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Short Communication

Comparative Analysis of Diagnostic Techniques for Entamoeba histolytica in Emergency Diarrhea Cases

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Antigen screening were conducted to stool samples from 60 patients admitted to our emergency department with diarrhea complaint between June 2009 and October 2009 by the methods of direct microscopic examination, trichrome staining, ELISA (Enzyme-linked immunosorbent assay), respectively. As a result of examination of total 60 samples with direct microscopic, trichrome staining and ELISA method, it was detected positive in 7(11.3%), 6(9.7%) and 8(12.9%) samples, respectively. The presence of *Entamoeba histolytica* has been accepted exactly in the samples in which ELISA test results were positive and necessary treatment of patients has been started immediately. Due to precise pathogen protozoan discrimination has not been performed with the direct microscopic examination, it was emphasized that unnecessary drug therapy would be prevented as a result of detection of presence of *E. histolytica* specific antigen by ELISA in the samples sent to the laboratory with the diagnosis of amoebiasis by concerned physician.

Key words: *Entamoeba histolytica*, diarrhea, diagnosis.

INTRODUCTION

Amoebic dysentery (amoebiasis) which is the agent of *Entamoeba histolytica* is widely seen around the world. About 50 million people has become infected a year and eventually over 100,000 people lose their lives (Singh et al., 2009; WHO, 1997; Stanley et al., 2003; Tanyuksel et al., 2003). It was detected that the incidence of this parasitosis were varied between 0.4 and 13% in researches done in our country (Tuncay et al., 2007; Nar et al., 2003; Eren et al., 2005; Oguzturk et al., 2001). Amoebiasis come into being in consequence of taken of quad-core mature cysts from water, foods, goods or hands by orally. *E. histolytica* trophozoites are placed into the colon mucosa and submucosa then forms a bloody-mucus diarrhea table. It forms abscesses by moving through blood to liver, lungs, brain and other tissues (Petri et al., 1999). While pathogenic *E. histolytica*, one of two morphologically similar species of *Entamoeba*, forms amebic colitis and liver abscess, non-pathogen *Entamoeba dispar (E. dispar)* does not give rise to a disease. It has been reported that approximately 90% of *Entamoeba* species detected in humans was *E. dispar* and *E. histolytica* (Uyar et al., 2009; Braga et al., 1998; Hague et al., 1998).

Searching for adhesion specific antigen in stool by ELISA is a sensitive and specific method for the diagnosis of *E. histolytica* (Uyar et al., 2009; Braga et al., 1998; Hague et al., 1998; Leo et al., 2006).

As a result of both pathogenic/non-pathogenic discrimination is not fulfilled and identification of leukocytes, macrophages and other amoebas as *E. histolytica* in stool, false-positive results occur. Incorrect assessment of the preparate also leads to needlessly writing and use of drugs in *E. dispar* cases. Put a correct diagnosis of patients admitted to the emergency department with complaints of diarrhea is of great importance for the adjustment of treatment. Therefore,

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Table 1. Statement of *E. histolytica* appearance in different diagnostic method with the statement of the same sample appearance in different diagnostic method.

Examination method	Positiven n (%)	Negativen n (%)	Total n (%)
Direct microscopy (DM)	7(11.3)	53(88.7)	0(100)
Trichrom	6(9.7)	54(90.3)	60(100)
ELISA	8(12.9)	52(87.1)	0(100)
DM and Trichrom*	2(3.33)	58(96.7)	0(100)
DM and ELISA*	5(8.3)	55(91.7)	0(100)
Trichrom and ELISA*	6(9.7)	6(90.3)	0(100)
DM, Trichrom, ELISA*	1(1.67)	59(98.3)	0(100)

*Statement of appearance /non-appearance of the same sample in two diagnostic methods.

we would like to investigate whether the use of ELISA method in this study has an advantage to direct microscopic examination and paint dispar trichrome methods in discrimination between pathogenic *E. histolytica* and non-pathogenic *E. dispar.*

It was aimed that contribute to give also the appropriate treatment to the patient with a reliable method that will be found.

MATERIALS AND METHODS

60 patients admitted to the Department of Emergency Medicine of Inonu University Medical Faculty with diarrhea between September 2009 - January 2010 were included in our study.

Each diarrheal patient's stool sample taken by emergency service doctors passed on Department of Parasitology laboratory by auxillary staff without waiting, the analysis was carried out by using native-lugol (direct microscopy) and trichrome methods .

E. histolytica specific antigen was investigated from all samples of these investigations also determined by ELISA (WAMPOLE *E. histolytica* 2 ELISA TechLab)

RESULTS

Entamoeba spp. cysts were seen in 7 of 60 (12.9%) samples by direct microscopic examination. The presence of *E. histolytica* specific antigen was detected in 8(12.9%) samples as for ELISA examination in all samples simultaneously. However, *Entamoeba spp*. was positive with trichrome staining method in 6 samples. While both of 3 diagnostic positivity methods were determined in only 1% of samples, the presence of *E. histolytica* specific antigen was determined by ELISA method in 5 of 7 samples *Entamoeba spp*. was seen in direct examination (Table 1).

DISCUSSION

Amoebiasis diagnosis could be stated with indicating the form of cyst and/or trophozoite forms of agent in microscopic stool examination. It was stated that the ratio of parasite exposure to cyst and trophozoite was 33 – 50% with only once microscopic examination, but this rate increased to 75% as a result of the examination of three stools at different times (Garcia et al., 1993). The sensitivity of microscopy ranged from 10-60% in the best conditions and presence of leukocytes or non-patho-gen species in feces can lead to false positive results (Haque et al., 2006). After the advice decision of World Health Organization in 1997 “Apply the tests which is oriented to a definite diagnosis of *E. histolytica*”, for the differential diagnosis of *E. histolytica* and *E. dispar* an important way is taken in the use of diagnostic methods as the detection of specific *E. histoytica* antigens (WHO, 1997). There are advantages of these methods at some points according to direct microscopy. We classify these advantages as high sensitivity and specificity of ELISA kits, available feature of quick finalization, not needed to experienced personnel as in the evaluation of direct microscopy and avoid cross-reaction against other parasites (Leo et al., 2006; Garcia et al., 1993; Haque et al., 2006; Tanyuksel et al., 2005).

In one of the research done with the subject ELISA method was applied by Gonzalez-Ruiz and their colleagues in order to determine *E. histolytica* antigens in stool for the diagnosis of amoebiasis and it was observed that ELISA method does not cross-react with other parasites. It was found that the sensitivity of this method was 87% and specificity was 100% and it is recommended that it would be implemented as a method of diagnosis. Also in several studies conducted in Turkey it was observed that direct microscopic examination has less sensitivity than ELISA and low consistency between them. A study in the south of our country positive results has found in 20.4% of total 88 sample with trichrome staining and with research of *E. histolytica* / *E. dispar* common antigen in 29.5%. While both positive diagnostic method was detected in 14 (15.99%) of samples, only direct examination positive in 4(4.5%) samples and also only ELISA positive in 12(13.6%) samples were obtained (Delialioglu et al., 2004). 9378 stool samples were examined between January 2004 and May 2006 in a study conducted in zmir, *E. histolytica*

were seen only 18 of them who ELISA applied 33 of 41 patients who *E. histolytica* / *E. dispar* was detected, although revealed by direct microscopic examination and culture methods, it has been reported that 15 patients were *E. dispar* undetectable by ELISA (Tuncay et al., 2007) . In our study, the number of samples detected by direct microscopy, but not found by ELISA was 2 (3.33%), respectively (Nar et al. 2006). While *Entamoeba spp.* were found in 9.1% of 77 people have gastrointestinal complaints with direct microscopic examination in Ankara, it was stated 6.25% positivity in research of *E. histolytica* specific antigen.

However, 24% cysts / trophozoites were detected with microscopy in a study conducted by Tanyüksel et al. (2005) in Ankara, positive results were determined by ELISA based on *E. histolytica* specific antigen detection in 13% of 380 samples. Only 5 (8.3%) of 8 *E. histolytica* cases detected by ELISA in our study were seen by direct microscopy. Mengelioglu et al. (2009) 20 has achieved positive results in ELISA test in 59.1% of stool samples with *E. histolytica* cysts. This ratio was also 71.4
% in our study. It was considered that possible parasite in the samples resulted as negative was *E. dispar* and / or other non-pathogenic protozoa. Today, the necessity of making *E. histolytica* and *E. dispar* separation is inevitable. Because when the patient is diagnosed *E. dispar,* it is not required to be treated, on the other hand if patients is diagnosed with *E. histolytica* they are required to be treated as an emergency. As a result, detection of pathogenic *E. histolytica* positivity with specific ELISA method in the stool samples in which *Entamoeba spp.* cysts / trophozoites forms were seen with direct microscopic point of view gives more healthy diagnosis opportunity. In addition, it shall be prevented to receive unnecessary drug treatment of patients through the use of this diagnostic method.

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