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Original Article

Prevalence of *Entamoeba Histolytica* and *Giardia Lamblia* Infection Among Diabetic and Non Diabetic Patients of 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%, on non-diabetic individuals 34.66% and 34.99% were found infected by *E. histolytica* and *G. lamblia*; respectively. 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. 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 the young (25-45 years) were more infected than old aged group (above 55).

KEYWORDS: Protozoan parasitic infestation, different techniques, diabetic and non-diabetic patients, Bangladesh.

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INTRODUCTION

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. Most of protozoan parasites are 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. Muttalib *et al.*, (1976)[1] showed that about 99% of non-diabetic children in rural areas of Bangladesh are infected by intestinal parasites. Intestinal protozoan parasite infections are amongst the most common ones in world, 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[2].

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[3-4]. In Bangladesh, the protozoan parasites, *Entamoeba histolytica* and *Giardia lamblia* are common[5-12]. 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)[13] 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)[14] 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[15], 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.

MATERIALS AND METHODS

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

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.

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).

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:

B. Formal-ether concentration technique (Cheesbrough, 1987):[16]

The formal-ether technique is recommended as the best over all technique for the concentration of parasites in faces. The formalin fixes the parasite and sediment and the faucal debris are separated in a layer between the ether and formal water.

C. Zinc sulphate floatation technique (Cheesbrough, 1987):[17]

The zinc sulphate technique was recommended for concentrating the cysts of *E. histolytica* and *G. lamblia.* 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. Number of *E. histolytica* and *G. lamblia* cysts counted per gram of faces.

The presence of parasite cysts was detected. The findings were confirmed with the help of Cheesbrough, 1987 and Chatterjee, 2004. All the findings of stool samples were recorded in the respective questionnaire.

RESULTS

The present 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. 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 collected blood and stool samples were detected by ELISA, Formol Ether Concentration technique and Zinc Sulphate method.

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-1).**

Table-1: 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
	Diabetic	697	111	15.93	123	17.65	31	4.45	0.025**	*E. histolytica*
ELISA (Blood serum)	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).

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*. 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 **(Fig.1).**

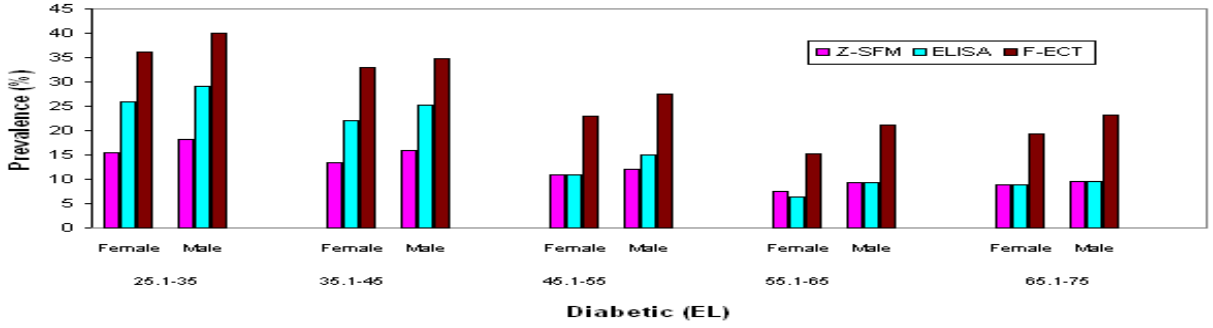

Fig- 1: The prevalence of *E. histolytica* infections in different age groups, in sexes among diabetic patients by different techniques. **EH = *Entamoeba histolytica***

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. 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* showed non-significant result **(Table-1).**

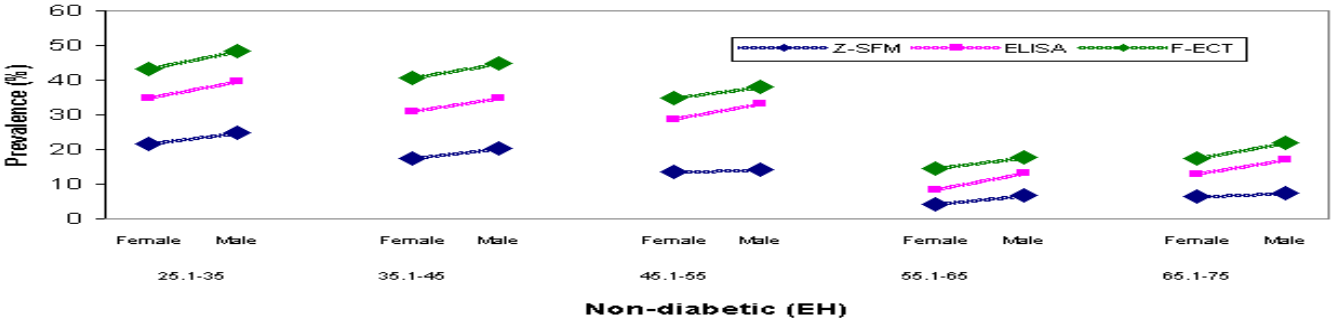

Fig-2: The prevalence of *E. histolytica* infections in different age groups, in sexes among the non-diabetes patients by different techniques. **EH = *Entamoeba histolytica***

Table-2: 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)				Double infestation *E. histolytica* and *G. lamblia*		P-value of proportion test	
			E. histolytica		*G. lamblia*					
			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).

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* **(Fig. 3).**

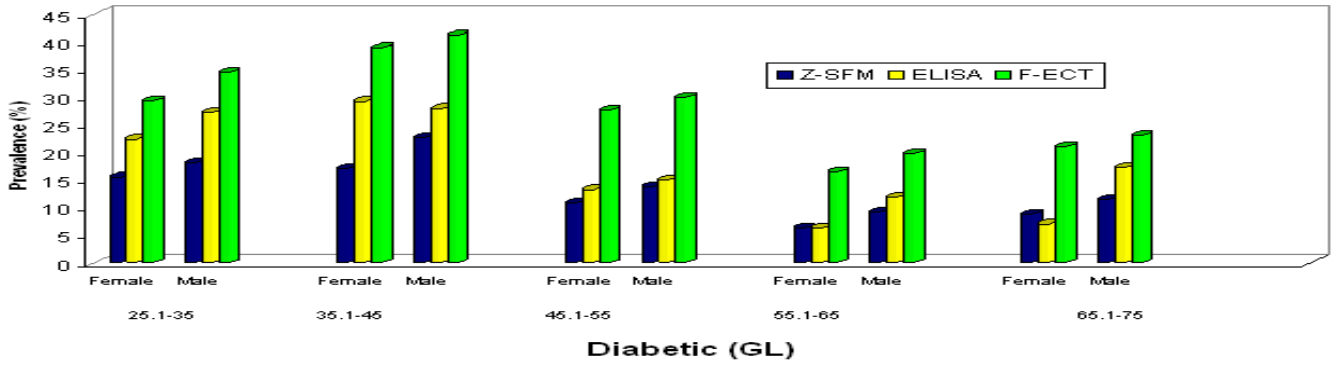

Fig-3: The prevalence of *G. lamblia* infections in different age groups, in sexes among diabetic patients by different techniques. **GL= *G. lamblia.***

Table-3: 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		Stool				P-value of proportion test	
				ELISA (Positive cases)		F-ECT (Positive cases)		Z-SFM (Positive cases)			
				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;

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-3).**

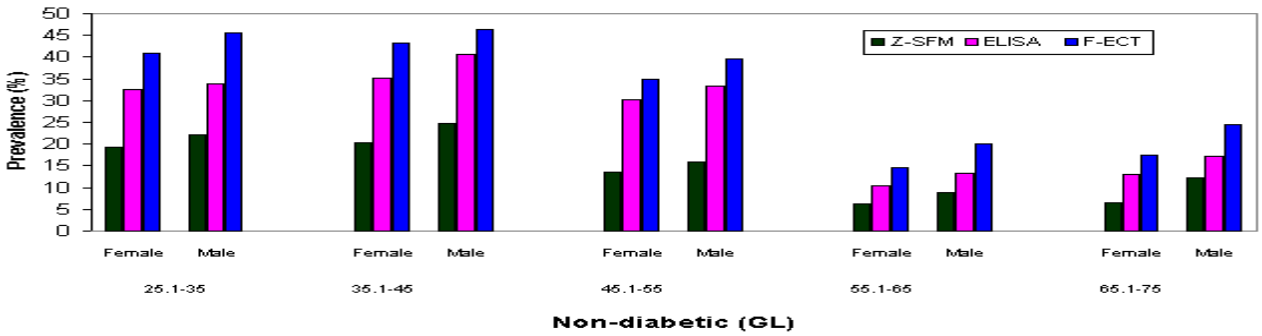

Fig-4: The prevalence of *G. lamblia* infections in different age groups and in females and males among non-diabetes patients by different techniques.

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 35-45 years old **(Fig. 1-4)**.

DISCUSSION

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[4]. Congestion, soil character, source of water, socio-economic conditions, low living condition, unhygienic surroundings allow for transmission of the amoebic infection. The distribution is worldwide, although the preponderance of morbidity and mortality is experienced in Central and South America, Africa and India[18].

Amoebiasis is defined as asymptomatic, invasive intestinal or extra intestinal diseases due to *E. histolytica* infection. Asymptomatic cyst is the most frequent manifestation of intestinal *Entamoeba* infection and 90% of *Entamoeba histolytica* infections are asymptomatic[19]. 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[20]. Additionally, asymptomatic carriers are more likely to spread the disease than symptomatic persons with invasive disease, as the latter individuals seek medical attention[21].

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[22]. The prevalence of disease in the developing world is due to fecal-oral spread of infection via contaminated food and water[23-24]. The finding of the present study clearly indicate that 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 patients was consistent with the present findings.

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 *E. histolytica* 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.*

Three different techniques 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 assy (ELISA) for the detection of *E. histolytica* and *G. lamblia* associated antigens in blood. 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[25].

In the present study, the three techniques used to diagnose infection with *E. histolytica* and *G. lamblia* 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. 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.

In conclusion, all three test (ELISA, formal-ether concentration and zinc solphate floatation techniques) studies proved to be reliable diagnostic tools. Both formal-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. Improvement of sensitivity can be obtained by combining the tests.

CONCLUSION

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. 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.

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