



A Preliminary Study Giardiasis in a Tertiary Care Hospital

KEYWORDS

Preliminary, Intestinal parasitic infection, a retrospective study.

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ABSTRACT *Background:* Intestinal parasitic infection is one of the major health problems in several developing countries, including India. The world health organization (WHO) estimates that there are 800-1000 million cases of ascariasis, 700-900 million hookworm infection, 500 million trichuris, 200 million giardiasis, and 500 million amoebiasis. *Aims:* The aim of this study was to determine the prevalence of *Giardia sp.* infections among the patients. *Material and Methods:* A total of 692 stool samples were examined for Protozoa and Helminths infection by routine microscopy. *Results:* There are eight different types of parasites detected. Out of which *Giardia lamblia* 28 (24.13%) were seen. *Conclusion:* It is needed to develop effective diagnostic, prevention and control strategies including health education and environmental hygiene.

INTRODUCTION

Intestinal parasitic infection is one of the major health problems in several developing countries, including India. The world health organization (WHO) estimates that there are 800-1000 million cases of ascariasis, 700-900 million hookworm infection, 500 million trichuris, 200 million giardiasis, and 500 million amoebiasis [1]. *Giardia duodenalis* (syn. *G. lamblia*, *G. intestinalis*) is a single cell parasite, inhabiting the small intestine. Like *Plasmodium* species causing malaria by infecting red blood cells, the genus *Giardia* belongs to the family protozoans [2]. The name lamblia has its origin from Vilem Lambl who described the trophozoite in humans in 1859, and the cyst form was discovered by Grassi twenty years later; however, Antony van Leeuwenhoek described the parasite in his own stool as early as in the 17th century [1-2]. *Giardia* has three morphologic forms; cysts, excyzoites and trophozoites [3]. Cysts are responsible for faecal-oral transmission, and are able to survive for a long period in the environment, especially in cold water in which experimental studies have shown survival for up to two months [4]. In the upper part of the small intestine, they release excyzoites containing four nuclei, which attach to the intestinal wall and rapidly divide into four trophozoites [3-4]. Trophozoites cause disease in the small intestine where they multiply by simple binary fission, though there is some evidence also of sexual reproduction [5]. The trophozoites have a characteristic duplication of organelles; four pairs of flagella enabling them to move, two identical nuclei, two median bodies and a ventral sucking disc which enables it to attach to the intestinal surface [2]. Trophozoites may be found in fresh faeces, but usually encyst, triggered by bile salts [6] or cholesterol depletion and micelle destruction [7], before being excreted in the stool. The objectives of this study were to determine the prevalence of *Giardia* infections among the patients who attended a tertiary care hospital at our area.

EPIDEMIOLOGY

Diarrhoea is probably the most common infectious disease worldwide, and following lower respiratory infections the second leading cause of death due to infections; in 2004 WHO reported an incidence of 4.6 billion episodes and 2.2 Million deaths due to diarrhoea per year, of these 1.8 million deaths in developing countries [8]. The most

common etiologic agents are species among the viruses rotavirus, calicivirus, astrovirus and enteric adenovirus; the bacteria *E. coli*, *Shigella*, *Salmonella*, *Campylobacter* and *Vibrio cholera*, and the parasites *Giardia*, *Entamoeba histolytica*, *Cryptosporidium*, *Cyclospora* and *Iso spora* [9]. Infections transmitted faecal-orally are more easily spread under conditions associated with poverty; such as decreased access to clean water, inappropriate sewage disposal, poor hygiene, crowding, close contact to farm animals and low educational level. A prevalence of 200 million cases of giardiasis in tropical countries has been estimated by WHO [10]. However, decreased access to reliable diagnostic tools in socioeconomic underdeveloped areas makes it difficult to assess the etiology of diarrhoea in clinical practice, and reporting systems are insufficient. Such estimates are also limited by the fact that few case-control studies have been performed in developing countries, and the clinical studies that are available show that prevalence varies greatly between and within countries.

PATHOGENESIS

Both parasite and host factors seem to be involved in the pathophysiological processes causing diarrhoea, malabsorption and malabsorption in giardiasis, although incompletely understood. In vitro studies on human samples have shown that *Giardia* attach by its adhesive ventral disc to the microvillus brush border of the intestinal epithelium, and cause barrier dysfunction by disrupting tight junctions and inducing epithelial apoptosis [11-13]. Further have experimental studies shown that activated CD8 T lymphocytes produce cytokines responsible for shortening of epithelial microvilli, which lead to malabsorption of electrolytes, nutrients and water as well as inhibition of the digestive enzymes lipase, protease and disaccharidase [14]. Disaccharidase insufficiency, and consequently failure in splitting and absorbing milk lactose, causes osmotic diarrhoea characteristic for temporary lactose intolerance commonly seen in giardiasis. Bacterial overgrowth in the small intestine may also play a part in the pathogenesis of the disease [15]. In clinical studies, inflammation and villous shortening in duodenal biopsies varies from 4% to 87% [16-17], and why there is such a high variability in mucosal reactions, as well as in clinical manifestations, is not known.

Genotypes and mixed infections have been proposed to be responsible for disease variability [18], but results from studies of the association between genotypes and severity of disease are not conclusive. An experimental study by Nash et al showed an association between strain variation and infectivity [19], and it seems that infection with a genotype less prevalent in a community induce more severe symptoms, however, both assemblage A and B have been associated with different symptom patterns in studies from different populations [20-30]. HIV infection does not seem to be associated with more severe disease [31,32]. Interestingly HIV infection stimulates the production of CD8 T-lymphocytes in the gut [33], and these lymphocytes are probably essential in the immune reaction against the Giardia parasite, as described above.

MATERIAL AND METHODS

4.1 Methodology

A retrospective study was carried out in the Parasitological section of the Department of Microbiology, Subharti Medical College & Hospital U.P, India, for a period of one year (January 2012 to December 2012).

4.2 Sample size

A total of 692 stool samples examined by routine microscopy.

4.3 Sample collection

The patients were provided wide mouthed clean, dry, properly labeled plastic container for collection of samples and recommend 5grams of solid or 10ml of liquid stool. The stool samples were examined within 1-2 hours of collection.

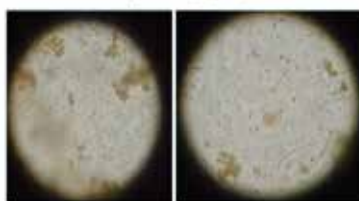
4.4 Microbiological examination

Each stool specimen was examined by the following techniques.

Macroscopic examination: The colour, consistency and the nature of the faeces were noted. The stool specimens were examined for the presence of worms like Ascaris, Enterobius, proglottids of Taenia, adult Hookworm and Trichuris, either with the naked eye.

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 (Himedia Pvt. Ltd Mumbai) and Lugol iodine (Himedia Pvt. Ltd Mumbai) solution. A cover slip was placed on it by taking care that the preparation was free of air bubbles and macroscopic debris. Unstained saline wet mount preparation was done to detect Protozoa trophozoites and Helminths eggs or larvae. Iodine wet mount was done to detect cysts [34].

Microscopic finding (Figure)



Cyst of Giardia

Trophozoites of Giardia

RESULTS

A total of 692 samples were examined, out of which

116(16.8%) samples was positive for parasitic infection.

Children (21.55%) were less infected than adults (78.44%) and the infection rate is similar in female ≤ 15 year (15.68%) and ≥ 15 year (15.84%) (Table 1).

The infection was higher in age groups between 16yr-50yr (65.52%) (Table.2).

The high number of giardia infection seen between 21-40yrage group.

Table 1: Age and gender wise distribution of positive samples (n= 116).

Category	Total tested	Positive	Percentage (%)
Age < 15 years	183	25	13.67
Male	132	17	12.87
Female	51	08	15.68
Age > 15 years	509	91	17.88
Male	244	49	20.09
Female	265	42	15.85

Table 2: Age wise distribution pattern of Protozoa and helminths infection in children and adults.

Age of patient	Name of Parasites							
	E.H/E.D	G.I	E.coli	H.w	B.h	I.belli	S.s	H.nana
0-5	8	3	1	-	1	1	-	1
6-10	-	4	-	-	-	-	-	-
11-15	2	-	3	1	1	-	-	-
16-20	6	2	4	3	-	-	2	-
21-30	16	6	4	2	-	-	-	-
31-40	5	8	-	8	-	-	-	-
41-50	7	2	-	1	-	-	-	-
51-60	3	2	-	4	-	-	-	-
≥ 60	2	1	-	2	-	-	-	-

E.H/E.D-Entamoebahistolitica/Entamoebadispar, G.IGiardialambliia, E.coli-Entamoebacoli, H.W-Hookworm, B.h-Blastocystishominis, I.belli-isosporabelli, S.s-Strongyloidesstercoralis, H.nana-Hymenolepis nana. Among eight

deferent type of parasites detected, the most common parasite identified were Entamoebahistolitica 49(42.24%), followed by Giardia lamblia 28(24.13%), Ancylostomaduodenale 21(18.10%) and Entamoeba coli 12(10.34%) also seen. The other parasite present as Strongyloidesstercoralis 2(1.73%), Blastocystishominis 2(1.73%), Isospora belli 1(0.86%) and Hymenolepis nana 1(0.86%) (Table 3).

Table 3: Distribution pattern of different intestinal parasite (n=116).

Name of parasite	No. of positive	Percentage (%)
Entamoebahistolitica	49	42.24
Giardia lamblia	28	24.13
Isospora belli	1	0.86
Blastocystishominis	2	1.73
Entamoeba coli	12	10.34
Ancylostomaduodenale	21	18.10
Strongyloidesstercoralis	2	1.73
Hymenolepis nana	1	0.86

DISCUSSION

Parasitic infestations are the major causes of morbidity and mortality in developing countries like India. The data on their prevalence and the sensitivity of various diagnostic methods help the clinicians and the microbiologists in the diagnosis and the management of the patients. The prevalence of *Giardia* sp. infection found in our study is (24.13%). It seems alarmingly high in comparison to international scenario [36-42]. Various studies have shown different prevalence rates of the parasitic infestations in different parts of India. But most of the studies had less sample sizes. The isolation of Protozoal cysts was higher than that of the Helminths ova. Our study showed that the most common intestinal parasite observed was *E. histolytica* (42.24%). Prakash, Tandon, and Shrivastava have also reported 35.6% and 18.4% positivity for the same [43-44]. The prevalence of *E. histolytica* has been observed as a common finding in tropical and subtropical countries and is responsible for diarrhoea and amoebic liver abscess in several studies [45]. These intestinal parasites are commonly transmitted by infected drinking water and food. In India, the water supply poses a big problem due to faecal contamination of the same. The most common Helminths infection in our study was *A. lumbricoides* (18.10%) which was similar to studies by Shrivastava where 22.2% of stool samples demonstrated *A. lumbricoides* [44]. However, in their study, the prevalence of *E. histolytica* was highest (42.24%) followed by *G. lamblia* (24.13%), *A. lumbricoides* (18.10%). The presence of oocysts of *Isospora belli* (0.86%) was an unusual finding in our study, as it is usually associated with AIDS patients and is responsible for chronic diarrhoea. Dalvi et al. have reported *I. belli* as the most common pathogen among HIV associated diarrhoea [46]. The prevalence of parasitic infections was more common in males (20.1%) as compared to that in females (15.8%), in age >15 year (Table-1). Marothi Y et al. [47]. Showed that the infections had a female preponderance. Various studies have shown the varying sex prevalence of the parasitic infections. However, the sex predominance for the parasite infections has still not been confirmed. The reason for the male preponderance in our study may relate to the daily activity rather than the sex predominance. Kang G et al. [48], The maximum number of parasites which was shown in a single sample was 3 (*Entamoebahistolytica* cysts, *Isospora belli* oocysts and *Giardia lamblia*). The diagnosis of parasitic infections in humans is challenging and it requires skills to identify and to differentiate them from one another. The routine diagnostic procedures lack sensitivity. The concentration methods should be performed routinely for the examination of parasites. Concentration permits the detection of the organisms which are present in small numbers: these may be missed by using direct wet mounts. The organisms that can generally be identified by using concentration procedures include: Helminths eggs and larvae; cysts of *Giardia lamblia*, *Entamoebahistolytica* / *Entamoebadispar*, *Entamoeba coli*, *Endolimax nana*, *Blastocystishominis* and *Iodamoebabutschii*; and the oocysts of *Isospora belli*.

CONCLUSION

The prevalence of *Giardia* Sp. Infections is (24.13%).

The infection of *Entamoeba coli* (10.34%), a commensal parasite is indicative of the populations precarious sanitary conditions and of elevated environmental contamination high listing the need for education, focused on hygiene measures.

Protozoal infection is more common than Helminths infection.

The Formol-ether technique to increase the diagnostic sensitivity.

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