Original Research Article

Study of prevalence of entamoeba histolytica in clinically suspected patients of dysentery in a tertiary care hospital, south Gujarat

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Abstract

Background: Entamoeba histolytica produces an invasive disease called amoebiasis that causes significant morbidity and mortality among high-risk groups. **Aims and Objective:** To assess the prevalence of Entamoeba histolytica in a suspected dysentery patients. **Methodology:** It was a cross –sectional study carried out for period of 2 months, a total of 63 samples were collected, from which 43 samples were taken from symptomatic patients and rest 20 samples were taken as control for test. Examination was done microscopically to detect the cyst of Entamoeba histolytica in lugol's iodine and normal saline mount and ELISA for stool antigen detection using Human Entamoeba histolytica antigen (EH Ag) ELISA kit (In Bios co. Cat No. MBS2607330) **Results:** From 43 samples, 27 were positive by ELISA (62.79%) and 14 samples were positive (32.55%) by microscopically. Because of the specificity of ELISA, sample positive by ELISA were considered as confirm positive cases. It is more prevalent in males and in age group of 0-10 years as compared to females and adults respectively. **Conclusion:** High prevalence in this area indicates that it is a major public health issue which needs to be looked after. It is necessary to develop some control strategies and effective prevention including public health education and environmental sanitation.

Key Word: E. histolytica, dysentery, risk factors, stool antigen, microscopic examination, ELISA.

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INTRODUCTION

Dysentery is a disorder characterized by frequent passage of watery stools containing blood and mucus. Amoebic dysentery is the infection of human intestinal and extra intestinal organs[1] caused by pathogenic strain of *E.histolytica*. It is a protozoan that is found worldwide and one of the major causative agent of dysentery.[2,3,4,5,6] Amoebiasis accounts for 3rd leading cause of death in humans after malaria and schistosomiasis. The prevalence of amoebiasis varies between the countries accounting for more than 10% in developed countries while between 50% prevalence is reported from developing countries.[7] The disease is more common in tropical regions of the world where hygiene and sanitary condition is often approximate.[8] It is highly endemic throughout the poor and socio-economically deprived communities. Poor hygiene, ingestion of raw material, compromised drinking water quality are some of the important risk factors. The genus *Entamoeba* contains many species, six of which (*E.histolytica, E.moshkovskii, E.poleckii, E.coli, E.hartmanni*) reside in the human intestinal lumen. Initially *Entamoeba histolytica* was thought to be a single species, but isoenzyme and molecular studies have led to the reclassification into two morphologically identical species: the pathogenic *E.histolytica* and the non-pathogenic *E.dispar*.[9] Global statistics on the prevalence of *E.histolytica* infection indicates that 90% of individuals remain asymptomatic while the other 10% develop clinically overt symptomatic diseases.The parasite host interaction determines the course of disease

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which ranges in spectrum from luminal colonization to amoebic colitis (dysentery or diarrhoea) and invasive extra intestinal amoebiasis, in which the liver abscess is the most common presentation.[8] It also causes abscess in lung, brain and skin infection etc.[10] The trophozoites have been found associated with the micro-ulcerations of the mucosa. Symptoms at this stage include non-specific colitis with edematous mucosa and hemorrhage. Ulcers are typically "flask-shaped". Ulceration of mucosa is the hallmark of invasive disease. Ulcers develop mostly in caecum and ascending colon.[11] Recently, it was found that the gut microbiome of the people with amoebic diarrhea is enchried in *Prevotellacopri*, indicating dysbiosis as one of the reason to the development of colitis.[12] Many tropical developing countries lack clean supply of domestic water as contamination may take place either at the source of water or at home due to poor sanitation. Another risk factor is the availability and usage of toilets. When defecation is done in open places, cyst of *Entamoeba histolytica* may be carried to water bodies or may be carried by mechanical vectors such as flies and contaminate food as well as water sources.[13] Various other factors such as overcrowding, insufficient education, poverty, contaminated water supply and unsanitary conditions contributes to the transmission of amoebiasis from person to person by feco-oral route. Therefore there is a need for its early and accurate diagnosis and for the prevention of its endemicity.[7] This parasite causes significant morbidity and mortality in the developing countries, accounting for about 50 million symptomatic cases and 100,000 deaths worldwide/year. In India, 15-20% of the population are affected.[14] However, the true distribution of the disease in most of the countries is not clear. This has been particularly difficult due to the existence of different species which are morphologically identical but genetically different namely *E.histolytica*, which is pathogenic. *E.moshkowskii* and *E.dispar*, which is non-pathogenic species. The differentiation of *E.histolytica* and *E.dispar* is necessary which will allow for the effective cure of patients with anti-amoebic drugs thus preventing the development of resistant types. It will also reduce the management costs and help to estimate the real prevalence of *E.histolytica*.[8,15] Traditionally the diagnosis of *Entamoeba histolytica* relies upon microscopic examination but due to its drawback of not being able to differentiate between pathogenic *E.histolytica* and non-pathogenic *E.dispar* there is a high degree of divergence from real prevalence.[7] Antigen-based enzyme-linked immunosorbent assays (ELISA) and PCR are more reliable to differentiate between the two thus making the correct diagnosis. Stool antigen assays have been reported to be better than microscopy with a sensitivity of 80-85% and specificity of 99% when compared with culture and isoenzyme analysis in areas of high endemicity.[5] PCR techniques are not widely available and even nonfeasible in developing countries. Therefore, stool ELISA is considered as a suitable substitute for the diagnosis of *E.histolytica* infections.[7] Most of the patients who attends our hospital are of rural and poor socio-economic class where sanitary hygiene are minimal. Intestinal parasitic infection is still a common and significant public health problem in this area.[16] Hence, early detection and differentiation of pathogenic *E.histolytica* from non-pathogenic strains by detection of antigen in stool plays a crucial role in clinical management of patients with amoebiasis.

AIMS AND OBJECTIVES

To assess the prevalence of Entamoeba histolytica in suspected dysentery patients.

MATERIALS AND METHODS

After taking the permission from Institutional Human Ethics Committee (IHEC), a cross-sectional study was carried out for a period of 2 months under ICMR-STS 2017.

For the selection of patients following criteria were used:

Inclusion Criteria

- Patients of all age groups having dysentery despite of its etiology

Exclusion Criteria

- Patients with known causes of blood in stools like rectal polyps, inflammatory bowel diseases, bleeding diathesis etc.
- Unlabelled and improperly labelled specimen
- Specimen that have leaked out of container
- Material collected insufficient in volume

The following data were recorded on admission for all patients-age, sex, residence, socio-economic status, source of water at home for drinking, treatment of drinking water, household sanitary and hygiene conditions, personal hygiene such as washing hands before eating and after excreta disposal. In the study a total of 63 samples were collected, out of which 43 were from clinically suspected patients of dysentery and 20 samples were taken as control for test, irrespective of age and sex, from OPD and wards of GMERS Medical College and hospital, Valsad. Fresh stool samples were collected and then transported to laboratory as soon as possible in sterile stool container without contamination with urine, water or disinfectant. All the samples were examined macroscopically for the presence of mucus and blood. General microscopic examination was done by using both normal saline and Lugol's iodine for the presence of cysts, trophozoites of Entamoeba histolytica

and charcot-Leyden crystals in fresh stool specimens. Samples were then tested for stool antigen using Human Entamoeba histolytica antigen (EH Ag) ELISA kit (In Bios co, Cat No. MBS2607330). The kit employs double sandwich ELISA technique. The minimum detectable amount of human EH Ag by this kit is up to 0.06 ng/ml(sensitivity) and it has no cross reaction with other factors(100%specificity). Testing method was performed according to kit manufacturer's instructions. Samples, reagents and standard were prepared according to kit insert and technical support from company.

Human EH Ag standard sample:

- Standard diluent 1.0 ml was added into human EH Ag lyophilized standard sample and was kept still for 30 min. After the sample was completely dissolved, it was slightly mixed and was marked label 1 on the tube, then dilution were done as per the need.(It was recommended in the kit instructions to use following concentration value to standard curve: 20,10, 5, 2.5, 1.25, 0.625, 0.312… ng/ml).

Legend of standard sample dilution method:

- Ten clean tubes were taken and were labelled[1,2,3,4,5,6,7,8, 9,10] respectively. Into each tube 300µl standard sample diluent was added. 300µl diluent was pipetted from tube1 to tube[2] and was mixed well. Further 300µl diluent was taken from tube[2] and added in tube[3] and was mixed well. Above steps were repeated up to tube[7]. As the kit is for quantitative detection we had use tube[8] and tube[9] for further dilution and Standard sample dilution in tube [10] was kept as negative control.

Result determination

1. Optical density (OD) value of each sample and specimen was minus with that of the blank well. (OD values of standard sample used for preparation of curve and negative control shown in table 1)
2. Standard curve was drawn manually (shown in figure 1.) taking the concentration value of samples as abscissa and OD readings as vertical coordinate. Smooth line was used to connect each coordinate point of standard sample. The concentrations of samples were found by checking the sample OD reading.

Results were calculated according to OD value, samples having OD value less than negative control were considered as negative.

Table 1: OD value of Standard sample, Negative control and blank well

Standard sample no.	OD*	Concentration of *E. histolytica antigen*
		ng/ml
1	2.011	20
2	1.394	10
3	1.008	5
4	0.812	2.5
5	0.756	1.25
6	0.712	0.625
7	0.676	0.312
8	0.624	0.156
9	0.586	0.078
10	0.523	0.039
NC†	0.514	-

*OD- Optical density †NC- Negative control

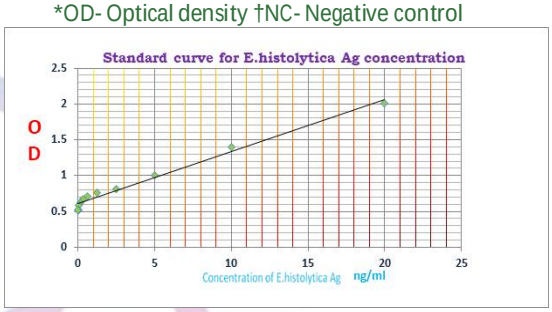

Figure 1: Standard curve for *E. histolytica* Ag concentration calculation

RESULTS

Out of 43 samples, 27 (62.79%) were positive by ELISA and from these only 14 (32.55) samples turned out to be positive microscopically. All the samples which were taken as control were negative by both methods as shown in figure 2. Most of the microscopically positive samples showed more than 20 ng/ml of concentration of antigen. Highest numbers of positive cases (37.03%) was seen in 0-10 years of age followed by 41-50 years group (18.5%) and males (70.37%) are affected more than female (29.62%), Age and sex wise distribution of positive samples shown in table 2. Distribution of positive samples (cases) according to factors contributing to transmission is shown in table 3.

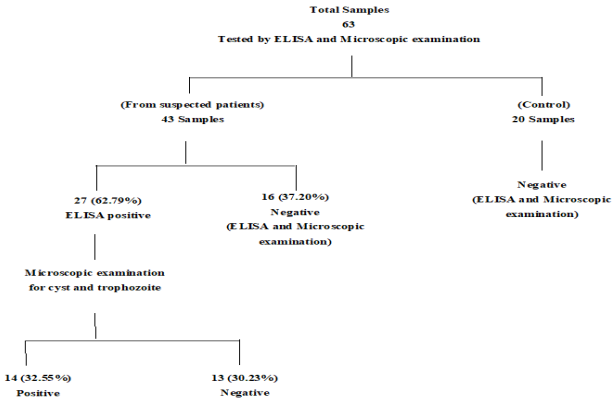

Figure 2: Result of tested samples

Table 2: Age and sex wise distribution of positive samples

Age group (years)	No. of positive samples- male	No. of positive samples- female	Total positive samples
0-10	7	3	10 (37.03%)
11-20	3	1	4 (14.81%)
21-30	2	1	3 (11.11%)
31-40	1	1	2 (7.40%)
41-50	4	1	5 (18.51%)
51-60	2	0	2 (7.40%)
>60	0	1	1 (3.70%)

Table 3: Distribution of positive sample according to factors contributing to transmission (n=27)

Source of water	Treatment of water	Sanitary hygiene (use of latrine)	Hand hygiene
Bore hole-21(77%) Tap- 2 (7.4%) RO treated – 4 (14.9%)	No – 23(85.1%) Yes- 4 (boiling) (14.9%)	Yes - 20(74%) No – 7 (16%)	Yes- 22(9 only with water) (81%) No – 5(19%)

DISCUSSION

In the present study out of 43 samples 27 (62.79%) were positive while in microscopic examination 14 (32.55%) were positive, this may be because of the low load of pathogens present in the stool sample which cannot be detected by microscopy. Previously a study conducted in same institute showed 45.23% of intestinal parasitic infection caused by *Entamoeba histolytica* based on microscopic examination.[16] In the study done by Tasleem Akhtar *et al*, a total of 14/288 (0.0486%) stool samples were positive for *Entamoeba histolytica* antigen on ELISA test.[7] Similar study conducted by Saeed A. Al-Harthi *et al* revealed only 2.6% positive samples for *Entamoeba histolytica* antigen and the study conducted by Moustafa Abdelaal Hegazi *et al*, showed the prevalence of *Entamoeba histolytica* to be 20% based on results of *Entamoeba histolytica* antigen detection kit.[17,18] Ghanshyam Kavathia *et al* in a cross-sectional study found out that *Entamoeba histolytica* infection was commonest in all of the protozoal infection as it constituted about 64.58% of all protozoal infections.[19]Our study shows comparatively higher prevalence than other studies. From a study in Kyuso district (Kenya), it was found that several large outbreaks of amoebic dysentery had resulted from the contamination of municipal water supplies with human wastes indicating water supply as one of the important risk factor for transmission of amoebiasis.[13] This problem is found to be more in rural areas which even do not have any municipal water network and sewage system. Most of the positive cases (77%) have borehole as their main source of water for drinking and most of them (85.1%) were not used any method to treat it before use. A study conducted in kyuso zone, showed that there is significant association between use of dry riverbed wells or earth dams as a source of water for drinking and the prevalence of *Entamoeba histolytica* in such patients when compared to those people who used other sources of water like borehole or tap water.[13] However his was not found to be consistent with our studies. Reason for such inconsistence may be due to the fact that amoebic cyst may survive for several days and weeks in borehole water. Moreover cyst is not affected by chlorine in amounts used for water disinfection. Sand filters are quite effective in removing

amoebic cysts. Therefore water filtration and boiling are more effective than chemical treatment of water against amoebiasis.[20] In the same study done in kyuso zone, have shown significant association between the use of latrines and prevalence of *Entamoeba histolytica* which is differ from our study .In our study most of the positive cases (74%) use latrines for defecation but latrine are simple pit type so it is difficult to maintain standard of cleanliness . Those who do not have latrines (16%) often defecates openly in the bushes which leads to the contamination of water sources and food stuffs.[13] Even though people uses the latrine but fail to maintain the standard of cleanliness and it leads to transmission of causative agent mechanically. Our study shows higher prevalence in males (70.37%) as compared to females (29.62%). These results are similar to study conducted by Zahida Tasawar *et al*, where the results regarding the relationship between gender and *Entamoeba histolytica* in humans had showed that the infection was found to be more prevalent in male host (22.36%) as compared to female host (20.9%), however the difference was statistically non-significant[9], Surinder kumar *et al* in a study examined the prevalence of *Entamoeba histolytica* and found that the prevalence of *Entamoeba histolytica* was higher in males than females, however it was not statistically significant.[1] In another study conducted by Erdogan Malatyali *et al* regarding the prevalence of *Entamoeba histolytica* in village of Sivas by ELISA method, it was found that infection rates among females was about 1.8% which was not significantly higher as compared to that among males 1.2%[15] but differ from our study. One of the convincing reasons which can be there behind higher prevalence in male may be that males in this area usually have to carry out their daily activities outside. As a result they are highly exposed to unhygienic environmental conditions.[1] Other reasons which are proposed are that males usually have decreased immune response as compared to females. These differences can be attributed to: (1) ecological, pathogens exposure is different because of sex-specific behavior or morphology (2) physiological, usually it is hormonal in origin. Some other cause of sex differences in occurrence of infection is attributed to differences in endocrine-immune interactions. Sexually mature male vertebrates have more susceptibility to infection and they usually carry higher parasite burdens, because sex steroids, specifically androgens in males and estrogens in females, modulate several aspects of host immunity. Along with affecting host immunity, sex steroid hormones are also able to alter genes and behavior which usually influences susceptibility and resistance to infection.[9] The lower prevalence of *Entamoeba histolytica* in adults as compared to children could be easily explained on the basis that adult people have higher immunity as compared to children and many of the defense systems that protect adults from many diseases are underdeveloped in children. In addition children do not take care of their personal hygiene such as lack of washing hands before meal, lack of fecal hygiene and playing in dust, this makes them highly susceptible to diseases.[9] In present study it has also been noted that all the suspected patients were labor workers or belongs to labor workers family and have limited income, because of continuous efforts of Governments, NGOs and other agencies that works for social welfare public awareness for cleanliness, to maintain hygiene , how to prevent transmission of disease etc. has been increased. In response to that people even they reside in slum area have start using latrine, pure water, and personal hygiene but they fail to maintain it. For the high prevalence amoebic dysentery in this area it may be possible that the soil of this area favors the survival of *E.histolytica* and it needs to be investigated.

CONCLUSION

The present study showed very high prevalence of *Entamoeba histolytica* in this area suggesting that it is one of the most common causative agents of dysentery in vulnerable group of population. With such a high prevalence there is a need of preventive measures to be taken such as educating the people in regards to personal hygiene, environmental sanitation and the effective method for purification of water to break the chain of transmission of diseases.

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