	Name of parasites	Total samples (blood)	Winter season(November, December, January, February)						Proportion test between strong and weak infection
				Strong positive	(%)	Weak positive	(%)	Total positive	(%)	
	Diabetic	*E. histolytica*	133	6	4.51	4	3.01	10	7.52	Ns
		G. lamblia		6	4.51	7	5.26	13	9.77	Ns
	Proportion test between *E.histolytica* and *G.lamblia*			ns		ns		Ns		
	Non-diabetic	*E. histolytica*	88	8	9.09	6	6.82	14	15.91	Ns
		G. lamblia		8	9.09	5	5.68	13	14.77	Ns
	Proportion test between *E.histolytica* and *G.lamblia*			ns		ns		Ns		
	Total diabetic patient		133	12	9.02	11	8.27	23	17.92	Ns
	Total non-diabetic patient		88	16	18.18	11	12.5	27	30.68	
	Proportion test between diabetic and non-diabetic			ns		ns		Ns		
	Total diabetic and non-diabetic samples		221	28	12.67	22	9.95	50	22.62	Ns

* Significant ** highly significant; ns =not significant at 5% level

Positive control=2.773; Strong positive=2.4
Negative control=0.461; Weak positive=0.525

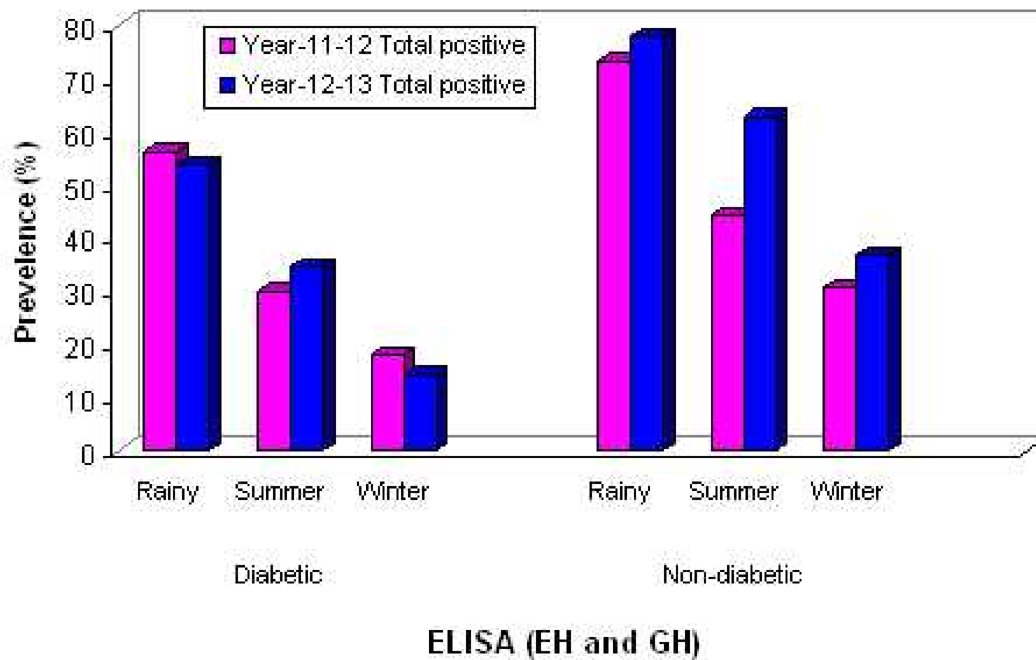
In winter season, (2012-13) by ELISA, out of 119 (diabetic) blood serum samples, 2.52% and 4.20% for *E. histolytica* antibody; and 3.36% and 4.20% for *G. lamblia* antibody; respectively. On the other hand, 106 (non diabetic) blood serum samples, 9.43%; and 8.49% for *E. histolytica*; and 10.38% and 8.49% for *G. lamblia* respectively; (Table-24B).

Table-24(B): The prevalence of *E. histolytica* and *G. lamblia* infection in winter season among the diabetic and non-diabetic patients detected by ELISA (2012-13)

Name of technique	Patient Groups	Name of parasites	Total samples (blood)	Winter season (November, December, January, February)						Proportion test between strong and weak infection
				Strong positive	(%)	Weak positive	(%)	Total positive	(%)	
ELISA	Diabetic	*E. histolytica*	119	3	2.52	5	4.20	8	6.72	Ns
		G. lamblia		4	3.36	5	4.20	9	7.56	Ns
	Proportion test between *E. histolytica* and *G. lamblia*			ns		Ns		Ns		
	Non-diabetic	*E. histolytica*	106	10	9.43	9	8.49	19	17.92	Ns
		G. lamblia		11	10.38	9	8.49	20	18.87	Ns
	Proportion test between *E. histolytica* and *G. lamblia*			ns		Ns		Ns		
	Total diabetic patient		119	7	5.88	10	8.40	17	14.29	Ns
	Total non-diabetic patient		106	20	18.87	18	16.98	39	36.79	Ns
	Proportion test between diabetic and non-diabetic			ns		Ns		Ns		
	Total diabetic and non-diabetic samples		225	27	12	28	12.44	56	24.89	Ns

* Significant ** highly significant; ns not significant at 5% level

Positive control=2.529; Strong positive=2.5
Negative control=0.004; Weak positive=0.445



EH = *E. histolytica* and GL = *G. lamblia*.

Fig-12(C): Comparison of total positive infection in three seasons by ELISA.

The results on diabetic and non-diabetic patients with *E. histolytica* antibody and *G. lamblia* antibody were non significant (Table-24A-24B).

5.9 Seasonal variation of parasitic infection among diabetic and non-diabetic individuals detected by F-ECT and ZSFM:

According to F-ECT, total 128 diabetic stool samples were examined, in rainy season (2011-2012), the overall prevalence of *E. histolytica* (cyst and trophozoites) was 43.75%; 16.41% mild, 22.66% moderate and 4.69% were severely infected. On the other hand,

101 non diabetic stool samples, the overall prevalence was 45.54% for *E. histolytica*; 7.92% mild, 26.73% moderate and 10.89% were sever infection (Table-25A).

Table-25(A): The prevalence of *E. histolytica* infections in rainy season among the diabetic and non-diabetic patients detected by F-ECT and Z-SFM.

Seasonal variation (Rainy season)	Name of parasites	Patient groups	Total samples examined (stool)	Name of techniques				Proportion test between F-ECT and Z-SFM
				F-ECT		Z-SFM		
				Positive cases	%	Positive Cases	%	
Rainy season (July, August, September, October) (2011-12)	*E. histolytica*	Diabetic	128	21+	16.41	9+	7.03	P>0.05
				29++	22.66	14++	10.94	P>0.05
				6+++	4.69	3+++	2.34	P>0.05
		Non-diabetic	101	8+	7.92	9+	8.91	P>0.05
				27++	26.73	10++	9.90	P>0.05
				11+++	10.89	3+++	2.97	P>0.05
Total diabetic positive cases			128	56	43.75	26	20.31	P<0.05*
Total non-diabetic positive cases			101	46	45.54		21.78	P<0.061
Proportion test between diabetic and non-diabetic				p>o.o5		(22) p>o.o5		
Total diabetic and non-diabetic			229	102	44.54	49	21.40	P<0.05*

p<0.05 = significant at 5% level,

p>0.05 = not significant at 5% level

Intensity of infection (W.H.O. 1987)

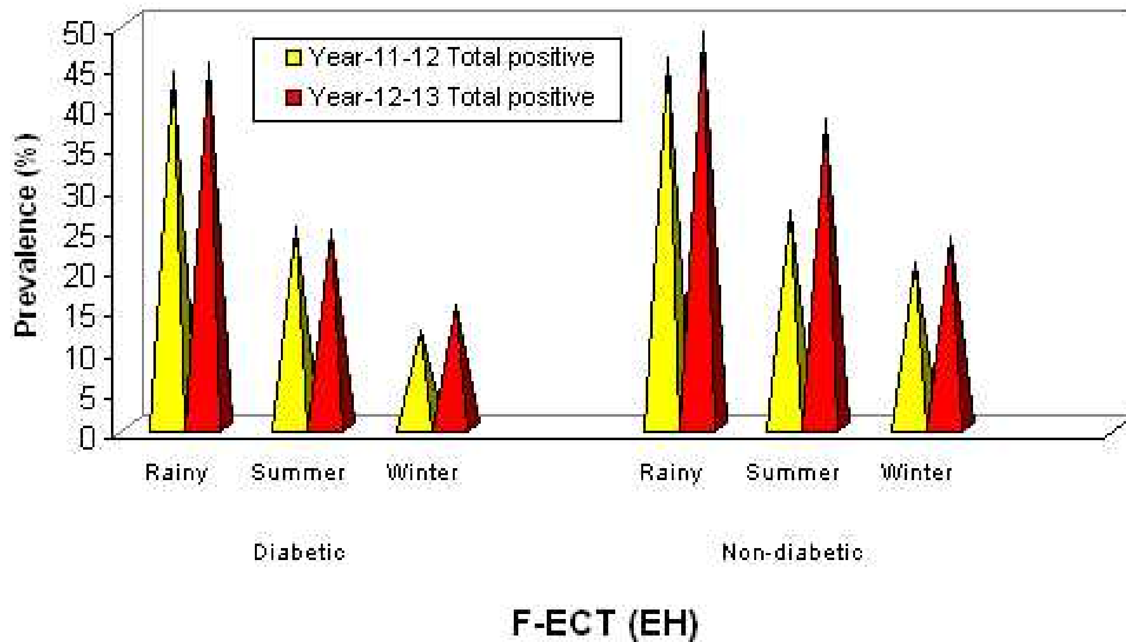
C p g= cyst count per gm

	E. histolytica	***G. lamblia***
+=Mild	c p g=1-149/gm	c p g=1-99/gm
++=Moderate	c p g=150-250 /gm	c p g=100-199/gm
+++=Sever	c p g=251/gm and above	c p g=200/gm and above

Table-25(B): The prevalence of *E. histolytica* infections in rainy season among the diabetic and non-diabetic patients detected by F-ECT and Z-SFM.

Seasonal variation (Rainy season)	Name of parasites	Patient groups	Total samples examined (stool)	Name of techniques				Proportion test between F-ECT and Z-SFT
				F-ECT		Z-SFM		
				Positive cases	%	Positive Cases	%	
Rainy season (July, August, September, October) (2012-13)	*E. histolytica*	Diabetic	93	9+	9.68	5+	5.38	P>0.05
				26++	27.96	9++	9.68	P<064
				7+++	7.53	2+++	2.15	P>0.05
		Non-diabetic	117	17+	14.53	11+	9.40	P>0.05
				31++	26.50	15++	12.82	P>0.05
				9+++	7.69	4+++	3.42	P>0.05
Total diabetic positive cases			93	42	45.16	16	17.20	P<0.054*
Total non-diabetic positive cases			117	57	48.72	30	25.64	P<0.040*
Proportion test between diabetic and non-diabetic				p>0.05		p>0.05		
Total diabetic and non-diabetic			210	99	47.14	46	21.90	P<0.05*

According to Z-SFM, the overall prevalence (diabetic) of *E. histolytica* (cyst and trophozoites) was 21.09%. The prevalence was 7.03% mild, 11.72% moderate and 2.34% sever infection. The overall prevalence among the non-diabetic individuals was 21.78%; while 8.91% mild, 9.90% moderate and 2.97% were severely infected (Table-25A).



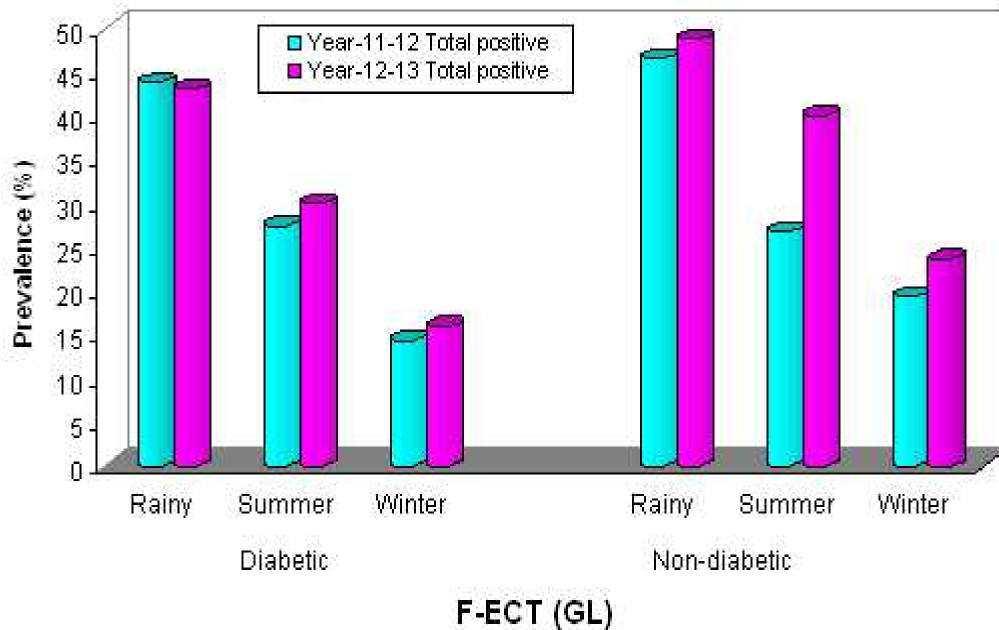
EH= *E. histolytica*

Fig-13(A): The prevalence of *E. histolytica* infections in three seasons among the diabetic and non-diabetic patients by F-ECT.

During rainy season (2012-2013), according to F-ECT, total 93 diabetic stool samples examined, the overall prevalence of *E. histolytica* (cyst and trophozoites) was 45.16%. On the other hand, 117 non diabetic stool samples, the overall prevalence was 48.72% for *E. histolytica* (cyst and trophozoites) (Table-25B).

In case of Z-SFM, the overall prevalence (diabetic) of *E. histolytica* (cyst and trophozoites) was 17.20%. The overall prevalence (non-diabetic) was 25.64% (Table-25B).

In rainy season (2011-2012), according to F-ECT, total 128 diabetic stool samples were examined, the overall prevalence of *G. lamblia* (cyst and trophozoites) was 43.75%; 14.06% mild, 24.22% moderate and 5.47% were severe infection. On the other hand, 101 (non diabetic) stool samples, the overall prevalence was 46.53%; 8.91%mild, 26.73% moderate and 10.89% were sever infection (Table-26A).



GL = *G. lamblia*

Fig-13(B): The prevalence of *G. lamblia* infections in three seasons among the diabetic and non-diabetic patients by F-ECT.

According to Z-SFM, the overall prevalence (diabetic) was 21.09% for *G. lamblia* (cyst and trophozoites); On the other hand, the overall prevalence (non-diabetic individuals) was 21.78%; (Table-26A); (Fig. 13B).

Table-26A: The prevalence of *G. lamblia* infections in rainy season among the diabetic and non-diabetic patients detected by F-ECT and Z-SFM.

Seasonal variation (Rainy season)	Name of parasites	Patient groups	Total samples examined (stool)	Name of techniques				Proportion test between F-ECT and Z-SFT
				F-ECT		Z-SFM		
				Positive cases	%	Positive cases	%	
Rainy season (July, August, September, October) (2011-12)	*G. lamblia*	Diabetic	128	18+	14.06	9+	7.03	P>0.05
				31++	24.22	14++	10.94	P<064
				7 +++	5.47	4+++	3.13	P>0.05
		Non-diabetic	101	9+	8.91	8+	7.92	P>0.05
				27++	26.73	11++	10.89	P>0.05
				11+++	10.89	3+++	2.97	P>0.05
Total diabetic positive cases			128	56	43.75	27	21.09	P<0.047*
Total non-diabetic positive cases			101	47	46.53	22	21.78	P<0.053*
Proportion test between diabetic and non-diabetic				P>0.05		p>0.05		
Total diabetic and non-diabetic			229	103	44.98	49	21.39	P<0.005**

P<0.05 = significant at 5% level

p>0.05 = not significant at 5% level

Intensity of infection (W.H.O. 1987)

C p g= cyst count per gm

	E. histolytica	***G. lamblia***
+=Mild	c p g=1-19/gm	c p g=1-99/gm
++=Moderate	c p g=150-250 /gm	c p g=100-199/gm
+++=Sever	c p g=251/gm and above	c p g=200/gm and above

Table-26(B): The prevalence of *G. lamblia* infections in rainy season among the diabetic and non-diabetic patients detected by F-ECT and Z-SFM.

Seasonal variation (Rainy season)	Name of parasites	Patient groups	Total samples examined (stool)	Name of techniques				Proportion test between F-ECT and Z-SFT
				F-ECT		Z-SFM		
				Positive cases	%	Positive cases	%	
Rainy season (July, August, September, October) (2012-13)	*G. lamblia*	Diabetic	93	7+	7.53	6+	6.45	P>0.05
				28++	30.11	14++	15.05	P<064
				5+++	5.38	2+++	2.15	P>0.05
		Non-diabetic	117	13+	11.11	10+	8.55	P>0.05
				33++	28.21	16++	13.68	P>0.05
				11+++	9.40	5+++	4.27	P>0.05
Total diabetic positive cases			93	40	43.01	22	23.66	P>0.05
Total non-diabetic positive cases			117	57	48.72	31	26.50	P<0.045*
Proportion test between diabetic and non-diabetic				P>0.05		P>0.05		
Total diabetic and non-diabetic			210	97	46.19	53	25.24	P<0.012*

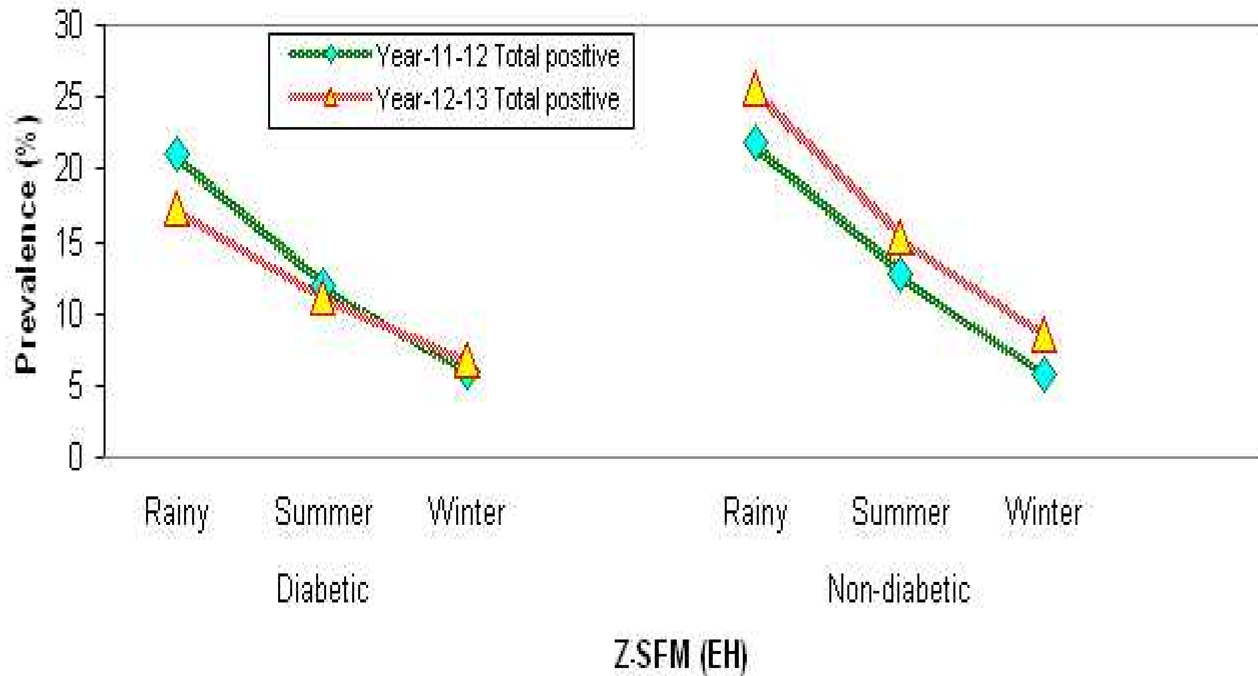
In rainy season (2011-2012), according to F-ECT, total 128 diabetic stool samples, the overall prevalence of *G. lamblia* (cyst and trophozoites) was 43.01%. On the other hand, 117 (non diabetic) stool samples, the overall prevalence was 48.72% (Table-28B).

According to Z-SFM, the overall prevalence (diabetic) was 23.66%. On the other hand, the overall prevalence (non-diabetic) was 26.50% (Table-28B).

In the rainy season (2011-2013), diabetic and non-diabetic individuals with *E. histolytica* and *G. lamblia* were significant.

During the period (2012-13), the prevalence was higher than that of the previous year (2010-2011) (Table-25-26).

In summer season (2010-2011), according to F-ECT, total 117 (diabetic) stool samples examined, the overall prevalence of *E. histolytica* (cyst and trophozoites) was 24.79%. On the other hand, 86 (non diabetic) stool samples were examined, theoverall prevalence was 26.74% for *E. histolytica* (Table-27A).



EH = *E. histolytica*

Fig-14(A): The prevalence of *E. histolytica* infection in three seasons among the diabetic and non-diabetic patients by Z-SFM.

According to Z-SFM, the prevalence (diabetic) was 11.97% for *E. histolytica* (cyst and trophozoites). On the other hand, the prevalence (non-diabetic individuals) was 12.79% for *E. histolytica* (Table-27A); (Fig. 14A).

Table-27A: The prevalence of *E. histolytica* infections in summer season among the diabetic and non-diabetic patients detected by F-ECT and Z-SFM.

Seasonal variation (summer season)	Name of parasites	Patient groups	Total samples examined (stool)	Name of techniques				Proportion test between F-ECT and Z-SFT
				F-ECT		Z-SFM		
				Positive cases	%	Positive cases	%	
Summer season (March, April, May and June) 2010-11	*E. histolytica*	Diabetic	117	14+	11.97	8+	6.84	P>0.05
				12++	10.26	5++	4.27	P>0.05
				3+++	2.56	1+++	0.85	P>0.05
		Non-diabetic	86	10+	11.63	5+	5.81	P>0.05
				7++	8.14	3++	3.49	P>0.05
				6+++	6.98	1+++	1.16	P>0.05
Total diabetic positive cases			117	29	24.79	14	11.97	P>0.05
Total non-diabetic positive cases			86	23	26.74	9	12.79	P>0.05
Proportion test between diabetic and non-diabetic				P>0.05		P>0.05		
Total diabetic and non-diabetic			203	52	25.62	23	11.33	P>0.05

P<0.05 = significant at 5% level;

P>0.05 = not significant at 5% level

Intensity of infection (W.H.O. 1987)

C p g= cyst count per gm

	E. histolytica	***G. lamblia***
+=Mild	c p g=1-149/gm	c p g=1-99/gm
++=Moderate	c p g=150-250 /gm	c p g=100-199/gm
+++=Sever	c p g=251/gm and above	c p g=200/gm and above

Table-27 (B): The prevalence of *E. histolytica* infections in summer season among the diabetic and non-diabetic patients detected by F-ECT and Z-SFM.

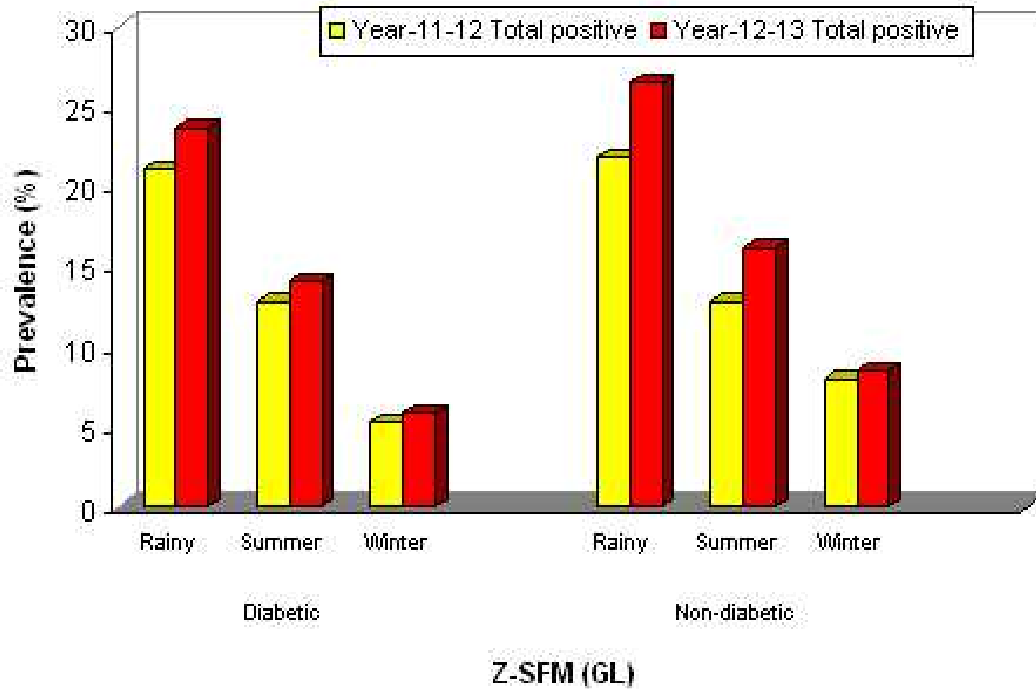
Seasonal variation (summer season)	Name of parasites	Patient groups	Total samples examined (stool)	Name of techniques				Proportion test between F-ECT and Z-SFT
				F-ECT		Z-SFM		
				Positive cases	%	Positive cases	%	
Summer season (March, April, May and June) 2012-13	*E. histolytica*	Diabetic	107	9+	8.41	6+	5.61	P>0.05
				13++	12.15	5 ++	4.67	P>0.05
				4+++	3.74	1+++	0.93	P>0.05
		Non-diabetic	105	20+	19.05	9+	8.57	P>0.05
				16++	15.24	5++	4.76	P>0.05
				4+++	3.81	2+++	1.90	P>0.05
Total diabetic positive cases			107	26	24.30	12	11.21	P>0.05
Total non-diabetic positive cases			105	40	38.10	16	15.24	P>0.05
Proportion test between diabetic and non-diabetic								
Total diabetic and non-diabetic			212	66	31.13	28	13.21	P<0.07

In summer season (2011-2012), according to F-ECT, out of 107 (diabetic) stool samples, the prevalence was 24.30% for *E. histolytica* (cyst and trophozoites). On the other hand, 105 (non diabetic) stool samples, the prevalence was 38.10% for *E. histolytica* (Table-27B).

According to Z-SFM, the prevalence (diabetic) was 11.21% for *E. histolytica* (cyst and trophozoites). On the other hand, the prevalence (non-diabetic individuals) was 15.24% (Table-27B).

In different groups and different techniques, the changes of infection intensity were non significant. Prevalence of final year, the infection was slightly higher than the initial year (Table-27A-27B); (Fig. 14A).

According to F-ECT out of 117 (diabetic) stool samples were examined, in summer season (2011-2012), the overall prevalence of *G. lamblia* (cyst and trophozoites) was 27.35%. On the other hand, 86 (non diabetic) stool samples, the overall prevalence was 26.74% for *G. lamblia* (Table-28A).



GL = *Giardia lamblia*

Fig-14(B): The prevalence of *G. lamblia* infection in three seasons among the diabetic and non-diabetic patients by Z-SFM.

According to Z-SFM, the overall prevalence (diabetic) was 12.82% for *G. lamblia* (cyst and trophozoites). The overall prevalence (non-diabetic individuals) was 12.79% for *G. lamblia* (Table-28A).

Table-28A: The prevalence of *G. lamblia* infections in summer season among the diabetic and non-diabetic patients detected by F-ECT and Z-SFM.

Seasonal variation (summer season)	Name of parasites	Patient groups	Total samples examined (stool)	Name of techniques				Proportion test between F-ECT and Z-SFT
				F-ECT		Z-SFM		
				Positive cases	%	Positive cases	%	
Summer season (March, April, May and June) 2011-12	*G. lamblia*	Diabetic	117	16+	13.68	8+	6.84	P>0.05
				13++	11.11	5++	4.27	P>0.05
				3+++	2.56	2+++	1.71	P>0.05
		Non-diabetic	86	10+	11.63	6+	6.98	P>0.05
				7++	8.14	4++	4.65	P>0.05
				6+++	6.98	1+ + +	1.16	P>0.05
Total diabetic positive cases			117	32	27.35	15	12.82	P>0.05
Total non-diabetic positive cases			86	23	26.74	11	12.79	P>0.05
Proportion test between diabetic and non-diabetic								
Total diabetic and non-diabetic			203	55	27.09	26	12.81	P<0.05*

P<0.05 = significant at 5% level;

P>0.05 = not significant at 5% level

Intensity of infection (W.H.O. 1987)

C p g= cyst count per gm

	E. histolytica	***G. lamblia***
+=Mild	c p g=1-149/gm	c p g=1-99/gm
++=Moderate	c p g=150-250 /gm	c p g=100-199/gm
+++=Sever	c p g=251/gm and above	c p g=200/gm and above

Table-28(B): The prevalence of *G. lamblia* infections in summer season among the diabetic and non-diabetic patients detected by F-ECT and Z-SFM.

Seasonal variation (summer season)	Name of parasites	Patient groups	Total samples examined (stool)	Name of techniques				Proportion test between F-ECT and Z-SFT
				F-ECT		Z-SFM		
				Positive cases	%	Positive cases	%	
Summer season (March, April, May and June) 2012-13	*G. lamblia*	Diabetic	107	12+	11.21	8+	7.48	P>0.05
				15++	14.02	6 ++	5.61	P>0.05
				5+++	4.67	1+++	0.93	P>0.05
		Non-diabetic	105	20+	19.05	9+	8.57	P>0.05
				18++	17.14	6+ +	5.71	P>0.05
				4+++	3.81	2+++	1.90	P>0.05
Total diabetic positive cases			107	32	29.91	15	14.02	P>0.05
Total non-diabetic positive cases			105	42	40	17	16.19	P>0.05
Proportion test between diabetic and non-diabetic								
Total diabetic and non-diabetic			212	74	34.91	32	15.09	P<0.041*

According to F-ECT, (2011-2012), out of 107 (diabetic) stool samples, the overall prevalence was 29.91% for *G. lamblia* (cyst and trophozoites) On the other hand, 105 (non diabetic) stool samples, the prevalence was 40% for *G. lamblia* (Table-28B).

According to Z-SFM, the overall prevalence (diabetic) was 14.02% for *G. lamblia* (cyst and trophozoites). The overall prevalence (non-diabetic individuals) was 16.19% for *G. lamblia* (Table-28B) (Fig. 14B).

In different groups and by different techniques, the changes of infection intensity were non significant. In the final year, the infection was slightly higher than the initial year (Table-27-28).

According to F-ECT, in winter season (2011-2012), out of 133 (diabetic) stool samples were examined, the overall prevalence of *E. histolytica* (cyst and trophozoites) was 12.03%. On the other hand, 88 (non diabetic) stool samples, the overall prevalence was 20.45% for *E. histolytica* (Table-29A).

Table-29A: The prevalence of *E. histolytica* infections in winter season among the diabetic and non-diabetic patients detected by F-ECT and Z-SFM.

Seasonal variation (winter season)	Name of parasites	Patient groups	Total samples examined (stool)	Name of techniques				Proportion test between F-ECT and Z-SFT
				F-ECT		Z-SFM		
				Positive cases	%	Positive cases	%	
Winter season (November, December, January, February) (2011-12)	*E. histolytica*	Diabetic	133	11+	8.27	5+	3.76	P>0.05
				5++	3.76	3++	2.26	P>0.05
				S(-------)		S(---- - -)		--
		Non-diabetic	88	10+	11.36	3+	3.41	P>0.05
				8++	9.09	2++	2.27	P>0.05
				S(-------)		S(--------)		
Total diabetic positive cases			133	16	12.03	8	6.02	P>0.05
Total non-diabetic positive cases			88	18	20.45	5	5.68	P>0.05
Proportion test between diabetic and non-diabetic				P>0.05		P>0.05		
Total diabetic and non-diabetic			221	34	15.38	13	5.88	P<0.07

P<0.05 = significant at 5% level;

P>0.05 = not significant at 5% level

Intensity of infection (W.H.O. 1987)

C p g= cyst count per gm

	E. histolytica	***G. lamblia***
+=Mild	c p g=1-149/gm	c p g=1-99/gm
++=Moderate	c p g=150-250 /gm	c p g=100-199/gm
+++=Sever	c p g=251/gm and above	c p g=200/gm and above

Table-29(B): The prevalence of *E. histolytica* infections in winter season among the diabetic and non-diabetic patients detected by F-ECT and Z-SFM.

Seasonal variation (winter season)	Name of parasites	Patient groups	Total samples examined (stool)	Name of techniques				Proportion test between F-ECT and Z-SFT
				F-ECT		Z-SFM		
				Positive cases	%	Positive cases	%	
Winter season (November, December, January, February) (2012-13)	*E. histolytica*	Diabetic	119	12+	10.08	6+	5.04	P>0.05
				6++	5.04	2++	1.68	P>0.05
				S(-------)		S(------)		--
		Non-diabetic	105	17+	16.04	6+	5.66	P>0.05
				8++	7.55	3++	2.83	P>0.05
				S(-------)		S(-------)		--
Total diabetic positive cases			119	18	15.13	8	6.72	P>0.05
Total non-diabetic positive cases			106	25	23.58	9	8.49	P>0.05
Proportion test between diabetic and non-diabetic				P>0.05		P>0.05		
Total diabetic and non-diabetic			225	43	19.11	17	7.56	P<0.07

According to Z-SFM, the overall prevalence (diabetic) of *E. histolytica* (cyst and trophozoites) was 6.02% for *E. histolytica* (cyst and trophozoites) On the other hand, the prevalence (non-diabetic individuals) was 5.68% for *E. histolytica* (Table-29A).

According to F-ECT, (2012-2013)out of 119 (diabetic) stool samples, the overall prevalence was 15.13% for *E. histolytica* (cyst and trophozoites) On the other hand, 105 (non diabetic) stool samples were examined, the prevalence was 23.58% for *E. histolytica* (Table-29B).

According to Z-SFM, the prevalence (diabetic) was 6.72% for *G. lamblia* (cyst and trophozoites). On the other hand, the prevalence (non-diabetic individuals) was 8.49% for *G. lamblia* (Table-29B).

According to F-ECT, (2012-2013)out of 119 (diabetic) stool samples, the overall prevalence was 15.13% for *E. histolytica* (cyst and trophozoites) On the other hand, 105 (non diabetic) stool samples were examined, the prevalence was 23.58% for *E. histolytica* (Table-29B).

According to Z-SFM, the prevalence (diabetic) was 6.72% for *G. lamblia* (cyst and trophozoites). On the other hand, the prevalence (non-diabetic individuals) was 8.49% for *G. lamblia* (Table-29B).

According to F-ECT, total 133 (diabetic) stool samples were examined in winter season (2011-2012), the overall prevalence of *G. lamblia* (cyst and trophozoites) was 14.29%. On the other hand, 88 (non diabetic) stool samples were examined, the prevalence was 19.32% for *G. lamblia* (Table-30A).

According to Z-SFM, the overall prevalence (diabetic) was 5.26% for *G. lamblia* (cyst and trophozoites). The overall prevalence among non-diabetic individuals found 7.95% (Table-30A).

According to F-ECT, total 119 diabetic stool samples, (2011-2012), the overall prevalence of *G. lamblia* (cyst and trophozoites) was 15.97%. In the case of 106 non-diabetic the overall prevalence was 23.58% (Table-30B).

According to Z-SFM, the overall prevalence (diabetic) was 5.88%. The overall prevalence among the non-diabetic was 8.46% (Table-30B).

Table-30(A): The prevalence of *G. lamblia* infections in winter season among the diabetic and non-diabetic patients detected by F-ECT and Z-SFM.

Seasonal variation (winter season)	Name of parasites	Patient groups	Total samples examined (stool)	Name of techniques				Proportion test between F-ECT and Z-SFM
				F-ECT		Z-SFM		
				Positive cases	%	Positive cases	%	
Winter season (November, December, January, February) (2011-12)	*G. lamblia*	Diabetic	133	10+	7.52	4+	3.01	P>0.05
				6++	4.51	3++	2.26	P>0.05
				S(-------)		S(-------)		--
		Non-diabetic	88	11+	12.5	4+	4.55	P>0.05
				6++	6.82	3++	3.41	P>0.05
				S(-------)		S(-------)		--
Total diabetic positive cases			133	19	14.29	7	5.26	P>0.05
Total non-diabetic positive cases			88	17	19.32	7	7.95	P>0.05
Proportion test between diabetic and non-diabetic				P>0.05		P>0.05		
Total diabetic and non-diabetic			221	36	16.29	14	6.33	P<0.07

P<0.05 = significant at 5% level;

P>0.05 = not significant at 5% level

Intensity of infection (W.H.O. 1987)

C p g= cyst count per gm

	E. histolytica	***G. lamblia***
+=Mild	c p g=1-149/gm	c p g=1-99/gm
++=Moderate	c p g=150-250 /gm	c p g=100-199/gm
+++=Sever	c p g=251/gm and above	c p g=200/gm and above

Table-30(B): The prevalence of *G. lamblia* infections in winter season among the diabetic and non-diabetic patients detected by F-ECT and Z-SFM.

Seasonal variation (Winter season)	Name of parasites	Patient groups	Total samples examined (stool)	Name of techniques				Proportion test between F-ECT and Z-SFM
				F-ECT		Z-SFM		
				Positive cases	%	Positive cases	%	
Winter season (November, December, January, February) (2012-13)	*G. lamblia*	Diabetic	119	13+	10.92	5+	4.20	P>0.05
				6++	5.04	2++	1.68	P>0.05
				S(-------)		S(-------)		--
		Non-diabetic	106	15+	14.15	5+	4.72	P>0.05
				10++	9.43	4++	3.77	P>0.05
				S(-------)		S(-------)		--
Total diabetic positive cases			119	19	15.97	7	5.88	P>0.05
Total non-diabetic positive cases			106	25	23.58	9	8.49	P>0.05
Proportion test between diabetic and non-diabetic				P>0.05		P>0.05		
Total diabetic and non-diabetic			225	44	19.56	16	7.11	P<0.07

Interpretation:

The prevalence of parasite was very high in the rainy season. On the other hand, the prevalence was very low during winter season and minimum in summer season. The present epidemiological study showed that amoebiasis and giardiasis were present throughout the year but seasonal peak of transmission occurred in September during monsoon season and minimum incidence was observed in March in summer season. The present study showed that in rainy season, trophozoite with cyst of *E. histolytica* was 44.54% and *G. lamblia* were 44.98%. The average relative humidity 55%-80% and average temperature 15-30ºC remain conductive to amoebiasis and giardiasis transmission. The present observation showed that the minimum prevalence was observed in summer season, when the average maximum temperature was 36.92ºC and average minimum temperature was 24.2ºC. The maximum prevalence was observed in rainy season when the average maximum relative humidity was 68.75 and average minimum relative humidity was 47.25%, the average rainfall was 75.85 mm. It was also determined that, in the rainy season, the environment become unhygienic and polluted,

water usually flow over the environment. Ingestion of cysts that are transmitted primarily by the faecal-oral route with contamination of drinking water, vegetables and food, and eating of uncooked vegetables and fruits (Table-18-32).

5.10 Comparison of sensitivity and specificity between ELISA and Z-SFM:

31. Sensitivity and Specificity of different diagnostic tests for the detection of *E. histolytica*:

Sensitivity of the antigen detection or enzyme link immunosorbent assay (ELISA) and zinc sulphate floatation method (Z-SFM) determined by the formol-ether concentration technique (F-ECT). Z-SFM sensitivity and specificity also measured with the comparison of ELISA. Sensitivity and specificity measure in different type of samples. In diabetic samples ELISA was 46.52% sensitive and 95.29% specific and Z-SFM showed sensitivity 23.53% and 92.16% specificity. Z-SFM is less sensitive then ELISA when compared to F-ECT. Among the non-diabetic sample highest sensitivity (58.37%) and specificity (88.83%) found in ELISA.

Table: 31.1. (A). Comparison between the results of ELISA and F-ECT in diabetic blood and stool samples.

	F-ECT positive	F-ECT negative	Total
ELISA positive	**87**	24	111
ELISA negative	100	**486**	586
Total	187	510	697

Sensitivity of ELISA: 46.52%
Specificity of ELISA: 95.29%

Table: 31.1. (B). Comparison between the results of ELISA and Z-SFM in diabetic blood and stool samples.

	F-ECT positive	ELISA negative	Total
Z-SFM positive	**44**	40	84
Z-SFM negative	143	**470**	613
Total	187	510	697

Sensitivity of Z-SFM: 23.53%
Specificity of Z-SFM: 92.16%

Table: 31.1. (C). Comparison between the results of Z-SFM and F-ECT in diabetic blood and stool samples.

	Z-SFM positive	Z-SFM negative	Total
ELISA positive	**24**	87	111
ELISA negative	60	**526**	586
Total	84	613	697

Sensitivity of ELISA: 28.57%
Specificity of ELISA: 85.81%

Table: 31.2. (A). Comparison between the results of ELISA and F-ECT in non-diabetic blood and stool samples.

	F-ECT positive	F-ECT negative	Total
ELISA positive	**152**	14	166
ELISA negative	57	380	437
Total	209	394	603

Sensitivity of ELISA: 58.37%

Specificity of ELISA: 88.83%

Table: 32.2. (B). Comparison between the results of Z-SFM and F-ECT in non-diabetic blood and stool samples.

	F-ECT positive	F-ECT negative	Total
Z-SFM positive	**59**	32	91
Z-SFM negative	150	**362**	512
Total	209	394	603

Sensitivity of Z-SFM: 28.23%

Specificity of Z-SFM: 91.88%

Table: 31.2. (C). Comparison between the results of ELISA and Z-SFM in non-diabetic blood and stool samples.

	Z-SFM positive	Z-SFM negative	Total
ELISA positive	**23**	143	166
ELISA negative	68	**369**	437
Total	91	512	603

Sensitivity of ELISA: 25.27%

Specificity of ELISA: 72.07%

Table: 31.3. (A). Comparison between the results of ELISA and F-ECT in diabetic and non-diabetic blood and stool samples.

	F-ECT positive	F-ECT negative	Total
ELISA positive	**207**	70	277
ELISA negative	189	**834**	1023
Total	396	904	1300

Sensitivity of ELISA: 52.27%

Specificity of ELISA: 92.26%

Table: 31 .3. (B). Comparison between the results of Z-SFM and F-ECT in diabetic and non-diabetic blood and stool samples.

	F-ECT positive	F-ECT negative	Total
Z-SFM positive	**96**	79	175
Z-SFM negative	300	**825**	1125
Total	396	904	1300

Sensitivity of Z-SFM: 24.24%
Specificity of Z-SFM: 91.26%

Table: 31. 3. (C). Comparison between the results of ELISA and Z-SFM in diabetic and non-diabetic blood and stool samples.

	Z-SFM positive	Z-SFM negative	Total
ELISA positive	**45**	232	277
ELISA negative	130	**893**	1023
Total	175	1125	1300

Sensitivity of ELISA: 25.71%
Specificity of ELISA: 79.38%

32. Sensitivity and Specificity of different diagnostic tests for the detection of *G. lamblia*:

Sensitivity of the antigen detection or enzyme link immunosorbent assay (ELISA) and zinc sulphate floatation method (Z-SFM) determined by the formol-ether concentration technique (F-ECT). Z-SFM sensitivity and specificity also measured with the comparison of ELISA. Sensitivity and specificity measure in different type of samples. In diabetic samples ELISA was 47.98% sensitive and 94.39% specific and Z-SFM showed sensitivity 30.81% and 93.59% specificity. Z-SFM is less sensitive then ELISA when compared to F-ECT. Among the non-diabetic sample highest sensitivity (58.29%) and specificity (88.27%) found in ELISA.

Table: 32.1. (A). Comparison between the results of ELISA and F-ECT in diabetic blood and stool samples.

	F-ECT positive	F-ECT negative	Total
ELISA positive	**95**	28	123
ELISA negative	103	**471**	574
Total	198	499	697

Sensitivity of ELISA: 47.98%

Specificity of ELISA: 94.39%

Table: 32.1. (B). Comparison between the results of Z-SFM and F-ECT in diabetic blood and stool samples.

	F-ECT positive	F-ECT negative	Total
Z-SFM positive	**61**	32	93
Z-SFM negative	137	**467**	604
Total	198	499	697

Sensitivity of Z-SFM: 30.81%
Specificity of Z-SFM: 93.59%

Table: 32.1. (C). Comparison between the results of ELISA and Z-SFM in diabetic blood and stool samples.

	Z-SFM positive	Z-SFM negative	Total
ELISA positive	**29**	94	123
ELISA negative	64	**510**	574
Total	93	604	697

Sensitivity of ELISA: 31.18%
Specificity of ELISA: 84.44%

Table: 32.2. (A). Comparison between the results of ELISA and F-ECT in non-diabetic blood and stool samples.

	F-ECT positive	F-ECT negative	Total
ELISA positive	**123**	45	169
ELISA negative	88	**346**	434
Total	211	392	603

Sensitivity ELISA: 58.29%

Specificity of ELISA: 88.27%

Table: 32.2. (B). Comparison between the results of Z-SFM and F-ECT in non-diabetic blood and stool samples.

	F-ECT positive	F-ECT negative	Total
Z-SFM positive	**38**	59	97
Z-SFM negative	173	**333**	506
Total	211	392	603

Sensitivity of Z-SFM: 18.01%

Specificity of Z-SFM: 84.95%

Table: 32.2. (C). Comparison between the results of ELISA and Z-SFM in non-diabetic blood and stool samples.

	Z-SFM positive	Z-SFM negative	Total
ELISA positive	**24**	145	169
ELISA negative	73	**361**	434
Total	97	506	603

Sensitivity of ELISA: 24.74%

Specificity of ELISA: 71.34%

Table: 32.3. (A). Comparison between the results of ELISA and F-ECT in diabetic and non-diabetic blood and stool samples.

	F-ECT positive	F-ECT negative	Total
ELISA positive	**192**	100	292
ELISA negative	217	**791**	1008
Total	409	891	1300

Sensitivity of ELISA: 46.94%

Specificity of ELISA: 88.78%

Table: 32.3. (B). Comparison between the results of Z-SFM and F-ECT in diabetic and non-diabetic blood and stool samples.

	F-ECT positive	F-ECT negative	Total
Z-SFM positive	**111**	79	190
Z-SFM negative	298	**812**	1110
Total	409	891	1300

Sensitivity of Z-SFM: 27.14%

Specificity of Z-SFM: 91.13%

Table: 32.3. (C). Comparison between the results of ELISA and Z-SFM in diabetic and non-diabetic blood and stool samples.

	Z-SFM positive	Z-SFM negative	Total
ELISA positive	**51**	241	292
ELISA negative	139	**870**	1008
Total	190	1110	1300

Sensitivity of ELISA: 26.84%

Specificity of ELISA: 78.38%

DISCUSSION

The present study is the first time done on protozoan parasitic anaemia among diabetic with comparison to non-diabetic patients. The prevalence of parasitic infestations was investigated among the diabetic and non-diabetic peoples in Bangladesh to observe the accuracy of deferent techniques for identification of *Entamoeba histolytica* and *Giardia lamblia*. Protozoan parasites have a series of distinct developmental stages in their life cycle. Based on their stage of development, unique life cycle between stool specimen collection and observation, parasites may be observed in different stages and morphology, namely cysts and trophozoites. These morphology dependent stages of parasites were observed in different techniques of stool specimens. Several techniques, such as Formol Ether concentration technique (F-ECT) and Zinc Sulphate Floatation method (Z-SFM) are very common used in clinical and pathological diagnosis for detection of stool's parasites.

The present study was carried out to compare the F-EC technique with ELISA of blood serum and Z-SF method in stool microscopy for detection of intestinal protozoan parasites and also find out the prevalence of different protozoan parasites in different sex and age group of host, to find out the monthly variation of the parasite infestation in study period.

6.1. Application of ELISA in serodiagnosis of invasive amoebiasis and giardiasis:

Intestinal infection with *E. histolytica* and *G. lamblia* is an important cause of dysentery worldwide especially where sanitary conditions are poor. The persons infected with *E. histolytica* each year, only about 10% of them become symptomatic and develop colitis and liver abscess. Patients infected with *E. histolytica* show a wide range of condition. Some patients show mild diarrhea with abdominal cramps, eventually resulting fulminant colitis. Because of the tissue damage that can occurs perforation of the intestine and the amoeba may disseminate to other parts of the body. This organism exists either as a

trophozoite or a cyst form. Their cyst is stable; once it enters the intestine, it begins to divide into trophozoite that binds to the intestinal mucosa via galactose binding lectine on the surface of the organism. This lectin is referred to as the galactose adhesion. Once the trophozoite have attached, they release tissue damaging enzymes and proteins that lyse the mucosal cell. Amoebic dysentery is a disease that is likely to be underestimated because of a lack of reliable diagnostic laboratory test (Khanum *et al.,* 2012).

E. histolytica is the etiological agent of human amoebiasis. The term *'histolytica'* literally means 'tissue dissolving' referring its carnivorous habits. It's usually colonize in the large bowel may destroy mucosal layer causing amoebic colitis or may invade other organs via circulation causing abscess especially in the liver (Tsutsumi, *et al.,* 1984). The WHO ranks *E. histolytica* as the third leading parasitic cause of death. It is now clear that tissue destruction results both directly from amoebic factors and from the host's inflammatory response (Huston, 2004). Most ELISA- based method for *E. histolytica* and *G. lamblia* employ complex procedures, leading to relatively high expenditure, labour, costs and turn around times. A simple, rapid, cost-effective and yet sensitive and specific ELISA method has now been developed for the detection of *E. histolytica* and *G. lamblia* in blood.

Clinical amoebiasis results from the spread of the normally luminal parasite into the colon wall and beyond; the key development in understanding this complex multistage process has been the publication of the *E. histolytic* genome, from which has come an explosion in the use of micro arrays to examine changes in gene expression that from changes in growth conditions (John *et al.,* 2006).

The intestinal protozoan parasite *G. lamblia* has a worldwide distribution and is the most commonly diagnosed flagellate in the intestinal tract, a reported global prevalence of approximately 30% (Root *et al.*, 1983). The most common mode of transmission of *G. lamblia* is by the direct faecal-oral route from contaminated hands, food or water, which maintain widespread endemic areas of infection. Giardiasis is endemic in developing countries and particularly affects children. Undeveloped countries the parasite is

commonly found in persons living in closed communities (Yoeli *et al.,* 1972), in those who are immuno-compromised (Smith *et al.,* 1998), and in children attending day care centers (Ozturkean *et al.,* 1993). There are also documented out breaks of giardiasis due to contaminated water supplies (Green *et al.,* 1988).

The infection rate of *E. histolytica* and *G. lamblia* are considered to be associated with the socioeconomic status of their host; because, these organisms are being spread through ingested food, contaminated water or by venereal transmission. The transmission rate of *E. histolytica* and *G. lamblia* cyst are also associated with their host's sewage disposal, source of drinking water and latrine structure. As amoebic dysentery and giardiasis are water borne disease, then water is the major factor of the transmission of *E. histolytica* and *G. lamlia* cyst. The prevalence of *E. histolytica* and *G. lamlia* is higher among supply water drinker than that of tube well water user, because the supply water is not sterilized from *E. histolytica* and *G. lamlia* may be contaminated with supply water in any site, from the central distribution of water to its any supply water or unhygienic reservoir.

There are every chance of contamination of sewage water with drinking water, which contains *E. histolytica* and *G. lamlia* cyst. Also if source of supply is not preserved purified or purified poorly then it may also content *E. histolytica* and *G. lamlia* cyst, where on the other hand tube well water users cannot have *E. histolytica* and *G. lamlia* at their source of supply or at the distribution system. Jahan *et al.,* (1987) found all positive cases drinking the supply water.

6.2. Parasitic infestation:

In the cross sectional study, total 1300 blood and 1300 stool samples were examined were 697 samples were collected from diabetic patients and 603 samples were collected from non-diabetic peoples in the study period (November 2010 to October 2012). All samples were examined by three different (ELISA, F-ECT and Z-SFM) techniques.
The result of the present study revealed that the overall prevalence that had been detected by ELISA was 43.77% whereas, by F-ECT and ZSFM, the prevalence was 61.93% and

28.08% respectively. So, it was found that the infection rate of the parasite detected by F-ECT was obviously higher than other two techniques.

Alam, *et. al.,* (2013) showed the several diagnostic tools were employed which include ELISA, acid fast staining and trichrome staining techniques. She also reported that, diagnosis of protozoan parasitic cases heavily rely on suitable approached and used of modern molecular tools like PCR, Real time PCR can be more sensitive for accurate identification of pathogens causing diarrhea in any clinical setting. She had found ELISA approached more sensitive and further optimization of classical staining techniques can be an added advantage to diagnosis protozoan parasites efficiently.

The present study showed the higher infection rate in non-diabetic people due to high population density, improper sanitation, lack of knowledge of personal hygiene and unhygienic food preservation. Though the prevalence of *E. histolytica* and *G. lamlia* infection are much high in non-diabetic people than those of diabetic people. This kind of study was performed by Islam (1983), who showed the prevalence of giardiasis was (60%) in non-diabetic people and that of amoebiasis was 40%.

Khanum, *et al.,* (2013) reported the overall prevalence of infestation was 23.14% where *E. histolytica* and *G. lamlia* were found as 4.86% and 3.71% respectively.

Banu *et al.,* (2011) found the highest prevalence of protozoan parasites (49.62%) in rural area (Kamrangirchar) and the lowest (18.22%) in urban area (Savar). The prevalence in slum areas was more than 30% which was comparatively lower than rural but higher than the urban area. Rahman (2009) reported overall protozoan infection as 43% in his study among the non-diabetic people of Chittagang Hill Tracts which was almost similar with the present study. Hyder *et al.,* (1998) reported gross prevalence (33.3%) of parasites among non-diabetic and adult population in different rural areas of Mymenshing district of Bangladesh, which was also closely related with our findings. Rao *et al.,* (2003) reported overall prevalence was 57% among non-diabetic people of Nepal, which was related with our study. Uddin *et al.,* (2005) studied the prevalence of protozoan parasitic

infestation among non-diabetic peoples in two areas, Kutombopur and Savar and reported overall prevalence as 71.01% with 65.2% in Kutombopur. The findings were higher than the present work due to differences in study population and prevailing socioeconomic condition.

According to Black *et al.,* (1980), Stanton *et al.,* (1989) reported prevalence of infection ranges from 24% to 52% depending on the methods of examining stool samples and on whether the stools came from healthy individuals or from people with diarrhea. More or less same findings were recorded by Islam *et al.,* (1975).

Khan (1994) studied in the patients of different diagnostic centre in Dhaka city, Islam *et al.,* (1983) reported *E. histolytica* and *G. lamblia* in the patients of Barisal Medical College and and Hospital. Nahar (2011) studied on outdoor patients of IBN Sina hospital, Dhaka and reported *E. histolytica* and *G. lamblia.*

6.3. Single parasitic (protozoan) infestation:

In the present study, the parasitic infestation in non-diabetic recorded was 34.66% *E. histolytica* and the highest prevalence 34.99% was found for *G. lamblia* in Formal Ether concentration technique. The lowest 16.09% was for *G. lamblia* in non-diabetic and 15.09% was for *E. histolytica* in Zinc Sulphate Floatation method. The prevalence of *G. lamblia* by ELISA was 28.03% in non- diabetic and 27.53% for *E. histolytica* which was comparatively lower than Formal Ether concentration technique but higher than the Zinc Sulphate Floatation method.

Khanum (1999) detected 39.25% by F-ECT whereas; by the ELISA and Z-SFM the prevalence were 31.25% and 22.92% respectively. Azian *et al.,* (2007) in a study on aborigin community in Pehng, Malysia, noted that the prevalence of *E. histolytica* and *G. lamblia* were 29.2% and 26.2% respectively.

Khanum (2001) reported *G. lamblia* was 3.49% by saline wet mount preparation, 3.41% by iodine wet mount preparation and KHO detected 4.03%; and the prevalence of *E. histolytica* was 2.24% by saline wet mount preparation, 2.15% by iodine wet mount preparation and 2.33% by KHO.

The prevalence of *E. histolytica* recorded were 37% by Kuntz (1960), 55% by Muttalib (1975), Rahim reported 71% *E. histolytica* in 2009. This indicates that infection level become lower in succeeding years. While in Bangladesh this rate is slightly higher (khan, 1994).

Hyder (1998) reported gross prevalence (33.3%) of parasitic infection among the non-diabetic peoples and adult population in different rural areas of Mymenshing district which was also similar to the present study. Uddin *et al.,* (2005) studied the prevalence of parasitic infestation among the non-diabetic peoples in two rural areas, Kutombopur, and Savar and reported overall 71.01% prevalence and 62.5% in Kutombopur, which was higher than the rural area. Rao *et al.,* (2003) reported overall 57% prevalence among tribal non-diabetic people of Nepal. Al-Madani (1995) stated 46.5% prevalence among the Asian female house-keepers in Aba District, Saudi Arabia.

The findings were higher than the present work due to differences in study population and prevailing socioeconomic condition and ecological condition. Shakur and Ehsan (1993) observed the prevalence of intestinal parasites (71.8%) in a hospital based study. The prevalence of this parasite was different than of the present investigation and this difference might be due to the study population, location, prevailing environment and socio-economic status. According to WHO (1987) as much as 60% of the world's population were infected with gut parasites. Globally reported the commonest parasitic infections were *E. histolytica* (27.53%) and *G. lamblia* (24.51%).

6.4. Age Groups:

Muazzem *et al.* (1968) found 3% *G. lamblia* infection in children. More or less similar findings were recorded by Shakur *et al.,* (1993) where, intestinal parasites were 70% in the children infected by *G. lamblia.* Haque and Sheikh (1976) found 2.93%-2.98% prevalence of giardiasis among 1-5 age groups of children. In 1983, Hossain *et al.,* found the prevalence in urban hospital patients in aged 5-9 was 21%. Gilman *et. al.* (1985) found higher prevalence in 5 to 10 years old village children. Saha *et al.,* (1981) recorded 16.7%, 4% and 14% the prevalence of *G. lamblia* were in Mirzapur, Bhaluka and Kaliganj respectively.

In the present study it was found that the prevalence of *E. histolytica* and *G. lamblia* infection detected by three techniques which were higher than that of the previous studies carried out in Bangladesh. This may be due to the geographical, ecological and soci-economic variation. Poverty, illiteracy, lack of knowledge of personal hygiene, improper sanitation, contaminated drinking water etc. are the major factors which are responsible for varied rates of prevalence of *E. histolytica* and *G. lamblia.*

Opara *et al.,* (2007) reported 21.1% intestinal protozoan infection in urban primary school children (5-16 years) in Imo State, Nizeria which was almost similar to the present finding of the present study.

Oguoma (2007) studied on Nigerian people, the prevalence of protozoan parasites was 11.61% by zinc sulphate floatation method and by formol ether concentration method 34.09% 11-20 years old are more infected. In the present study, among non-diabetic peoples the highest prevalence (44.76%) of *G. lamblia* was observed in 35-45 years of age category; and 45.69% was observed for *E. histolytica* among non-diabetic peoples who were in 25-35 years of age category. The lowest prevalence of *G. lamblia* (7.74%) was observed among diabetic peoples in 55-65 years of age category; and 5.38% *E. histolytica* was observed among diabetic peoples in 55-65 years of age category.

Considering the age groups, diabetic and non-diabetic patients of the 25.1-35 and 35.1-45 years age category was found to be more susceptible for parasitic infestation. About 40.13% of the diabetic and 44.76% of the non-diabetic patients of 35.1-45 age groups were infected by *G. lamblia* which was much higher than average 28.41% and 34.99% (diabetic and non-diabetic) of the studied samples for this particular parasite.

About 38.05% of the diabetic and 45.69% of the non-diabetic patients of 25.1-35 age groups were infected by *E. histolytica* which was much higher than average 26.83% and 34.66% (diabetic and non-diabetic) of the studied samples for this particular parasite.

About 38.05% of the diabetic and 45.69% of the non-diabetic patients of 25.1-35 age groups were infected by *E. histolytica* which was much higher than average 26.83% and 34.66% (diabetic and non-diabetic) of the studied samples for this particular parasite.

Banu (2011) reported the highest prevalence of *G. lamblia* (20%) among the adolescent girls of the 12-13 years age group in Bangladesh. Hussain *et al.* (2003) reported that *G. lamblia* (11%) and *E. histolytica* (8.5%) among the people of Hilly areas. Ahmed and Talukder (2002) recorded the prevalence of *G. lamblia* (32.7%) and *E. histolytica* (14.9%) among the school children (aged 9-16 years) from rural and urban areas of Bangladesh.

6.5. Male and Female:

From the present study the higher prevalence of protozoan parasites was found in male than female by three different techniques. Males are more infected than females although there was no statistical significance ($P>0.05$). According to the sex, the present study showed that, the prevalence of amoebiasis and giardiasis among male were higher than that of female, as the male population are more exposed to external environment, they usually use public toilet, take food and water from outside Similar prevalence was recorded by Wanke (1988) where, the male:female ratio of *E. histolytica* positive respondents was 60:24.

Khanum *et al.,* (2013) reported that the prevalence of intestinal parasites was higher in female (30.56%) than in male (22.29%). In another study by Uddin *et al.,* (2005), highest prevalence 87.50% was also noted among the female non-diabetic peoples aged 12-14 years. Khanum (2010) reported that the high rate of infection with *Entamoeba* spp. found in female (12.29%) in 11-12 years old children detected by dipstick test. ELISA showed 5.02% prevalence of *E. histolytica* in female. But according to Haque 1976 the infection is higher in males.

6.6. Symptomatic and Asymptomatic:

In the present study, by ELISA method, in diabetic blood samples, the prevalence of *E. histolytica* was 18.68% in symptomatic types and 12.91% in asymptomatic types; which 31.45% of non-diabetic symptomatic types and 21.21% of asymptomatic types were positive for *E. histolytica* antibody. In diabetic blood samples, the prevalence was 21.15% in symptomatic types and 13.8% in asymptomatic types; and 31.18% of non-diabetic symptomatic types and 22.94% of asymptomatic types were positive for *G. lamblia* antibody.

The world wide prevalence of *E. histolytica* has been described as 14.3% which, 16% in Asia, 12% in America and17% in Africa (Belding, 1995). Several microscopy-based epidemiological studied in Iran have shown *Entamoeba* 2.2% to 30% infection. Current microscopic-based studies, showed a high prevalence of *E. histolytica* in asymptomatic carriers. This was true in the tropical areas of the south, where previous surveys showed that up to 30% by *E. histolytica* in asymptomatic individuals residing in rural areas with poor sanitation were infected by use ELISA. William (1996) observed *E. histolytica* infection in an urban refugee camp in Dhaka, Bangladesh, in 5% to 30% of patients aged 25 year.

Khanum (2005) reported that the prevalence of *E. histolytica* was 8.6% in asymptomatic stool samples. Dipstick test showed 10.32% infection in asymptomatic stool samples and

27.35 in symptomatic stool samples. The highest prevalence found in symptomatic stool samples. ELISA also detected higher prevalence 9.09% in symptomatic stool samples.

6.7. Sensitivity and Specificity:

The present study measured the sensitivity and specificity of antigen detection test, ELISA and microscopy, Z-SFM compared with microscopy, F-ECT for *E. histolytica*. In diabetic samples ELISA was 46.52% sensitive and 95.29% specific and Z-SFM showed sensitivity 23.53% and 92.16% specificity. Z-SFM is less sensitive then ELISA when compared to F-ECT. Among the non-diabetic sample highest sensitivity (58.37%) and specificity (88.83%) found in ELISA.

Uddin *et al.,* (2005) studied the measured of sensitivity, specificity and correlation of microscopy and culture compared with antigen detection test, ELISA for *E. histolytica*. Sensitivity of microscopy (18.04%) and culture (19.39%) both are lower in cases of asymptomatic carrier. In cases of diarrheal samples, both microscopes (23.44%) and culture (26.56%) showed litter bit higher sensitivity and in case of amoebic liver abscess (ALA) they are equally sensitive (22.22%). Culture was more specific than microscopy both in case of asymptomatic (96.52%) and symptomatic (96.52%) stool samples. Culture has a good correlation with ELISA, 91.57% in case of asymptomatic and % in case of symptomatic stool samples. Microscopy also has good correlation but slightly lower then the culture, 87.94% and 81.01% for asymptomatic and diarrheal samples respectively. In abscess aspirate samples microcopy and culture showed lower correlation with ELISA, 48.27% for both for the diagnosis of amoebiasis.

Khanum *et al.,* (2005) measured the sensitivity, specificity of microscopy and Dipstic test compared with antigen detection test, ELISA for *E. histolytica*. The sensitivity of microscopy was 23% and specificity was 91%. Dipstick test showed 100% sensitivity and 92% specific. Dipstick test was more sensitive and specific than microscopy when compared with ELISA for the diagnosis of amoebiasis.

Khanum *et al.,* (2001) measured the sensitivity, specificity of microscopy and ELISA using cut-off point 0.25 compared with antigen detection test, ELISA for *G. lamblia*. The sensitivity of microscopy was 90.9% and specificity was 97%. ELISA using cut-off point 0.25 showed 70.4% sensitivity and 97% specific. Microscopy was more sensitive and specific than ELISA using cut-off point 0.25 when compared with ELISA for *G. lamblia*. In 1993, Goldin *et al.,* showed the sensitivity of the ELISA was 94.2% and 98% specific for the diagnosis of giardiasis. Al-Tukhi *et al.,* 1993 showed the sensitivity of the ELISA was 84% and 97% specific. Similar findings were reported by Hassan *et al.,* in 1995 for the diagnosis of *Giardia* infection.

The present study measured the sensitivity, specificity and correlation of antigen detection test, ELISA and microscopy, Z-SFM compared with microscopy, F-ECT for *G. lamblia*.

In diabetic samples ELISA was 47.98% sensitive and 94.39% specific and Z-SFM showed sensitivity 30.81% and 93.59% specificity. Z-SFM is less sensitive then ELISA when compared to F-ECT. Among the non-diabetic sample highest sensitivity (58.29%) and specificity (88.27%) found in ELISA.

6.8. Seasonal variation of parasitic infection:

In the present study, the parasite of prevalence was very high in the rainy season. On the other hand, the prevalence was very low during winter season and minimum in summer season. The present epidemiological study shows that amoebiasis and giardiasis were present throughout the year but seasonal peak of transmission occurred in September during monsoon season and minimum incidence was observed in March in summer season. The present study shows that in rainy season trophozoite with cyst of *E. histolytica* were 44.54% and *G. lamblia* were 44.98%. Khanum *et al.,* (2013) studied the seasonal pattern showed that highest (30%) prevalence occurred in rainy season and lowest (17.19%) in winter season.

6.9. Double parasitic (protozoan) infestation:

In the present study, the extent of double infection was lower than that of single infection. The more prevalent combination (*E. histolytica* and *G. lamblia*) was observed in 13.10% of non-diabetic peoples and lowest was 5.64% in diabetic peoples.
In diabetic patients, by ELISA the prevalence of double infections (*E. histolytica*+ *G. lamblia*) was 4.18% in females and 4.73% in males were found and 7.52% in females and 8.58% in male by F-ECT and 2.51% in females and 3.55% in males by Z-SFM.
By ELISA in diabetic patients and non-diabetic individuals showed significant result; and other two techniques (F-ECT and Z-SFM) in single and double infestation of (*E. histolytica* + *G. lamblia*) protozoan parasites showed not significantly.

Khanum *et al.* (1999) worked on the intestinal protozoan parasites infection of age group 2-16 years, irrespective to sex, from lower income group employee in Dhaka city and noted 11.5% of double infection (*E. histolytica*+ *G. lamblia*).

Banu *et al.,* (2011) found 3.12% prevalence of *E. histolytica* and *G. lamblia* combination among the non-diabetic peoples which was closely related with the present investigation. Opara *et al.,* (2007) found 3.4% of *E. histolytica* and *G. lamblia* double infection among the school children which was lower than that of the findings of the present study. Kaur and Kaur and Sween (2007) reported 17.64% infestation of *E. histolytica* and *G. lamblia* combination which was much higher than of the findings of the present study.

Rahman (2009) reported 1.28% of *E. histolytica*+ *G. lamblia* double infection only from the female subjects which were much lower than present study findings (7.52%). The difference may be due to the study population and environmental obligation.

6.10. Immunopatholigy of *Entamoeba histolytica* and *Giardia lamblia* infection:

Denis and Chadee (1988) reported, infections with *E. histolytica* that are confined to the intestinal lumen an antibody response but peripheral blood lymphocytes are unresponsive to amoebic antigens. In asymptomatic individuals, amoebic antigens or soluble amoebic products can pass out of the intestinal lumen and elicit a systemic immune response. It may be that specific amoebic proteins cause a local transient immunosuppressant in the gut facilitating amoebic invasion.

In the present study, it was observed that, 15.93% in diabetic patients and 27.53% in non-diabetic samples were seropositive for *E. histolytica* antibody; and 17.65% of diabetic and 28.03% of non-diabetic samples were seropositive for *G. lamblia* antibody, which indicate that the prevalence was higher (positive) in non-diabetic people may be due to good immune reactivity in non-diabetic people in comparison to diabetic patient. On the other hand, less immune reactivity against *E. histolytica* and *G. lamblia* in diabetic patients are evident. As diabetes are a chronic non-communicable disease, if uncontrolled it gradually infect all the vital organs of the body. It eventually involves immune system of the body. The less immune reactivity against *E. histolytica* and *G. lamblia* in diabetic patient was consistent with this finding (Tally and O'Connor S, 1991).

Again, Ozturkean (1993), repoted that diabetic patients were more vulnerable to various types of infection as their body's immune system became weak and cannot fight against those infectious agents.

Patients infected with *E. histolytica* show a wide range of conditions. Some patients show mild diarrhea with abdominal cramps, eventually resulting fulminate colitis. Because of the tissue damage that can occurs perforation of the intestine may result and the amoeba may disseminate to other parts of the body. This organism exists either as a trophozoite or a cyst form. Their cyst is stable; once it enters the intestine, it begins to divide into trophozoite that binds to the intestinal mucosa via galactose binding lectine on the surface of the organism. This lectine is referred to as the galactose adhisin. Once the trophozoite

has attached, they release tissue damaging enzymes and proteins that lyses the mucosal cell. Amoebic dysentery is a disease that is likely to be underestimated because of a lack of reliable diagnostic laboratory test.

Talley and O'Connor S, (1991) worked on diabetic and non-diabetic peoples in Woden Valley Hospital, Canberra, and reported a low hemoglobin level in diabetic peoples compared with the non-diabetic peoples.

6.11. Amoebic killing by macrophages and lymphocytes:

Denis and Chadee (1988) reported, indirect evidence for a role of macro- phages in the killing of amoebae has been obtained from several studies. Macrophages can be activated by lymphokines to kill *E. histolytica* trophozoites.

This killing mechanism is contact-dependent and is mediated by both oxygen- and non-oxygen-dependent pathways. *E. histolytica* is susceptible to hydrogen peroxide. Studies with the xanthine — oxidase system —generating O_2 H_2O_2 and hydroxyl radicals — have shown that killing is inhibited by catalase but not by superoxide dismutase or scavengers of hydroxyl radical and singlet oxygen.

Although virulent amoebae have a cytolytic effect, the interaction between *E. histolytica* and lymphocyte obtained from patients recovering from amoebic liver abscess leads, in contrast, to lysis of the amoebae. The former effect (lysis of the lymphocytes) is only observed when the lymphocytes are obtained early in the course of acute hepatic amoebiasis or from normal controls. The cytotoxic properties of lymphocytes from recovered patients may depend on lymphocytotoxins, since direct contact of lymphocytes and amoeba does not always appear to be essential for the lytic effect. Supernatant fluid from such lymphocytes stimulated with amoebic antigen has been claimed to be equally effective. However, such supernatant fluids have recently been found to inhibit amoebic protein synthesis rather than lysing amoebae when added to axenic cultures of *E. histolytica*. Careful characterization of the effector cell is necessary in this type of study, since a small number of macrophages may be responsible for the lytic effect. In a recent

study, lectin-activated lymphocytes of the phenotype were shown to kill amoebae by a contact-dependent mechanism. Study of the mechanisms involved is warranted. Antibody-dependent cell-mediated cytotoxicity (ADCC), a mechanism that has been shown to operate against other parasites, may also occur in amoebiasis as red blood cells sensitized with amoebic anti- gens are lysed by the combined action of immune serum and mononuclear cells from normal blood. In other studies, lysis of amoebae was not observed with either immune serum and complement or ADCC using live amoebae as their target cells (Denis and Chadee, 1988)

Collectively, these observations suggest that (1) invasive amoebiasis triggers a substantial immunosuppressive response in the host, affecting both the afferent and accessory functions of macrophages, that is seemingly triggered by amoebic proteins secreted in the environment, and

(2). Killing of virulent amoebae by macrophages is dependent upon sequential activation of these cells by different soluble factors, similar to the activation pattern for the acquisition of tumoricidal activity. Furthermore, it appears that resident tissue macrophages, mainly Kupffer and spleen cells, are refractory to activation for the acquisition of cytotoxic activity against *E.histolytica.*

In diabetic cases, immune cell suppresses and cell cannot work properly. Immune regulatory mechanism defective, so less production and optimum level antibody is not found in diabetic patients. *E. histolytica* infections, in diabetic patients are less than non-diabetic patients. The higher level of immune responses against *E. histolytica* and *G. lamblia* were observed in non-diabetic subject. The reason behind this increased immunity against infection. On the other hand, the comparatively prevalence in diabetic subjects are the result of diminished reactivity against infection, as the diabetic subjects are bit immuno- suppressed resulting from less activity of the immune responsive cells.

The serological diagnosis by ELISA method, the prevalence was a bit higher positive in non-diabetic people may be due to good immune reactivity in non-diabetic people in

comparison to diabetic patient. On the other hand, less immune reactivity against *E. histolytica* and *G. lamblia* in diabetic patients are evident. As diabetic are a chronic non-communicable disease, if uncontrolled which gradually intact all the vital organs of the body and untreated. It eventually involves immune system of the body that was more evident by delayed healing of a wound. The less immune reactivity against *E. histolytica* and *G. lamblia* in diabetic patient was consistent with this finding. Again, diabetic patients were more vulnerable to various types of infection as their body's immune system became weak and cannot fight against those infectious agents (Denis and Chadee, 1988).

SUMMARY

In the present investigation, out of 1300 blood and stool samples, 697 from diabetic and 603 from non-diabetic samples were collected for the identification of different protozoan parasite species. The study was undertaken to find out the prevalence of *Entamoeba histolytica* and *Giardia lamblia* among the people of diabetic and non diabetic peoples (25-70 years) in Bangladesh. This investigation was a cross sectional study with a sample size of 1300 diabetic patients and non-diabetic individuals and conducted during the period of June 2011 to July 2013. The entire study was carried out in the Department of Immunology (Immunology Laboratory) Bangladesh, Institute of Health Science (BIHS) Hospital, Mirpur, Dhaka.

Diarrhoeal diseases are one of the leading causes of mortality and morbidity worldwide especially in diabetic and non-diabetic individuals, children, travelers and immuno-compromised patients. Parasites can be protozoa having their choices of niche in human body. Most of protozoan parasites can be excreted with stool in both cyst and trophozoite stages.

Poor sanitation, illiteracy makes quite a good number of victims of diarrhea and other gastrointestinal discomforts. The first five year plan of Bangladesh (1973-78) reported that 645 of the children of the country suffered from intestinal parasitic infection. Intestinal protozoan parasite infections are amongst the most common ones in worldwide, particularly in tropical and subtropical developing countries. *Entamoeba histolytica,* which might cause the life threatening liver abscess, is estimated to cause sever disease in 48 million people and killing 70 thousand each year. Multiple infections with several different parasites like *Giardia lamblia* and amoebae are common and their harmful effects are often aggravated by coexistent malnutrition or micronutrient deficiencies.

Bangladesh is mostly a plain land and embedded with rivers and canals. The soil humidity and temperature contributes a lot towards parasitic infection. With an ever-increasing

population leading to overcrowding and unhygienic practices, these parasites pose a serious threat that is compounded by limited resources. Competency in the diagnosis and proficiency of laboratories in such peripheral setting remain questionable and may be attributed to these limited resources. Simple and cost-effective diagnostic methods may provide a solution to these difficulties.

Parasitic infestation in diabetic and non-diabetic peoples:

Protozoa are among the most important pathogens that can be cause infections in immuno-commprosied hosts. Over recent decades, parasitic protozoa have been recognized as having great potential to cause waterborne and food borne disease. The organisms of greatest concern in food production worldwide are *Entamoeba histolytica* and *Giardia lamblia*. Although other parasitic protozoa can be spread by food or water, current epidemiological evidence suggests that these two present the largest risk. Their occurrence depends on factors that include season, the age and other demographic characteristics of a population.

The use of appropriate technique plays an important role in the detection of the parasitic infections. ELISA with blood samples, formol-ether concentration technique (F-ECT) and zinc sulphate method (Z-SFM) were applied to faecal samples for the detection of prevalence of *Entamoeba histolytica* and *Giardia lamblia*.

Single and double parasitic (protozoan) infestation:

Out of 697 diabetic patients, by ELISA 15.93% and 17.65% were found infected by *E. histolytica* and *G. lamblia*; while out of 603 non-diabetic individuals 27.53% and 28.03% were infected by *E. histolytica* and *G. lamblia*; respectively.
According to the results of F-ECT on diabetic patients, 26.83% and 28.41%, on non-diabetic individuals 34.66% and 34.99% were found infected by *E. histolytica* and *G. lamblia*; respectively.

According to the results of Z-SFM, on diabetic patients 12.05% and 13.34%, on non-diabetic individuals 15.09% and 16.09% were found infected by *E. histolytica* and *G. lamblia*; respectively. A specimen was considered positive for *E. histolytica* and *G. lamblia* if either cysts or trophozoites or both were present.

In overall observation, it is evident from the present study that, formol-ether concentration technique is most suitable and sensitive method for detection of protozoan infections. According to the blood and stool samples were examined by different techniques, the prevalence of *E. histolytica* and *G. lamblia* were found higher among the peoples without diabetes than the diabetic patients.

The presence of two intestinal parasites species at a time in a single host was considered as double infection. Double parasitic infestation (8.03%) was found in diabetic patients and 13.10% was found in non-diabetic individuals.

Sex wise prevalence of *Entamoeba histolytica* and *Girdia lamblia* in blood and stool samples among diabetic and non-diabetic peoples based on different diagnostic techniques:

Total 697 diabetic blood serum samples were examined by ELISA, females were 359 and 338 were males. The prevalence of *E. histolytica* was 14.48% in females and 17.46% in males. In the case of *G. lamblia*, the prevalence was 15.88% in females and 19.53% in males. On the other hand, out of 697 diabetic stool samples, the prevalence of *E. histolytica* was 25.07% in females and 28.70% in males; and *G. lamblia* was 27.02% in females and 29.88% in males were found by F-ECT. *E. histolytica* was found 11.14% in females and 13.02% in males; and *G. lamblia* 11.70% in females and 15.09% in males by Z-SFM.

According to ELISA, total 603 non-diabetic blood serum samples were examined; the prevalence of *E. histolytica* was 25.55% in females and 29.72% in males. In the case of *G. lamblia*, the prevalence was 26.50% in females and in 29.72% males. On the other hand, out of 603 non-diabetic stool samples, the prevalence of *E. histolytica* was 32.81% in

females and 36.71% in males; and *G. lamblia* was 32.81% in females and 37.41% in males were found by F-ECT *E. histolytica* was found 14.20% in females and 16.08% in males; and *G. lamblia* 14.81% in females and 17.83% in males by Z-SFM.

In diabetic patients, the prevalence of double infections (*E. histolytica*+ *G. lamblia*) was 4.18% in females and 4.73% in males were found by ELISA; and 7.52% in females and 8.58% in male were found by F-ECT and 2.51% in females and 3.55% in males by Z-SFM.

In case of non-diabetic individuals, the prevalence of double infections (*E. histolytica*+ *G. lamblia*) was 7.57% in female and 10.14% in male was found by ELISA; and 11.67% in female and 14.69% in male were found by F-ECT and 4.73% in female and 6.64% in male by Z-SFM.

Males were (slightly) more infected than females. But three techniques show that both male and female were non significant result. By this comparative study it can be said that in almost each cases *G. lamblia* (both female and male) had slightly higher response than *E. histolytica* (both female and male). The infection rate of *E. histolytica* and *G. lamlia* are considered to be associated with the socioeconomic status of their host; because, these organism are being spread through ingested food, contaminated water or by venereal transmission. The transmission rate of *E. histolytica* and *G. lamlia* cyst also associated with their host's sewage disposal, source of drinking water and latrine structure. The prevalence of amoebiasis and giardiasis among male was higher, than that of female, as the male population are more exposed to external environment, they use public toilet, take food and water from outside and cannot maintain proper personal hygiene when they are outside of their residence.

Parasitic infestation among the diabetic and non diabetic peoples (symptomatic and asymptomatic):

By ELISA method, out of 697 diabetic blood samples (from symptomatic), 18.68% was positive and (from asymptomatic) blood samples, 12.91% was for *E. histolytica* antibody. Similarly, non-diabetic (from symptomatic), 31.45% was positive and (from asymptomatic)

blood samples, 21.21% was for *E. histolytica* antibody. Out of 697 diabetic blood samples (from symptomatic), 21.15% was positive and (from asymptomatic) blood samples, 13.8% was for *G. lamblia* antibody. Non-diabetic (from symptomatic), 31.18% was positive and (from asymptomatic) blood samples, 22.94% was for *G. lamblia* antibody.

By F-EC technique, 31.87% diabetic symptomatic types and 21.32% asymptomatic types were positive for *E. histolytica*. Similarly, 37.63% non-diabetic symptomatic types and 29.87% asymptomatic types were positive for *E. histolytica*. Among diabetic 30.49% of symptomatic types and 26.13% of asymptomatic types were positive for *G. lamblia*. Among non-diabetic individuals, 38.71% of symptomatic types and 29% of asymptomatic types were positive for *G. lamblia*.

By Z-SF method, 13.46% of symptomatic diabetic patients and 10.51% of asymptomatic types were positive for *E. histolytica*. Similarly, 19.35% of non-diabetic symptomatic types and 8.23% of asymptomatic types were positive for *E. histolytica*.
Among diabetic patients, 14.56% of symptomatic and 12.01% of asymptomatic types was positive for *G. lamblia*. Similarly, 20.16% of non-diabetic symptomatic types and 9.12% of asymptomatic types were positive for *G. lamblia*.

Age wise prevalence of *E. histolytica* and *G. lamblia* in stool and blood samples among diabetic and non-diabetic peoples based on different diagnostic techniques:

Age may be an important factor to cause parasitic infection. Ages of diabetic and non-diabetic individuals were categorized in five groups in the present study. Age of the diabetic and non-diabetic individuals, appear to be an important variable for the infestation of intestinal parasites; nevertheless, the infestation of double parasite was not highly remarkable in the present study.

Among all five age groups, among diabetic patients by ELISA, the highest prevalence of *E. histolytica* was 27.43% in the age group of 25-35 years and the lowest 7.74% in 55-65 years. In the case of *G. lamblia,* the highest prevalence was 28.66% found in the age group

of 35-45 years and the lowest 9.03% in 55-65 years. In case of double infection (diabetic) of *E. histolytica* and *G. lamblia,* the highest prevalence was 7.64% in the age of 35-45 years and the lowest prevalence was 1.29% in 55-65 years.

Considering all of the five age groups, non-diabetic individuals of age group 25-35 years was more infected (37.09%) as compared with other age groups and lowest 10.75% was found in 55-65 year group for *E. histolytica*. Similarly the highest prevalence was 37.76% found in the age group of 25-35 years and the lowest 11.83% in 55-65 years for *G. lamblia*.

In case of non-diabetic the double infection of *E. histolytica* and *G. lamblia,* highest (13.29%) in the age of 35-45 years and the lowest prevalence was 3.23% in 55-65 years. Strength of relationship or linear of relationship between age and prevalence of infection was found to be significantly related. Among diabetic patients, the prevalence of *E. histolytica* was negatively correlated with ages ($r = -0.93^{**}$, $p<0.019$) which implied that as the age increased, protozoan parasite infection tends to decrease.

Among diabetic patients, the prevalence of *G. lamblia* was found negatively correlated with ages ($r = -0.83$, $p>0.075$). Age group was not significantly associated ($p>0.075$) with infection by *G. lamblia* parasite. On the other hand, infection by *E. histolytica* was found to be highly significant and associated ($p<0.019$) with age groups. Age of the diabetic patients, double infestation was not significant: ($r = -0.74$, $p=0.152$).

In non-diabetic patients, the prevalence of *E. histolytica* was negatively correlated with age ($r = -0.963^{**}$, $p<0.008$). In *G. lamblia* cases (non-diabetic patients), the prevalence was found negatively correlated with age groups ($r = -0.843$). Age group was not significantly associated ($p>0.073$) with infection by *G. lamblia* parasite. On the other hand, infected by *E. histolytica* was found to be significantly associated ($p<0.008$) with age groups.

According to F-ECT, among all five age groups, in diabetic patients, the highest prevalence of *E. histolytica* was 38.05% in the age group of 25-35 years and the lowest 38.05% in 55-

65 years. *G. lamblia*, infection was highest (40.13%) in the age group of 35-45 years and the lowest (18.06%) in 55-65 years.
In case of double infection (diabetic), the highest prevalence was 16.56% in the age of 35-45 years and the lowest was 1.94% in 55-65 years.

Considering all of the five age groups, among non-diabetic individuals of age group 25-35 years was more infected (45.69%) as compared with other age groups and lowest infection (16.13%) was found in 55-65 year group for *E. histolytica.* Similarly the highest (non-diabetic) prevalence (44.76%) was found in the age group of 35-45 years and the lowest 17.20% in 55-65 years for *G. lamblia*. In case of double infection (non-diabetic) the highest prevalence was19.58% in the age of 35-45 years and the lowest prevalence was 4.30% in 55-65 years.

According to Z-SF, among all five age groups, in diabetic patient, the highest prevalence of *E. histolytica* was 16.81% in the age group of 25-35 years and the lowest 8.39% in 55-65 years. *G. lamblia* infection was highest (19.75%) in the age group of 35-45 years and the lowest (7.74%) in 55-65 years for *G. lamblia*. In case of double infection (diabetic), the highest prevalence was 5.73% in the age of 35-45 years and the lowest was 0.65% in 55-65 years.

Considering all of the five age groups, among non-diabetic individuals of age group 25-35 years was more infected (23.18%) as compared with other age groups and lowest infection (5.38%) was found in 55-65 year group for *E. histolytica*. Similarly the highest (non-diabetic) prevalence (22.38%) was found in the age group of 35-45 years and the lowest 7.53% in 55-65 years for *G. lamblia*. In case of double infection (non-diabetic), the highest prevalence was 9.09% in the age of 35-45 years and the lowest prevalence was 1.08% in 55-65 years.

Considering the age groups, in non-diabetic individuals, the highest prevalence of *E. histolytica* was 38.05% in the age group of 25-35 and in the case of *G. lamblia,* it was 40.13% found in the age group of 35-45 years detected by F-ECT. In comparison of three

techniques for blood and faecal samples, in case of single and double parasitic infections, the prevalence always found higher among non-diabetes than the diabetic patients.

Seasonal variation of parasitic infection among diabetic and non-diabetic individuals detected by three techniques:

The present epidemiological study showed that amoebiasis and giardiasis were present throughout the year but seasonal peak of transmission occurred in September during monsoon season and minimum incidence was observed in March in summer season.

In rainy season (2011-12), by ELISA, total 128 (diabetic) blood serum samples were examined, strong positive was 21.09% and 6.25% was weak positive for *E. histolytica* antibody. In the case of *G. lamblia* antibody, strong positive was 22.66% and 6.25% are weak positive. On the other hand, 101 (non diabetic) blood serum samples, the highest prevalence was 24.75%; and 10.89% was weak for *E. histolytica* antibody. *G. lamblia* antibody was strong positive (25.74%) and 11.88% was weak positive.

In rainy season (2012-13) by ELISA, total 93 (diabetic) blood serum samples were examined, strong positive was 20.43% and 5.38% was weak positive for *E. histolytica* antibody. In the case of *G. lamblia* antibody, strong positive was 21.51% and 6.45% are weak positive. On the other hand, 117 (non diabetic) blood serum samples, the highest prevalence was 32.47%; and 6.83% was weak for *E. histolytica* antibody. For *G. lamblia* antibody, 33.33% was strong and 5.13% was weak. In the rainy season, the present study showed that, infestation in diabetic patients and non-diabetic individuals with *E. histolytica* antibody and *G. lamblia* antibody were highly significant. During the year of the study (2012-13), the prevalence was more positive than that of the previous year (2011-12).

In summer season (2011-12), by ELISA, 117 (diabetic) blood serum samples were examined, strong positive was 7.69% and 6.84% was weak for *E. histolytica* antibody; and 7.69% and 7.69% were for *G. lamblia* antibody respectively. On the other hand, out of 86

(non diabetic) blood serum samples, 12.79%; and 9.30% were *E. histolytica* antibody; and 13.95% and 8.14% were for *G. lamblia* antibody respectively (Table-23A).
The results on diabetic patients and non-diabetic individuals with *E. histolytica* antibody and *G. lamblia* antibody were non significant. In summer season, (2012-13) by ELISA, 107 diabetic blood serum samples, strong positive was 6.54% and 9.35% was weak for *E. histolytica* antibody. In the case of *G. lamblia* antibody, strong positive was 8.41% and 10.28% was weak. On the other hand, 105 non diabetic blood serum samples, the strong positive was 20.95%; and 9.52% was weak for *E. histolytica* antibody. For *G. lamblia* antibody 23.81% was strong positive and 8.57% was weak positive. The results on diabetic patients with *E. histolytica* and *G. lamblia* were non significant; and non-diabetic patients with *E. histolytica* and *G. lamblia* were significant.

In winter season, (2011-12) by ELISA, out of 133 (diabetic) blood serum samples were examined, strong positive 4.51% and 3.01% were weak for *E. histolytica* antibody; while *G. lamblia* antibody strong positive were 4.51% and 5.26% was weak. On the other hand, 88 (non diabetic) blood serum samples, 9.09%; and 6.82% for *E. histolytica* antibody; and 9.09%; and 15.68% for *G. lamblia*; respectively. In winter season, (2012-13) by ELISA, out of 119 (diabetic) blood serum samples, 2.52% and 4.20% for *E. histolytica* antibody; and 3.36% and 4.20% for *G. lamblia* antibody; respectively. On the other hand, 106 (non diabetic) blood serum samples, 9.43%; and 8.49% for *E. histolytica*; and 10.38% and 8.49% for *G. lamblia*; respectively. The results on diabetic and non-diabetic patients with *E. histolytica* antibody and *G. lamblia* antibody were non significant.

According to F-ECT, total 128 diabetic stool samples were examined, in rainy season (2011-2012), the overall prevalence of *E. histolytica* (cyst and trophozoites) was 43.75%; 16.41% mild, 22.66% moderate and 4.69% were severely infected. On the other hand, 101 non diabetic stool samples, the overall prevalence were 45.54% for *E. histolytica*; 7.92% mild, 26.73% moderate and 10.89% were sever infection.

According to Z-SFM, the overall prevalence (diabetic) of *E. histolytica* (cyst and trophozoites) was 21.09%. The prevalence was 7.03% mild, 11.72% moderate and 2.34%

sever infection. The overall prevalence among non-diabetic individuals found 21.78%; while 8.91% mild, 9.90% moderate and 2.97% were severely infected.

In rainy season (2012-2013), according to F-ECT, total 93 diabetic stool samples were examined; the overall prevalence of *E. histolytica* (cyst and trophozoites) was 45.16%. On the other hand, 117 non diabetic stool samples, the overall prevalence were 48.72% for *E. histolytica* (cyst and trophozoites).

In case of Z-SFM, the overall prevalence (diabetic) of *E. histolytica* (cyst and trophozoites) was 17.20%. The overall prevalence (non-diabetic) was 25.64%.
In rainy season (2011-2012), according to F-ECT, total 128 diabetic stool samples were examined, the overall prevalence of *G. lamblia* (cyst and trophozoites) was 43.75%; 14.06% mild, 24.22% moderate and 5.47% were severe infection. On the other hand, 101 (non diabetic) stool samples, the overall prevalence was 46.53%; 8.91%mild, 26.73% moderate and 10.89% were severly infection. According to Z-SFM, the overall prevalence (diabetic) was 21.09% for *G. lamblia* (cyst and trophozoites); On the other hand, the overall prevalence (non-diabetic individuals) was 21.78%.

In rainy season (2011-2012), according to F-ECT, total 128 diabetic stool samples, the overall prevalence of *G. lamblia* (cyst and trophozoites) was 43.01%. On the other hand, 117 (non diabetic) stool samples, the overall prevalence was 48.72%.
According to Z-SFM, the overall prevalence (diabetic) was 23.66%. On the other hand, the overall prevalence (non-diabetic) was 26.50%. In the rainy season (2011-2013), diabetic and non-diabetic individuals with *E. histolytica* and *G. lamblia* infection were significant. During the period (2012-13), the prevalence was higher than that of the previous year (2011-2012). In different groups and different techniques, the changes of infection intensity were non significant. Prevalence of final year, the infection was slightly higher than the initial year.

The parasite of prevalence was very high in the rainy season. On the other hand, the prevalence was very low during winter season and minimum in summer season. The present epidemiological study shows that amoebiasis and giardiasis were present throughout the year but seasonal peak of transmission occurred in September during monsoon season and minimum incidence was observed in March in summer. The present study shows that in rainy season trophozoite with cyst of *E. histolytica* were 44.54% and *G. lamblia* were 44.98%. The average relative humidity 55-80% and average temperature 15-30ºC remain conductive to amoebiasis and giardiasis transmission. Our observation shows that the minimum prevalence was observed in summer season when the average maximum temperature was 36.92ºC and average minimum temperature was 24.2ºC. The maximum prevalence was observed in rainy season when the average maximum relative humidity was 68.75 and average minimum relative humidity was 47.25%, the average rainfall was 75.85 mm, average wind speed was 5.4 Km/hr. and average sunshine was 237.57 hours. It was also determined that, in the rainy season, the environment become unhygienic and polluted water usually flow over the environment. Ingestion of cysts that are transmitted primarily by the faecal-oral route with contamination of drinking water, vegetables and food, and eating of uncooked vegetables and fruits.

Sensitivity and Specificity of different diagnostic tests for the detection of *E. histolytica* and *G. lamblia:*

Sensitivity of the antigen detection or enzyme link immunosorbent assay (ELISA) and zinc sulphate floatation method (Z-SFM) determined by the formol-ether concentration technique (F-ECT). Z-SFM sensitivity and specificity also measured with the comparison of ELISA. Sensitivity and specificity measure in different type of samples. In diabetic samples ELISA was 46.52% sensitive and 95.29% specific and Z-SFM showed sensitivity 23.53% and 92.16% specificity. Z-SFM is less sensitive then ELISA when compared to F-ECT. Among the non-diabetic sample highest sensitivity (58.37%) and specificity (88.83%) found in ELISA.

It revealed that, F-ECT is most suitable method for detection of single and as well as double protozoan infections. The ELISA technique is the best of all techniques, which represents the present infection as well as the recent infection of amebiasis and giardiasis. The F-ECT is recommended as the best technique to get the concentration of the parasites present in faeces. Z-SFM used to diagnose infection with *E. histolytica* and *G. lamblia*, which is a quick and comparatively simpler examination and gives a precise and reliable diagnosis. A person can easily detect parasitic infection by using Z-SFM without centrifuging.

CONCLUSION

1. The present study revealed that a sizeable portion of the diabetic and non-diabetic patients of the country are infected with protozoan parasites. Among the two protozoan parasites patients which decrease with the increase of age due to better immune response of the people.

2. The finding of the present study clearly indicates that the test results (test sensitivity), the prevalence of investigation in non-diabetic people was significantly higher (69.65%) than the diabetic patients (55.24%) which reaffirms the fact that less immune reactivity against *E. histolytica and G. lamblia* in diabetic patient was consistent with the present findings.

3. Single parasitic infections were found highly prevalent compared to the double parasitic infections among diabetic and non-diabetic patients which led higher incidence of single parasites among the double parasites.

4. In the light of present study, it may be concluded that the people of our country have high prevalence with gastrointestinal protozoan parasitic infections. Compared with current studies and earlier studies it was found that the incidence of parasitic infection was relatively lower in urban areas than rural areas. Low socio-economic condition, poor sanitary practices, poverty, and most important is lack of health education may be related to the wide prevalence of intestinal protozoan parasitic infection in our country.

5. In current study sample size was taken by random sampling procedure. Total 1300 cases were observed and it was pointed that males were more infected than females, but it was not statistically significant. The prevalence of infection was highest in month of May and was lowest in January. In the remaining month the prevalence fluctuated from moderate to low. So, climate and season was the important for investigating an infectious disease. E.g. amoebiasis and giardiasis have a high prevalence during summer and during flood.

6. In conclusion, all three tests (ELISA, formal-ether concentration and zinc sulphate floatation techniques) studied proved to be reliable diagnostic tools. Both formol-ether concentration and zinc sulphate floatation techniques are rapid and easy to use with a high sensitivity and specificity, and both can easily be applied in routine clinical laboratories. The ELISA technique is the best of all techniques, which represents the present infection as well as the recent infection of amebiasis and giardiasis. Improvement of sensitivity can be obtained by combining the tests.

7. The ELISA is not as rapid as the other two tests but may provide an excellent tool for follow-up of treated patients, since quantitative results are obtained. Moreover, only a small amount amoebic antigen is required for the ELISA compared to the F-ECT and Z-SFM. F-ECT showed high prevalence of *Entamoeba* spp., which may be *E. histolytica* or *E. dispar* in total population. Furthermore, the *E. histolytica* –specific ELISA was shown to be a sensitive and specific method for the rapid detection of the *E. histolytica*.

8. In total populations there were the present of *E. dispar* (non-pathogenic amoeba)/ *E. histolytica* complex infection. These results indicate that the presence of either four-nucleus amoeba or trophozoites in the stool of a patient with diarrhea is not equal to amoebiasis (the presence of the pathogenic *E. histolytica*). This is important when one considers the currently available treatments, some of which have undesirable side effects. So, accurate identification of *E. histolytica* is required to treat an infected patient. In this case ELISA procedure is a reliable diagnostic technique for the detection of *E. histolytica* in total *Entamoeba* infection.

9. In the present study, three different techniques was used for detection of intestinal protozoan parasites and it was observed that ELISA has high detection rate compared to other two techniques but ELISA is not able to identified the protozoan trophozoite and cyst. So, the need for rapid, inexpensive methods to diagnose amoebiasis and giardiasis have led to the recent development of enzyme-linked immunosorbent assay (ELISA) for the detection of *E. histolytica and G. lamblia* associated antigens in blood. Microscopic stool

examination was first carried out by wet mount smear and then by formal-ether concentration technique and zinc sulphate floatation technique. The F-ECT is recommended as the best technique to get the concentration of the parasites present in faeces. Z-SFM used to diagnose infection with *E. histolytica and G. lamblia*, which is a quick and comparatively simpler examination and gives a precise and reliable diagnosis.

RECOMMENDATION

The following recommendations are being made in the light of the present study to control parasitic infestation, prevention of anaemia, improvement of nutritional status and socioeconomic conditions of diabetic and non-diabetic patients:

To prevent and control the parasitic infestation:

- The intestinal parasitic infestations are partly due to the ignorance and unhygienic condition of the habit and habitat of the diabetic and non-diabetic patients. They should be educated and be made aware about the causes and transmission of the intestinal parasites.
- The diabetic and non-diabetic patients as well as their family members should be motivated to involve themselves in the health care activities and for practicing personal hygiene such as washing hands before taking meals and after defaecation, nail clipping, regular use of footwear etc.

Behavior change through communication:

BCC is a multi-level tool for promoting and sustaining the desired behavior in individuals and communities by using a variety of communication channels and creating demand for information and service.

The media can influence through food advertisements. Mass media can encourage new behaviors. Multimedia approaches that combine face-to-face and mass media are appropriate for nutrition education.

Food advertising is also a pervasive and influential aspect of media. Advertisements for so called ‘junk foods’ and non-nutritious fast foods should be regulated. Children and diabetic and non-diabetic patients have to be taught to recognize the purpose of

advertising strategies such as those providing important information and those solely promoting a particular brand. Children and diabetic and non-diabetic patients also need to learn to recognize that some advertising may not be supportive of good nutrition.

In addition to government agencies, other sectors such as industry and trade, consumer organizations, insurance companies, sports organizations etc. can utilizes the mass media.

Control of micronutrient deficiency:

- Improve of consumption of micronutrient-rich foods through home gardening or provision of food through community kitchens or poultry farming.
- Providing iron and folic supplements to diabetic and non-diabetic patients through the community outreach programmed.
- Fortification of food with micronutrients, which may be the cost effective approach in overcoming the micronutrient deficiencies, especially among the economically deprived population.

Compared the different techniques:

Both three techniques, used to diagnose infection with *E. histolytica and G. lamblia* in the present study have advantages and disadvantages. The assay appeared to be useful as an epidemiologic tool. But it is unlikely to replace microscopic examination of stool for trophozoites and cysts as a routine diagnostic test, however because other potential pathogens would escape detection. The present study suggests that the combination of microscopy and ELISA could prove to be a useful means for diagnosis of *E. histolytica and G. lamblia* infection.

Legal sector:

- Ban on sale of non-iodized salt.
- Rights-based approach to provision of health services to diabetic and non-diabetic patients.

LITERATURE CITED

Abd-Alla, M. D. Jackson, T. F., Reddy, S. and Ravdin, J. I. 2000. Diagnosis of invasive amebiasis by enzyme-linked immunosorbent assay of saliva to detect amebic lectin antigen and anti-lectin immunoglobulin G antibodies. *J. Clin. Microbiol.* **38**: 2344- 2347.

Abd-Alla, M. D. Jackson, T. G. and Ravdin, J. I. 1998. Serum IgM antibody response to the galactose-inhibitable adherence lectin of *Entamoeba histolytica*. *Am. J. Trop. Me. Hyg.* **59:** 431-434.

Abd-Alla, M. D. Ahmed, M. and Ravdin J. I. 1992. Use of Enzyme- linked Immunosorband Assay to detect anti-adherance protein antibodies in sera of patient with invasive amoebiasis in Cairo, Egypt. *Am. J. Trop. Med. Hyg.* **47**: 800-804.

Adamu, H., Endeshaw, T., Kifle, A. and Petros, B. 2006. The prevalence of intestinal parasites in paediatric diarrhoeal and non- diarrhoeal patient in Addisabab spiral with special emphasis on opportunistic parasitic infection and with insight into the demographic and Socio-economic factors. *Ethio. J. Health*. **20**(1): 39-46.

Agundis-Mata, C., Blanco, F., Toledo, J., Reta, F., Kumate, J. and Isibasi, A. 1996. identification of a 35 KDa glycoprotein from *Entamoeba histolytica* by sera from patients with amoebic liver abscess and with mouse monoclonal antibody. *Immunol. Investig.* **25**: 519**-**529**.**

Alam, S., Khanum, H., Frhana, R. and Haque, R. 2013. Prevalence of different protozoan parasites in patients visiting at ICDDR'B Hospital, Dhaka. *J. Asiat. Soc. Bangladesh, Sci.* **39**(1): 117-123.

Alaofe, H., Zee, J., Dossa, R. and O'Brien H.T. 2008. Intestinal parasitic infections in non-diabetic adolescent girls from two boarding schools in southern Benin. *Trans. R. Soc. Trop. Med. Hyg.* **102**(7): 653-661.

Ali- Shtayeh, M.S., Hamdan, A.H.Y., Shaheen, S.F., Abu-Zeid, I. and Faidy, Y.R. 1989. Prevalence and seasonal fluctuations of intestinal parasitic infections in the Nablus area, West Bank of Jordan. *Ann. Trop. Med. Parasitology*. **83**(1): 67-72.

Ali, R., Perez, M. J. and Martin, E. 2003. Evaluation of 2 immunologic techniques (ELISA, DIF) for the diagnosis of *Giardia lamblia* in faeces. Enferni. *Infece. Microhiol. Clin*. **12**(7):337-340.

Allison, M. C., Green, E. L, Bhattacharya, D. N., Smith, A. and Pounder, R. E. 1988. A microscopic and immunodiagnostic search for giardiasis in patients with gastrointestinal disorders. *Scand. J. Gastroenterol*. **23:** 209-212.

Al- Madani, A.A. and Mahfouze, R.M. 1995. Prevalence of intestinal parasitic infectionsn among Asian female housekeepers in Abha District, Saudi Arabia. *Southeast Asian J. Trop. Med. Public health*. **26**(1): 135-137.

Al-Tukhi, M. H., Ackers, J. P., Al-Ahdal, M. N. Tana, M. A., and Peters, W. 1993. ELISA for detection of *anti-Giardia* specific Ig M: response in serum. *J. Trop. Med. Hyg*. **96**(6): 333-336.

Amin, O. M. 2002. Seasonal prevalence of intestinal parasites in the United States during 2000. *Am. J. Trop. Med. Hyg.* **66:** 799-803.

Aristizabal, H., Acevedo, J. and Botero, M. 1991. Fulminat amebic colitis. *World. J. Surg.* **15:** 216-221.

Attia, R. A., Rik, H. and motawea, S. 1995. Impotance of *Entamoeba histolytica* antigen in serodiagnosis and follow up of patients with amoebic liver abscess. *J. Egypt. Soc. Parasitol*. **25:** 485-490.

Avron, B., Bracha, R., Deutsch, M. R. and Mirelman, D. 1983. *E. invadens* and *Entamoeba histolytica* separation and purification of precysts and cysts by centrifugation on discontinuous density gradients of Percoll. *Experimental parasitology.* **55:** 265-269.

Azian, N.M.Y., San, Y.M., Gan, C.C., Yusri, M.Y., Nurulssyamzawaty, Y., Zuhaizam, A.H., Maslawati, M.N., Norparina, I. and Vythilingam, I. 2007. Prevalence of intestinal protozoa in an aborigine community in Pahang, Malaysia. *Trop. Biomedicine.* **24**(1): 55-62.

Bagan, S. J. V., Gisbert, S. C. and Milian M. A. 1988. Pathology of the oral mucosa in patients with type I diabetes mellitus: study of 44 cases. *Med. Cutan. Ibero. Lat. Am.* **16:** 419-421.

Banu, H., Khanum, H. and hossain, A. 2011. Parasitic infestation among the adolescent girls of Bangladesh. *Bang. J. Zool*. **16:** 419-421.

Banu, H., D'silva, J. and Islam, N. 2003. Epidemiological factors and parasite infection in children. *Bangladesh J. Zool.* **31**(2): 243-246.

Bapat, M. M. and Bhave, G.G. 1990. Counter immunoelectrophoresis in the immunodiagnosis of amoebiasis and giardiasis. *J. Postgrad. Med.* **36**: 124-127.

Barua, P., Khanum, H., Haque, R., Arju, T. and Uddin, M. S. 2014. Comparing direct microscopy and in-vitro culture for diagnosis of *Blastocystis homonis* infection among the infants of a diarrheal-affluent area of Mirpur, Dhaka, Bangladesh.

Bartholomew, G. A., Rodu, B. and Bell, D. S. 1987. Oral candidiasis in patients with diabetes mellitus: a thorogh analysis, *Diabetes. Care.* **10**: 607-612.

Belding, D. L. 1995. Textbook of clinical parasitology, 4th ed. Appleton- Century-Crofts, Inc., New York, N. Y.

Black, R.E., Merson, M. H., Rahman, A. S. M. M., Yunus, M., Alim, A. R. M. A., and Curlin, G.T. 1980. A two year study of Bacterial, Viral and Parasitic agents associated with diarrhea in rural Bangladesh. *J. Infect. Dis.* **142**(5): 660-664.

Blessmann, J., Ali, I. K. M., NU, P, A. T., Dinh, B. T., Viet, T. Q. N., Van, A. L., Clark, C. G. and Tannich, E. 2003. Longitudinal Study of Intestinal *Entamoeba Histolytica.* Infections in Asymptomatic Adult Carriers. *J. Clin. Microbiol.* **41**: 4745-4750.

Bordley, L. K. 2003. Intestinal parasitic infections, anaemia, and under nutrition among non-diabetic adolescents of Madhya Prodesh. *Indian Council of medical Research*. **28**(1): 21-29.

Boreham, J., I. K. Ali, P. A. NU, B. T. Dinh, T. Q. Viet, A.L. Van, C. G. Clark and E. Tannich. 1981. Longitudinal study of intestinal *Entamoeba histolytica* and *Giardia lamblia* infection in asymptomatic adult Carriers. *J. Clin. Microbial*. **41**: 4745-4750.

Braga, L. L., Mendonca, Y., Paiva, C. A., Sales, A., Cavalcante, A. L. M. and Mann, B. J 1998. Seropositivity for and Intestinal Colonization with *Entamoeba histolytica* and *Entamoeba dispar* in Individuals in Northeastern Brazil. *J. Clin. Microbiol.* **36**: 3044-3045.

Brooks G. F., Butel, J. S. and Morse S. A. 1998. Viruses & Parasites. In Jawetz, Melnic and Adelberg's Medical Microbiology. 21st ed. Connecticut, USA. *Appleton* and *Lange* Pp507-527.

Bruchard, G. D. and Mirelman, D. 1991. Characterization of *Entamoeba histolytica* strains isolated from patients with HIV infection. *Infection.* **19:** 164-169.

Bruckner *et al.,* 1997. A single-step immuno-chromatographic test for the detection of *Entamoeba histolytica* antigen in blood samples. *J. Immunol. Methods.* **196**: 193-198.

Bruckner, D. A. 1992. Amebiasis. *Clin. Microbial Rev.* **5**: 356-369.

Caballero-Salcedo, A., Viveros-Rogel, M., Salvatiaerra, B., Tapia-Conyer, R., Sepulveda-Amor, J., Gutierrez, G. and Ortiz-Ortiz, L. 1994.

Carison, J. R., Sullivan, P. S., Harry, D. J., Stork, M. A., Thorton, S. A. and Dupont, H. L., 1988. Enzyme immunoassay for the detection of *Giardia lamblia. Eur. J. Clin Microbiol. Infect. Dis.* **7**: 538-540.

Cates, J. L., Finestone, A. and Bogash, M. 1973. Phimosis and diabetes mellitus. *J. Urol*. **110**: 406-407.

Chatterjee, K.D. 2004. Parasitology (Protozoology and Helminthology) in relation to clinical medicine. Chatterjee Medical Publisher, Calcutta. Pp 238.

Cheesbrough, M. 1987. Medical Laboratory Manual for Tropical Countries. Blackworth Co Publishers. Pp 570.

Cheesbrough, M. 2005. District Laboratory Practice in Tropical countries, Part 2. (Cambridge Low Price Editions) Cambridge University Press. Pp 299-309.

Chowdhury, M.R. 1981. Intestinal parasite infection in non-diabetic individual of Dhaka population, Bangladesh. *Armed Forces Med. J.* **4** (1): 5-12.

Chowdhury, O. P., Das, D., Munoz, M. L. and Das, P., 1996**.** Biochemical and Immunological characterization of soluble antigens of *Giardia lamblia*. Parasiotology research. **83**(6): 604-610.

Chowdhury, G., Prakash, V., Kumar, A., Shahi, S. K. and Sharma, M. 1991. Protective immunity to *Entamoeba histolytica* infection in subjects with antiamoebic antibodies residing in a hyperendemic zone. *Scand. J. Infect. Dis.* **23**: 771-776.

Chowdhury, M.R. 1999. Immunity. In: Modern Medical Microbiology (5th ed.) Dhaka, Bangladesh. Pp 97-127.

Clark. C. G. and Diamond, L.S. 1993. *Entamoeba histolytica*: a method for isolate identification. *Exp. Parasitol*. **77:** 450-455.

Clark. C. G. and Diamond, L.S. 1991. Ribosomal RNA genes of pathogenic and non pathogenic *Entamoeba histolytica* are distinct. *Mol. Biochem. Parasitol*. **49**: 297-302.

Clark. C. G., Cantellano, M. E. and Bhattacharya, A. 2000. *Entamoeba histolytica*: an overview of biology of the organism. (*In J. I.* Ravdin (ed.), Amebiasis). Imperial College press, London, UK. Pp 1-45.

Craig, A.H. Tsai, L.M. Seroepidemiology of amebiasis in Mexico.1970. *Am. J. Trop. Med. Hyg*. **50**: 4129.

Crompton, D.W.T. and Savioli, L. 1993. Intestinal parasitic infection and urbanization. WHO Bull. **71**: 1-7.

Cummins, A. J., Moody, A. H., Lalloo, K. and Chiodini, P. L. 1994. Rapid latex agglutination test for extraluminal amoebiasis. *J. Clin. Pathol.* **47**: 647-648.

Danciger, P., and Lopez, V.K., 1975. Detection of specific anti- *Giardia* antibodies in the serodiagnosis of symptomatic giardiasis. *J. Diarrhoeal. Dis Res*. **10**(3):151-155.

Das, A.R. 1990. Detection and diagnosis of protozoan infection by different techniques in a slum area of Dhaka city. D.C.M. Dissertation, NIPSOM, Dhaka. Pp 60.

David, L. and Belding. 1992. Text book of Clinical parasitology. Second edition. Pp101-105.

Dellalioglu, N.,Aslan, G., Sozen, M., Babur, C., Kanik,, A. and Emekdas, G. 2004. Detection of *Entamoeba histolytica* /*Entamoeba dispar* in stool specimens by using enzyme-linked immunosobent assay. *Mem Inst Oswaldo Cruz.* **99:** 769-772.

Denis, M. and Chadee, K. 1988. Immunopathology of *Entamoeba histolytica* infections. Institute of Parasitology of McGill University, Canada. Pp 247-252.

Diamond, L.S. Diamond, L.S. 1961. Amoebiasis: Nutritional implications. *Rev. Infact. Dis.* **4**: 843-850.

Diamond, L.S., Harlow, D. R., Philips, B. P., *et al.,* 1979. *Entamoeba histolytica*. Arch. Invest. *Med. (Mex.).* **9** (Suppl. 1): 329.

Diamond, L.S. and Clark, C.G. 1993. A redescription of *Entamoeba histolytica* Schaudinn 1903 (Emended Walker 1911) separating it from *Entamoeba dispar* (Brumpt 1925). J. Eukaryot. *Microbiol.* **40**: 340-344.

Dryden, M. W., Payne, P. A. and Smith, V. 2005. Comparison of common fecal floatation techniques for the recovery of parasite eggs, cysts and oocysts. *Vet Ther* **6**: 15-28.

D'Silva, J., Banu, H. and Islam, n. 2003. Protozoan infection in children and their personal habits. *Dhaka Univ. J. Biol. Sci.* **12**(2): 193-198.

Dutt, P., Mehta, S. and Vinayak, V. K. 1958. Enzyme-Linked immunosorbent assay for copro-diagnosis of giardiasis and characterissation of a specific *Giardia lamblia* antigen in stools. *J. Med. Microbiol*. **34**(5): 271-275.

Feleke, M. 2010. Comparision of formal-acetone concentration method with that of the direct iodine preparation and formol-ether concentration methods for examination of stool parasites. *Ethiop. J. Health Dev.* **24**(2): 148-151.

Felnger, P. 1977. Serological diagnosis of extraintestinal amoebiasis. A comparision of stool ELISA and other immunological tests. *Tropical parasitol.* **28**: 485-491.

Fotedar, R., Stark, D., Beebe, N., Marriott, D., Ellis, J. and Harkness, J. 2007. Laboratory diagnostic techniques for *Entamoeba* species. *Clinical Microbiology*. **20**(3): 511-532.

Furness, B. W., Beach, M. J. and Roberts, J. M. 2000. Giardiasis surveillance-United States, 1992-1997. *Morbidity and Mortality Weekly Report, CDC Surveillance, Summer 2000,* **49**(7): 1-13.

Gally, J. A. 1973. Structure of Immunoglobulins. In Antigens. Sela, M., ed. Vol 1. York: Academic press. Pp162.

Garcia, L. S. Bruckner, D. A. 1997. Diagnostic medical parasitology. 3rd ed. A. S. M. Press, Washington, D. C.

Gardner, B. B. 1980. Comparision of direct wet mount and trichome staining techniques for detecting *Entamoeba* species trophozoites in stools. *Journal of clinical microbiology*. **12**(5): 656-658.

Gibb, D. and Brown. L. 1995. Vaginal symptoms and insulin dependent diabetes mellitus. *N. Z. Med. J.* **108:** 252-253.

Gilman, R. H. Chong, Y. H., Davis, C., Greenberg, B., Virik, H.K. and Dixon, H. B. 1985. The adverse consequences of heavy *Entamoeba* infection. *Trans. R. Soc. Trop. Med. Hyg.* **77**: 432-8.

Gatti, S., Cevini, C., Marchi, L., Novati, S. and Scaglia, M. 1995. *Entamoeba histolytica* autochthonous isolates from mentally retarded Italian patients. *Parasitol Res.* **81**: 148-151.

Gatti, S., Mahdi, R., Bruno, A., Cevini, C. and Scaglia, M. 1998. A survey of amoebic infection in the Wonji area of central Ethiopia. *Ann Trop Med Parasitol.* **92**: 173-179.

Gatti, S., Swierczynski, G., Robinson, F., Anselmi, M., Corrales, J., Moreira, J., Momtalvo, g., Bruno, A., Maserati, R., Bisoffi, Z. and Scaglia, M. 2002. Amebic infections due to the *Entamoeba histolytica* - *Entamoeba dispar* complex a study of the incidence in a remote rural area of Ecuador. *Am. J. Trop. Med. Hyg.* **67**: 123-127.

Ghosh, P. K., Gupta, S., Leon, L. R., Ghosh, R., Ruiz Ordaz, B. H. and Ortizortiz, L. 1998. Intestinal amoebiasis: antibody- secreting cells and humoral antibodies. *J. Diarrhoeal Dis. Res.* **16**: 1-7.

Goldin, A. J., Hall, A., Sarker, R. N., Warhurst, D. C. and Miles, M. A. 1993. Diagnosis of *Giardia lamblia* infection in Bangladeshi infants : faecal antigen capture ELISA. *Trans. R. Soc. Trop. Med. Hyg.* **87**: 428-432.

Goncalvesa, l. M. C., Dasilva, V. L., De-andradea, C. M., Reinhardb, K., Darochaa, G. C., Lebailly, M., Bouchetc, F., ferreiraa, L. F. and araujoa A. 2004. Amoebiasis distribution in the past: first steps using an immunoassay technique. *Transactions of the royal Society of Tropical Medicine and Hygiene.* **98**: 88-91.

Gonin, P. and Trudel, L. 2003. Detection and Differentiation of *Entamoeba histolytica* and *Entamoeba dispar* Isolates in Clinical Samples by PCR and Enzyme-Linked Immunosorbent Assay. *J. Clin. Microbiol.* **41**: 237-241.

Gonzalez-Rulz, A., Haque, R., Rahman, T., Aguire, A., Jaramillo, C., Castanon, G., Hall, A, Guhl, F., 1994. A Monoclonal antibody for distinction of invasive and non invasive clinical isolates of *Entamoeba histolytica*. *J. Clin. Microbil*. **30**: 2807-2813.

Green E. L., Allison, M. C., Bhattacharya, D. N., Smith, A. and Pounder, R. E. 1988. A microscopic and immunodiagnostic search for giardiasis in patients with gastrointestinal disorders. Scand. gastroenterol **23**: 209-212.

Hall, A., Anwar, K.S., Tomkins, A.M. and Rahman, L. 1999. The distribution of protozoan parasites in hosts: a study of 1765 people in Bangladesh. *Trans. R. Soc. Trop. Med. Hyg.* **93**: 505-510.

Haque, N. and Sheikh, A 1976. Incidence of intestinal parasite in children of different socio-economic population of Dhaka City. *Bang. Med. Res. Council*. *Bull.* **11** (11): 1-7.

Haque, R., Neville, L. M.., Wood, S., and Pertri, W. A. J. R..1994. Short report: detection of of *Entamoeba histolytica* and *E. dispar* directly in stool. *Am. J. Trop. Med. Hyg*. **50:** 595-596.

Haque, R., Ali, R. B. Sack. B. M. Farr, G. Ramakrishnan W. A. Petri, J. R. 2001. Amebiasis and mucosal IgA antibody against the *Entamoeba histolytica* adherence lectin in Bangladeshi children. *J. Infect. Dis.* **183:** 1787-1793.

Haque, R., Ali, I. K. M., Akhter, S. and Pertri- Jr., W. A. 1998. Comparison of PCR, isoenzyme analysis, and antigen detection for diagnosis of *Entamoeba histolytica* infection. *J. Clin. Microbiol.* **36:** 449-452.

Haque, R., Mondal, D., Kabir, M., Roy, S. and William, A.P. 1993. Diagnosis of pathogenic *Entamoeba histolytica* infection using a stool ELISA based on monoclonal antibodies to te galactose-spacific adhesion. *J. Infect. Dis*. 167-249.

Haque, R., Mondal, D. B., Akther, S., Barry, M. F., Sackr. B. and Petri, WS. A. 2003. Epidemiology and clinical characteristics of acute diarrhea with emphasis on *Entamoeba histolytica* in infections Preschool Children in an urban slum of Dhaka, Bangladesh. Am. J. Trop. Med. Hyg., **69(4):**398-405.

Haque, R., Mondal, D., Kabir, M., Roy, S. and Petri W. A. Jr. 2006. *Entamoeba histolytica* infection in children and protection from subsequent amebiasis. *Infect Immun*. 74: 904-909.

Haque, R., Roy, S. Siddique, A. Mondal, U., Rahamn, S. M., Mondal, D and Petri W. A. Jr. 2007. Multiplex real-time PCR assay for detection of *Entamoeba histolytica*, *Giardia intestinalis* and *Cryptosporidiumspp. Am. J. Trop. Med. Hyg.* **76**(4): 713-717.

Haque. R. A. S. Faruque, P. Hahn, D. M. Lyerly, and W. A. Petri, J. R. 1997. *Entamoeba histolytica* and *Entamoeba dispar* infection in children in Bangladesh. *J. Infect.Dis.* **175:** 734-736.

Haque, R., Nevilie, L. M., Hahn, P. Petri, W. A., J. R. 1995. Rapid diagnosis of *Entamoeba* infection by using *Entamoeba* and *Entamoeba hitolytica* stool antigen detection Kits. *J. Clin. Microbiol*. **33:** 2558-61.

Hassan, G.M., Monwar, G.M., Saha, S. and Begum, A. 1995. Impact of sanitation and health education on intestinal parasite infection among primary school aged children of Sherpur, Bangladesh. *Trop. Doct.* **33(3):** 139-143.

Hautaner, A., and Rezaeian, M. 1978. Detection of serum an enzyme-linked immunosorbent assay. *Parasitol. Res.* **97**: 209-212.

Hossain, M. M., Ljungstrom, I., Glass, R. I., Lundin, L., Stollan, B. J. and Huldt, G. 1983. Amoebiasis and giardiasis in Bangladesh: Parasitological and Serological studies. *Trans. R. Soc. Trop. Med. Hyg.* **77(4):** 552-554.

Huda, C. G. Chappell, C. L, 1976. *Entamoeba* antigen and *Giardia* antigen detection in non-diabetic patients with chronic gastrointestinal disturbances. *J. Fam. Pract*. **35**(1): 49-53.

Hung, C. C., Chen, M. Y. 1999. Invasive amoebiasis: an emerging parasitic disease in patients infected with HIV in an area endemic for amoebic infection. *AIDS.* **13:** 2421-2428.

Hussain, M.M., Ali, M.K. and Rashid, M.A. 2003. Aetiology of anaemia in Bangladesh. Institute of Epidemiology Disease Control and Research (IEDCR). *Yearly Health Situation Rep.* **28**: 61-62.

Huston, C. D. 2004. Parasite and host contributions to the pathogenesis of amebic colitis. *Trends in Parasitology.* **20**: 23.

Hyder, S.M.Z., Chowdhury, S.A. and Chowdhury, A.M.R. 1998. Prevalence of anaemia and intestinal parasites in a rural community of Bangladesh Research and Evaluation Division BRAC. Res. Monogr. Ser. No. 12.

Islam, A.F.M., Sadeque, M., Biswas, K., Ahmed, N. Mahmood, G. 1975. Intestinal parasite and anaemia in student of Dhaka University. *Bang. Med. Res. Council. Bull.* **7**(1): 40-45.

Islam, A., Stoll, B. J., Ljungstrom, I., Biswas, J., Nazrul., H. and Huldt, G. 1983. *G. lamblia* infections in a cohort of Bangladeshi mothers and infants followed for one year. *J. Pediatr.* **103**: 996-1000.

Isaac- Renton, J.L. 1991. Laboratory diagnosis of giardiasis. *Clin.Lab. Med.* **11**(4): 811-827.

Jackson, T. F. H. G. 2000. Epidemiology (In ed. J. I. Ravdin, Amebiasis). Imperial College Press, London, United Kingdom. Pp 47-63.

Jacson, T. F., Gathiram, V. and Simjee, A. E. 1985. Seroepidemiological study of antibody responses to the zymodemes of *Entamoeba histolytica. Lancet.* **1**: 716-719.

Jacson, T. F., Visser, P. S. and Anderson, C. B. 1990. *Entamoeba histolytica*: naturally occurring infections in baboons. *Arch. Investing. Med.* **1**: 153-156.

Jacobsen, K.H., Ribeiro, P.S., Quist, B.K. and Rydbeck, B.V. 2007. Prevalence of intestinal parasites in non- diabetic young Quichua Children in the Highlands of Rural Ecuador. *J. Health Popul. Nutr*. **25(4):** 24-32.

Jahan, W. and Goldin, A.J., 1987. A capture Elisa detects *Giardia lamblia* antigens in formalin-treate faecal samples. *Trans. R. Soc. Trop.Med.Hyg*. **86**:164-165.

Janoff, E. N., Carft, J. C., Pickering, L. K., Novotny, T., Blaser, M. J., Knisley, C. V. and Reller, L. B. 1989. Diagnosis of *Giardia lamblia* infections by detection of parasite specific antigens. *J. Clin. Microbiol.* **27**(3): 431-435.

Joel klein, H., Flores, R. and Wen. J**.** 2011.Gene duplication in the genome of Parasitic *Giardia lamblia*. BMC Evol Biol. **10**:49.

John, P. A. and Mirelman, D. 2006. Progress in research on *Entamoeba histolytica* pathogenesis Department of biological chemistry weizmen institute of Science, Rehovot 76100, Israel.

Kang, G., Mathew, M.S., Rajan, D. P., Daniel. J. D., Mathan, M. M. Mathan, V. I and Muliyil, J. P. 1998. Prevalence of intestinal parasites in rural Southern Indians. *Trop Med. Int. Hlth*. **3:**70-75.

Kappus, K. D. 1934. Intestinal parasitism in the United States on a continuing problem. *Am. J. Trop. Med. Hyg.* **50**(6): 705-13.

Karanis, P., Kourenti, C. and Smith, H., 2007. Waterborne transmission of protozoan parasites: A worldwide review of outbreaks and lessons learnt. *Journal of water Health* **5(1):** 1-38.

Kaur, H. and Sween. 2007. Identification of protozoan parasites by Staining Techniques. *Dhaka univ. J. Biol. Sci.* Pp 01-09.

Kean, B. H. 1988. A history of amebiasis (*Tn J.* I. Ravdin (ed.), Amebiasis: human infection by *E. histolytica*). John Wiley & Sons, Inc., New York, N. Y. 1-10 Pp.

Kebede, A., Verweij, J. J. and Polderman, A. M. 2004a. The use of real-time PCR to identify *Entamoeba histolytica* and *Entamoeba dispar* non-diabetic people and primary-school children in Ethiopia. *Trop. Med. Parasitol Trop. Med. Parasitol.* **98:** 43-48.

Kebede, A., Verweij, J. J. and Polderman, A. M. 2004b. short communication: Misleading microscopy in amoebiasis. *Trop. Med. Parasitol Trop. Med. Parasitol.* **9**: 651-652.

Kelsall, B. L., Jackson, T. G., Gathiram, V., Pearson, R.D. and Ravdin, J. I. 1994. Secretory immunoglobulin A antibodies to the galactose-inhibitable adherence protein in the saliva of patients with amebic liver disese. *Am. J. Trop Med. Hyg.* **51**: 454-459.

Khan, A. A.1994. Amoebiasis and giardiasis problem in Bangladesh overview and control personal community. Pp 54-60.

Khanum, H., Rahman, F. and Farhana, R. 2013. Occurrence of intestinal parasites among the teachers, students and staffs of Dhaka University. *J. Asiat. Soc. Bangladesh, Sci.* **39(2):** 239-246.

Khanum, H., Chowdhury, S. and Bhuiyan, Z. J. 1999. Infestation of three intestinal worms in children of three selected areas, Bangladesh. *Pakistan J. of Zool*. **31(4):** 391-396.

Khanum, H., Ahmed, S., Uddin, M.H., Rahman, A.B.M.M., Dey, R.R. and Farhana, 2008. Prevalence of intestinal parasites and anaemia among the slum male children in Dhaka city. *Dhaka Univ. j. Biol. Sci*. **17**(2): 137-145.

Khanum, H., Ahmed, S., Uddin, M.H., Rahman, A.B.M.M., Dey, R.R. and Farhana, 2005. Prevalence of intestinal parasites and anaemia among the slum Female children in Dhaka city. *Dhaka Univ. j. Biol. Sci*. **7**(3): 130-135.

Khanum, H., Ahmed, S., Uddin, M.H., Rahman, A.B.M.M., Dey, R.R. and Farhana, 1998. Prevalence of intestinal parasites and anaemia among the slum female and male non-diabetic individuals in Dhaka city. *Dhaka Univ. J. Biol. Sci*. **1**(2): 17-25.

Khanum, H. 2010. Intestinal parasitic infestation among the outdoor non-diabetic patients of Dhaka University Medical Centre, Bangladesh. *Asian Pacific Journal of Tropical Medicine.* Pp 745-747.

Khanum, H., Musa, S., Ferdousi, Z. and Arefin, N. 2000. Observation on the prevalence of geohelminth ova in green leaves/vegetables from different markets of Dhaka city. *Bangladesh J. Nutr*. **13:** 69-73.

Khanum, H. Haque, R. and Badruzzahan, K. A. 2001. Prevalence of *Entamoeba histolytica* in patients and serodiagnosis of invasive amoebiasis, Bangladesh. *J. Zool.* **28**(1): 63-68.

Khanum, H., Shanjida, S. Uddin M. H. and Islam, M. S., 2012. Protozoan parasites in a wastewater treatment plant of Bangladesh. *J. Zool.* **31:** Pp 05-08.

Khatun, T. 2000. Observation on occurrence of intestinal protozoa and helminth parasites in human in Dhaka city of Bangladesh. Pp 24-30.

Klein, J. 2011. The Numerous Foundation. Kids Health.

Knisley, C. V., Engelkirk, P. G., Pickering, L. K., West, M. S. and Janoff, E. N. 1989. Rapid detection of Giardia antigen in stool with the use of enzyme immunoassays. *Am. J. Clin. Pathol.* **91**(6): 704-708.

Krogstad, D. J., Healy, G. R. and Herron, C. A. 1978. Amebiasis: epidemiologic studies in the United States, 1971-1974. *Ann. Intern. Med. Hyg.* **88**: 89-97.

Kuby, J. 1997a. Cell-mediated and humoral effector responses. In: *Immunology* 3rd ed. New York: WH Freeman and Company. Pp 398.

Kuby, J. 1997b. Immunoglobulins; Structure and Functio. In: *Immunology* 3rd ed. New York: WH Freeman and Company. Pp 126.

Kuby, J. 1997c. Overview of the immune system. In: *Immunology* 3rd ed. New York: WH Freeman and Company. Pp 3-24.

Kuntz., R.E. 1960.Intestinal protozoa inschool going children of Dhaka city. *Am. J. Trop. Med. Hyg*. **9**: 168-174.

Lascurain, L. R., Mora, T. M., Acosta, A. G. and Santos, P. J. I. 1993. The prevalence of antibodies aagainst Giardia lamblia. In umbilical cord serum and in maternal peripheral blood. Bol. Med. Hosp. Infant. Mex. 50(1): 27-31.

Lesh, F. A. 1875. Massenhafte Entwickelung von Amoben in Ddickdarm, Virchows Arch. Patho. Anat. Clin. Med. 65,196-211. English Translation in: Massive development of Amebas in the large intestine. *Aam. J. Trop. Hyg.* **24:** 383-392, 1975.

Liotta, A., Cardella, F., FerraraD., *et. al.,* 1987. Vaginal infections in population of diabetic children and adolescents, *Pediatr Med. Chir*. **9**: 305-308.

Lotter, H., Jackson, T. F. H. G. and Tannich, E. 1995. Evolution of three serological tests for the detection of antiamebic antibodies applied to sera of patients from an area endemic for amebiasis. *Trop. Med. Parasitol.* **46**: 180-182.

Lotter, R., Mannweiler, E. and Tannich. E. 1993. Crude or recombinant proteins applied to latex agglutination, complement fixation and enzyme-linked immunoabsorbant assays for the serodiagnosis of invasive amoebiasis. *Trop.Med. Parasitol*. **44**: 335-340.

Lee, K.J., Ahn, Y. K. and Young, T. S. 2000. A small scale survey of intestinal parasites Infection among children and non-diabetic peoples in Legaspi city. *Morbid Mortal Weekly Rep. Surveill. Summ*. **51**: 1-25.

Lee, St., Heiner, D. C. and Monogr, W. D. 1986. Development of Serum IgG subclass levels in children. In: Allergy. Dukor, P., Kallos, P., Trnka, Z., Waksman, B. H. eds.*Karger. Basel*. **19**: 108

Lopez, *et al.,* 1980. Evaluation of ELISA for detection of *Giardia lamblia*-specific copro-antigen employing monospecific antibodies. *Jpn. J. Med. Sci. Biol*. **43**(6): 209-217.

Margolis, L., Esch, G. W., Holmes, J. C., Kuris, A. M. and Schad, G. A. 1982. The use of ecological terms in parasitology (Rport of an Ad Hoc Committee of the American Society of Parasitologists). *J. Parasitol*. **68**(1): 131-133.

Markell, E. K., John, D. T. and Krotoski, W. A. 1999. Lumen-Dwelling Protozoa. 8th ed. The W. B. Saunders Co., Philadelphia, Pa.

Mirelman, D., Nuchamowitz, Y., Stolarsky, T. 1997. Comparision of use of enzyme-linked immunosorbent assay based Kits and PCR amplification of RRNA genes for simultaneous detection of *Entamoeba histolytica* and *E. dispar. J. Clin. Microbiol*. **35**: 240-257.

Mohamed, D. A., Terryf. G. H. and Jonathan, I. R. 2004. Identification of the *Entamoeba histilytica* galactose inhibitable lectin epitopes recognized by human immunoglobulin an antibodies following cure of amebic liver abscess: American Society for microbiology. **72** (7):3974-3980.

Mondal, D., PetriJr, W. A., Sack, R. B and Haque, R. 2006. *Entamoeba histolytica* – associated diarroeal illness is negatively associated with the growth of preschool children: evidence of a prospective study. *Trans. R. Soc. Trop. Med. Hyg*. **100**(11): 1032-1038.

Morell, A., Skvaril, F. and Barandun, S. 1976. Serum concentrations of IgG subclasses. *Clin. Immunobiol*. **3**: 37

Muazzem, M.G. and Ali, T. 1968. Incidence of intestinal parasites among children of East Pakistan. Medicus. **25**: 215-221.

Muller, S. A. 1966. Dermatologic disorders associated with diabetes mellitus. *Mayo. Clin Proc.* **41**: 689-703.

Muttalib, M.A., Islam, N., Gani, J. A., Khan, K., Azizullah, A. and Islam, B. 1970.

Muttalib, M.A., Islam, N., Gani, J. A., Khan, K., Azizullah, A. and Islam, B. 1975. Intestinal parasites in the University of Dacca Student. *J. Trop. Med. Hyg.* **78**: 10-11.

Muttalib, M.A., Islam, N. and Islam, S. 1976. Prevalence of intestinal parasites in rural children of Bangladesh. *Bangladesh Med. J.* **4** (1): 11-26.

Nagakura, K., Tachibana, H., Kaneda, Y., Suzuki, H., and Takeuchi, T. 1990. Amebiasis in institutions for the mentally retarded in kanagawa Prefecture, Japan. *Jpn J. Med. Sci. Biol.* **43:** 123-131.

Nahar, S. 2011. Prevalence of intestinal parasites among the outdoor patients of IBN sina hospital, Dhaka. Pp 45-46.

Nesbitt, R. A., Mosha, F. W., Katki, H. A., Ashraf, M., Assenga, C. and Lee, C. M. 2004. Amebiasis and comparison of microscopy to ELISA technique in detection of *Entamoeba histolytica* and *Entamoeba dispar*. *J. Natl Med. Assoc.* **96**: 671-677.

Noble and Noble. 1980. The Biology of Parasites. Vol. 1, 5th ed. Lea and Febiger.

Nuruzzaman, M, Huda, Q. 1974. Parasitic infestation in gastrointestinal tract disorders. *Bangladesh. Med. J.* **2:** 119-123.

Oguoma, V. M. and Ekwunife, C. A. 2007. The need for a better method: Comparision of direct smear and formol- ether concentration techniques in diagnosing intestinal parasites. *The Internat J. of Trop. Med.* **3:** 2.

Ohnishi, K., and Murata, M. 1997. Present characteristics of symptomatic amebiasis due to *Entamoeba histolytica* in the east-southeast area of Tokyo. *Epidemiol. Infect*. **119**: 363-367.

Opara, F.n., Udoye, A.A., Okere, P.U., Osuala, F.O.U. and Iwuala, M.O.E. 2007. The prevalence of intestinal protozoan infections in primary school children in Owerri municipality, Imo state, Nigeria. *J. Parasitic Dis*. **31**(1): 44-48.

Orlandi, P. A., Chu, D. M. T., Bier, J. W. and Jacson, G. J. 2002. Parasites and the food supply. *Food Technology*. **56**(4): 72-81.

Ozturkean, S., Topcu, S. and Bokici, M. Z. 1993. Incidence of oral candidiasis in diabetic patienta. *Mikrobiyol Bull*. **27**: 352-356.

Pal, S., Sengupta, K., Manna, B., Sarkar, S., Bhattacharya, aaaaas. And DAS, P. 1996. Comparative evaluation of somatic and excretory antigens of *Entamoeba histolytica.* In serodiagnisis of non-diabetic human amoebiasis by ELISA. *Indian J. Med. Res.***104**: 152-156.

Parija, S. C. and Karki, B. M. 1999. Detection of circulating antigen in amoebic liver abscess by counter-current immunoelectrophoresis. *J. Med. Microbiol*. **48**: 99-101.

Pillai, D. R., Kain, K. C. 1999. Immunochromatographic strip-based detection of *Entamoeba histolytica* - *Entamoeba dispar* and *Giardia lamblia* coproantigen. *J. clin. Microbiol.* **37**: 3017-3019.

Proctor, E. M. 1991. Laboratory diagnosis of amebiasis. *Clin. Lab. Med.* **11**: 829-859.

Rahman, M.M. 2009. Prevalence of intestinal parasites and their effects on croos section (economy and area based) of people residing in Chittagong and Chittagong Hill Tracts. M. Phil. Dissertation, University of Cittagong, Bangladesh. Pp 147.

Rahim, A. S. Haque, R., Mondal, D., Karim, A, Molla, I. H. Ahmad, B. D. and Petri W. A. Jr. 2009. Prospective case-control study of the association between common enteric protozoal parasites and diarrhea in Bangladesh. *Clin Infect. Dis*. **48**(9): 1191-1197.

Randall, G. R., Goldsmith, R. S., Shek, J. 1984. Use of enzyme-linked immunosorbent assy (ELISA) for detection of *Entamoeba histolytica* antigen in fecal samples. *Trans. R. Soc. Trop. Med. Hyg.* **79**: 593.

Ravdin. J. I., Terry, E. H. G. and Ahmed, E. S. 1990. Association of serum antibodies to adherence lectin with invasive amoebiasis and asymptomatic infection with pathogenic *Entamoeba histolytica. J. Infec. Dis.* **162**: 768-772.

Ravdin, J. I. 1995. Amebiasis. *Clin. Invest.Dis.* **20**: 1453-1464.

Ravdin, J. I. 2000. Amebiasis. Series on Tropical Medicine: Science and Practice. Vol: 2: Imperial College Press. Pp186.

Restrepo, S. L., Restrepo, Z., Villareal, C. L. E., Aguirre, A. and Restrepo, M. 1996. Diagnostic tests for amoebic liver abscess: comparison of enzyme-linked immunosorbent assay (ELISA) and counterimmunoelectrophoresis (CIE). *Rev. Soc. Bras. Med. Trop.* **29**: 27-32.

Roa, V. G., Aggrawal M. C.., Yadav R., Das S. K., Sahare L. K., Bondley M. K. and Minocha R.K. 2003. Intestinal parasitic infections, anaemia and under nutrition among Tribal non-diabetic peoples of Modhya Pradesh. *Indian J. Community Med*. **28**(1): 1-5.

Roche, J. and Benito, A. 1999. Prevalence of intestinal parasite infections with special reference to *Entamoeba histolytica* on the island of Bioko (Equatoria Guinea).

Roitt, I. M. and Delves P. J. 2001. Innate Immunity. In: *Roitt's Essential Immunology.* 10th ed. Oxford. *Blackwell Science Ltd.* Pp 1-20.

Roitt, I. M. and Delves P. J. 2001. Specific Acquired Immunity. In: *Roitt's Essential Immunology.*10th ed. Oxford. *Blackwell Science Ltd.* Pp 21-36.

Root, D. M., Cole, F. X., Willilanson, J. A. 1978. The development and standardization of an ELISA method for the detection of *Entamoeba histolytica* antigens in fecal samples. *Arch. Invest. Med. (Mex).* **9** (Suppl. 1): 203.

Saha, B. and Chowdhury, A.B. 1981. Protozoan infection in under five children in Rangpur and Dinajpur dristricts. *Bangladesh Med. J.* **16**(2): 7-11.

Sayeed, M. A., Ali, L. A. M. Z., Banu, A. and Khan, A. K. A. 1997. Effect of Socioeconomic Risk Factors. On the difference in prevalence of diabetes between rural and urban population in Bangladesh. *Diabetes Care*. **H9**: 551-555.

Schimd, D. T., G. and Roberts, L. S. 1981. Foundation of parasitology. The C. V. Mosby Company, St. Lousis Toronto. London.

Shakur, M.S. and Ehsan, M.A. 1993. intestinal parasites: a frequent association and contributing factor of schooling going children. *Bangladesh J. Child Health*.**17**(1): 10-13.

Shetty, N. and Prabhu, T. 1988a.Evolution of faecal preservation and staining methods in the diagnosis of acute amoebiasis. *J. Clin. Pathol*. **41**: 694-699.

Shetty, N. Das, P., Pal, S. C. and Prabhu, T. 1988b. Observations on the interpretation of amoebic serology in endemic areas. *J. Trop. Med. Hyg.* **91**: 222-227.

Singh, R, John, T. J., Cherian, T. and Raghupathy, P. 1994. Immune response to virus and parasite vaccine at p, 12 & 15 months of age. Indian. *J. Med. Res.* **100**: 155-159.

Smith, H. V. 1998. Detection of parasites in the environment. *Parasitology.* **117**: S113- S141.

Smith, F. R., Curran, A. S., Raciti, K. A. and Black, F. L. 1982. Reported Meals in persons immunology primed by Prior vaccination. *J. Pediatr.* **101**: 391-393.

Speelman, P., Ljungstrom, I. 1986. Protozoal enteric infections among expatriates in Bangladesh. *Am. J. Trop. Med. Hyg.* **35**: 1140-1145.

Standing Committee of Analysis, Environmental Agency, United Kingdom. 1999. Isolation and identification of *Cryptosporidium* oocysts *Giardia* cysts in waters.Methods for the Examination of Waters and Associated Materials (London; HMSO) Pp 44.

Stanley S. L. Jr. 2003. Amoebiasis. *Lencet. Mar.* **22**: 361(9362): 1025-1034.

Stoll, B. J., Glass, R. I. Huque, M. I., khan, M. U., Holt, J. E. and Banu, H. 1982. Surveillance of non-diabetic patients attending a diarrhoeal disease hospital in Bangladesh. *Br. Med. J.* **285**: 1185-1188.

Stanton, B., Silimperi, D.R., Khatun, K., Kay, B., Ahmed, S., Khatun, J. and Alam, K. 1989. Parasitic, bacterial and viral pathogens isolated from diarrhoeal and routine stool specimens of urban Bangladeshi children. *J.Trop. Med. Hyg.* **92**: 46-55.

Sultana, S. 1994. Study on prevalence intestinal protozoan parasites *Giardia intestinalis* (Alexeieff. 1914) and *Entamoeba histolytica* (Schaudinn, 1903) infestation Among children in three rural areas of Bangladesh (Mirzapur, Bhaluka and Kaligonj). *M. Sc. Thesis, Univ. Dhaka.*

Tachibana, H., Kobayashi, S., Nagakura, K., Kaneda, Y., and Takeuchi, T. 2000. Asymptomatic cysts of *Entamoeba histolytica* but not *Entamoeba dispar* in institutions for the mentally retarded in Japan. *Parasitol. Int.* **49:** 31-35.

Talley J. N. and O'Conor S. 1991. Clinical examination. A systemic to physical diagnosis. Canberra, Australia. Pp 320-326.

Talukder, A. K. 2002. Faecal sample from 500 healthy army recruits aged 17-20 years were examined as well as concentration method. D. C. M. dissertation, NIPSOM. Pp 20-25.

Taylor, B., Miller, E., Farrington C. P., Petropoulos M. C., Favot-Mayaud, I. and Li. J. *et. al.,*1999. autism and measles, mumps, and rubella vaccine: no epidemiological evidence for a causal association. *Lancet.* **353**: 2026-2029.

Thompson-Jr., J. E. and Glasser, A. J. 1986. Amebic abscess of the liver. Diagnostic features. *J. Clin. Gastroenterol*. **8**: 550-554.

Tsutsumi, V., Mena-lopez, R., Anaya-Velazquez, F. and Martinez-Palomo, A. 1984. Cellular basis of experimental amoebic liver abscess formatin. *Am. J. Pathol.* **117**: 81-91.

Turgeon, M. L. 1996. Antigen and antibodies. In: *Immunology and serology in Laboratory medicine.* 2nd ed. St. Louis, Missouri, USA: *Mosby-year book Inc.* Pp 15-34.

Uddin, A. 2007. Status of gastrointestinal protoxan parasites in Chittagogn city and adjoining areas. M.Sc. Thesis. University of Cittagong, Bangladesh.

Uddin, M.H., Rahman, M. M. and Khanum, H. 2005. Hemoglobin level among non-diabetic peoples and it's relation to intestinal parasites. *Banglades J. Zool*. **33**(2): 183-187.

UNICEF. 1997. Prevalence of intestinal parasites in healthy adult and adolescent population of a rural community. The dancing horizon: human development prospects for Bangladesh, Dhaka.

Vazquez, J. A. and Sobel, J. D. 1995. Fungal infections in diabetes. *Infect Dis. Clin. North Am.* **9**: 97-116.

Vignesh, R. Balakrishnan, P., Shankar, E. M., Murugavel, K. G., Hanas, S., Cecelia, A. J., Thyagarajan, S. P., Solomln, s. and Kumarasamy, N. 2007. High proportion of isosporiasis among HIV-infected patients with diarrhea in Southern India. *Am. J. Trop. Med. Hyg*. **77:** 823-824.

Vreden, S. G., Visser,L. G., Verweij, J. J., Blotkamp, J., Stuiver, P. C., Aguirre, A. and Polderman, A. M. 2007. Outbreak of amebiasis in a family in the Netherlands. *Clin. Infect. Dis.* **31:** 1101-1104.

Walderich, B., Burchard, G. D., Knobloch, J. and Muller, L. 1998. Development of monoclonal antibodies specifically recognizing the cyst stage of *Entamoeba histolytica*. *Am. J. Trop. Med. Hyg*. **59:** 342-351.

Walsh, J. A. 1986. Problems in recognition and diagnosis of Amoebiasis; estimation of the global magnitude of morbidity and mortality. *Rev. Infect. Dis.* **18:** 228-238.

Walsh, J. A. 1988. Human infection by *Entamoeba histolytica* prevalence of *Entamoeba Histolytica*. *Ravdin J. I.,* 93-106.

Wang, L. C. 2004. Improvement in the identification of intestinal parasites by a concentrated merthiolate-iodine-formaldehyde technique. *J. Parasitol*. **84:** 457-458.

Wanke, C., Bulter, J. and Islam, M. 1988. Epidemiologic and clinical features of invasive amoebiasis in Bangladesh. Acase control comparion with other diarrheal diseases and postmortem findings. *Am.J. Trop. Med. Hyg***. 38:** 355-441.

Weinke, T., Friedrich- Janicke, B., Hopp, P. and Janitschke, K. 1989. Prevalence and clinical importance of *Entamoeba histolytica* in two high-risk groups: travelers returning from the tropics and male homosexuals. *J. Infect. Dis.* **161:** 1029-1031.

Wats, F. N. 1979. Behavioral aspects of the management of diabetes mellitus: Education, Selfcare and Metabolic control. *Behavior Research and Therapy*. **18**: 171-180.

Whittle, H. C. and Aaby, P. 1996. Viruses & Parasites. In: *Oxford Text Book of Medicine.* Weatherall D. J. Ledingham J. G. G., Warrell, D. A. eds. Vol 1. 3rd Ed. Oxford, UK: Oxford University Press Pp375-381.

William, A. and Petri, J. R. 1996. Amebiasis and *Entamoeba histolytica* Gal/Gal lectin. From lab Beach to beside. *Journal of Investigative Medicine*. **44:** 2.

World Health Organization/W.H.O., 1991. Environmental health in urban development. *Technical Report Series,* No. 807, World Health Organization, Geneva.

World Health Organization/W.H.O., 1997. The sex and age distribution of the world populations: The1996 revision. New York: United Nations.

World Health Organization/W.H.O., 1994. Bench Aids for Diagnosis of Intestinal Parasites. Geneva: World Health Organization.

World Health Organization/W.H.O.1990. Listed of estimated annual death ranked six parasites diseases, World Health Organization. Geneva. Switzerland.

World Health Organization/W.H.O.1969. Amoebiasis: report of a W. H. O. Expert committee W. H. O. *Tech. Rep. Ser.* No. 421.

World Health Organization/W.H.O. 2002. Reducing risks promoting healthy life. WHO Rep.

WHO.1987. Public Health Significance of intestinal parasitic infections. *Bull. World Health Org.* **65(5):** 575-588.

Yadav, S. K., Jain, A. K., Srivastava, V. K. and Gupta, J. P. 1990.Comparison of stool microscopy and serology (ELISA) in epidemiology of amebiasis. *Indian J. Gastroenterol.* **9:** 25-26.

Yang, J. and Kennedy, M. T. 1979. Evaluation of enzyme-linked immunosorbent assay for the sero diagnosis of amoebiasis. *J. Clin. Microbiology*. **10:** 1778-785.

Yoeli, M., Most, H., Hammand, J. and Scheinesson, G. P. 1972. Parasitic infections in a closed community, results of a 10 year survey in Willowbrook State School, New York. *Transsactions of the royal society of tropical medicine and hygiene*. **66(5):** 764-776.

Zaid, R. B. Islam, M. N., Ahsan, K., *et. al.,* 1999. Pattern of mucocutaneous yeast infection among diabetics- A study on 460 cases at BIRDEM. *J. Diab. Endocr*. **27**(1): 16-18.

ANNEXURE

Dhaka University (Dept. of Zoology) and Institute of Health Science (BIHS) Hospital.

Prevalence of *Entamoeba histolytica* and *Giardia lamblia* infection among diabetic and non diabetic patients of Bangladesh.

QUESTION NAIRE:

Code: | D | N | D | **Patient's ID:** | | | | |

Date :

1. Name :
2. Age :
3. Sex : (Male or Female)
4. Height and Weight :
5. Address :
6. Medication
7. Duration of Diabetes mellitus
8. Blood sugar states constant or not
9. Bowel habit pattern
10. Seasonal variation present or not regarding bowel habit
11. Food habit (Every day)

(a). Protein (b) Fat (c) Carbohydrate

(i). Morning (ii). Noon (iii). Evening (Rice, bread, vegetables and others eating).

CLINICAL FEATURE:

12. History of Fever
13. History of Anaemia
14. history of Hepatomegaly
15. History of Splenomegaly
16. Symptomatic or Asymptomatic

(A). Test Method: *Entamoeba histolytica* and *Giardia lamblia* Antibody, IgG (ELISA) Method:

(a). Symptomatic cases
(!) Positive
(!!) Negative

(b). Asymptomatic
(!) Positive
(!!) Negative

(B). Stool for *Entamoeba histolytica* and *Giardia lamblia*: Positive / Negative (Method: F-ECT AND Z-SFM)

(a). Trophozoite
(b). Cyst