Prevalence of amoebiasis in a model research community and its confirmation using stool antigen elisa for *Entamoeba histolytica*

Tasleem Akhtar, Aamir Ghafoor Khan, Israr Ahmed, Rubina Nazli and Jamila Haider
Chemistry Department, Shaheed Benazir Bhutto University, Sheringal, Dir Upper, Pakistan

Abstract: *Entamoeba histolytica (E. histolytica)* produces an invasive disease called amoebiasis, which commonly produces diarrhea with or without blood in both children and adults, leading to high morbidity and mortality. *Entamoeba dispar (E. Dispar)* is a non invasive, non pathogenic organism. Both *Entamoeba histolytica and Entamoeba Dispar* look alike on microscopy and therefore cannot be differentiated unless checked on ELISA, PCR or other specific method. To calculate the actual prevalence of pathogenic amoebiasis in children by comparing the stool microscopy with ELISA stool antigen i.e. gold standard. Across sectional, comparative study. Children under five years in a community village Budhni, District Peshawar. A sample of 288 children aged <5 years were randomly selected. Information's were collected on the age and gender of the children. Fresh stool specimens were examined microscopically and with stool antigen kit of ELISA for detection of *Entamoeba histolytica*. The specificity and sensitivity of microscopic method was calculated against ELISA. Data was analyzed using statistical computer software package SPSS version 10.0. A total of 288 stool specimens were collected and examined for *Entamoeba histolytica*. Out of these 36(12.5%) stools were positive for *E. histolytica*on microscopy while 14(4.9%) were positive on ELISA. Out of 14 ELISA positive samples, 10 samples were also positive on microscopy while 4 were ELISA positive but microscopy negative. About 22 samples, which were positive on microscopy were negative on ELISA indicating that these samples might have been of *E. Dispar* which is non pathogenic protozoa. The sensitivity and specificity of microscopic method was 71.4% and 90.5% respectively, as against stool antigen test. Actual prevalence of *Entamoeba histolytica* is low in the area. Stool ELISA was able to differentiate between pathogenic *Entamoeba histolytica* and the non-pathogenic *Entamoeba dispar* and thus can minimize unnecessary antiamoebic treatment in these children.

Keywords: Amoebiasis, *Entamoeba histolytica*, Microscopy, *ELISA* Stool antigen detection test.

INTRODUCTION

Amoebiasis is caused by intestinal protozoan parasite *E. histolytica* and is the third leading parasitic cause of death in humans after malaria and schistosomiasis. Worldwide, it is responsible for 40,000-100,000 deaths a year (Sebastiaan *et al*., 2007).

The prevalence of amoebiasis varies between countries. High prevalence of more than 10% is reported in developed countries (Stanley, 2003) while between 50% prevalence is reported from developing countries and over 90% of infections in patients with dysentery is due to E. *histolytica* (Caballero-Salcedo *et al.,* 1994). Various factors, such as poor education, poverty, overcrowding, contaminated water supply and unsanitary conditions contribute to the fecal-oral transmission of these diseases. Amoebiasis can easily transmit from person to person therefore there is a need for its early and accurate diagnosis and for the prevention of its endemicity.

The diagnosis of *E. histolytica* infection traditionally relies upon microscopic examination of fresh or fixed stool specimens but the drawback of microscopy is that it cannot differentiate among pathogenic *E. histolytica* and non pathogenic *E. dispar* and there is a high degree of divergence (Fotedar *et al*., 2007). Antigen- based enzyme-linked immunosorbent assays (ELISA) and PCR are more reliable to differentiate in the two and thus make 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 analysisin areas of high endemicity (Haque *et al.,* 1998). PCR techniques are not widely available and remain nonfeasible in many developing countries therefore, stool ELISA is considered as a suitable substitute for the diagnosis of *E. histolytica* infections.

In Pakistan microscopic method is widely used for the identification of *E. histolytica* but due to diagnostic overlap between pathogenic *Entamoeba histolytica* and the non pathogenic *Entamoeba dispar* the correct disease prevalence of pathogenic species of *E. histolytica*is not known. This study was undertaken to find out prevalence of amoebiasis in our model research community, village Budhni, using stool microscopy and further confirming it with more specific ELISA method for detection of *Entamoeba histolytica.*

SUBJECT AND METHODS

This cross-sectional comparative study was conducted on children under five years in village Budhni. This village is established by Pakistan Medical Research Council

**Corresponding author:* e-mail: rubinanazli44@gamil.com

(PMRC) in 1984 as a model community for its research studies. It is about 30KM away from Peshawar and has an estimated population of 7555 and total families 1467. For research purpose the whole village is divided into 9 geographical blocks and each house is marked with a specific number. Family Folder of each family is maintained and updated regularly by the lady health visitors of the centre. The population of children under five is 823 in this village. The study was approved by the institutional ethical committee. The nature of the study was explained to the parents and informed consent taken. The mother/ proxy of child were interviewed by a trained health care worker according to pre-designed questionnaire. A sample of 288 children both male and female were randomly selected and included in the study. A plastic vial with a spoon and sterile tight-fitting lid was given to the mother of child for collection of stool specimen during house to house visit. The mother was instructed to avoid stool contamination with urine. Each container was marked with waterproof ink with an identification number. Fresh stool samples were delivered to lab immediately for microscopy and other examinations.

To achieve reliable results a mini lab was established in the Community Research Centre in village Budhni equipped with basic requirement. Microscopic examination was done by using both normal saline and Lugol's iodine for identification of cyst and trophozoites of *Entamoeba histolytica* in fresh stool specimens. Samples were then tested for stool Antigen using ELISA kit(produced by Entamoeba Celisa Path, Cellabs Pty Ltd., Brookvale, Australia). Stool ELISA was run as per package insert. A positive result was defined as an optical density reading < 0.05 after subtraction of the negative control optical density.

Data collected was analyzed using statistical computer software SPSS program. The percentage of results was calculated. The *E. histolytica* was considered to be true positive if the stool ELISA test was positive, and considered true negative if ELISA was negative (could be *E. dispar* positive). Contingency tables (2x2) were used to compare two methods for identification of *E. histolytica* and to calculate sensitivity and specificity of microscopic method as compared to stool antigen test.

RESULTS

The demographic characteristic of study population is shown in table 1. A total of 288 children were included in the study; out of which 175(61%) were males and 113(39%) females. The mean age was 33.8±16.1 months (table 1).

The overall prevalence of *E. histolytica*on microscopy was 12.5%, with significantly (P<0.05) higher affection in males (72.2%) as compared to females (27.8%). *E. histolytica* had highest prevalence (52.7%) in 2 to 3 years age group and lowest (13.9%) in over 4 years. The difference was significant (P<0.05) among the two age groups (table-2).

Microscopy results were compared with *E. histolytica* identification kit using ELISA method (table-3). Out of 288 stools tested 36(12.5%) were positive for *E. histolytica* on microscopy but using ELISA, the antigen was detected in 10/36 (27.8%) samples only. There were 4(28.6%) cases, which were negative on microscopy, but were positive on ELISA stool antigen test, thus making a total of 14/288 stool specimens, which were positive for *E. histolytica* on ELISA test. The sensitivity and specificity of microscopic method was calculated against ELISA and was found to be 71.4% and 90.5% respectively.

DISCUSSION

In the present study ELISA stool test verified 14 infections due to *E. histolytica* while stool microscopy picked 36 cases, showing large wrongly diagnosed amoebic infections in our country. *E. histolytica* is mostly diagnosed on microscopy of stool, but this method is neither sensitive nor specific to distinguish between *E. hitolytica* and *E. dispar*. Also microscopy is very much dependent on the skill of the technician; therefore its results vary from study to study. It is remarkable that, whenever an alternative method to differentiate between *E. hitolytica* and *E. dispar* were used, majority (88%) was found to be *E. dispar* and only 10% were due to *E. histolytica* (Hooshyar *et al*., 2004).

In Pakistan, many studies have reported the prevalence of intestinal parasites and *E.hislytica* but unfortunately all have used microscopic identification of *E. histolytica*. A study from Swat reported a prevalence of 70% (Khan *et al.*, 2005), from Karachiit was 48.9% (Siddiqui *et al.*, 2002)**,** from Quetta 29% (Wadood *et al*., 2005), Multan 21.7% (Tasawar *et al.*, 2010) and from Vehari 20.2% (Mehmood *et al*., 2009) while lower figures were reported from Islamabad 1.4% (Ashok *et al*., 1995). The prevalence of *E. histolytica* in our study was lower as compared to those reported from most parts of the country.

During last few years there has been remarkable development in molecular based diagnostic methods to detect *E. histolytica* (Tanyuksel and Petri, 2003). Many studies have documented the ability of EIA method to differentiate between pathogenic *E. histolytica* from potentially non-pathogenic *E. dispar* infection (Tanyuksel *et al.*, 2005). PCR and ELISA had comparable sensitivities when performed directly on fresh stool specimens, identifying 87% and 85% respectively, of *E.*

Table 1: Demographic characteristics of study population.

Characteristics	Number	Percentage
Sample size	288	-
Gender Male Female	 175 113	 60.8 39.2
Age (months) Mean ± SD (33.88 ± 16.13)	-	-

Table 2: Distribution of *Entamoebahistolytica* positive children on microscopic examination of stool specimens according to age and gender.

Age (months)	*Entamoebahistolytica* Positive					
	Male		Female		Total	
	N	(%)	N	(%)	N	(%)
0-12	5	19.2	1	10	6	16.7
13-24	7	26.9	3	30	10	27.8
25-36	6	23.1	3	30	9	25
37-48	4	15.4	1	10	5	13.9
49-60	4	15.4	2	20	6	16.7
Total	26	100	10	100	36	100

Table 3: Evaluation of microscopic findings for Entamoebahistolytica versus stool antigen detection kit for *H. histolytica* identification using ELISA method.

Microscopy	*Entamoebahistolytica* (ELISA)			Sensitivity %	Specificity %
	Positive	Negative	Total		
Positive	10	26	36	71.4	90.5
Negative	4	248	252		
Total	14	274	288		

histolytica infections as identified by isoenzyme analysis. The correlation of results by ELISA and PCR for identification of *E. histolytica* in stool was 93% and all three techniques for specific identification of *E. histolytica* in fresh stool showed excellent correlation. Only Tech Lab *E. histolytica* antigen detection test was found to be rapid and technically simple (Haque *et al*., 1998).

In another German study, the sensitivity and specificity of ProSpecT ELISA was 73.5% and 97.7%, in stool specimens respectively, compared to microscopy for *E. histolytica/ E. dispar*in travelers returning from vacations abroad (Jelinek *et al.,* 1996). The results of this study are almost similar to our study.

Haque *et al* (1998) reported that the overall correlation between results of the TechLab antigen detection test and PCR was 94%. In a study, ELISA test was compared with microscopy for identification of *E. histolytic* and showed ELISA to be 96% sensitive and 93% specificas compared to stool microscopy (Haque *et al.*, 1994), while another study, showed ELISA to be 97% specific and 100% sensitive (Haque *et al*., 1993).

The routine use of ELISA test to differentiate *E. histolytica* from *E. dispar* would help to determine the true prevalence of the two species in our country and will also help the doctors in deciding about the treatment. In conclusion, we show a low prevalence of amoebiasis infection in our region and demonstrated that *E. dispar*is the predominant species found among children. The routine use of ELISA test should be used to determine the true prevalence of these two species in our country to decide about disease burden and its future treatment.

ACKNOWLEDGMENT

The authors are thankful to Pakistan Medical Research Council for funding this project. Thanks are due to mothers of participating children for providing information's and stool specimens. Field and lab staff of PMRC is acknowledged for their hard work and help for completion of this project.

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