



Detection of *Entamoeba histolytica* Coproantigen Among Children with Dysentery in Ahvaz, Southwest Iran

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Abstract

Entamoeba histolytica is one of the important parasitic diseases in many parts of the world, especially the tropical and subtropical regions. The parasite is transmitted through contaminated water and vegetables. The exact diagnosis of infection with the parasite is crucial in many medical laboratories since there are many false positive and negative results in their reports. Therefore, the current study aimed at evaluating and comparing microscopic and coproantigen ELISA (the enzyme-linked immunosorbent assay) results to reach an appropriate test for the correct diagnosis of amoebiasis in children. One hundred stool samples were collected from children under 15 years old with dysenteric diarrhea from April to September 2018. Microscopic tests and coproantigen ELISA were performed on all the samples. The results showed that 5% of the samples had *E. histolytica*/*E. dispar* cysts. The findings of ELISA to detect coproantigen did not show any specific *E. histolytica* antigen in the samples. Hence, all the patients received chemotherapy for shigellosis. *E. histolytica* infection is not the main causative agent for dysenteric diarrhea in children in the studied area, and laboratory experts should be trained to prevent false-positive reports.

Keywords: *Entamoeba histolytica* Infection, Coproantigen, Dysenteric Diarrhea, Children

1. Background

Entamoeba histolytica is a pathogen parasite causing acute enteritis with dysenteric symptoms in susceptible individuals. The parasite spreads to other parts of the body via the bloodstream and causes amoebic abscesses in the liver, lung, brain, and skin (1). Approximately 10% of the world population (more than 500 million) are infected with *E. histolytica*, 1% of which develop the invasive form of the disease with an annual death toll of 100,000 (2). Amoebiasis is the leading cause of death from a parasitic infection after malaria in the world with the annual death toll of 100,000 in the tropical areas and developing countries (3, 4). According to microscopic studies, the infection's prevalence is 2.2% to 30% in different parts of Iran (5). Some other studies showed that the prevalence of *E. histolytica* and *E. dispar* in Central, Northern, and Southern parts of Iran were 0.78%, 3.9%, and 4.6%, respectively (6). There are three other non-pathogenic species of *Entamoeba*- i.e., *E. dispar*, *E. moshkovskii*, and the recently described *E. bangladeshi*, which their trophozoites and cysts are morphologically very similar to those of *E. histolytica*

(7). One of the important views on the diagnosis of the parasite is the establishment of precise, rapid, and accessible diagnostic tests in all laboratory centers. There are several methods to detect *E. histolytica* in serum and stool, including microscopic detection of cysts and trophozoites in stool samples, as well as coproantigen detection, PCR, and antibody detection in sera (7). Microscopic examination has a low sensitivity (60%) due to the difficulty of distinguishing between pathogenic and non-pathogenic *Entamoeba* species and the lack of expert practitioners in many laboratories (8). Also, the *E. histolytica* cyst is not easily differentiated from white blood cells in stool samples (9). Antibody detection in serum samples of the infected patients is not reliable since only 70% - 80% of patients are seropositive, and, on the other hand, more than 25% of people are seropositive in endemic areas (10, 11). Real-time PCR and isoenzyme analysis of culture are two sensitive methods to distinguish the pathogen from nonpathogen species, but they are not practical in many laboratories due to lack of expert staff, high cost, and their time-consuming nature (8, 12). According to the World Health Organization recommendations, an accessible, rapid, and sensitive test should

be considered for the more precise diagnosis of *E. histolytica*, especially in developing countries (1, 13). The proper diagnosis of *E. histolytica* in patients is of great importance to prevent unnecessary treatment and apply appropriate drugs (14). The detection of *E. histolytica* antigen in the stool (coproantigen) by the ELISA (enzyme-linked immunosorbent assay) technique is easy, rapid, and more sensitive than microscopic tests and is practical in all diagnostic laboratories (5, 10, 15). Distinguishing *E. histolytica* from other causes of dysentery, such as shigellosis, is a critical problem in many diagnostic laboratories.

2. Objectives

Therefore, the current study aimed at differentiating *E. histolytica* from shigellosis by detecting coproantigen in stool samples of children with dysentery for the proper diagnosis and treatment.

3. Methods

Totally, 100 stool samples were collected from children under 15 with dysenteric diarrhea in a pediatric hospital from April to September 2018. This project was approved by the Ethics Committee of Ahvaz Jundishpur University of Medical Sciences (code no.: ajums REC.1393014). The samples were transferred to the Parasitology Department of Medical School and microscopically examined for the detection of trophozoites or cysts of *E. histolytica*. The samples were stained with trichrome staining methods and examined for trophozoites and cysts of *E. histolytica*. Horse albumin was used to fix stool smears. After microscopic examination, the samples were stored at -20°C until used for ELISA (Biomerica, Germany catalog no. 7078). The kit had been coated with a monoclonal antibody against *E. histolytica* specific antigen (ESA), and after adding the sample, the other antibody against *E. histolytica* was used (sandwich ELISA). Tetramethyl benzidine and peroxidase were used as the substrate and enzyme to create a yellow-color complex. The optical density was read at the wavelength of 450 nm by the ELISA reader machine. According to the instructions of the kit manufacturer, at the wavelength of 450 nm, the positive control should be greater than 0.5 and the negative control less than 0.15 OD. The borderline for positive or negative samples was 0.15 OD.

4. Results

The current study results indicated that the prevalence of this disease in Iran, as mentioned in the introduction,

should be amended and updated. Microscopic examination of samples revealed that 5% (five out of 100) of stool samples contained *E. histolytica*/*E. dispar* cysts. The results of the ELISA showed that none of the stool samples were positive, and the OD of all samples was under 0.15.

5. Discussion

The current study results showed no positive samples for *E. histolytica* infection among 100 child patients with dysenteric diarrhea, using the coproantigen ELISA test. The prevalence of infection was previously investigated. In a two-year study, Safi et al. (16), reported that 1.83% out of 14,614 patients with gastrointestinal (GI) symptoms were infected with *E. histolytica*/*E. dispar*. Yosefi et al. (17), showed that 1.7% out of 100 samples obtained from HIV⁺ patients contained *E. histolytica* cysts. Rafiei et al. (18), studied the contamination of surface waters and presented that 50% of 44 river and surface water samples from Ahvaz and 6.3% of water samples from Shush, Khuzestan Province, Southwest Iran, were contaminated with *Entamoeba* spp. (19).

The prevalence of intestinal parasite was also studied in other parts of Iran. Zebardast et al. (9), reported that 153 stool specimens out of 1520 (10%) were infected with intestinal parasites in patients with GI disorders in Tehran. The parasites included *Blastocystis* spp. (4.73%), *Giardia intestinalis* (2.30%), *E. coli* (1.38%), *Endolimax nana* (0.92%), *Cryptosporidium* spp. (0.06%), *E. dispar* (0.06%), *Dientamoeba fragilis* (0.06%), *Iodamoeba butschlii* (0.06%), *Chilomastix mesnili* (0.06%), *Hymenolepis nana* (0.19%), and *Dicrocoelium dendriticum* (0.13%). They did not observe *E. histolytica* in any of the specimens. The prevalence of *E. dispar* is 10 times more than *E. histolytica*, and it is estimated that *E. dispar* is the main enteric amoeba in the Central and Northern areas of Iran. Feiz Haddad et al. (20), showed that 10.68% of stool samples referred to the central laboratory of Dezful contained *E. histolytica*/*E. dispar*. It seems that the *E. histolytica* infection has a rare prevalence in many parts of Iran (21). Solaymani et al. (22), along with other researchers, confirmed that all Iranian asymptomatic cyst passers were infected with *E. dispar*; a nonpathogen species of *Entamoeba* genus (6, 22, 23). Savadkoobi et al. (24), reported that 6% out of 537 children with dysenteric symptoms presented *E. histolytica* infection with positive microscopic stool examinations in Babol City, North of Iran. The prevalence of *E. histolytica* infection was 1% among patients with GI diseases in Tehran hospitals (25). A meta-analysis on findings from 1988 to 2009 estimated the average prevalence of 1.3% for *E. histolytica* in Iran. This finding indicated a recently decreased infection rate of *E. histolytica* in many

parts of Iran. Promotion of a healthy lifestyle, the increase of people's health knowledge, and the consumption of safe water in many parts of the country are the main reasons for decreasing *E. histolytica* infection in Iran (26). Reports from different parts of the world indicate the infection rate of *E. histolytica* as 10.6% in Jordan (27), 11% in New Delhi (28), 5.3% in Turkey (29), 9.2% in Saudi Arabia (30), 65.7% in Nigeria (31), and 66.6 in Nepal (32).

In many medical laboratories, *E. histolytica* in stool specimens is commonly diagnosed by a direct microscopic examination in order to save time and expenditure, and ease of performance. Unlike helminths, the direct method is not suitable for the diagnosis of protozoan infection; the idea confirmed by many studies. The technical level and the experience of the laboratory practitioner, as well as useful training, are the crucial criteria for proper identification of protozoa in stool samples (33-35). Uslu et al. (14), reported that the direct microscopic method provides false-positive results in half of the patients infected with *E. histolytica*. Only 40% of the children infected with *E. histolytica* were diagnosed microscopically, and the rest were positive by other sensitive methods, such as antigen detection and isoenzyme analysis of cell culture. The microscopic technique could not diagnose many cases of *E. histolytica* infection, which were positive by antigen detection and isoenzyme analysis of cell culture (36). Several studies show that the detection of *E. histolytica* antigen in stool by ELISA technique is a more reliable, sensitive, and specific method, faster and easier to perform in many medical laboratories (34, 37-39). The sensitivity and specificity of coproantigen test are evaluated by many studies. el-Hamshary et al. (40), reported that coproantigen ELISA in 93 patients susceptible to amoebiasis was more sensitive and specific than microscopic methods to differentiate between pathogen and nonpathogen *Entamoeba* species. Baumann et al. (41), showed that 14 out of 15 suspected patients were diagnosed with amoebiasis (93% sensitivity) using coproantigen ELISA kits. Singh et al. (42), reported that the diagnosis of amoebiasis using coproantigen ELISA kits had an 89% sensitivity and 100% specificity. Urdaneta et al. (43), showed that coproantigen ELISA is a more reliable method than the microscopic examination with 98.3% sensitivity and 97.6% specificity. The positive and negative predictive values were 96.2% and 97.6%, respectively, to detect *E. histolytica* in stool samples.

There was a 100% correlation between the antigen detection kit and the conventional nested PCR results for *E. histolytica* diagnosis in suspected patients (22, 23, 35). Gharibi et al. (44), presented that among 200 patients with dysenteric diarrhea, 17, 30, and 23 were positive for *E. his-*

tolytica/E. dispar using microscopic, coproantigen, and PCR techniques, respectively. They reported that the more positive samples were detected by coproantigen ELISA.

5.1. Conclusions

According to the current study findings, there are many *E. histolytica* misdiagnoses in medical diagnostic laboratories in the studied region, and the rate of *E. histolytica* infection is very low among children with dysenteric diarrhea. Therefore, useful training of laboratory personnel to promote experience and use of a combination of culture and coproantigen detection techniques are strongly recommended. The small sample size was one of the limitations of the current study; therefore, larger sample sizes should be considered in further studies.

Footnotes

Authors' Contribution: Ahmad Shamsizadeh did the study design, diagnosis of patients, and collection of samples. Roya Nikfar did diagnosis of patients, collection of samples, and writing of the manuscript. Mahmoud Rahdar did microscopic test and ELISA test, writing, and corresponding author

Conflict of Interests: The authors declared no conflict of interest.

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