Original Article

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Microbiology Section

The Prevalence of Intestinal Parasitic Infestations and the Evaluation of Different Concentration Techniques of the Stool Examination

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ABSTRACT

Aims and Objectives: The intestinal parasitic infections which are prevalent in the developing countries may even be more important than the bacterial infections. In India, malnutrition, unhygienic conditions, the improper disposal of sewage, the non-availability of potable water supplies in the rural and the urban areas, the indifferent attitude of the population towards personal hygiene, their low socio-economic status and the low literacy rates are responsible for the high rates of intestinal parasitic infections.

In view of the above facts, the present study was undertaken to assess the prevalence of the intestinal parasitic infections in the urban and the rural populations which came under a tertiary care teaching hospital.

Material and Methods: A total of 1000 stool samples were collected from the rural and the urban populations and each stool sample was examined by: 1. Gross examination 2. Direct microscopic examination by using saline and iodine preparations and by 3. Concentration techniques like simple slat flotation, Zinc sulphate centrifugal floatation, formol-ether concentration and modified formol-ether concentration.

Results: The prevalence of the intestinal parasitic infections was higher in the rural population. A male predominance was noted (33.29%) in both the populations. Children who were between 10-20 years of age had the highest prevalence of the parasitic infestations.

The common parasite which was isolated from both the populations was Entamoeba histolytica, with a prevalence rate of 65.57%, followed by Ascaris lumbricoides.

Conclusion: The modified formol-ether sedimentation procedure showed a high sensitivity for the parasitic detection. The supplementation of the routine method with floatation and the sedimentation technique will improve the diagnostic accuracy when this is compared to the routine method alone.

Key Words: Concentration techniques, Simple salt floatation, Zinc sulphate centrifugal floatation, Formol-ether concentration, Modified formol-ether method

INTRODUCTION

Parasitic infections are a major public health problem worldwide; particularly in the developing countries [1]. The prevalence of the intestinal parasitic infections varies from one region to another and it also depends largely on the diagnostic methods which are employed and the number of stool examinations which are done. In India, malnutrition, unhygienic conditions, the improper disposal of sewage and the non-availability of potable water supplies in the rural and the urban areas are responsible for the high rate of intestinal parasitic infections [2]. Globally, as many as 500 million people may harbour Entamoeba histolytica and several tens of thousands die each year as a consequence of fulminating colitis or amoebic liver abscesses [3]. The number of people who are affected by Giardia lamblia, whipworm, roundworm and hookworm in the developing world has been estimated to be 200, 500, 700 and 800 million respectively [4].

The conventional methods which are used for the detection of intestinal parasites from stool include the direct wet mount and the iodine mount. The conventional methods lack sensitivity in the detection of parasites in the stool specimens. The detection of parasites in the faecal specimens is enhanced by the use of concentration procedures. Various concentration techniques like simple slat floatation, Zinc sulphate centrifugal floatation, formol-ether concentration and modified formol-ether concentration are employed for the diagnosis and the epidemiologic surveillance of parasitic infections in humans. These techniques increases the detection of the helminthic eggs, larvae and the protozoan cysts. Certain techniques like formol-ether concentration have the advantages of less alteration to the organisms and an increased recovery of the Schistosoma spp. and operculated eggs. In view of the increasing polyparasitism in the developing countries, there is a need of sensitive diagnostic tools that are simple to apply and to concurrently detect different intestinal parasite species in the same stool sample.

The objectives of this study were to determine the prevalence of intestinal parasitic infections among the patients who attended a tertiary care teaching hospital and to compare 4 different concentration techniques with the conventional technique in diagnosing parasitic infections.

MATERIALS AND METHODS

A total of 1000 patients, both symptomatic and asymptomatic, were studied for 2 years from July 2009 to July 2011. Fresh stool specimens from 500 patients who attended the tertiary care teaching hospital, Mahadevappa Rampure Medical College, Gulbarga and 500 specimens from the Rural Community Health Centre, Rajapur, Gulbarga District, were collected in sterile containers and transported to the Department of Microbiology immediately. The stool samples which were contaminated with the patient's urine were rejected. The stool samples from the patients who had history of ingestion of kaolin, magnesia, powdered aluminum, barium, bismuth or iron were rejected. Both the formed and the unformed stools were examined freshly.

Each stool specimen was examined by the following techniques.

1. Macroscopic examination: The colour, consistency and the nature of the faeces were recorded. 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 or with the aid of a hand lens.

2. 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 saline or iodine solution. A cover slip was placed on it by taking care that the preparation was free of air bubbles and macroscopic debris.

3. The microscopic examination after the various concentration techniques:

a) Simple salt floatation: Briefly, about 1gm of faeces was emulsified with 3-4 ml of saturated salt solution in a 20ml conical glass test tube. It was stirred well and more salt solution was added till the container was nearly full, with the stirring being continued. Any coarse matter which floated up was removed and the tube was placed on a levelled surface with a glass slide being placed over the top of the tube, which was in contact with the fluid. It was allowed to stand for 30 minutes. The slide was removed and observed for the presence of eggs/cysts.

b) Zinc sulphate centrifugal floatation: 1g of the stool specimen was emulsified in 10 parts of tap water and it was strained through a wire gauze. The filtrate was collected in a Wassermann tube and centrifuged at 2,500 rmp. The supernatant was discarded and the sediment was re-suspended in water. This step was repeated till the supernatant became clear. To the sediment, 3-4 ml of 33% Zinc sulphate solution was added, it was mixed well and it was filled with ZnSO4 solution, about half an inch of the rim. Several loopfuls of the supernatant fluid were removed with a bacteriological loop and they were observed for parasites.

c) Formol-ether concentration: 1g of stool was emulsified in 7ml of 10% formol saline and it was kept for 10 minutes for fixation. It was then strained through a wire gauze. The filtrate was added to 3 ml of ether and it was centrifuged at 2000 rpm for 2 minutes. It was allowed to settle. The supernatant was removed and a wet mount was made of the deposit to look for parasites.

d) Formol-ether concentration which was modified by Allen and Ridely: 5 It was a modification of the formol-ether method where the centrifugation was done at 3000 rpm for 60 seconds instead of 2000 rpm for 2 min. The sediment was used for the parasitic examination.

RESULTS

A total of 1000 stool samples were examined, out of which 276 (27.6%) samples were positive for intestinal parasitic infestation, as was observed by the different parasitic diagnostic methods. A total of 132 (26.4%) out of the 500 stool samples from the tertiary care teaching hospital and 144 (28.8%) out of the 500 stool samples from the Rural Community Health Centre were positive for parasites [Table/Fig-1].

Overall, the prevalence of parasitic infections in males and females was 33.39% and 21.29% respectively [Table/Fig-2]. Children who were between 10-20 years of age had the highest prevalence of the parasitic infestations [Table/Fig-3].

Dual infections were seen in 55/276 patients. The most common dual infection was the infestation of the Entamoeba histolytica cysts with Ascaris eggs. Two patients showed triple parasitic infections. Both of them were infested with Entamoeba histolytica cysts, Ascaris eggs and Taenia spp eggs.

Routine diagnostic methods like wet and iodine mounts poorly

Parasite	Tertiary care hospital	Rural health center	Total	%
Entamoeba hystolytica Trophozoites and cysts	86	95	181	65.57%
Giardia cysts	12	12	24	8.69%
Ascaris lumbricoides eggs	17	18	35	12.68%
Hookworm eggs	11	13	24	8.69%
Taenia spp	3	2	5	1.81%
Hymenolepis nana eggs	1	2	3	1.08%
Trichuris trichura eggs	1	1	2	0.72%
Enterobius vermiculars	1	0	1	0.36%
Strongyloides larva	0	1	1	0.36%
Total	**132**	**144**	**276**	

[Table/Fig-1]: Prevalence of parasitic infestation

Hospital	Male		Female		Total	
	No	%	No	%	No	%
Tertiary Care Teaching Hospital	87/297	29.29	45/203	22.16	132/500	26.4
Rural Community Health Centre	87/224	38.83	57/276	20.65	144/500	28.8
Total	**174/521**	**33.39**	**102**	**21.29**	**276/1000**	**27.6**

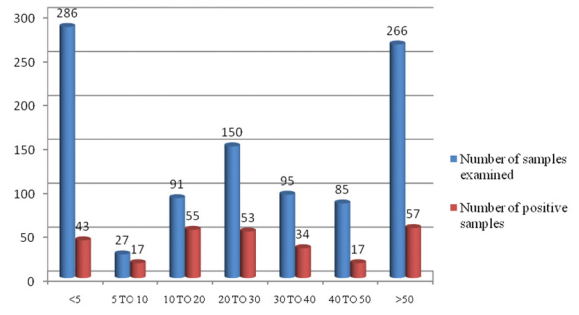
[Table/Fig-2]: Sex wise distribution of parasitic infections

Age	Rural health center			Tertiary care hospital		
	No	Positive	%	No	Positive	%
<5	144	24	16.66	142	19	13.38
5 to 10	15	9	60	12	8	66.66
10 to 20	43	27	62.79	48	28	58.33
20 to 30	71	26	36.61	79	27	34.17
30 to 40	49	18	36.73	46	16	34.78
40 to 50	41	9	21.95	44	8	18.18
>50	137	31	22.62	129	26	20.15
Total	**500**	**144**		**500**	**132**	

[Table/Fig-3]: Showing age wise prevalence of parasitic infections

Procedure	No positive for parasites (Total No 276)	%
Routine wet and iodine mount	105	38
Simple salt floatation	116	42
Zinc sulphate centrifugal floatation	152	55
Formol-ether concentration	157	56.88
Formol-ether concentration modified by Allen and Ridely 5	179	64.85

[Table/Fig-4]: Sensitivity of different parasitic examination methods



[Table/Fig-5]: Age wise prevalence of parasitic infections

demonstrate parasitic infections with a sensitivity of 38% (105/276). The modified formal ether concentration technique was found to be most sensitive method in this study. The method could demonstrate 179/276 (64.85%) parasitic infestations [Table/Fig-4].

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. Various studies have shown different prevalence rates of the parastitic infestations in different parts of India. But most of the studies had less sample sizes. In this study, 1000 samples were included, both from the rural and the urban areas to know the prevalence of the disease.

The present study showed that the rural population had a higher prevalence of the parasitic infections (28.8%) as compared to the urban population (26.4%), with a p value of >0.05. This finding was in agreement with the findings of Marothi Y et al., [6]. The most common parasitic infestation was that of Entamoeba hystolytica (65.57%), followed by that of Ascaris lumbricoides [Table/Fig-1]. This finding was comparable to the results of Marothi Y et al., [6] and Bisht D et al., [7]. The prevalence of parasitic infestations was more common in males (33.39%) as compared to that in females (21.29%, [Table/Fig-2]. Marothi Y et al., [6] showed that the infestations had a female preponderance. Various studies have shown the varying sex prevalence of the parasitic infestations. 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., [8], in their study, showed that he commonest parasitic infection was Hookworm (61.5%), followed by Giardia (53.8%) and Cryptosporidium (39.7%). But the present study did not show any Cryptosporidium spp in any sample. The results of Kang G et al., [8], showed that older children and adults had a higher prevalence of parasitic infections as compared to preschool children. These results were comparable to those of the present study [Table/Fig-5]. The prevalence of dual infections was high in the present study (19.9%) as compared to that in Marothi Y et al' study et al., [6] (1%). The numbers of the triple parasitic infestations were also high in the present study (0.7%) as compared to that in Marothi Y et al., study [6]. The maximum number of parasites which was shown in a single sample was 3 (Entamoeba histolytica cysts, Ascaris eggs and Taenia spp eggs).

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: helminth eggs and larvae; cysts of Giardia lamblia, Entamoeba histolytica / Entamoeba dispar, Entamoeba coli, Endolimax nana, Blastocystis hominis and Iodamoeba butschii; and the oocysts of Isospora belli. The present study showed that there was a significant increase in the number of parasites which were detected by following the application of the concentration methods. The inclusion of two or three different concentration techniques with different principles into the routine diagnostic tests increased the sensitivity. Moges F et al., [9] compared the formol-ether concentration technique with the routine iodine preparation and the Formol acetone concentration techniques. They showed that the formol-ether concentration technique was more sensitive as compared to the other methods. The present study found that 64.5% of the cases were detected by the formol-ether method, thus making it the most sensitive method. The inclusion of the modified formol-ether and the simple salt flotation techniques in the routine practice increases the sensitivity of the parasite detection. All the three methods are cost effective and they can be performed in rural settings with minimum basic infrastructures.

CONCLUSION

The prevalence of parasitic infections remains high. Because of malnutrition, unhygienic conditions, the improper disposal of sewage and the non-availability of potable water supplies in the rural areas, the prevalence of the parasitic infections remains high. The modified formol-ether technique and the simple salt flotation technique can be used in combination to increase the diagnostic sensitivity.

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