Gastroenterology and Hepatology from bed to bench. ***2008;1(1):45-50***

REVIEW ARTICLE

Update of knowledge for best Amebiasis management

Ehsan Nazemalhosseini Mojarad [1, 2], Mohammad Rostami Nejad[1], Ali Haghighi[2]

[1] *Research Center for Gastroenterology and Liver Diseases, Shahid Beheshti University M.C., Tehran, Iran*

[2] *Department of Medical Parasitology & Mycology, School of Medicine, Shahid Beheshti University M.C., Tehran, Iran*

ABSTRACT

Amebiasis is the infection of the human gastrointestinal tract by Entamoeba histolytica, a protozoan parasite that is capable of invading the intestinal mucosa and may spread to other organs, mainly the liver. The detection of Entamoeba histolytica from the nonpathogenic but identically appearing parasites Entamoeba dispar and Entamoeba moshkovskii is an important goal of the clinical microbiology laboratory. Currently, there is no low-cost laboratory test available for the differentiation of E. histolytica from E. dispar infections. It is likely that at least 90% of the infections previously ascribed to E. histolytica are actually E. dispar, while only the remaining 10% are infected with E. histolytica in its new sense. The present manuscript review recent advances in this regard. The purpose of this study is to alert physicians (and perhaps with their help, laboratories) to the importance of distinguishing between the two species of amoebae.

Keywords: *Amebiasis, Entamoeba histolytica, Entamoeba dispar, Entamoeba moshkovskii*.

(Gastroenterology and Hepatology from bed to bench 2008;1(1):45-50).

INTRODUCTION

The protozoan parasite Entamoeba histolytica causes an estimated 50 million cases of amebiasis and 40,000 to 100,000 deaths annually, placing it second only to malaria as a cause of death resulting from parasitic protozoa (1). The detection of Entamoeba histolytica, the causative agent of amebiasis, from the nonpathogenic but identically appearing parasites Entamoeba dispar and Entamoeba moshkovskii is an important goal of the clinical microbiology laboratory. Microscopy, culture/zymodeme analysis, and molecular biology-based techniques are used for the diagnosis of E. histolytica. Each detection test has different advantages and disadvantages. Currently, there is no low-cost laboratory test available for the differentiation of E. histolytica from E. dispar infections. The development of this valuable diagnostic tool for use in clinical laboratories and large-scale epidemiological studies has been made a priority (2) and is the subject of intense research (3). Tools that allowed accurate differentiation of the two species were clearly needed, and in the past decade differentiation based on DNA amplification has been a research focus of many groups. Species-specific primers that amplify regions of several different genes have been used (4). Classic microscopic examination of the parasite E. histolytica in stool cannot differentiate E. histolytica from E. dispar or E. moshkovskii (5-7). Microscopic techniques are unable to differentiate between two species, however, they are at best only

Received: 10 August 2007 *Accepted*: 18 December 2007
Reprint or Correspondence: Ehsan Nazemalhosseini Mojarad. Department of Medical Parasitology & Mycology, School of Medicine, Shahid Beheshti Medical University, Tehran, Iran. **E-mail**: ehsan_nazem_m@yahoo.com

10 to 60% sensitive and confounded with false-positive results due to misidentification of macrophages and nonpathogenic species of Entamoeba. Culture along with isoenzyme (zymodeme) analysis enables differentiation of E. histolytica from E. dispar or E. moshkovskii and was considered the gold standard for diagnosing amebic infection during the past decades. New approaches to the detection of E. histolytica are based on the detection of an E. histolytica-specific antigen and DNA. Several groups have reported the detection of amebic antigen in stool samples, serum, liver abscess pus samples, and saliva using enzyme-linked immunosorbent assay methods (8-12).

PATHOLOGY and CLINICAL MANAGEMENT

Amebiasis is the infection of the human gastrointestinal tract by Entamoeba histolytica, a protozoan parasite that is capable of invading the intestinal mucosa and may spread to other organs, mainly the liver. Entamoeba dispar, an ameba morphologically similar to E. histolytica that also colonizes the human gut, has been recognized recently as a separate species with no invasive potential (2,5,13,14). Depending on the affected organ, the clinical manifestations of amebiasis are intestinal or extraintestinal. There are four clinical forms of invasive intestinal amebiasis, all of which are generally acute: dysentery or bloody diarrhea, fulminating colitis, amebic appendicitis, and ameboma of the colon. Dysenteric and diarrheic syndromes account for 90% of cases of invasive intestinal amebiasis. Patients with dysentery have an average of three to five mucosanguineous evacuations per day, with moderate colic pain preceding discharge, and they have rectal tenesmus. In patients with bloody diarrhea, evacuations are also few but the stools are composed of liquid fecal material stained with blood. While there is moderate colic pain, there is no rectal tenesmus. Fever and systemic manifestations are generally absent. These syndromes constitute the classic ambulatory dysentery and can easily be distinguished from that of bacterial origin, where the patient frequently complains of systemic signs and symptoms such as fever, chills, headache, malaise, anorexia, nausea, vomiting, cramping abdominal pain, and tenesmus (15).

Although E. histolytica can infect almost every organ of the body, the most frequent form of extraintestinal amebiasis is the amebic liver abscess. This condition, which results from the migration of trophozoites from the colon to the liver through the portal circulation, is more common in adults than in children and more frequent in males than in females for 10 and 3 times respectively (16,17). In general, the onset is abrupt, with pain in the right hypochondrium radiating toward the right shoulder and scapular area. The pain usually increases with deep breathing, with coughing, and while stepping on the right foot during walking. When the abscess is localized to the right lobe, symptoms include an irritative cough that is sometimes productive and a pleuritic type of chest pain. Abscesses in the upper left lobe can cause epigastric, sometimes dyspneic pain, at times spreading to the base of the neck and to one or both shoulders. Fever between 38 to 40°C is found in 85 to 90% of patients with amebic liver abscess. The patient commonly has chills and profuse sweating in the afternoon and at night. Other symptoms include anorexia, nausea, vomiting, diarrhea (with or without blood), and dysentery.

On physical examination, the cardinal sign of amebic liver abscess is painful hepatomegaly. Digital pressure and fist percussion will often produce intense pain in the liver region. On palpation, the liver is soft and smooth, in contrast to the rough, hard, irregular character of the liver in patients with cirrhosis and hepatocarcinoma. Jaundice is present in 8% of the patients who

respond well to treatment. When jaundice is severe, multiple abscesses should be suspected. Diarrhea or dysentery is seen in less than one-third of patients. Complications of amebic liver abscess include perforation to the pericardial space, pleura, or peritoneal cavity (15,17).

The diagnosis of invasive intestinal amebiasis is still based on the microscopic identification of E. histolytica trophozoites in rectal smears or recently evacuated stools and on the results of rectosigmoidoscopy. Trophozoites are most likely to be found in the bloody mucus and in the yellowish exudates covering the mucosal ulcerations obtained during rectosigmoidoscopy. Diagnostic problems arise when only cysts are identified in stools of healthy or diarrheic individuals. A commercially available laboratory test based on the identification of specific E. histolytica antigens in stool (3) is able to discriminate E. histolytica from E. dispar cysts (W. A. Petri, unpublished observations). However, the high cost and lack of knowledge of this test have hindered its use in clinical laboratories, especially in countries where amebiasis is endemic. Until these new diagnostic tests are widely available to clinical laboratories, these samples should be reported as containing E. histolytica/E. dispar (2,17).

DIAGNOSIS

How should physicians respond to a laboratory diagnosis of Amebiasis? First, they need to know whether the laboratory reporting E. histolytica has identified the pathogenic organism, an event which seldom occurs at present. Perhaps at the urging of physicians, more and more laboratories will recognize the importance of distinguishing between the two species. If a laboratory does not yet differentiate pathogenic from nonpathogenic species, then any reported E. histolytica should be treated, even in asymptomatic patients, because symptoms may appear in that person later and because the patient may carry infection to others. In this regard, clinical judgment should be modified by the realization that the pathogen is considerably rarer than previously believed (1,2,13). The World Health Organization has recommended that Entamoeba histolytica "should be specifically identified and if present should be treated" (1). The diagnosis of amebic liver abscess is sometimes difficult (1). In areas of endemic infection or when there is a history of travel to such places, amebic abscess should be suspected in patients with spiking fever, weight loss, and abdominal pain in the upper right quadrant or epigastrium and in patients with tenderness in the liver area. The presence of leukocytosis, a high alkaline phosphatase level, and an elevated right diaphragm suggest a hepatic abscess. The diagnosis is confirmed by ultrasonography or by computed tomography (CT) scans. The CT scan is the most precise method for identifying hepatic abscesses, especially when they are small, and following intravenous injection of contrasting agents, it is of great value in the differential diagnosis of other focal lesions of the liver (16). Serological tests for antiamebic antibodies are positive in approximately 75% of patients with invasive colonic amebiasis and in over 90% of patients with amebic liver abscesses. Most studies have focused on a single factor in an attempt to dissect the multiple mechanisms used by the parasite that ultimately result in tissue destruction (17). Some problems exist to distinguish between IBD and colitis associated with amoeba according to both symptomatic and endoscopic appearance of the colon. Sometimes IBD can co-exist with amebiasis. This, of course, leads to confusion in the diagnosis and treatment of the disease (4). Preliminary data obtained from the application of these methods confirm the presence of E. dispar in most asymptomatic amebic infections, although E. histolytica asymptomatic colonization is not uncommon (18).

EPIDEMIOLOGY

Intestinal and extra-intestinal amebiasis remains a significant health concern worldwide, especially in developing countries. Asymptomatic cyst passing is the most common manifestation of the intestinal Entamoeba infection. An estimated 10% of world's population are infected with E. histolytica / E. dispar, and between 40,000 to 110,000 individuals die of invasive amebiasis annually (1,2). It has been known that many people who are apparently infected with E. histolytica never develop symptoms and then infections clear spontaneously (19,20). In previous study on amebiasis in Ethiopia, a high prevalence of E. histolytica was found, while upon polymerase chain reaction (PCR)-confirmation only E. dispar was shown (21). In another study in Ethiopia on 108 stool samples, PCR was positive for E. histolytica in one specimen and for E. dispar in 77 while 30 samples were negative for both species (22). Data from some parts of Iran showed that 92.1% of the isolates were E. dispar and 7.9% were E. histolytica or mixed infections (23,24). We concluded that PCR is a preferable tool for differential diagnosis of microscopic positive E. histolytica/E. dispar strains. Our results together with a similar work in north-east of Iran (25) and a study in north, center and south of Iran (23,24), explain that E. dispar is the main agent of infection and amebiasis is quite rare in Iran.

The realization that Entamoeba histolytica and Entamoeba dispar are two distinct but morphologically identical species (5) has had a major impact on all aspects of amebiasis research, most notably epidemiology (1,2). It is very important to keep in mind that according to the older data, many E. histolytica infections were most probably confused with E. dispar due to limited data obtained from microscopic examinations. Epidemiological studies have shown that low socioeconomic status and unsanitary conditions are significant independent risk factors for infection. In addition, people living in developing countries have a higher risk and earlier age of infection than do those in developed regions. Invasive amebiasis due to E. histolytica is more common in developing countries. In areas of endemic infection, a variety of conditions including ignorance, poverty, overcrowding, inadequate and contaminated water supplies, and poor sanitation favor direct fecal-oral transmission of amebas from one person to another. It is now known that even in areas where invasive amebiasis is common, E. dispar is by far the most prevalent species (26).

DIVERSITY AMONG ISOLATES

Since the first description of amebiasis in 1878 by Lo¨sch (27), we still do not have a proper answer to the question of why disease and symptoms develop in only 5 to 10% of those infected with E. histolytica. It has been speculated that a spectrum of virulence levels among the E. histolytica strains and variability in the host immune response against amebic invasion contribute to the outcome of amebic infection. While variation in human immune responses against amebic infection is not understood, the polymorphic structure of E. histolytica has recently been unveiled (28), but is E. dispar really nonpathogenic, and should it on this basis be completely dismissed as a subject for further investigation? It has been shown to be capable of producing variable focal intestinal lesions in animals and of destroying epithelial cell monolayers in vitro. There is also some evidence that pathological changes may occur in some humans, though, invasive lesions and symptomatic infections have to date not been reported. Whether these characteristics are variable among strains is unknown (29). We propose that molecular typing and analysis of genotypes of E. histolytica isolates from a variety of geographic locations should help in determining geographic origins of isolates and routes of transmission.

CONCLUSION

The acceptance of Entamoeba histolytica and Entamoeba dispar as distinct species has had a major impact on our views of amebiasis, in particular its clinical management and epidemiology. It is likely that at least 90% of the infections previously ascribed to E. histolytica are actually E. dispar, while only the remaining 10% are infected with E. histolytica in its new sense. However, it also appears that many E. histolytica infections never progress to become symptomatic and are spontaneously lost. This observation raises some important questions. Are the organisms that produce invasive, symptomatic disease genetically distinct from those that give rise to asymptomatic infections? Or do all E. histolytica isolates have the potential to become invasive? Do certain invasive isolates show tropism for specific organs, with some preferentially ending up in the intestinal wall while others reach extraintestinal sites? To address the possibility of a relationship between parasite variation and infection outcome, the ability to differentiate isolates of E. histolytica is necessary (1,5,8). The World Health Organization has recommended that Entamoeba histolytica "should be specifically identified and if present should be treated" (1,2). Until that time, epidemiological data on amebiasis were mainly based on microscopic detection of E. histolytica/E. dispar cysts without the differentiation between the two species. Moreover, many cases will be missed, as the sensitivity of microscopy is known to be low (8). As a result, accurate data on the prevalence of E. histolytica is not available and therefore there is a need to obtain such data using specific and more sensitive tools. When diagnosing a patient who has a medical history and clinical findings suggestive of infectious enteritis, physicians usually start their investigation by ordering a stool culture and one or more stool specimens to be examined for protozoa and other parasites. If the initial culture result is negative and the laboratory identifies Entamoeba histolytica, treatment for that parasite will probably be given, and subsequent investigation will be curtailed. However, recent discoveries suggest that this approach may be incorrect most of the time, because we now know that what has been considered E. histolytica actually includes two distinct species, the much more common of which is always nonpathogenic. The purpose of this short account is to alert physicians (and perhaps with their help, laboratories) to the importance of distinguishing between the two species of amoebae.

ACKNOWLEDGEMENT

The authors express their appreciation to Dr. Mohammad Reza Zali the Director of ShahId Beheshti Research Center for Gastroenterology and Liver Diseases, for helpful suggestions on this manuscript.

REFERENCES

1. WHO/PAHO/UNESCO. A consultation with experts on amebiasis. Epidemiol Bull 1997;18:13-14.

2. Anonymous**.** *Entamoeba* taxonomy. WHO Bull 1997;75:291–92.

3. Haque R, Ali IKM, Akther S, et al. Comparison of PCR, isoenzyme analysis, and antigen detection for diagnosis of *Entamoeba histolytica* infection. J Clin Microbiol 1998;36:449–52.

4. Garcia LS, editor. Diagnostic medical parasitology. 4th edition. ASM Press, Washington, 2001.

5. Diamond LS, Clark CG. A redescription of *Entamoeba histolytica* Schaudin 1903 (amended Walker 1911) separating it from *Entamoeba dispar* (Brumpt 1925). J Eukaryot Microbiol 2003;40:340–44.

6. Gathiram V, Jackson TF. Frequency distribution of *Entamoeba histolytica* zymodemes in a rural South African population. Lancet 1985;719–21.

7. Gonzalez-Ruiz A, Haque R, Aguirre A, et al. Value of microscopy in the diagnosis of dysentery associated with invasive Entamoeba histolytica. J Clin Pathol 1994;47:236–39.

8. Zaki M, Clark CG. Isolation and characterization of polymorphic DNA from. Entamoeba histolytica. J Clin Microbiol 2001;39(3):897-905

9. Haque R, Faruque AS, Hahn P, et al. Entamoeba histolytica and *Entamoeba dispar* infection in children in Bangladesh. J Infect Dis 1997;175:734–36.

10. Krogstad DJ, Spencer HC, Healy GR, et al. Amebiasis: epidemiologic studies in the United States, 1971–1974. Ann Intern Med 1978;88:89–97.

11. Strachan WD, Chiodini PL, Spice WM, et al. Immunological differentiation of pathogenic and nonpathogenic isolates of *E. histolytica.* Lancet 1988;561–63.

12. Haque R, Mollah NU, Ali IKM, et al. Diagnosis of amebic liver abscess and intestinal infection with the TechLab *Entamoeba histolytica* II antigen detection and antibody tests. J Clin Microbiol 2000;38**:**3235–39.

13. Clark CG. Entamoeba dispar, an organism reborn. Trans R Soc Trop Med Hyg 1998;92:361–64.

14. Martinez-Palomo A, Espinosa-Cantellano M. Amoebiasis: new understanding and new goals. Parasitol Today 1998;14:1–3.

15. Martinez-Palomo A, Espinosa Cantellano M. Intestinal amoebae. In: Cox FEG, Kreier JP, Wakelin D, editors. Topley & Wilson's microbiology and microbial infections. Edward Arnold, New York, N.Y., 1998.

16. Sepu´lveda B, Manzo N. Clinical manifestations and diagnosis of amebiasis. In: Martinez-Palomo A, editor. Amebiasis. Elsevier Science Publishing, Amsterdam, the Netherlands, 1986;p:169-88.

17. Cantellano E, Palomo M. Pathogenesis of intestinal amebiasis: from molecules to disease. Clin Microbiol Rev 2000;13(2):318–31.

18. Braga LL, Lima AAM, Sears CL, et al. Seroepidemiology of *Entamoeba histolytica* in a slum in Northeastern Brazil. Am J Trop Med Hyg 1996;55:693–97.

19. Tachibana H, Kobayashi S, Nagakura K, et al. Asymptomatic cyst passers of Entamoeba histolytica but not Entamoeba dispar in institutions for the mentally retarded in Japan. Parasitol Int 2000;49:31–35.

20. Ibne Karim M, Zaki M, Clark CG. Use of PCR amplification of tRNA gene linked short tandem repeats for genotyping Entamoeba histolytica. J Clin Microbiol 2005;43(12):5842-47.

21. Kebede A, Verweij JJ, Petros B, et al. Misleading microscopy in amoebiasis. Trop Med Int Health 2004;9(5):651–52.

22. Kebede A, Verweij J, Dorigo-Zetsma W, et al. Over diagnosis of amebiasis in the absence of Entamoeba histolytica among patients presenting with diarrhoea in Wonji and Akaki, Ethiopia. Trans R Soc Trop Med Hyg 2004;97:305-7.

23. Hooshyar H, Rezaian M, Kazemi B. Distribution and differential diagnosis of Entamoeba histolytica from Entamoeba dispar by the PCR-RFLP method in Central Iran. Ann Saudi Med 2003;23(6):363-66.

24. Hooshyar H, Rezaian M, Kazemi B, et al. The distribution of Entamoeba histolytica and Entamoeba dispar in northern, central, and southern Iran. Parasitol Res 2004;94:96-100.

25. Nazemallhoseini Mojarad E, Haghighi A, Azimi Rad M, et al. Prevalence of Entamoeba histolytica and Entamoeba dispar in Gonbad City, Iran. Iranian J Parasitol 2007;2(2):48-52.

26. Tanyuksel M, Petri WA JR. Laboratory diagnosis of Amoebiasis. Clin Mic Rev 2003;16(4):713-29.

27. Lesh FA. Massive development of amebas in the large intestine. Fedor Aleksandrovich Lesh (Losch). Am J Trop Med Hyg 1975;24:383-92.

28. Haghighi A, Kobayashi S, Takeuchi T, et al. Remarkable genetic polymorphism among Entamoeba histolytica isolates from a limited geographic area. J Clin Microbiol 2002;40:4081-90.

29. Zaki M, Meelu P, Sum W, et al. Simultaneous differentiation and typing of Entamoeba histolytica and Entamoeba dispar. J Clin Microbiol 2002;40:1271-76.