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Stool analysis results to know and ensure the prevalence of common types of parasites in pediatric patients of C.N.B.C.A.K of M.G.M Medical College, Indore- A Tertiary care centre

Authors
Dr Shailendra Singh Thakur[1], Dr Vipin Todase[2]
[1]Assistant Professor, Dept of Pathology, M.G.M Medical College, Indore, M.P state, India
[2]Senior Resident, Dept of Pathology, M.G.M Medical College, Indore, M.P state, India
*Corresponding Author
Dr Vipin Todase
Email: *vips.todase@gmail.com*

Abstract
The objective of the current study was to determine the incidence of common types of parasites encountered in pediatric patients of Chacha Nehru Bal Chikitsalaya and Anusandhan Kendra of M.G.M Medical College, Indore. The current study is a retrospective study which includes the results of 1068 stool sample and occult blood sample. The results obtained during last two years (2016–2017), were compared to the earlier reports of other regions. Attempts were made to find out the cases and trends of parasite incidence and to locate any differences between the current study results and the earlier reports.
Keywords: *stool, parasite , pediatric, concentration technique.*

Introduction

Stool analysis is a simple, common laboratory test done in pediatric patients to screen for parasites in cases of diarrhea and other gastrointestinal disorders. Sometimes the test is used to confirm the presence of a specific parasites related to a specific clinical situation like the Schistosoma parasite in cases of hepatosplenomegaly or hookworm parasite in case of iron deficiency anaemia[1,2,3] (WHO, 1991; Kochhar, 2004; Halton et al., 2005). Parasitic infections are a major public health problem worldwide; particularly in the developing countries[4]. The parasites commonly found in stool specimen are protozoa, trophozoites or cysts, complete worms or segments of worm can be seen [5,6]. (Cox, 1993; Panjarathinam, 2007). However, in cases of Strongloides stercoralis worms ova or larvae are known to be present [7] (Iqbal, 2008).

Stool sample can also be examined for occult blood especially to confirm or exclude more serious gastrointestinal problems like duodenal or gastric ulcers or gastrointestinal malignancies. 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 [8].

Several studies conducted on stool smear stained with eosin, iodine and Zehl-Nelson stains, further showed the presence of Entamoeba histolytica, Entamoeba coli, Giardia lamblia and Cryptosporidium parvum. Other intestinal parasite found including: Ascaris lumbricoides, Trichuris

trichiura, Schistosoma mansoni, Hymeolepis nana, Ancylostoma duodenale, Enterobius vermicularis, Taenia saginata and Schistosoma hematotium [9] (Abdel-Hafez et al., 1986). In the present study, the prevalence of common types of intestinal parasites encountered in hospital is studied and compared with earlier reports.

Materials and Methods

A total of 1068 patients, both symptomatic and asymptomatic, were studied for 2 years from July 2016 to July 2017. Fresh stool specimens from 500 patients who attended the Chacha Nehru Bal Chikitsalaya and Anusandhan Kendra of M.G.M Medical College, Indore, Madhya Pradesh and were collected in sterile containers. The stool samples which were rejected are those which contaminated with the patient's urine, 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.

Following techniques are used on stool specimen examined.

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: A small amount of stool sample was mixed in 1-2 drops of saline or iodine solution on a 1mm thick microscopic slide, a cover slip was placed on it by taking all necessary precaution.

3. The microscopic examination after the various concentration techniques:

a) Simple salt floatation: 3-4 ml of saturated salt solution and about 1gm of faeces was emulsified within a 20ml conical glass test tube, stirred well and with the stirring being continued more salt solution was added till the container was nearly full. 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.

Occult blood was tested by following the instructions and procedures given by the manufacturers as shown in the inserted leaflet of available kit. All Records were maintain and and the results were analysed using computer softwares.

Results

A total of 1068 stool samples were examined, out of which 309(28.9%) samples were positive for intestinal parasitic infestation, as was observed by the different parasitic diagnostic methods. Overall, the prevalence of parasitic infections in males and females was 54.3% and 45.6% respectively [Table-2].

Children who were between 10-15 years of age had the highest prevalence of the parasitic infestations [Table-3].

Dual infections were seen in 34/309 patients i.e 11 %. The most common dual infection was the

infestation of the Ankylostma duodenale with Ascaris eggs. Two patients showed triple parasitic infections. Both of them were infested with Entamoeba vermicularis cysts, Ascaris eggs and Ankylostma duodenale. Routine diagnostic methods like wet and iodine mounts poorly demonstrate parasitic infections with a sensitivity of 40.77% (126/309).

The formal ether concentration technique was found to be most sensitive method in this study. The method could demonstrate 173/309 (55.98%) parasitic infestations [Table/Fig-4][10].

Table 1 Prevalence of parasitic infection

Parasite	Total	Percentage
Ankylostoma duodenale eggs	192	62.14
Enterobius vermiculars	54	17.49
Entamoeba hystolytica Trophozoites and cysts	19	06.14
Ascaris lumbricoides eggs	32	10.36
Giardia cysts	10	03.23
Taenia spp	00	00
Trichuris	02	0.64
Strongyloides larva	00	00
Total	309	

Table 2 Gender wise distribution of parasitic infection

.N.B.C. and A.K. Hospital, Indore	Male	Female	Total	Percentage of infected cases
Total no of cases	561/1068	507/1068	1068	309/1068= **28.93%**
Percentage	52.53	47.47	100	
No.of Infected cases	168/309	141/309	309	
Percentage	54.37	45.63	100	

Table 3 Age wise distribution in pediatric patients.

Age	Total number of cases	Positive cases	Percentage
1 – 5 year	437	128	29.29
5-10 year	347	103	29.68
10-15 year	284	78	31.45
Total	1068	309	28.93

Table 4 Sensitivity of procedures done

Examination procedure done	Number of positive cases for parasite(total number 309 cases)	Percentage
Routine wet and iodine mount	126	40.77
Simple salt floatation	142	45.95
Zinc sulphate centrifugal Floatation	157	50.80
Formol-ether concentration	173	55.98

Discussion

The data on their sensitivity and prevalence of the diagnostic methods help the consulting doctor and the pathologist in the diagnosis and the management of the patients. Studies in different regions of India shows different prevalence rates of the parasitic infestations in different parts of India. Parasitic infestations are the major causes of morbidity and mortality in developing countries like India. But majority of the studies had less sample sizes. In this study, 1068 samples were included among the patient attending the C.N. B. C and A.K of M.G.M. medical college, Indore.

The most common parasitic infestation was that of Hookworm i. e ankylostoma duodenale (65.57%), followed by that of Ascaris lumbricoides [Table -1]. This finding was comparable to the results of Bisht D et al.[12]

The prevalence of parasitic infestations was more common in males (54.37%) as compared to that in females (45.63%) [Table-2]. Marothi Y et al.[11] 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.[13] In their study, showed that the commonest parasitic infection was Hookworm (61.5%), which relate to our study, followed by Giardia (53.8%) and Cryptosporidium (39.7%).

The prevalence of dual infections in the present study (11%) as compared to that in Marothi Y et al study et al.(1%). The numbers of the triple parasitic infestations were also high in the present study (0.6%) as compared to that in Marothi Y et al. study[11]. The maximum number of parasites found in a single sample was 3 (Entamoeba histolytica cysts, Ascaris eggs and ankylostoma duodenale).

In humans, the diagnosis of parasitic infections is difficut and it requires experience and skills to identify and to differentiate them from one another. The routine diagnostic procedures lack sensitivity.

For the examination of parasites concentration methods should be performed routinely. Concentration permits the detection of the organisms which are present in small numbers: these may be missed by using direct wet mounts. Generally, by using concentration procedures, the organisms that can be identified include: helminth eggs and larvae; cysts of Giardia lamblia, Entamoeba histolytica / Entamoeba dispar, Entamoeba coli, Endolimax nana, etc.

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.[14] compared the formol-ether concentration technique with the routine iodine preparation and the Formol acetone concentration techniques. The present study found that 55.98 % of the cases were detected by the formol-ether method, thus making it the most sensitive method. Moges F et al. [14] showed that the formol-ether concentration technique was more sensitive as compared to the other methods. 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 settings with minimum basic infrastructures.

Conclusion

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

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