

Comparison of Detection of *Giardia lamblia* and *Entamoeba histolytica* Using Microscopy and Antigen Detection Rapid Cards in Patients with Diarrhea

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ABSTRACT

Background and objectives: Intestinal infections by protozoan parasites like *Giardia lamblia* and *Entamoeba histolytica* is still much prevalent, more so in developing countries. Microscopy remains the mainstay of its diagnosis despite its limitations. Therefore, we have tried to assess the usefulness of using antigen based rapid card test to that of the routine stool microscopy in this study and see its associated risk factors.

Materials and methods: Stool samples were collected from enrolled patients with history of loose motion. On these, routine stool microscopy was followed by a rapid combo test for antigen detection of *Entamoeba*, *Giardia* and *Cryptosporidium*. The associated risk factors of these patients were then checked and analyzed.

Results: A total of 2,434 stool samples were included in the study without duplication, of which 790 samples were found positive for an intestinal parasite by microscopy and 249 were positive by the rapid triage card test for one of the three intestinal protozoan parasites. Both cysts and trophozoites of *G. lamblia* and *Entamoeba* spp. were recovered from these samples. Stool microscopy for *Giardia* was positive in 17 samples and 60 for *Entamoeba* spp. However, by rapid card test, 114 samples were found to be positive for *G. lamblia*, 129 for *Entamoeba* spp. and 51 for *Cryptosporidium* spp. The additional detection of these intestinal pathogen by the rapid cards were found to be statistically significant ($p < 0.005$).

Conclusions: The results show that routine screening for intestinal parasitosis is important as many persons can have silent infection. In addition, a combination of another test especially rapid test along with microscopy helps to identify such cases.

Keywords: Diarrhea, *Entamoeba histolytica*, Giardiasis, Malabsorption, Rapid test.

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INTRODUCTION

Intestinal parasitic infections are one of the major causes of morbidity and mortality worldwide. Amebiasis, giardiasis, hookworm infection, ascariasis, and trichuriasis are the most common parasitic infections seen among the community. They are responsible for iron-deficiency anemia, chronic diarrhea, portal hypertension and malnourishment in children along with other comorbidities.¹ Giardiasis caused by *Giardia duodenalis* (otherwise, *G. intestinalis*, *G. lamblia*), is one of the established intestinal protozoan flagellate of the humans.²⁻⁴ Giardiasis is widespread globally and it is common in both children and adults. The prevalence of *Giardia* infection is more in underdeveloped and developing countries. Poor socioeconomic conditions such as inadequate sanitation, poor hygienic practices and illiteracy are identified risk factors for intestinal parasitic diseases in the communities.⁵ World Health Organization has included *giardiasis* in the "neglected diseases initiative".⁶ The life cycle of *Giardia* species involves two forms of the parasite such as trophozoite and cystic forms. *Giardia* transmission occurs through the ingestion of the infective cyst stage shed in human or animal feces.⁷ The cyst has potential to remain infectious for several weeks.³ Another important protozoan parasite also follows a similar pattern but causes invasive diarrhea is *Entamoeba histolytica*.

The mode of transmission of both is via fecal-oral route by ingestion of cysts. After ingestion of cysts, the incubation period is approximately 9–15 days.⁸ The clinical manifestations for giardiasis vary from asymptomatic to acute watery, foul smelling diarrhea, nausea, epigastric pain and weight loss.⁸ Although many acute infections may cure spontaneously, it may also lead to the development of subacute or chronic infections.⁹ *E. histolytica* usually

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presents with an invasive disease mostly in the large intestine as amebic dysentery or if it spreads, as abscesses in liver, lungs or rarely in the brain.

Giardiasis as well as amebiasis can be identified by a simple stool microscopic examination. However, no single test or combination of two or more methods can detect all infections. To maximize the diagnostic sensitivity, three stool samples collected on alternate days is recommended due to intermittent shedding of *G. lamblia* and *E. histolytica* in stool.¹⁰ However, continued negative stool examination do not rule out both these infections. In addition, microscopy is not able to differentiate between the trophozoites/cysts of *E. histolytica*/*E. dispar*/*E. moshkovskii*. The purpose of this study is to evaluate the distribution of symptomatic as well as asymptomatic giardia and entamoeba infections and factors associated with it.

MATERIALS AND METHODS

Setting and Study Design

It is an observational study conducted in a tertiary care hospital in south India over a period of 4 years.

Inclusion Criteria

A cohort study carried out in a tertiary care hospital in south India from January 2016 to December 2019. Stool samples collected for suspected intestinal parasitic infections with loose motion from various departments like medicine, surgery, pediatrics, pulmonary medicine, gynecology, were examined microscopically by wet mount examination using direct saline and iodine mounts. They were also stained with permanent stool stains like trichrome and examined. Formal ether sedimentation concentration technique was also performed on the samples to check for any missed out intestinal parasitic cysts and eggs and examined microscopically. After this procedure, antigen detection triage cards (CerTest *Crypto + Giardia + Entamoeba* combo card, CerTest Biotech, Zaragoza, Spain) were used to test all the stool samples. This rapid card can detect *E. histolytica*, *G. lamblia* and *Cryptosporidium* spp. at one-step in the stool sample. Other species like *E. dispar/E. moshkovskii* believed to have controversial role in causing amebiasis can only be confirmed by polymerase chain reaction (PCR). Therefore, those samples coming positive for *Entamoeba* spp. by the rapid card test were confirmed by PCR for the species.

Exclusion Criteria

Only one stool sample from each patient was taken. Repeated stool samples from which *Giardia* and *Entamoeba* were already identified was excluded from the study during this period.

All results were calculated as means or percentages. A value of $p < 0.005$ was taken as significant.

RESULTS

The total number of stool samples received in the Microbiology laboratory was 2,434. Total of 790 samples were found positive for an intestinal parasite by microscopy and 249 were positive by the rapid triage card test for one of the three intestinal protozoan parasites (Table 1). Of the total samples positive, stool microscopy for *Giardia* was positive in 17 samples. All the positive samples have cyst forms with three samples having trophozoite forms as well (Fig. 1). Among the positive cases, six cases were from pediatric age group. Fourteen out of 17 positive cases were from rural areas. Most of the patients were from medical gastroenterology department. Among these positive patients, three were symptomatic and detected while doing the routine investigations for other health issues.

Microscopy for *Entamoeba* spp was positive in 60 cases during the study period. All the positive samples have cyst forms among

which five samples have trophozoite forms as well. Forty-five out of 60 positive cases were from rural areas. Most of the patients (71%) were from medicine ward admitted with complaints of loose stools sometimes mixed with white mucus, whereas the remaining were distributed among the other departments which we have included in our study. Of these 60 positive samples with *Entamoeba* infection, one sample was found to have *Entamoeba* spp. and *Giardia* sp. coinfection. All the positive cases were identified by direct wet mount preparation of stool samples by saline and iodine mount followed by trichrome staining. Besides these two protozoan parasites, other intestinal parasites which were recovered include eggs of *Ascaris lumbricoides*, hookworm, *Enterobius vermicularis*, larvae of *Strongyloides stercoralis*, oocysts of *Cryptosporidium* spp., *Cystoisospora belli* etc. by microscopy. The distribution of positivity for these two parasites seen year wise is given in Table 2.

However, by rapid card test, a total of 114 samples were found to be positive for *G. lamblia* whereas 129 were found positive for *Entamoeba* spp. and 51 for *Cryptosporidium* spp. (Table 1 and Fig. 2). All samples positive for microscopy were rapid card positive. These were found to be statistically significant ($p < 0.005$). These 129 samples positive for *Entamoeba* spp. were confirmed using our laboratory standardized protocol of nested multiplex PCR for *E. histolytica/E. dispar/E. moshkovskii*.¹¹ It was seen that of the total 129 samples positive by rapid card test, 87 of them were confirmed as *E. histolytica*, 31 as

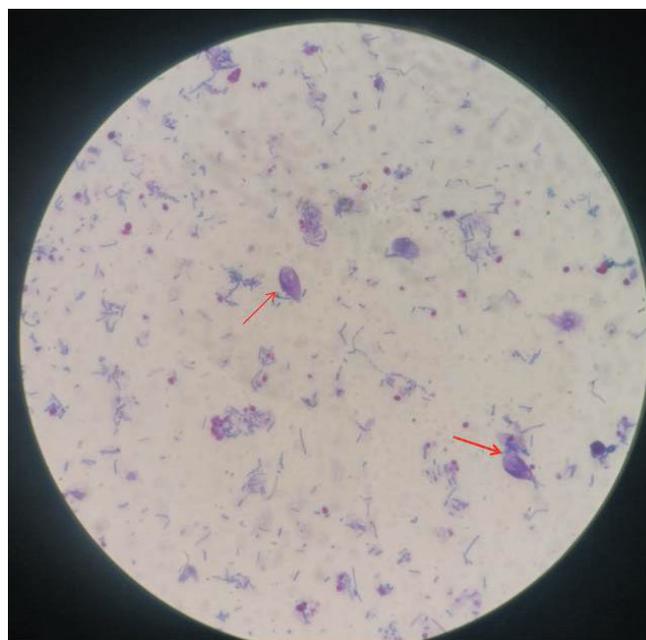


Fig. 1: *Giardia lamblia* trophozoites in trichrome stain smear

Table 1: Positivity of the intestinal parasites by microscopy and triage card test

Year	Total samples	Total positives	Positive for <i>Giardia</i>		Positive for <i>Entamoeba</i>		Others
			Microscopy	Rapid card	Microscopy	Rapid card	
2016	686	181	3	25	18	27	108
2017	651	242	4	51	31	46	110
2018	622	197	7	26	10	41	113
2019	475	170	3	12	1	15	139
Total	2434	790	17	114	60	129	490

Table 2: Yearwise distribution of *Giardia lamblia* and *Entamoeba* spp. positive samples by microscopy

Year	Total number of stool samples	<i>Giardia</i> spp.	<i>Entamoeba</i> spp.
2016	686	3	18
2017	651	4	31
2018	622	7	10
2019	475	3	1


Fig. 2: Detection of the three intestinal parasites using rapid test (A) shows the negative test; (B) shows a sample testing positive for *Giardia lamblia* antigen

E. dispar and 11 as *E. moshkovskii*. This is also an important finding, which creates a big impact on the further management of the patient.

It was seen that associated comorbidities like irritable bowel syndrome, malabsorption, and growth stunting were seen in nine patients among those whose stool sample were positive for *G. lamblia*. Similar observation was also seen with those positive for *Entamoeba* spp. by microscopy but were not found to be statistically significant ($p = 0.016$).

DISCUSSION

This study aimed to estimate the frequency, epidemiology and associated factors of *Giardia* and *Entamoeba* infections. We performed a stool survey in a tertiary care hospital in south India from January 2016 to December 2019. We aimed at procuring the socio-demographic data from these patients. The identification of *Giardia* spp. in stool samples by microscopy is the gold standard method for the diagnosis of giardiasis.¹² Stool examination, because of its simplicity, has an important place in the diagnosis of giardiasis, especially in regions with high infection rates and limited diagnostic facilities.¹³ In this study; fecal samples were processed using floatation and sedimentation methods. The overall positivity of the infection by microscopy was 0.7% during the study period. However, a total of 114 were positive for *Giardia* antigen by rapid triage cards. These were seen even in patients who were asymptomatic. This is a significant finding in our study as this infection may be seen among the asymptomatic too.

In the vulnerable target group of children under five years of age, *G. lamblia* is the third most common agent of diarrheal disease.¹⁴ Infection with *Giardia lamblia* has also been linked to the development

of irritable bowel syndrome and chronic fatigue.¹⁵ The prevalence of giardiasis in humans ranges from 2 to 3% in industrialized countries, up to 30% in low-income and developing countries.¹⁶

People living in rural or under developed areas are more prone to the ingestion of infective form of parasites as compared to those who live in urban/suburban or well developed areas.¹⁷ A study by Fantinatti et al.¹⁸ attributed the high prevalence of giardiasis in study population to multiple factors such as poor sanitation and occurrence of anthro-po-zoonotic transmission cycles. In our study, 83% of the patients with *Giardia* infection are from rural areas. In a study by Quihui-Cota et al.¹⁹ 23% of giardiasis was prevalent in pediatric age group. Also, in a study by Mukherjee et al.²⁰ no significant association was found with seasonality which is similar to our study.

In case of *E. histolytica* infection, overall positivity of infection was 3% by microscopy during the study period, which is lesser than other parts of country. Studies from different geographical areas of the world reported that intensity of intestinal infection including *E. histolytica* was significantly higher among children,^{21,22} which is in contrast to our findings where majority of positive cases were from adult patients. Similar picture is seen among those patients who tested positive for *Entamoeba* spp. by rapid triage test. Seasonality has not much effect on the positivity of the patients and infection but it has more to do with the sanitation condition of the household and the surrounding areas ($p < 0.001$).

CONCLUSION

Routine screening of intestinal parasites is important to detect silent infections. This will be more beneficial for those having underlying comorbidities or conditions or treatment, which induces immunosuppression.

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