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Prevalence of intestinal parasitic infections among patients attending a tertiary care South Delhi hospital

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Abstract

Background: Intestinal parasitic infections (IPIs) are amongst major problems and endemic in developing countries. Various geographical, socioeconomic and other factors i.e. climate, hygiene, and age etc. play important role in prevalence of IPIs.

Material and Methods :A cross-sectional study was conducted at HAHC Hospital and HIMSR, Delhi, from Aug. 2021 to Aug. 2022. Total 300 stool specimens were collected and examined by direct wet mount followed by Formal ether concentration technique for all stool specimens. Modified Trichrome stain and ZN stain were also used for detection of protozoal cysts.

Results: Prevalence of intestinal parasitic infection was found to be 21%. Most common parasite was Entamoeba histolytica (57.33%), followed by Giardia lamblia (28.0%). Other common parasites included Ascaris lumbricoides (5.34%), Cryptosporidium sp. (5.34%),Hymenolepis nana (1.33%), Trichuris trichura (1.33%) and Hookworm (1.33%). No significant difference was noted for prevalence among males (21.66%) and females (20.00%). Highest prevalence was noted in the age group of 0-10 years (29.0%) followed by 11-20 years (21.21%). Formalether concentration technique resulted in significant increase in sensitivity for detection of parasites (23.66% vs 17.66% without concentration).

Conclusion : Entamoeba histolytica and Giardia lamblia are commonest intestinal parasites. Concentration techniques and other staining are important for increasing sensitivity of detection.

Keywords: Intestinal parasitic infections (IPI's), stool concentration techniques, Entamoeba histolytica, Giardia lamblia

Introduction

Intestinal parasitic diseases constitute a major global health burden in developing countries mainly due to overcrowding, differences in environmental sanitation [1], and other environmental and sociocultural factors enhancing parasitic transmission [2, 3]. In third world countries the intestinal parasites are endemic and widely prevalent due to poor sanitation, under-nutrition, personal hygiene and lack of awareness about safe potable drinking water in low socio economic groups with substandard housing conditions apart from warm and humid climate [4]. The prevalence of IPI's not only varies in different parts of the world but also in different regions of same country [5].

These infections are associated with very high morbidity and mortality causing over 33% of deaths worldwide. [6,7]. The World Health Organization (WHO) estimates that approximately 3.5 billion people are exposed to IPIs and causing disease in 450 million people worldwide , resulting in 40–100 thousand deaths yearly [8,9]. Most of these infections involve children (upto 50%) though it can affect people of all ages. Most of these IPIs in children can be attributed to their poor hygiene and sanitation, malnutrition and low immunity. Despite a significant problem in developing countries, many of the diseases caused due to IPI's have been categorized as neglected tropical diseases. Hence millions of children are at risk of acquiring IPIs and require effective preventive and treatment measures periodically.

Ascariasis, hookworm infection, Amoebiasis, Giardiasis and Trichuriasis are the most common intestinal parasitic infections (IPIs) in developing countries, world-wide [10]. Entamoeba histolytica and Giardia lamblia are amongst two most common intestinal protozoal infections globally.

According to the World Health Organization report, there were 1.5 billion Ascaris lumbricoides infections followed by 700–900 million Hookworm infections and 500 million Trichuris trichuria infections seen in upto 24% of the world people. [6,11].

Apart from a very high morbidity, IPIs are associated with the annual loss of about 39 million disability-adjusted life years, which are responsible for vast health and financial problem. Furthermore, the most important problem of IPI's is around 90% of infected individuals remain asymptomatic, transmitting infection directly or indirectly [12]. When symptomatic, IPIs cause malnutrition, weight loss, anaemia, abdominal pain, diarrhoea, dysentery and other gastrointestinal problems.

Diarrhea is loose, watery stools three or more times a day. Diarrhea may be acute, persistent, or chronic: **Acute diarrhea** is a common self limiting condition typically lasting for 1 or 2 days.

Persistent diarrhea lasts longer than 2 weeks and less than 4 weeks.

Chronic diarrhea lasts for more than 4 weeks which be continuous or intermittent [13].

The diarrhea may be due to bacterial or parasitic infections generally and less frequently due to fungal pathogens.

The different methods for diagnosing the intestinal parasites include direct wet mount examination (by saline mount and iodine mount) which is the conventional method most commonly used for the detection of intestinal parasites from stools specimens. These methods are used to visualize eggs, cysts, trophozoites and larvae of various parasites, although these are less sensitive for detection of parasites in stool specimens, especially when the load is less [14]. The sensitivity of detection can be increased by using various concentration techniques and combining conventional microscopy with other staining methods. Important concentration techniques include formal-ether sedimentation, formalin-acetone sedimentation, saturated salt floatation and zinc sulfate floatation techniques. These techniques increase sensitivity of detection by increasing detection of various parasitic stages like helminthic eggs, larvae and protozoal cysts/ oocysts.

There is no single best test available for parasitic diagnosis, hence these infections remain under reported. In resource limited developing countries with high burden of IPIs as significant public health problem, simple, cheap and sensitive diagnostic techniques are required for optimum diagnosis.

Further the sensitivity increases by adopting combination methods for optimum diagnosis of IPI's. Limited data is available from South Delhi region regarding prevalence of intestinal parasitic infections and also effect of diagnostic sensitivity with concentration techniques along with other stains. Therefore, such studies are very important to assess local prevalence of Intestinal parasitic infections and to formulate effective prevention and treatment strategies on a regular basis.

Materials and Methods

A cross sectional study was conducted at the Microbiology laboratory, from a tertiary care hospital of South Delhi, during the period August 2021 to August 2022. A total of 300 fresh stool specimens without any preservatives were collected from patients presenting with or without GI/ other symptoms to various OPDs and from inpatients presented to Microbiology laboratory, as per suggestion of treating physician. No extra charges were taken for the tests. The study population comprised of individual of all age groups and gender who attended our hospital during the study period. Patients were informed regarding collection of morning stools avoiding urinary contamination. Stool specimens from the patients giving history of intake of any antiparasitic drugs in the preceding two weeks or contaminated with urine were excluded. Stools specimens is (formed/unformed) were examined immediately after receiving in the laboratory.

Fresh stool specimens were collected and processed within 1-2 hours of collection.

A clean, dry, wide – mouth sterile plastic container with a tight - fitting lid was used for collection of stool specimen. A properly labeled container with unique ID number was given to patients after instruction for specimen's collection to collect around 5-10 grams of solid or 10ml of liquid stool.

Stool specimens were examined for enteric parasites by the following methods:

Macroscopically, stool specimens were examined for color, consistency, OBT (Occult Blood Test), presence/absence of mucus and/or blood, any adult

worm and/or parasite segments, pH followed by test for reducing sugars.

B. Microscopic examination

1. Direct wet mount -: About 1-2 mg of stool specimen was emulsified in 1-2 drops of normal saline (0.85%) and Lugol's iodine solution on either sides of a single slide. A cover slip was then placed and the slide was examined under 10 X and 40 X objective of light microscope.

2. Formal-ether sedimentation and staining: Concentration using Formol ether was done for all the specimens along with modified ZN staining and Trichrome staining of two separate fresh smears, irrespective of the result of wet mount examination. Cyst of E. histolytica/E dispar/E. moshkovskii was differentiated from nonpathogenic Entamoeba coli on the basis of shape size and number of nucleus. Further as cyst of E.histolytica/E dispar/E. moshkovskii cannot be differentiated by microscopy these were presumptively reported as E histolytica. All slides and smears, as a part of general routine, were examined multiple times by students and junior and senior faculties.

Results

Of total 300 stool specimens examined, 180 were from males and 120 were from female patients. Of these, 63 specimens [21%] demonstrated presence of some kind of parasites (ova/ cyst/ trophozoite) and hence were found to be positive for intestinal parasitic infections. (Figure 1). Percent positivity for males and females was 21.66% and 20 % respectively with no significant gender wise difference (P value= 1). [Table 1]

29.0%, (n=38) of total positive specimens were from age group 0-10 years, showing the highest prevalence for intestinal parasitic infection followed by age group of 11-20 years showing 21.21% (n=7) which was significant. (P < 0.05).[Table 2 and Table 3]

Overall, Entamoeba histolytica was found to be the most common parasite 57.33%, followed by Giardia lamblia 28.0%, Ascaris lumbricoides 5.34%, Cryptosporidium 5.34%,Hymenolepis nana 1.33%, Trichuris trichura 1.33% and Hookworm 1.33% respectively as shown in [Figure-2.]

Infection with more than one parasite was noted in (n=14/63) 22.22 % of positive specimens.

Formal-ether sedimentation technique showed a significant increase in sensitivity for detection of Intestinal parasites, 23.66% (n=71/300) in comparison of direct wet mount 17.66% (n=53/300) (P < 0.05) , additionally detecting 23.35% of intestinal parasites. Apart from this 4 acid fast oocysts of Cryptosporidium sp. were found with modified ZN staining.

Fig.1: Prevalence of parasitic infection

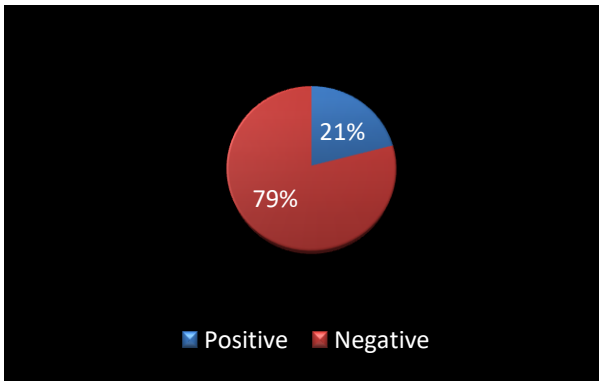

Table 1: Gender wise prevalence of parasitic infection.

S.No	Gender	No. of patients examined	No. of infected patients	% of infected patients
1.	Male	180	39	21.66
2.	Female	120	24	20.00
	Total	300	63	21.00

Table 2: Age wise prevalence of parasitic infection

S.No.	Age (years)	No. of patient examined	No. of infected patients	% of infected patient
1.	0-10	131	38	29.0
2.	11-20	33	7	21.21
3.	21-30	43	6	13.95
4.	31-40	26	1	3.84
5.	41-50	19	3	15.78
6.	51 & above	48	8	16.66
	TOTAL	300	63	21.00

Figure 2: Distribution of various intestinal parasites

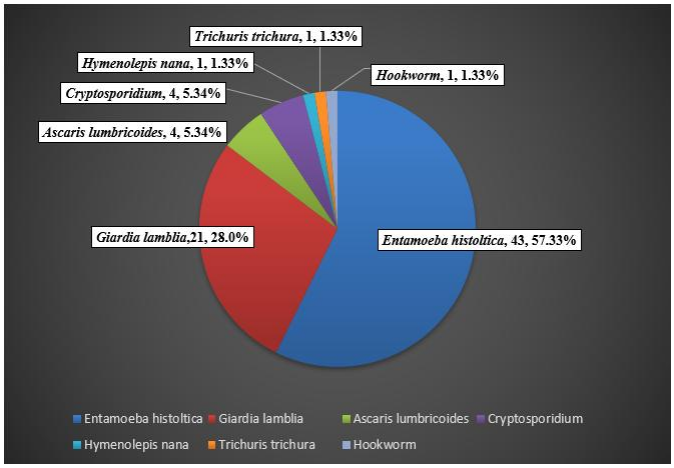

Table 3: Distribution of intestinal parasites

Name of parasites	Age Distribution 0-10 years	11-20 years	21-30 years	31-40 years	41-50 years	51 years & above	Total	% Percentage
Entamoeba histolytica	30	2	4		2	5	43	57.33
Giardia lamblia	11	3	1	1	2	3	21	28.0
Ascaris lumbricoides	3					1	4	5.34
Cryptosporidium	2	1				1	4	5.34
Trichuris trichura	1						1	1.33
Hymenolepis nana		1					1	1.33
Hookworm						1	1	1.33
Total	47	7	5	1	4	11	75	100%

Discussion

Most studies from developing countries have reported wide variation in the prevalence of intestinal parasites.

This study showed an overall prevalence of 21.0% for intestinal parasites in our study population. Various authors from India have quoted a variable range of prevalence for intestinal parasitic infections ranging from 10-35%. [5,15]. A comparatively higher prevalence was quoted by the study conducted by Manisha Patel et al., in year 2020 (34%) [5]. Some of the previous study from this part of India has reported prevalence as high as 71% [16]. Authors from abroad have also reported similar prevalence ranging from 19% to 50% [17,18,19].In our own previous study conducted in year 2012, a slightly higher prevalence of 26.1% was noted [20] . However, a comparatively lower prevalence from Indian studies have been reported by similar studies of Bhawna Kumari et al., and Mishra Shobha et al., [15] ,that is 13.41% and 15.19% respectively [15, 21].

Poverty, poor environmental sanitation , under nutrition, lack of personal hygiene and awareness about safe potable drinking water due to substandard housing are important reasons for such variation,

apart from different testing methods employed, difference in the number of specimens examined per patient, inclusion or exclusion of concentration techniques in the workup of specimens [17,18,19]. Although the prevalence of our study is comparable to prevalence reported from various parts of India but this is definitely higher than the prevalence reported from developed countries [22,23] . This variation is probably due to socio-economic differences as well as difference in climatic conditions, time, place, health awareness, and living standards.

A slightly higher prevalence was noted in males as compared to females (21.66% vs 20.0%) but no significant difference was observed for gender wise prevalence of parasitic infections (P value= 1), which is in agreement with other reports from India and other countries regarding gender independence of parasitic infections [24,25,26] . This is in contrary to the study findings of Patel Gupta et al and Parmeshwarappa KD et al who reported a higher prevalence in males as 37.7% vs 28.2% in females and 33.39% vs 21.29% in females respectively.

In our study, the prevalence of intestinal parasites varied across age groups. Pre-school and school-going children have been found to be at the greatest risk for intestinal parasitic infections which is also shown by most of studies reported. Very few authors reported a higher prevalence in older age groups [27,28].

Based on the age, prevalence of parasitic infection was found to be highest among patients aged 0-10 years (29%) followed by 11-20 years (21.21%). Similar findings are reported from different parts of India [28] . This was significantly higher in children (P < 0.05). High parasitic infection found among school aged children and young adults might be due to their unhygienic behavior and lack of sanitation especially in lower socioeconomic groups.

The most common parasite identified in our study was E. histolytica/E dispar/E. moshkovskii, seen in 14.33 % (43/300) of all specimens and 57.33% (43/75) amongst all positive specimens. This was followed by G lamblia in 7.0% (21/300) of all specimens screened and 28.0% (21/75) of all positive specimens. A. lumbricoides and Cryptosporidium spp. was found in 5.33% (4/75) followed by T. trichiura, H. nana and hookworm (Ancylostoma/Necator sp.) in 1.33% (1/75) of all positive specimens.

Similar to our study, Manisha Patel et al and Jeevitha Dhanabal et al also reported Entamoeba histolytica as the most prevalent parasite followed by Giardia lamblia [14, 29].

In our study, the prevalence for G. lamblia increased from 2% to 28.0% when compared to our previous study conducted in 2012 [20]. Although these two studies are from the same place but have a different prevalence which might be due to different time span of specimens collection and also due to probable consumption of contaminated water/food over the years. This also emphasizes the public health importance of such studies required to know the magnitude of these infections and also in formulating local preventive strategies and infection control measures.

Direct microscopy of fresh stool specimen, although less sensitive, is the gold standard technique for detecting IPIs and is commonly used in resource poor countries.

In our study the sensitivity of direct wet mount examination was 17.66% (n=53/300) which increased to 23.66% (n=71/300) with formalin-ether concentration technique for parasite detection. Concentration additionally detected 25.35% of the intestinal parasites when compared with only direct wet mount without concentration and hence significantly increased sensitivity for parasitic detection ($P < 0.05$).This emphasized that concentration methods like formalin-ether concentration technique increases sensitivity of detection for parasitic diagnosis in comparison to conventional wet mount techniques which would have been missed otherwise when the load is very less. Therefore these should be performed routinely along with use of other diagnostic methods in combination. Our finding supports the result of majority of Indian studies and also from abroad stating formalin-ether concentration technique as more sensitive than direct wet mount technique [14,21, 30, 31]

We also included the Modified Ziehl Neelsen staining technique and Modified Trichrome staining technique for better identification of the intestinal parasitic infections especially coccidean parasites and Microsporidia. Although we couldn't find Microsporidia spp. which may be due to difference in study population especially for immunocompromised patients. Furthermore, PCR is required for exact confirmation of screening findings.

Similar recommendations were suggested by Bhawna Kumari et al and also supported by various other studies [32-37]. All the studies done across India and outside India highlighted the significance of using Trichrome and Modified Ziehl Neelsens staining for the identification of intestinal parasites.

Multi-parasitism or presence of more than one species of intestinal parasite in a single specimen was seen in only 3.66 per cent (11 /300) of all stool specimens screened and comprised 14.66 per cent (11/75) of all positive specimens. Our finding is similar to the results of Praharaj Ira et al [6, 38].

The limitations of this study included the multiple sampling to increase sensitivity and lack of clinical correlation and molecular identification of protozoal parasites. These could not be done due to patient inconvenience and resource limitations.

Conclusion

The present study concluded an overall prevalence of 21% which supports the fact that intestinal parasitic infections are major public health problem in developing countries like India.

Data on prevalence are important to know the local magnitude of IPIs and plan effective preventive strategies especially among children who are at maximum risk. Such specific population groups at increased risk of IPIs should be the target for intervention measures such as chemoprophylaxis for treatment as well preventing transmission of infections. Although community-based prevalence data are most informative from a public health point of view, hospitals can also act as sentinel facilities because they capture greater morbidity and help provide essential data on these infections.

Apart from age-related trends, temporal trends and seasonality patterns might also be helpful in developing focused prevention programs against IPIs. The high prevalence of parasites like E. histolytica, G. lamblia, A. lumbricoides and Trichuris trichura signifies the need of health and hygiene education, public awareness regarding use of latrine, water

source protection from fecal contamination, proper sanitation and hygienic behavior along with the continuity to deworming program.

Authors' contributions

Adib khan was a major (main author/ 1st author) contributor in performing the stool examination , analyzing and interpreting the data and carrying out the tests and in writing the manuscript . Neetu shree was co author who helped in the manuscript and in the data interpretation . And, Shahin ansari helped in formating , editing and in generating the data. All authors read and approved the final manuscript

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