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Status of *Giardia* Infection with Other Intestinal Parasites among Paediatric Patients in Tertiary Medical College Hospitals

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Abstract

Background: Paediatric diarrhoea, the burning issue of nowadays, drastically affects the child mortality and morbidity. Apart from virus and bacteria, parasites also play an important role in causing diarrhoea at that age group. Giardiasis, the most prevalent among the parasitic infections, though neglected yet carries significance because of its long term health issues. The aim of the study was to identify the intestinal parasites by wet mount preparation and further detection of the flagellated protozoan parasite, *Giardia* by special staining - Trichrome staining technique.

Materials and methods: It was an observational cross sectional study which was carried between the periods of July 2019 to June 2020, where a total of 200 diarrhoeic patients up to 18 years were enrolled. Direct microscopic method was done where parasites were detected by wet mount preparation and further Trichrome stainning was performed for the detection of *Giardia.*

Results: Out of 200 faecal samples, cysts of *Giardia,* ova of *Ascaris Lumbricoides* (AL) *Trichuris Trichiura* (TT) and both AL and TT were identified in 2.5%, 1.5%, 0.5% and 1.0% samples respectively by wet mount preparation. By Trichrome staining method we detected 3.5% of *Giardia* cysts.

Conclusion: Staining technique could be applied in addition to wet mount preparation of stool samples. So that we could recover the undetected *Giardia* cyst in the aforesaid samples.

Key words: Diarrhoea; *Giardia*; Parasites; Trichrome staining.

Introduction

Diarrhoea is the second leading cause of child mortality worldwide. Globally, there are nearly 1.7 billion cases of childhood diarrhoeal disease every year, resulting death of approximately 525,000 children each year.[1] One in ten child deaths result globally from diarrhoeal diseases before their 5th birthday, mostly occurring in sub-Saharan Africa and south Asia. In south Asia, diarrhoea accounts for 26.1% of childhood death with a peak incidence in their early years of life.[2] Diarrhoeal diseases are a major public health problem that particularly affects children in developing countries where insufficient access of sanitation, hygeine and portable water supply.[3]

Although enteric viruses and bacteria remain the predominant etiological agents, intestinal protozoal parasites are also significantly related to diarrhoeal diseases which include *Giardia duodenalis, Cryptosporidium* spp., *Entamoeba histolytica, Blastocystis hominis* and *Dientamoeba fragilis*. Certers for Disease Control and Prevention (CDC) has reported that the approximately 33% people suffers from giardiasis in developing countries.[4] Giardiasis is more prevalent especially in children living in low hygiene settings with poor quality of water and overcrowded places.[5] About 200 million cases of *Giardia*-related diarrhoea are reported every year especially among the infants, young children and young adults which is associated with stunting growth, malnutrition, poor cognitive function and deficiencies in micronutrients including vitamin A and iron that results in anaemia.[6] Due to its remarkable threat on public health, giardiasis was included in the "Neglected disease initiative" in 2004.[7] Moreover it is one of the important causes of infectious diarrhoea, yet it has been neglected for ages in our country and there was no such study done on this pathogen so far in Chattogram, a metropolitan city of Bangladesh. So we intended to identify the parasite in diarrhoeic stool of paediatric age group. Thus we could reveal the parasitic cause of paediatric diarrhoeal disease and cooperate the clinician to provide appropriate treatment accordingly which would indirectly compensate the indiscriminate use of antibiotics.

Materials and methods

This was an observational cross sectional study, conducted from July 2019 to June 2020 at the Microbiology laboratory of Chittagong Medical

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College (CMC) Chattogram. After approval by Ethical Review Committee of Chittagong Medical College, Chattogram, the stool specimen was taken from indoor and outdoor paediatric diarrhoeic patients of Chittagong Medical College Hospital and Chattogram Maa-O-Shishu Hospital Medical College, Chattogram. Informed written consent was obtained from the patients or guardian. A total of 219 paediatric patients (Up to 18 years) were randomly selected for the study. If a child had diarrhoea, defined as passage of loose stool for 3 or more times in 24 hrs (Excluding bloody diarrhoea) and the specimen was collected in a clean, leak proof, wide-mouth container labeled appropriately. Only one specimen was collected from each subject, immediately transported to the laboratory where the sample was separated into two containers: one contained 10% formalin preserved faecal sample for direct wet mount preparation and the other contained unpreserved faecal sample. After wards formol-ethar concentration technique was followed with this unpreserved sample prior to staining procedure.

Results

Out of 200 diarrhoeic faecal samples, wet mount preparation was done to all samples where cysts of *Giardia* were detected in 2.5% cases. Moreover, helminthic eggs like ova of *A. lumbricoides* (AL) were found in 1.5%, *T. trichiura* (TT) in 0.5% and both AL and TT were identified in 1.0% samples. Again each sample was stained by Trichrome staining method by which we detected 3.5% of *Giardia* cyst.

Table I Detection of parasite by wet mount preparation (n=200)

Parasites	Frequency	Percentage (%)
Giardia	05	2.5
A. lumbricoides	03	1.5
T. trichiura	01	0.5
Both *A. lumbricoides* & *T. trichiura*	02	1.0
No parasite	89	94.5
Total	200	100

Above Table I show the wet mount findings among study samples, no parasite was found in 189 (94.5%) samples. *Giardia* was found in 5 (2.5%) samples only.

Table II Detection of Giardia by Trichrome staining (n=200)

Trichrome staining findings	Frequency	Percentage (%)
Giardia (+ve)	07	3.5
Giardia (-ve)	193	96.5
Total	219	100

Table II represents the Trichrome staining findings among study population. Giardia was found in 7 (3.5%) samples.

Discussion

In developing countries, intestinal parasite like *Giardia* is one of the significant causes of diarrhoea in children.[8] Though infections due to this parasite are self-limiting in immunocompetent individuals, but chronicity may results into malnutrition, growth faltering, cognitive function impairment, especially in case of children.[5] Because of these alarming effects on child's health, it draws attention to find out the incidence of protozoan parasites responsible for childhood diarrhoeic disease.

In the present study, faecal samples were collected from paediatric diarrhoeic patients ranging from 1 month to 18 years old. At first all samples were examined for direct microscopic examination by wet mount preparation. Afterwards Trichrome staining was done to all samples for detection of *Giarrdia*.

Among 200 samples 2.5% cases were found *Giardia* positive by wet mount preparation. Based on microscopic examination, the prevalence of *Giardia* infection was 1.66% among 0 to >50 age group in Iran.[9] A study on paediatric patients (<5 years) in a tertiary hospital was done where *Giardia* cysts were found in 4.14% cases.[10] In a slum area of Bangladesh, Giardia positive samples were found in 6.01% cases among the school going children.[11] Another study conducted in India showed that Giardia was detected 15.5% by direct wet mount preparation which is much higher than the current study findings.[12] This dissimilarity may be due to the large sample size (n=1680) and large age group distribution (3 to 45 years). Geographic area may also be a factor for this difference.

In wet mount film, the study showed ova of AL 3 (1.5%), TT 1 (0.5%) and both AL and TT 2 (1.0%) were detected. A study reflected, 0.9% TT but no AL which was based on hospitalized paediatric diarrhoiec children (<5 year) that goes

in accordance to our study.[10] Another research demonstrated 0.6% of TT ova but AL was 9%.[13] Furthermore, it was reported that the prevalence of AL and TT ova was 8.2% and 0.9% respectively.[14] In our study relatively low numbers of helminthes were detected probably because of urban setting of the study population who had better sanitation and hygiene practices. Another cause could be ingestion of antihelminthic drugs at a regular interval.

By Trichrome staining technique, 3.5% cases were found positive in our study. A surveillance study was done in Iran where 3.8% prevalence rate of giardiasis was found by staining method which is somewhat similar with our study.[15] Among the outdoor patients of ICCDDR'B it was reported 0.37% Giardia positive cases were detected which is not in accordance with our findings.[16]

Some pitfalls are responsible for the small number of trophozoite and/or cyst detection in this study. Though microscopy is the most common and cost-effective method, but still it is labour some and much time-consumimg. As the procedure is influenced by individual's skill and experience, therefore this method is impractical to standardize.[17] Moreover low parasite count could be missed by this procedure. It's often indicated to collect at least three stool samples at alternate days because of intermittent shedding of protozoan cysts.[18] But it's difficult and inconvenient to go for three samples from the same patient. Though conventional microscopy of more than one faecal sample is still being recommended to diagnose intestinal protozoa in the stool samples, its sensitivity is still found to be low even after multiple examinations. Also the parasite might be disguised itself by bile pigment and not visualized by wet mount examination[12]. But this study was done with single faecal sample that is why low concentration of parasite could be missed through microscopy. These could be the reason behind their relative low rate of detection.

Limitations

- The current study was tertiary hospital based study rather than rural or urban slum based. For that reason the variation in detection rate of aforesaid parasites was observed.
- Single faecal specimen was examined from each patient.
- Small sample size could be another reason of detecting low number of intestinal parasites which could not be actual picture of the country.

Conclusion

It can be concluded that, apart from viral and bacterial etiology, *Giardia* along with other intestinal parasites can also contribute in paediatric diarrhoeal disease. By routine stool examination, it's quite difficult to find out all the parasites. In this aspect staining procedure like Trichrome staining, could be an additional procedure to overcome the *Giardia* cyst that are missed in wet mount preparation.

Recommendation

- Multicenter large scale study is recommended.
- PCR can be done in highly suspected cases that could be a good opportunity to reveal the prevalence of this protozoan intestinal parasite in this region. Moreover, it would be a great help for research purpose as well.

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Contributors of authors

NJN: Conception, design, acquisition of data, interpretation of data, manuscript writing and final approval.

AHMSKC: Acquisition of data, data analysis, critical revision of the version and final approval.

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