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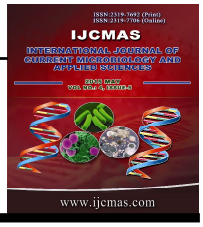

Original Research Article

Prevalence of *Giardiasis* in Patients Attending Tertiary Care Hospital in Northern India

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Keywords

Giardiasis, *Giardia intestinalis*, Diarrheal diseases, ELISA, prevalence

A B S T R A C T

Giardiasis caused by *Giardia intestinalis* is one of the major causes of diarrheal diseases through the world. Infection may be either asymptomatic or may cause diarrheal illness that may be acute or chronic. Being endemic in areas of poor sanitation it affects 33% of the people in the developing world. The study was carried in the Department of Microbiology, Hind Institute of Medical Sciences, Safedabad, Uttar Pradesh. A total of 756 stool sample were collected and screened for *Giardia* through microscopy (Iodine and saline mount), Formol ether concentration method and ELISA. Among 756 samples screened, 148 samples were positive. The samples which were positive through direct microscopy were 119 whereas through ELISA it was 148. The age group that showed the maximum amount of parasitic infection was among 16–20 years and least was found to be the age group of 60 years and above in either of the identification method.

Introduction

Giardiasis is one of the major causes of diarrheal diseases through the world. The flagellate protozoan *Giardia intestinalis*- (previously known as *G lamblia*) is the causative agent and most common intestinal parasite isolated worldwide (Daly *et al.*, 2010; Eisenstein *et al.*, 2008; Nishi *et al.*, 2009; Robertson *et al.*, 2007), being more common among children than in adults (Hill, 2005; Huang and White, 2006).

G. intestinalis infection may be either asymptomatic or cause diarrheal illness which may be acute or chronic. Frequency of its presence is found to be as high as80% of raw water supplies from lakes, streams, and ponds and around15% in filtered water samples (Robertson *et al.*, 2008; Ryu *et al.*, 2007). Being a common cause of chronic diarrhea, it results in growth retardation among children in developing countries.

Giardiasis usually is zoonotic with cross-infectivity between animals and humans. *Giardia intestinalis* has been isolated from the stools of beavers, dogs, cats, and primates. Other *Giardia* species include *G. muris* in rodents; *G. agilis* in

amphibians; *G. psittaci* and *G ardeae* in birds; and *G. microti* in voles and muskrats (Thompson *et al.,* 2008; Huston, 2006; Ballweber *et al.,* 2010).

Giardia species are endemic in areas with poor sanitation. It has been a major cause of morbidity in developing countries. Frequency of outbreak is more common due to contaminated food and water. Ingestion of as few as 10 *Giardia* cysts may be sufficient to cause infection. *G. intestinalis* is a significant pathogen among people with malnutrition, immune deficiencies, or cystic fibrosis.

Travelers to highly endemic areas, immunocompromised patients, and homosexual men include high-risk groups for *Giardiasis*. Cyst passage rates among certain group of sexually active homosexual men have been found to be as high as 20%. These groups of individuals were frequently symptomatic (Farthing, 1996; John, 2007).

Being a global disease, *Giardiasis* infects nearly 2% of adults and 6% to 8% of children in developed countries whereas effecting nearly 33% of people in developing world. In India the value significantly fluctuates from 3.8% to 23.5 %.The study described herein was designed to access the prevalence of *Giardiasis* among patients attending tertiary care hospital situated at a sub-urban area in Barabanki (Uttar Pradesh).

Materials and Methods

Methodology

A prospective study was carried out in the Department of Microbiology, Hind Institute of Medical College & Hospital U.P, India, for a period of one year (05-01-2014 to 28-02-2015).

Sample size

A total of 756 stool sample were collected from patients after getting their consent and examined by routine microscopy.

Sample collection

Patients were provided with a universal container which was properly labeled for collection of samples. They were asked to collect around 5grams of solid stool or 10ml if the consistency of the stool was liquid. The stool samples hence collected were examined within 1–2 hours of collection.

Microbiological examination

Each stool specimen was examined by the following techniques.

Macroscopic examination

The colour, consistency, presence of blood and mucus were observed.

Direct microscopic examination by using saline and iodine preparations

On a 1mm thick microscopic slide, a small amount of stool sample was emulsified in 1 - 2 drops of Normal saline (to demonstrate helminthic egg and larvae, motile trophozoites of intestinal protozoa) and Lugol's iodine solution (to demonstrate protozoan cysts). A cover slip was placed on the top ensuring the preparation to free of air bubbles and macroscopic debris.

Formol-ether concentration method

The sample which was negative for direct microscopic method of saline and iodine mount was further processed using Formol-ether concentration method as follows:

1–2 gm of feces was emulsified in 10 ml of water and the large particle was allowed to sediment. The supernatant was then spun at 2500 rpm /min for 2–3 min. The supernatant was discarded. 10% of Formal saline was added and mixed well, and allowed to stand for 10 minutes.

3 ml ether was added and the centrifuge tube was shaken well. It was then spun at 2500rpm/min for 2–3 min, thus forming four layers- a top layer of ether, a plug of debris at the interface, the formalin saline layer, and the sediment at the bottom. The debris was dispatched from the sides of the tube, and the top 3 layers were discarded. The sediment was suspended in a few drops of fluid and examined through wet mount and iodine preparation.

Enzyme Linked Immuno Sorbent Assay

The test was performed according to manufacturer's instructions in RIDASCREEN® (R-Biopharm AG, Darmstadt, Germany)

The thawed stool samples (100 mg) were mixed with 1 ml of sample dilution buffer and centrifuged at 5000 rpm for 5 min. The supernatant was taken for further tests. 100 µl of stool suspensions were pipetted in the microwells along with 100 µl each of positive and negative controls provided by the manufacturer.

Then 100 µl of enzyme-conjugated antibody was added, mixed thoroughly and incubated at room temperature (20–25°C) for 60 min.

The wells were washed 5 times with 300 µl of wash buffer each time. After washing for the last time, the plate was knocked out thoroughly onto a clean absorbent paper in order to remove any residual moisture.

Then 100 µl of substrate was added to each well and the plate was incubated at room temperature (20–25°C) in the dark for 15 min.

Then, 50 µl of stop reagent was added to each well and mixed properly. The absorbance of controls and patient samples was read at 450 nm using an ELISA micro-titer plate reader (Immunoskan-MS, Biological Diagnostic Supplies Limited, UK).

Results and Discussion

Out of 756 samples processed 148 were positive for *Giardia*. The number of samples which were positive for *Giardia* in direct microscopy was 119, through concentration method was 12 more and with ELISA positive sample was increased by 17 respectively.

The age group that showed the maximum amount of parasitic infection was among 16-20 years and least was found to be the age group of 60 years and above in either of the identification method. The prevalence of *Giardiasis* was seemed to be high on males in comparison to females. Out of total samples collected, 7 were found to be having co-infection with other parasites such as Hook worm and *Hymenolepis nana*.

Morbidity due to intestinal parasites has always been an important public health problem especially in the developing countries. Although the rate of mortality from diarrheal diseases in the developed countries has decreased but they continue to be one of the major causes of morbidity and mortality in the developing world.

One of the major cause is *Giardia* which has been documented to be transmitted either from person to person, animal to person or

from the environment to person. These transmission modes are well favored by high temperatures and moist climatic conditions, poor personal hygiene and unsanitary habits of individuals.

Table.1 shows the data of the overall distribution of *Giardia* positive outcome

Age Group [yrs.]	Direct Microscopy				Concentration Method				ELISA			
	MALE		FEMALE		MALE		FEMALE		MALE		FEMALE	
	No.	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*
0-15	51	42.85	22	18.48	57	43.51	23	17.55	62	41.89	29	19.59
16-20	12	10.08	04	3.36	12	9.16	05	3.81	12	8.10	05	3.37
21-59	17	14.28	09	7.56	21	16.03	09	6.87	24	16.21	11	7.43
60+	03	2.52	01	0.84	03	2.29	01	0.76	04	2.70	01	0.67
Total	**83**	**69.7**	**36**	**30.25**	**93**	**70.99**	**38**	**29.0**	**102**	**68.91**	**46**	**31.08**

Table.2 shows the data of various positive outcomes for their corresponding age group

Age Group [yrs.]	Total Samples	Total positive outcome	
		No.	*%*
0-15	564	91	16.13
16-20	48	17	35.41
21-59	124	35	28.22
60 +	20	05	25
TOTAL	**756**	**148**	**19.57**

Table.3 shows the gender wise prevalence of *Giardiasis*

GENDER	Total Samples	Total positive outcome	
		No.	*%*
MALE	502	102	20.31
FEMALE	254	46	18.11
TOTAL	**756**	**148**	**19.57**

In our study the prevalence of *Giardia* was 15.74% through direct microscopy, 17.32% through concentration method and 19.57% through ELISA respectively. *Giardia* species had the highest prevalence among the parasites in accordance with the other parasites (Eisenstein *et al.,* 2008; Nishi *et al.,* 2009).

Direct microscopy yielded 119 sample positive for *Giardia* which was increased by 12 more through Formal ether concentration method. The positivity rate of sample were further increased by 17 when ELISA was performed giving us the sum of 148 positive findings from the total of 756 samples. The prevalence of *Giardia* was 19.57 percent which was lower than similar study carried out by Singhal *et al.* (Sears, 2007). The drop in the percentage in our study may be because of various factors like area,

implication of proper sanitation and inclusion of different age group.

The maximum positivity was seen among the patients within 16–20 years. This was different from the study by Chou et al, where prevalence was much among the student below the age group of 12 years. Because of the lower frequency of patients within this age group showing higher positivity, lower socio-economic condition were most of the patients work along the highway eateries may be the major cause of highest frequency of positives in this age group.

The number of patients infected with *Giardiasis* was higher in male than in females similar to study conducted by Suman *et al.* (Walia *et al.,* 1986) and Julio *et al.* (Ballweber *et al.,* 2010). This increase in frequency among male may be due to their higher attendance in OPD and sociological factor where male is the sole bread winner of the family.

In sub-urban region where the study has been conducted, regular camps are required for screening of patients for parasitic infection and creating awareness among people about personal hygiene. ELISA may be used for the routine diagnosis of *Giardiasis* as an efficient and much faster technique when considered in large sample size. Though there has been a narrow fluctuation in sensitivity and specificity pattern of using ELISA technique, yet it proves to be a useful diagnostic tool where detection may be possible during initial stage of infection, which is sometimes not possible through direct microscopy or concentration method.

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