Conventional Microscopy Versus Molecular and Immunological Methods in the Diagnosis of Amoebiasis

Dear Editor,

Entamoeba histolytica, the causative agent of intestinal amoebiasis affects more than 50 million people worldwide. Amoebiasis is considered to be the most common parasitic infection particularly in the tropics and subtropics.[1] It is the second leading cause of the death from parasitic diseases worldwide.[2] Humans are the primary reservoir and infection happens to be by ingestion of mature quadri-nucleate cyst through contaminated food and water.[3] Treatment and management of infection with *E. histolytica* has been considerably affected since 90% of the infected individuals remain asymptomatic. Clinical diagnosis of amoebiasis also remains elusive in most of the cases due to contrasting illness course in different communities, varied clinical presentations and unavailability of infrastructures in the developing countries.

Difficulty In the diagnosis of amoebiasis is due to the presence of other harmless commensals such as Entamoeba dispar as reported by Brumpt in 1925 and other noninvasive amoebae such as *Entamoeba moshkowski, E. poleki, E. coli*, *and E. hartmanii*.[4-7]

The laboratory diagnosis of *E. histolytica* currently relies on the direct microscopic identification of the parasite. Other methods of diagnosis include the culture, using Boek and Drbohlav's biphasic amoebic medium, isoenzyme assay using different zymodemes, stool ELISA on monoclonal antibodies to galactose specific adhesin, rapid indirect haemagglutination assay (IHA) to detect serum antiamoebic antibodies and polymerase chain reaction (PCR) nested multiplex PCR targeting 16s like rRNA gene, realine PCR, single round PCR, and PCR-RFLP (restriction fragment length polymorphism).[8-12]

Of the available diagnostic techniques, the microscopic detection of the morphological forms of the parasite in stool samples is often used in developing countries. Limitation of the microscopic detection is that it is insensitive to differentiate between pathogenic strains of entamoeba from other nonpathogenic amoebae. Diagnosis by culture, though is much sensitive and specific, is laborious and time consuming which may require several weeks. Amoebic culture can also show false negative results which can be accounted to either delay in processing or probably antiamoebic therapy prior to stool collection. ELISA using monoclonal antibodies (MAbs) directed against pathogen specific epitopes of the galactose adhesin means to diagnose amoebiasis. Detection of antibodies to *E. histolytica* in patients by using indirect haemagglulination assay (IHA) may fail to distinguish past from present infection.

Results of several studies on detection and differentiation of *E. histolytica, E. dispar, E. moshkowski* and other harmless amoebae in clinical specimen using PCR showed the potential use of molecular methods in the diagnosis of amoebiasis.[13] A recent study which involved 218 stool samples has demonstrated the use and role of PCR in differentially diagnosing pathogenic *E. histolytica* (51) from morphologically resembling non- pathogenic *E. dispar* (39),[14] which otherwise by conventional microscopy cannot be differentiated. Shih-yu Liang *et al*. in their study have evaluated 130 fecal specimens and showed that molecular methods have 100% specificity towards differential identification of *E. histolytica* and other nonpathogenic amoebae.[15] Significance and advantages of DNA based techniques over other methods in identifying the parasites, quantify and provide important information on formulating and implementing the parasite control programms in both human and animal is highlighted in a recent article by Hunt PW.[14] Diagnosis of amoebiasis is usually performed on clinical grounds alone in most of the endemic countries having limited resources. Microscopic methods, though are cost-effective require well-trained laboratory personnel. This has remarkably affected the estimates of global prevalence of amoebiasis due to *E. histolytica*. The prevalence and the true epidemiology of amoebiasis are still unclear. Previous studies showing high rates of infection with *E. histolytica* may not be true as studies reported that *E. dispar* is about 10 times more common.[15]

The focus should now be on recent developments in the diagnosis and management of amoebiasis. With advance in the laboratory techniques that can differentiate pathogenic *E. histolytica* from other nonpathogenic amoebae studies must be encouraged to estimate the true prevalence of *E. histlytica* infection.

Clinicians and microbiologists must focus on specific diagnosis of *E. histolytica* infection by employing the advanced diagnostic tools, thereby avoiding unnecessary and unwarranted chemotherapy.

Ramana KV, Kranti PG[1]

Department of Microbiology, Prathima Institute of Medical Sciences, Nagunur, Karimnagar,
[1]Community Medicine, Kamineni Institute of Medical Sciences, Narketpally, Andhra Pradesh, India
E-mail: ramana_20021@rediffmail.com

References

1. Nuran D, Gonal A, Mehmet S, Babur C, Kanik A, Emekdas G. Detection of Entamoeba histolytica/Entamoeba dispar in stool specimens by using enzyme-linked immunosorbent assay. Mem Inst Oswaldo Cruz 2004;99:769-72.
2. Trol H, Marti H, Weiss N. Simple differential detection of Entamoeba histolytica and Entamoeba dispar in fresh stool specimens by sodium acetat-acetic acid-formalin concentration and PCR. J Clin Microbiol 1997;35:1701-5.
3. Orozco E. Pathogenesis in amebiasis. Infect Agents Dis 1992;1:19-21.
4. Kebede A, Verweij JJ, Petros B, Polderman AM. Short communication: Misleading microscopy in amoebiasis. Trop Med Int Health 2004;9:615-652.
5. Brumpt E. Differentiation of human intestinal amoebae with four-nucleated cysts. Trans R Soc Trop Med Hyg 1928;22: 101-14.
6. Clark CG, Diamond LS. The Laredo strain and other 'Entamoeba histolytica like' amoebae are Entamoebia mshkovskii. Mol Biochem Parasitol 1991;46:11-8.
7. Pariya SC, Rao RS. Stool culture as a diagnostic aid in the detection of Entamoeba histilytica in the faecal specimens. Indian J Pathol Micobiol 1995;38:359-63.
8. Haqe R, Kress K, Wood S, Jackson TF, Lyerly D, Wilkins T, *et al*. Diagnosis of pathogenic Entamoebia histolytica infection using a stool ELISA based on monoclonal antibodies to the galactose-specific adhensin. J Infect Dis 1993;167:247-9.
9. Haque R, Ali IK, Akther S, Petri WA Jr. Comparision of PCR, isoenzyme analysis, and antigen detection for diagnosis of Entamoeba histolytica infection. J Clin Microbiol 1998;36:449-52.
10. Caballero-Salcedo A, Viveros-Rogel M, Salvatierra B, Tapia-Conyer R, Sepulveda-Amor J, Gutierrez G, *et al*. Seroepidemiology of amoebiasis in Mexico. Am J Trop Med Hyg 1994;50:412-9.
11. Kebede A, Verweij JJ, Endeshaw T, Messele T, Tasew G, Petros B, *et al*. The use of real-time PCR to identify Entamoeba histolytica and E. dispar infections in prisoners and primary-school children in Enthiopia. Ann Trop Med Parasitol 2004;98:43-8.
12. Liang SY, Hsia KT, Chan YH, Fan CK, Jiang DD, Landt O, *et al*. Evaluation of a New Single-Tube Multiprobe Real-Time PCR for Diagnosis of Entamoeba histolytica and Entamoeba dispar. J Parasitol 2010;96:793-7.
13. Liang SY, Chan YH, Hsia KT, Lee JL, Kuo MC, Hwa KY, *et al*. Development of loop-mediated isothermal amplification assay for detection of Entamoeba histolytica. J Clin Microbiol 2009;47:1892-5.
14. Hunt PW. Molecular diagnosis of infections and resistance in veterinary and human parasites. Vet Parasitol 2011;180:12-46. Available from: http://www.sciencedirect.com/science/article/pii/S0304401711003803.
15. Mirelman D, Nuchamowitz Y, Stolarsky T. Comparision of use of enzyme linked immunosorbent assay-based kits and PCR amplification of RNA genes for simultaneous detection of entamoeba hoistolytica and E. dispar. J Clin Microbial 1997;35:2405-7.

Access this article online	
Quick Response Code:	Website: www.amhsr.org
	DOI: 10.4103/2141-9248.105679