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Antigen Detection of *Entamoeba Histolytica* Intestinal Infection: Cost-Associated Challenge in a Resource Poor Country

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Abstract

Purpose: Laboratory diagnosis of *Entamoeba histolytica* infection is still being made through compound light microscopy in resource limited countries despite the associated flaws. This study is aimed at applying and determining the usefulness of ELISA antigen detection technique for *E. histolytica* intestinal infection diagnosis in a resource poor nation.
Methods: A total of 150 subjects with acute and persistent diarrhoea had their stool specimens examined by compound light microscopy for *E. histolytica/dispar* and other intestinal parasites. Ninety-four of them (62.67%) had their stool specimens examined with ELISA antigen detection for *E. histolytica*
Results: Two (1.33%) of the subjects were positive for *E. histolytica/ dispar* on microscopy but none was positive for *E. histolytica* by ELISA antigen detection. Subjects positive by microscopy were identified as *E. dispar* (1.33%) carriers.
Conclusion: ELISA antigen detection technique is a preferred method of detection of parasites even in resource poor settings to avoid confusing results. At present, the best use of *E. histolytica* II kit in resource poor nations should be limited to screening of *E. histolytic/dispar* microscopically positive stool samples for *E. histolytica* infection.

Keywords: Amoebasis; Antigen detection; ELISA; Entamoeba histolytica; Laboratory diagnosis; Nigeria.

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Introduction

Entamoeba histolytica, the causative agent of amoebaisis, is believed to infect more than 10% of the world's population with majority of infection occurring in the developing world [1,2]. The parasite has been reported as the third major cause of death attributable to parasitic infections globally, after malaria and schistosomiasis [2,3]. However, the diagnosis of intestinal amoebiasis, the first stage of multi-systemic disease caused by *E. histolytica*, is plagued with many challenges.

In Nigeria, laboratory diagnosis of intestinal amoebiasis is often based on compound microscopy which does not accurately detect the amoeba species [4]. As of 1997, it was officially accepted by the World Health Organization (WHO) that *E. histolytica sensu lato* is composed of two morphologically identical species: the pathogenic *E. histolytica sensu stricto* and the non-pathogenic (but very common) *Entamoeba dispar* [5]. These two parasites cannot be correctly distinguished under compound microscopy alone unless the trophozoites of *E. histolytica* are seen engulfing red blood cells (heamatophagous trophozoites) [4]. Even though, the identification of heamatophagous trophozoite on compound microscopy is considered diagnostic, it is practically a rare finding and occurs mainly in acute dysentery [4,5].

Alternative diagnostic and specific approaches for amoebiasis had since been developed and these include enzyme linked immunosorbent assay (ELISA) antigen detection technique; culture followed by isoenzyme analysis on electrophoresis and polymerase chain reaction (PCR) based techniques [4]. None of these new approaches is currently being used in Nigeria. The aim of this work therefore is to apply and determine the usefulness of ELISA antigen detection technique for *E. histolytica* intestinal infection diagnosis among patients with diarrhoea in a resource poor nation like Nigeria.

Patients and Methods

Patients

This study was conducted at the University of Ilorin Teaching hospital (UITH), Ilorin, Nigeria between December 2005 and May 2007. The study was approved by the Ethical review committee of UITH, Ilorin. All consenting patients presenting to the various hospitals within the Ilorin metropolis with acute and persistent diarrhoea or dysentery within the period of study were enlisted. Patients with chronic diarrhoea and those on antimicrobials, antacids, laxative, soap and water enema within a two week period from the sampling or those who have just completed investigation with barium meal/enema were excluded from the study. Stool samples reaching the laboratory after two hours of collection were also not processed. The age and sex of patients were noted at the time of recruitment.

Single stool specimen was collected in a clean, dry, wide neck, sterile bottle from each of the participant and was divided into two parts. One part of the specimen for diagnosis by ELISA antigen detection was preserved at -20 ^{o}C until analysis, while the one for microscopy was processed immediately.

Methods

To a drop of normal saline on a microscope slide, was suspended a small amount of the stool specimen and was covered with cover slip. This was examined under a compound light microscope first under x10 objective lens and then under x 40 objective whose stage had been warmed with a 60 watt bulb for at least one min.

The stool sample was concentrated by the sedimentation technique using formol-ethyl acetate method [7]. A drop of the sediment obtained was examined in a drop of 2% iodine for cysts of *E. histolytica*. Cysts, ova and larvae of other intestinal parasites were also sought for and identified using diagrams in the image library of DPDx CD on

Laboratory Diagnosis of Parasitic Diseases by Centre for Disease Control and Prevention, USA [8].

The *E. histolytica* enzyme linked immunosorbent assay (ELISA) based antigen detection kit (*E. histolytica* II) made by TechLab (USA) was used according to manufacturer's instructions. The kit is based on monoclonal antibody-peroxidase conjugate specific for *E. histolytica* adhesin. According to the manufacturer, a positive result is recorded if optical density of >0.05 is obtained after subtracting the negative control optical density.

A total of 150 specimens were collected and all examined microscopically, while 94 randomly selected stool specimens, including the ones positive for *E. histolytica/ dispar* on microscopy, were examined with ELISA antigen detection method obtained from USA. Only 62.67% of the patients had their stool screened for *E. histolytica* with ELISA antigen detection kit due to cost associated factor as it takes about ₦2,000.00 (approximately $14.00) to process a single stool. The cost of obtaining in Nigeria an ELISA antigen detection kit is about ₦146,000 (approximately US$1000.00).

The data collected were presented using descriptive statistics.

Results

By microscopy, only 2 (1.33%) of the patients were positive for *E. histolytica/ dispar*. None of the patients was positive for *E. histolytica* by ELISA antigen detection technique implying that the prevalence of *E. dispar* was 1.33%.

Other intestinal parasites seen in the stool of some patients are presented in Table 1. The overall level of intestinal parasitic infection was 9.33%. *Ascaris lumbricoides* (5.33%) was the most predominant intestinal parasite seen. *Schistosoma mansoni* and hookworm were seen in 0.67% and 1.33% of the patients, respectively. Mixed infections were found in 0.67% with *A. lumbricoides* and hookworm being responsible for the co-infection. Intestinal parasitosis among the male and female patients with diarrhoea were found mainly among those aged 10 years and below (Table 2).

Discussion

In developing countries, diagnosis of *E. histolytica* infections remains unsatisfactory largely because of the inadequacies of the laboratory methods readily available. The most commonly used method is microscopy which had resulted in various prevalence rates in different localities and even within the same region over time [9-11]. In this study, only 1.33% of the patients were positive for *E. histolytica/dispar* using microscopy. The use ELISA antigen based diagnostic method has made it possible to detect that the microscopy result was indeed inaccurate as none of the patients was positive for *E. histolytica*. This result is an indication that the frequent reliance on microscopy for diagnosis of amoebiasis in resource poor settings like the situation in Nigeria could often lead to wrong diagnosis.

Table 1: Intestinal parasites seen in the stool specimens of patients with diarrhoea in Ilorin, Nigeria

Parasite type	Number positive	Rate (%)
Ascaris lumbricoides	8	5.33
Hookworm	2	1.33
Schistosoma mansoni	1	0.67
Ascaris lumbricoides + Hookworm	1	0.67
Entamoeba hisolytica/dispar	2	1.33
Total	14	9.33

n=150

Table 2: Age and sex related occurrence of intestinal parasites in the stool specimens of patients with diarrhoea in Ilorin, Nigeria

Age range (years)	Male Diarrhoea Patients		Female Diarrhoea Patients	
	Number examined	Number infected with parasites (%)	Number examined	Number infected with parasites (%)
<1	8	2 (3.03)	9	3 (3.57)
1-5	54	1 (1.52)	26	2 (2.38)
6-10	47	2 (3.03)	25	1 (1.19)
11-15	16	0 (0.00)	34	1 (1.19)
16-20	15	0 (0.00)	42	1 (1.19)
>20	10	0 (0.00)	14	1 (1.19)
Total	**66**	**5** (7.58)	**84**	**9** (10.71)

The inaccurate diagnosis of amoebiasis based on microscopy results has been documented in different studies. In a study conducted in North Eastern Brazil, 4.1% of patients were positive for *E. histolytica/dispar* with microscopy but with both PCR and antigen immunoenzymatic assay, none (0%) was positive for *E. histolytica* [12]. In another study conducted in Bangladesh, out of 98 patients with diarrhoea, 88 were diagnosed to be infected with *E. histolytica* by microscopy but only 53 were positive by isoenzyme analysis and 45 (85%) out of these were positive by the ELISA antigen detection method [13]. Another study in Bangladesh also showed that, out of 202 diarrhoea patients whose stool samples were examined by microscopy, 69 were positive for *E. histolytica* while 106 were negative. However, of the 69 positive stool specimens by microscopy for *E. histolytica,* only 56 were positive with ELISA antigen detection method [14]. Similarly, 2 out of 106 negative stool samples for *E. histolytica* by microscopy were positive with ELISA antigen detection method with the results of ELISA correlating well with the gold standard (culture followed by isoenzyme analysis).

To the best of our knowledge, there are no reported studies in Nigeria yet where ELISA antigen detection method or other molecular techniques had been used to diagnose amoebiasis for comparison. Nonetheless, from the present study and others cited above, more patients are often positive by microscopy than with ELISA and other methods adjudged more precise than microscopy for *E. histolytica*. This is probably because of the wrong identification of other *Entamoeba* species and white blood cells as *E. histolytica* with microscopic method [4]. This result therefore shows that most patients treated for amoebiasis based on microscopic diagnosis actually required no treatment hence, the need to shift to the use of ELISA antigen detection method and probably, PCR for diagnosis of *E. histolytica* by developing countries who already do not do so.

The high cost however of using ELISA antigen based technique for diagnosis of *E. histolytica* is a major challenge in resource poor countries. To buy and ship a tray of ELISA antigen detection kit used in this study to Nigeria cost about ₦146,000 (approximately $1,000.00) and by extension, an already impoverished patient may need to pay about ₦3000 (approximately $21.00) for a single stool examination. The cost of this procedure probably also explain the reason why this type of study has never been reported in this country. Therefore the support of government and non-governmental agencies in reducing the cost of ELISA antigen detection kit is highly advocated as a cheap and readily available kit would result in routine specimen testing thereby enhancing the quality of medical care and indirectly, the quality of life of the citizenry. Until this is done, the best use of *E.*

histolytica II kit in resource poor nations should be limited to screening of *E. histolytic/dispar* microscopically positive stool samples for *E. histolytica* infection among patients that can afford the cost of this test.

Conclusion

The use of microscopy in the diagnosis of amoebiasis in resource poor settings often leads to wrong diagnosis and hence wrong treatment. ELISA antigen detection technique correctly diagnoses amoebiasis and prevents the confusing result from microscopy that cannot specifically differentiate *E. histolytica* from *E. dispar.* However, the cost of each test is too expensive for most patients in Nigeria.

It is recommended that at present, the best use of *E. histolytica* II kit in resource poor nations should be limited to screening of *E. histolytic/dispar* microscopically positive stool samples for *E. histolytica* infection.

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