Original Article

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Microbiology Section

Intestinal Amoebiasis and Risk Factors Associated with its Transmission Among Paediatric Patients Attending a Tertiary Health Care Facility in Northern India

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ABSTRACT

Introduction: The occurrence of *Entamoeba histolytica* as a human intestinal parasite is a serious problem especially in developing countries. Despite the high prevalence of amoebiasis in various parts of India, unfortunately there is paucity of information with regards to the prevalence of intestinal amoebiasis in children.

Aim: To determine the prevalence of amoebiasis among children attending a tertiary care center in Lucknow and to study their socio-demographic profile.

Materials and Methods: This prospective cross-sectional study was carried out from January to June 2016, at Integral Institute of Medical Sciences and Research, Lucknow , Uttar Pradesh, India. Systemic random sampling was done to collect 185 stool samples from children aged 0-14 years before the institutional treatment. The stool samples were examined for cysts/trophozoites of *E. histolytica* using saline and iodine preparations. Statistical study of the data was done using chi-square test. The $p<0.05$ was taken as significant.

Results: A total of 31 of the 185 samples were positive, i.e., they contained cysts and/or trophozoites of *E. histolytica*. This study indicated that the prevalence of amoebiasis among children was 16.8% with an expected 95% confidence range of 11.4-22.2. Although, the number of males infected were more than the females, no significant association was found between prevalence and gender ($\chi^2=0.52$, $p=0.819$). Poor hygiene was significantly associated with the children suffering from intestinal amoebiasis ($p=0.021$). Significant association was also observed between lower socio-economic status and prevalence of *E. histolytica* among children.

Conclusion: The present study showed that intestinal amoebiasis is a major public health problem in children particularly in younger age group. Proper and effective diagnostic techniques for the detection of *Entamoeba histolytica* and treatment of infected individuals should be encouraged and made available in hospitals and rural health centers.

Keywords: *Entamoeba histolytica*, Parasite, Prevalence, Socio-demographic

INTRODUCTION

The occurence of *Entamoeba histolytica* as a human intestinal parasite is a significant issue particularly in developing countries. Several species of the genus *Entamoeba* infect humans. These include: *Entamoeba histolytica*, *Entamoeba dispar*, *Entamoeba coli*, *Entamoeba hartmani*, *Entamoeba polecki*, and *Entamoeba gingivalis* [1]. Among these, only *E. histolytica* is considered pathogenic and causes amoebiasis or amoebic dysentery [2].

While amoebiasis is rare in developed countries of the world yet the disease is common in the less developed and developing countries of the world. It is an important health problem, especially in developing countries [3]. This condition is more prevalent in certain areas of the world including West and South-East Africa, China, the whole South-East Asia, Mexican states and western parts of South America, and also the Indian subcontinent [4]. The high prevalence of *E. histolytica* infections is closely linked with poverty in these regions, poor environmental hygiene, poor personal hygiene, and poor health service providers having an inadequate supply of drugs and lack of adequate and proper awareness of the transmission mechanisms and life-cycle patterns of these parasites [5-7]. Children and pregnant women are mostly vulnerable to these infections [6]. Amoebiasis is the 3rd leading parasitic cause of death worldwide. It has an estimated worldwide prevalence of 500 million cases of symptomatic disease, and 40,000–110,000 deaths annually [8,9].

Despite the high prevalence of amoebiasis in various parts of India, unfortunately there is paucity of information with regards to the prevalence of intestinal amoebiasis in children in our area. Therefore this study was designed to access the prevalence of amoebiasis among children attending a tertiary care center in Lucknow, Uttar Pradesh and to study their socio-demographic profile. The outcome of the study would provide necessary information to the health care providers for prevention and control of intestinal amoebiasis.

MATERIALS AND METHODS

The present prospective study was conducted in the Department of Microbiology at Integral Institute of Medical Sciences and Research, Lucknow, Uttar Pradesh, India. The study lasted over a period of six months from January to June 2016. The study was approved by the Institutional Research Committee (IRC) and the Ethical Review Committee (ERC) with registration number IIMSR/IEC/2016/11.

Children aged 0-14 years that were in and out-patients of our hospital, constituted the study population. Before the commencement of the study, written consent was obtained from parents/guardians of the children. A well pre-designed questionnaire was used to obtain a detailed clinical history and various socio-demographic details such as age, gender, place of residence and socio-economic status. Apart from hand hygiene, sanitation and domestic hygiene was taken into account to measure hygiene status of study subjects. Accompanying parents/guardians who refused to give consent for their children participation were excluded from the study. Children who had taken antiparasitic drug during the last three months were also excluded from the study. A cross-sectional survey was conducted to ascertain the prevalence of *E. histolytica* in the children. Sample size of 185 was calculated on the basis of a recent similar study [10].

Systemic random sampling was done to collect 185 stool samples from children before the institutional treatment. No child had history of receiving anti-protozoan drugs within the past three months. A clean, dry, screw capped, wide mouthed plastic faecal container with scoop was provided to their parents and instructed on how to collect the samples. They were advised to collect 1-2 ml of faeces on the scoop without contaminating it with urine and water and insert it, into the bottle. All the containers along with specimen were properly labelled with the respective sample number and date. These samples were transported to the laboratory as soon as received, usually within two hours after collection.

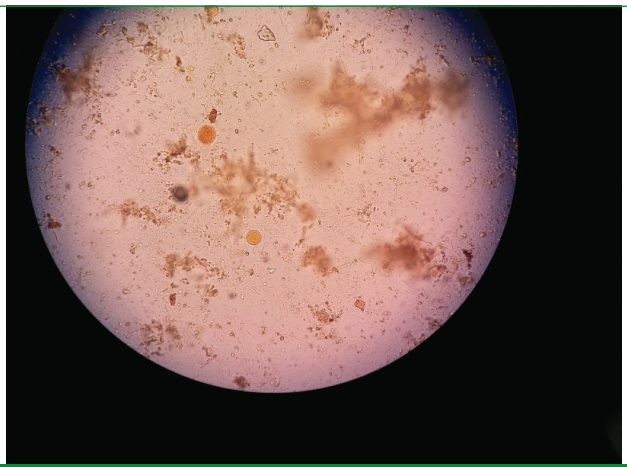
Sample Examination

All the faecal samples were first examined macroscopically for its colour, odour, presence of mucus, pus, visible parasites/segments and blood. The applicator sticks were used to test for consistency. Microscopic examination was subsequently done to examine cysts and trophozoites of *E. histolytica*, using normal saline and lugol's Iodine on grease free slides. The preparation was examined microscopically for the presence of trophozoites and cysts.

Formal ether concentration technique was used to concentrate cysts in the stool samples by the force of gravity. Such technique not only increases the number of parasites in the sediment but also making them more visible by removing organic and inorganic debris [11]. Stool sample was emulsified in 7 mL of 10% formal saline and was kept for 10 minutes. Then, it was passed through a wire gauge and filtrate collected in a centrifuge tube. Three milliliter of ether was added to it and the mixture was shaken vigorously for 1 min followed by centrifugation at 2000 rpm for 2 min. Then it was allowed to settle down. Debris was loosened with a stick; the upper part of the test tube was cleared of fatty debris. Supernatant fluid was decanted, leaving 1 or 2 drops. Deposit, after shaking, was poured on to a glass slide with the help of a fresh Pasteur pipette. A drop of iodine was put to aid the identification of cysts and covered with cover slip.

STATISTICAL ANALYSIS

Statistical study of the data was done using chi-square test. The $p<0.05$ was taken as significant.



[Table/Fig-1]: Iodine-stained wet mount showing cysts of *Entamoeba histolytica*.

RESULTS

The study was conducted on 185 samples collected from paediatric patients. A total of 31 of the 185 samples were positive, i.e., they contained cysts and/or trophozoites of *E. histoytica* [Table/Fig-1]. This study indicated that the prevalence of amoebiasis among children was 16.8% with an expected 95% confidence range of 11.4-22.2.

In the present study, out of 185 samples, 109 samples were collected from the inpatients with 17 (15.6%) positive samples and 76 were collected from outpatients with 14 (18.4%) positive samples. No significant difference was seen in proportion of *E. histolytica* in patients attending OPD and IPD units ($p = 0.613$).

Socio-demographic profile of all the study subjects was also studied. Though age-wise difference of positivity was not significant ($p=0.374$), the maximum number of children suffering from intestinal amoebiasis were in the age group of 5-10 years while least numbers were in the age group of under 5 years [Table/Fig-2]. In our study, the number of males infected was more than the females. Presence of *E. histolytica* was observed to be independent of gender. No significant association was found between prevalence and gender ($\chi^2=0.52$, $p=0.819$).

Age Group	Total	*E.histolytica*		Significance	
		No. Present	%	Chi-Sq	p-value
Under 5 years	82	11	13.4	1.968	0.374
5 to under 10 years	48	11	22.9		
10 and above	55	9	16.4		
Total	185	31	16.8		

[Table/Fig-2]: Age-wise distribution of *Entamobea histolytica* among pediatric patients.

Though area-wise difference of positivity was not significant in the present study, the maximum number of positive patients was residing in rural area followed by semi urban and urban area [Table/Fig-3].

Place	Total	*E.histolytica*		Significance	
		No. Present	%	Chi-Sq	p-value
Urban	25	2	8.0	1.699	0.428
Rural	129	24	18.6		
Semi Urban	31	5	16.1		
Total	185	31	16.8		

[Table/Fig-3]: Area-wise Distribution of *Entamoeba histolytica* among pediatric patients.

In the present study out of 185, 122 and 63 children were following poor and good hygiene practices respectively. *E. histolytica* was detected in 26/122 (21.3%) children with poor hygiene status whereas only 5/63 (7.9%) children with good hygiene status were found to be suffering from intestinal amoebiasis. Upon statistical analysis it was revealed that poor hygiene was significantly associated with the children harboring *E. histolytica* ($p=0.021$).

Study subjects were divided into socio-economic group on the basis of Modified BG Prasad's Socio-economic classification system. Significant association was also observed between lower socio-economic status and prevalence of *E. histolytica* among children. In the present study, most of the positive patients were from lower social class [Table/Fig-4].

Social Class	Total	*E.histolytica*		Significance	
		No. Present	%	Chi-Sq	p-value
Lower	122	26	21.3	5.328	0.021*
Middle	63	5	7.9		
Total	185	31	16.8		

[Table/Fig-4]: Association of *E. histolytica* with social class among paediatric patients.

DISCUSSION

Out of total 185 stool samples examined during the study period, 31(16.8%) cases of intestinal amoebiasis were found. The prevalence of *E. histolytica* recorded in this study is quite higher than those observed by some other researchers. Similar study carried out in Bareilly and Vadodara districts, low prevalence rates of 2.50% and 7.9% were reported respectively [12,13]. Similarly, Aher A and Kulkarni S in the year 2011 reported prevalence rate to be 3.9% in Ahmednagar district of Maharashtra [14]. Dhanabal J et al., from Chennai and Kaur R et al., from Delhi reported the prevalence rates to be 21.8% and 11% respectively, which are similar to the result of our study [15,16]. Study conducted by Bisht D et al., from Ghaziabad

showed the prevalence of *E. histolytica* to be 20.3% [17]. Prakash O et al., and Shrivastava JB et al., have also reported a high positivity rate of 35.6% and 18.4% respectively [18,19]. In another study conducted in urban slums of Lucknow, the presence of *E. histolytica* was found in 11.8% of the study population [20]. The prevalence rates of *Entamoeba histolytica* exhibit wide variation between geographic areas. Prevalence of 18.6% and 11% was reported in studies carried out in Nigeria and Degema respectively [21,22].

In our study, with respect to gender, male patients were more affected as compared to females. Males had prevalence rate of 17.20% (20/116) while females had the prevalence rate of 15.9% (11/69). This could be attributed to the fact that due to socio-cultural life style of the area, boys are more likely to interact with contaminated environment (food, water, among others) than girls. Similar findings have been reported by Yasmeen M and Singh S in the year 2015 [10]. Contrary to our finding, study conducted by Panda S et al., showed higher infectivity in females as compared to males [23]. Another study done by Nyenke C et al., showed females (12.3%) to be more infected than males (8.8%) [22].

Among age category represented in this study, *Entamoeba histolytica* was more prevalent among younger age group of 5-10 years (11/48, 22.9%) followed by 10-14 years (9/55, 16.4%). Similar findings have been reported by other researchers. If the host has increased resistance, it remains healthy and therefore the parasite is either driven away or assumes a benign relationship with the host, but if the host loses the competition, a disease develops [24]. Reuben CR et al., showed high prevalence of 31.0% among younger children between age group of 6-10 years whereas least level of infection was recorded among those within age groups of 11-15 and >16 years. This could be attributed to the fact that children within this younger age group are found to be playing on the soil with little or no care. They tend to be ignorant of the gains associated with general cleanliness and high level of personal hygiene. Lower rate of infestation in the older age group children could be due to the reason that they have better personal hygiene habits than younger children. They are quite matured and are so conscious of the need to take personal hygiene more seriously as compared to the other age [25]. The reason for the low occurrence of *E. histolytica* in under 5 children (11/82, 13.4%) can be attributed to their innate resistance due to the induced production of secretory immunoglobulin A (sig A) that can diminish the adhesion between *E. histolytica* trophozoites and epithelial cells, hence reducing new infection [26].

Socio-demographic and environmental factors were also studied in children. The prevalence of intestinal parasitic infestation depends upon various factors like, hygiene, availability of clean drinking water, poverty etc., [27]. Our study reflects that poor personal hygiene and low socio-economic status of the person aids in the transmission of parasite. Most of the positive patients were following low standards of personal hygiene (26/122, 21.3%) which was significantly (p=0.021) more than the subjects practicing good hygiene habits (5/63, 7.9%). Significant association was also observed between lower socio-economic status and prevalence of *E. histolytica* among children. In the present study, most of the positive patients were from lower social class (26/122, 21.3%). Lower income leads to poor living conditions and poor sanitary habits aggravating their wards growth and parasitic infections [28]. In our study maximum number of positive patients were residing in rural area (24/129, 18.6%) that was slightly more than semi urban (5/31, 16.1%) and moderately more than the urban area (2/25, 8.0%). Though area-wise difference of positivity was not significant (p = 0.428), this could be attributed to overcrowding and poor housing in the community which are predisposing factors for the spread of the infection [29].

LIMITATION

The major limitation of this study is inadequate sample size and short study period. Therefore data obtained is not representative of the total population but only of the study respondents. Apart from these limitations, carriers harboring *E. histolytica* could have been missed as we conducted study only on children presenting with some complaints. In future a similar study can be conducted with a larger sample size including asymptomatic ones, with a view to obtain data that could be generalized to the entire population.

CONCLUSION

The present study shows that intestinal amoebiasis is a major public health problem in children particularly in younger age group. Poor personal hygiene, rural residence and low socio-economic status are significantly associated with prevalence of *Entamoeba histolytica*. There is a need to promote more public health awareness programmes towards a better understanding of the source and adverse impacts of amoebiasis among children. Improvement of sanitation, provision of safe water, good toiletry habits and proper waste disposal would help in control of the disease. Proper and effective diagnostic techniques for the detection of *Entamoeba histolytica* and treatment of infected individuals should be encouraged and made available in hospitals and rural health centers.

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