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TURKISH INFLAMMATORY BOWEL DISEASE SOCIETY RECOMMENDATIONS ON SELECTED TOPICS OF CROHN'S DISEASE

TÜRK İNFLAMATUVAR BARSAK HASTALIKLARI DERNEĞİNİN CROHN HASTALIĞI İLE İLGİLİ SEÇİLMİŞ KONULARDA ÖNERİLERİ

What is the most accurate method in the diagnosis of amebic dysentery?

Amibik dizanterinin tanısında en doğru yöntem nedir?

Key words: Amebic dysentry, amebic colitis, diagnosis

Anahtar kelimeler: Amibik dizanteri, amibik kolit, tanı

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INTRODUCTION

Intestinal amebiasis was first defined by Fritz Schaudinn in 1903, and the agent causing this disease was called E. histolytica. E. histolytica is the most pathogenic microorganism among the Entamoeba species and may lead to invasive diseases such as amebic liver abscess and infections in the respiratory and genitourinary systems and the cerebrum.

Amebiasis is an important public health problem effecting 500 million people annually, particularly in the developing countries. The established risk factors for amebic infection are hot climate, low socioeconomic status, poor hygienic conditions, drinking dirty water and journey to the endemic zones (1-4).

Clinical, endoscopic and histopothologic distinction between amebic colitis and the other inflammatory bowel diseases are difficult. Amebic dysentery may accompany other underlying inflammatory diseases. In patients with inflammatory bowel disease, in case of unexplainable symptoms like fever, dyspnea or confusion and usually in immunocompromised cases, clinicians must be aware of the possibility of parasitic or fungal infections. Screening for parasitic or fungal infections prior to immunomodulatory treatment is usually expected to be unbenefical. Expert opinion is that screening the cases travelling to endemic regions will be appropriate (5).

In Turkey, where various geographic regions with socioeconomically different structures exist, especially in regions with lower socioeconomic conditions, amebiasis is an important health problem. In our country, which is an endemic region for amebiasis, the presence of amebic dysentery should be ruled out in severe colitis prior to immunomodulatory treatment and in the differential diagnosis of Crohn's disease.

METHODS

In the ECCO guidelines in 2009, related to the opportunistic infections, there are no comments with regard to amebic dysentery. Due to the fact that amebic dysentery is seen commonly in our country, particularly more frequent in some regions of our country, we used the 3e systematic literature search to answer the question: "Which test is the most accurate for the diagnosis of amebic dysentery?"

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Systematic literature search was preformed only in the Pubmed database. In addition, other publications were achieved by screening the literature references. “Amebic dysentery” was used as the key word. Only the medical literature in English was searched and the trials with patients older than 18 years of age were included in the study. In this systematic literature search, the sensitivities and the specificities of the diagnostic methods used in amebic dysentery were determined in the statistical analyses.

RESULTS

Fourteen studies related to the diagnostic methods for amebic dysentery were achieved as a result of 3e systematic search. Determination of sensitivities and specificities of the diagnostic methods and also establishment of the diagnostic priorities were targeted, while evaluating the studies. The types of 14 studies are stated in Table 1.

The Diagnostic Methods To Be Used In The Diagnosis of Amebic Dysentery

1. Microscopy

The microscopic techniques are wet preparation method, concentration method and permanent staining of fresh stool with trichrome or iron haemotoxylin stains. The sensitivity for the staining of fresh stool sample with direct saline (wet preparation method) is quite low (10%). The preparation should be evaluated in the first one hour in order to observe the erythrocytes inside the moving trophozoites. However, in cases who do not present with acute dysentery, trophozoites do not contain erythrocytes. Only cysts are presented in the stool samples of asymptomatic subjects. Cysts may be seen by the concentration method. However, some investigators advocate that for the diagnosis of E. histolytica, the permanent staining of fresh stool preparations with trichrome or iron haemotoxylin stains and the characteristics in microscopic findings could be enough. This issue was discussed by WHO in Mexico in 1997 and it was stated that E. histolytica and E. dispar could not be discriminated by the light microscope, owing to their similar morphologies (6).

Table 1. The types of studies analyzed

Type of the study	Number of studies
Cross-sectional study	3
Case control	8
Retrospective	3

Microscopy is a method with less sensitivity and specificity compared to other laboratory techniques. Sensitivity is about 60% and the rate of false positivity is high owing to the false perception of the macrophages as the trophozoites and the polymorphonuclear leukocytes as the cysts. In the two separate studies performed by Nesbitt et. al. and Delialiaoğlu et.al., stool microscopy and stool antigen methods were compared and the sensitivity of stool microscopy was found to be 43% and 54%, respectively (6, 7).

2. Amoeba Antigen in Stool

Antigen-based ELISA kits specific for E. histolytica are specific for the monoclonal antibodies targeting Gal/GalNAc-specific lectin (E. histolytica II; TechLab, Blacksburg, VA) and monoclonal antibodies targeting serine-rich antigen (Optimum S kit; Merlin Diagnostika, Bornheim-Hersel, Germany). Whilst the other ELISA kits, Entamoeba CELISA PATH kit (Cellabs, Brookvale, Australia), are specific for lectin-specific monoclonal antibodies. ProSpecT EIA (Remel Inc.; Alexon-Trend,Inc., Sunnyvale, CA) fixes the E. histolytica antigen in stool samples (8, 9).

This is a beneficial method in endemic regions where diarrheal diseases are common. Its usefullness is approved in developing countries where most of the amebiasis cases are observed. It is particularly useful in countries where the molecular techniques can not be used due to their costs (10). The disadvantage of this method is that it is 100 folds less sensitive compared to PCR (11).

3. Antibody Fixation

The role of serology is restricted in the diagnosis of amebiasis and the major reasons concerning this issue are the use of poorly characterized amebic antigen preparations, complex methodologies and high prevalence of seropositive uninfected patients in endemic areas (12). Nevertheless, in non-endemic regions with developed industry, the serology is beneficial in determining the E. histolytica infection. In non-endemic areas, high ELISA antibody titration has a sensitivity of 95% in the patients with amoeba positive stool. Furthermore an important advantage is that the test does not give cross reaction with non-pathogenic Entamoeba species (13).

4. DNA Extraction Methods

DNAs of E. histolytica, E.dispar ve E moshkovskii can be detected in stool or in the liver abscess

Table 2. The sensitivities and the specifities of the tests used in amebic dysentery-diagnosis

Number of studies	Diagnostic method	Sensitivity	Specificity
14	Microscopy	%57.8	%86.3
	Detection of stool antigen	%62.5	%96.5
	Antibody for Entameoba histolytica	%63.3	%87.6
	Conventional PCR	%91.6	%92.2
	Real-Time PCR	%97.1	%77.2

samples, due to the developments in molecular techniques such as PCR and Real-Time PCR. These are the reliable methods applied for the diagnosis of amebiasis in recent years. In a study comparing stool antigen and PCR, Haque et. al. found the conventional PCR sensitivity at a rate of 99% (8). Continuous monitoring of "amplicon" a PCR product is provided by the fluorescent marking technique in the Real-Time PCR method which has a high sensitivity. Sensitivity rates up to 99% have been obtained for Real-Time PCR in the trials compared with other methods (14).

The major disadvantages of DNA extracting methods which are restricting their usages are the difficulties in extracting DNA from stool samples, the time consuming and costly process of DNA amplification and extraction (10).

Evaluating the sensitivities of diagnostic methods used in the diagnosis of amebic dysentery, the most sensitive methods are the DNA extracting methods (the sensitivities for real time PCR and conventional PCR are 97.1% and 91.6%, respectively) (Table 2). The studies comparing conventional PCR with other methods determined that the specificity and the sensitivity of the conventional PCR are significantly high (10,14). Investigating the specificities of diagnostic tests for amebic dysentery, the most specific tests were detection of amoebic antigen in stool (96.5%) and the conventional PCR (92.2%) (Table 2). Despite its low sensitivity compared with the PCR, in the literature it is stated that the stool antigen detection is a reliable laboratory test, when PCR techniques cannot be used (10, 11).

CONCLUSION

In ECCO's comments, regarding the prevention and treatment of opportunistic infections and as an approach to inflammatory bowel diseases, it is stated that screening for amebic dysentery is not necessary in non-endemic regions. But in our country, especially in some regions where it is fairly widespread, the presence of this infection should be considered in the differential diagnosis of Crohn's disease and in severe colitis prior to immunomodulatory treatment. Based on these results national recommendations for the diagnosis of amebic dysentery are seen in the box.

Recommendation:

The tests to be chosen in the circumstances of our country for amebic dysentery screening in cases with Crohn's disease are as follows:

1. *The sensitivity of stool microscopy is low, and cannot identify the pathogenic and non-pathogenic species* ***(EL 2c, RG B).***
2. *Stool Ag test is recommended as a first line test, since it is a sensitive, specific, rapid and easy to do test and can make the distinction between of E. histolytica and E. dispar* ***(EL 2b, RG B).***
3. *If the clinical suspicion exists in cases with negative antigen detection in stool, the diagnosis should be confirmed via PCR (DNA extraction method)* ***(EL 2b, RG B)***

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