Detection of *Entamoeba histolytica, Entamoeba dispar* and *Entamoeba moshkovskii* in Patients with HIV

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Entamoeba histolytica is a harmful amoeba causing amoebic colitis and liver abscess, whereas both *Entamoeba dispar* and *Entamoeba moshkovskii* are non-pathogenic harmless commensals, hence leads to a confusing scenario when it comes to the definite diagnosis of amoebiasis based on morphology alone. Many studies have shown moderate to high prevalence of *E.histolytica* infection in patients with HIV from different parts of India .The studies on prevalence of *E. dispar* and *E. moshkovskii* in patients with HIV, however, are not properly documented. This is due to lack of proper diagnostic methods to distinguish *E. histolytica* from *E. dispar* and *E. moshkovskii* .It is strongly believed that both *E. dispar* and *E. moshkovskii* infection patients with HIV in other parts of India can also be detected, using recent molecular methods such as PCR . Hence, estimation of prevalence of intestinal amoebiasis solely based on detection and identification of *E. histolytica* in stool specimens by microscopy alone is therefore not reliable. The present paper will review prevalence of *E. histolytica, E. dispar* and *E. moshkovskii* in patients with HIV in India and recent methods for detection of these amoebae.

In this world of *maya* there is a famous saying that a snake could be mistaken for a rope and vice versa. In the world of parasitic infections *E. histolytica*, the causative agent of amoebiasis has a similar modus operandi. It is one of the clever terrorist in the world of parasite.

E. histolytica is a single celled protozoal parasite. It has two stages: cyst and trophozoite .The cysts measure 10–20 µm in diameter and typically contain four nuclei. The cysts which are resistant to acidification, chlorination and desiccation, and capable of surviving in a moist environment for several weeks, are spread via the ingestion of faecally contaminated food or water. The trophozoites measure usually 15 µm to 20 µm (range 8 µm to 30 µm).[1]

In 1875, in St. Petersburg, Russia, Fedor Losch was credited with the initial documentation of amoebae in stool.[2] Losch described the amoebae in the stool as having a "round, pear shaped or irregular form and which are in a state of almost continuous motion." In 1890, Sir William Osler reported the first North American case of amebiasis, when he observed amoebae in stool and abscess fluid from a physician who had previously resided in Panama. In 1913, in the Philippines, Walker and Sellards documented the cyst form of *E. histolytica* as the infective form of the parasite; in 1925, Dobell further described the organism's life cycle.

E. histolytica holds the second podium position placed just below malaria as a major killer amongst parasitic infections. It is a harmful parasite that causes intestinal infection (intestinal amoebiasis) and also extra-intestinal invasive amoebiasis (e.g. amoebic liver abscess, etc). Amoebiasis is transmitted via ingestion of cysts in fecally contaminated drinking water and foods, but also by direct contact with dirty hands or objects.

In most infected humans the symptoms of intestinal amoebiasis are intermittent and mild (various gastrointestinal upset, including colitis and diarrhea). In more severe cases the gastrointestinal tract hemorrhages, resulting in dysentery. In some cases the trophozoites will enter the circulatory system and infect other organs, most often the liver (hepatic amoebiasis), or they may penetrate the gastrointestinal tract resulting in acute peritonitis; such cases are often fatal.

In most of the laboratories of the world especially in developing countries like India, the intestinal amoebiasis is diagnosed by demonstration of either cysts or trophozoites or both in the stool by light microscopy.[3] The stool specimens of the symptomatic patients positive for the amoeba were recommended for specific ant amebic therapy.

What is new in the understanding of the amoebae?

However, there has been a rethinking on the understanding of the biology, pathogenesis and diagnosis of this parasite, *E. histolytica* , since 1993, when it was conclusively established the presence of a new amoebae, *E.dispar*, the cysts and trophozoites of which are similar in their morphology to those of *E. histolytica* when seen in the stool specimens by light microscopy. Similarly another amoebae, *E. moshkovskii* has also been documented in the year 1998 in human stool specimens which is also similar in morphology to that of *E. histolytica*.

E.histolytica is a harmful and disease causing amoeba, whereas both *E.dispar* and *E. moshkovskii* are non-pathogenic harmless commensals.[4,5] Hence leads to a confusing scenario when it comes to the definite diagnosis of amoebiasis when dependent on morphology alone. Because all these three amoebae include the pathogenic *E. histolytica* and non-pathogenic *E.dispar* and *E.moshkovskii* are similar in morphology. It means cysts and trophozoites of these three amoebae in the stool appear similar when examined by conventional light microscope. It is not possible to tell the amoebae present in the stool specimens is either *E.histolytica* or *E.dispar* or *E.moshkovskii*. The cysts of *E.dispar* and *E.moshkovskii* just look like that of *E.histolytica*, the trophozoite stage of *E.histolytica*, *E. dispar* and *E.moshkovskii* also look same, except in rare cases where *E. histolytica* trophozoite may have ingested RBC[6] (Table 1).

Table - 1 Differentiating of *E.histolytica, E.dispar, E.moshkovskii.*

Characteristics	*E.histolytica*	*E.dispar*	*E.moshkovskii*
Pathogenicity	Pathogenic	Nonpathogenic	Nonpathogenic
Disease caused	Amoebiasis which includes intestinal amoebiasis, amoebic liver abscess, etc)	Commensal ; Do not cause any disease	Commensal; Do not cause any disease
Cyst stage (Morphology)	Similar	Similar	Similar
Trophozoite Stage (Morphology)	Similar, except rarely contains ingested RBC	Similar, does not contains ingested RBC	Similar, does not contains ingested RBC
Virulence factor	Cysteine proteinase, Amoebapore & N-acetyl galactose inhibitable lectins	None	None
Prevalence	prevalent in almost all parts of the world	prevalent in almost all parts of the world	Prevalence reported only in North America, Italy, South Africa, Bangladesh and first time in India from our laboratory.
Treatment by Antiamoebic chemotherapy	Required	Not required	Not required

Significance of these new observations

The demonstration of look alike *E.dispar* and *E.moshkovskii* in stool specimens has led to rethinking on the diagnosis, epidemiology, and treatment of the infections caused by *E.histolytica*.

Diagnosis of intestinal amoebiasis

Estimates of intestinal *E. histolytica* infections have primarily been based on microscopy of stool. The sensitivity of light microscopy under the best of circumstances is only 60%. The stool microscopy alone cannot differentiate *E. histolytica* from those of morphologically identical but nonpathogenic species such as *E. dispar* and recently described *E. moshkovskii*.

The culture followed by isoenzyme analysis can detect *E. histolytica* and *E.dispar* but not *E.moshkovskii*. The culture followed by isoenzyme analysis takes long time take long time (usually weeks) to give report and it is labor intensive. Other tests like monoclonal antibody based ELISA which detects coproantigen (antigen in stool) are able to detect *E. histolytica* or *E.dispar* but are unable to detect *E.moshkovskii* in stool samples, also this test requires a minimum of 1000 trophozoite per well to give a positive result. The PCR based test like nested PCR can detect and differentiate *E. histolytica*/*E.dispar*/*E.moshkovskii* complex by exploiting the differences in there genomes. Many laboratories in the world including ours at JIPMER , Pondicherry are now employing the recent molecular techniques such as the nested polymerase chain reaction (PCR) for specific detection and differentiation of the real culprit *E. histolytica* from those of look alike amoeba by targeting there genomes.[7] The nested PCR test can detect the DNA of even a single trophozoite or cyst in 50mg of stool samples, which makes it a very powerful technique.

The nested PCR based study conducted in Department of Microbiology, JIPMER hospital on patients stool samples showing *E.histolytica* like amoeba by microscopy this revealed the fact that 81% of patients were actually infected with *E.dispar* and only 19% of patients were infected with *E.histolytica* the true pathogen implying that 81% patients would have been misdiagnosed and unnecessarily treated with antiamoebic chemotherapy.[7] The study clearly shows that how risk prone it will be if diagnosis is based on microscopy alone (Table 2).

Table -2 Approaches for diagnosis of amoebiasis

Diagnostic tools	Diagnosis of amoebaisis	Comments
Microscopy and culture	Unable to distinguish between *E.histolytica*, *E.dispar* & *E.moshkovskii*	Less sensitive and specific.
Coproantigen detection	Presently can detect *E.histolytica* and *E.dispar* but not *E.moshkovskii*.	Convenient technique to perform Stool samples should be fresh for better performance of the test. Requires approximately 1000 trophozoites per well to show positive result.
Isoenzyme analysis	Presently can detect *E.histolytica* and *E.dispar* but not *E.moshkovskii*.	The 'gold standard' Labour intensive Costly Time consuming(1-7 weeks)
Nested-PCR	Can distingu ish and detect separately *E.histolytica*, *E.dispar* & *E.moshkovskii*.	Requires high technical skill Labour intensive Less time consuming Highly specific and sensitive

Epidemiology of amoebiasis

Prior to description of *E.dispar* and *E.moshkovskii*, amoebiasis caused by so called pathogenic *E.histolytica* was known as a 10% disease because it has been estimated that approximately, 500 million people or 10 percent of world's population are infected. They carry *E.histolytica* in their intestinal tract .10 percent of these infected people or 50 million people each year suffer from active amoebic disease ; 10 percent of which (50 ,000 to 1, 00 , 000 people) die

every year due to the disease .But this concept needs revision with the reporting of a new look-alike species of amoeba *E.dispar* and *E.moshkovskii* . It is now being suggested that majority of 500 million people who were earlier believed to harbor *E. histolytica* in their intestinal tract may be harboring commensal and non-pathogenic *E.dispar* or possibly *E.moshkovskii*, but not pathogenic *E. histolytica*. *E.dispar* has been documented from most places of the world including India.[8-10] We have documented the prevalence of *E.dispar in* human stool samples in Pondicherry.[7]

Human isolates of *E.moshkovskii* to date have been documented from North America, Italy, South Africa, and Bangladesh, and they have never been associated with disease. Our laboratory has also documented the prevalence of *E.moshkovskii* in human stool for the first time in India.[7] We strongly believe that both *E.dispar* and *E.moshkovskii* infection in other parts of India can also be detected, if studies are carried out by using these recent molecular techniques.

Hence, estimation of prevalence of intestinal amoebiasis solely based on detection and identification of *E. histolytica* in stool specimens by microscopy needs a reevaluation. This concept needs revision with the reporting of a new look-alike species of amoeba *E.dispar* and *E.moshkovskii*. It is now being suggested that majority of 500 million people who were earlier believed to harbor *E. histolytica* in their intestinal tract may be harboring commensal and non-pathogenic *E.dispar* or possibly *E.moshkovskii* , but not pathogenic *E. histolytica* . *E.dispar* has been documented from most places of the world including India.[8-10] We have documented the prevalence of *E.dispar in* human stool samples in Pondicherry.[7]

Treatment of amoebiasis

The thrust area in the field of diagnostic amoebiasis is to break the deadlock of accurate diagnosis of amoebiasis and to know the true epidemiology of intestinal amoebiasis which would save the patients from unnecessary treatment with antiamebic drugs which have side effects and the expenditure on these drugs can be avoided.

As per the World Health Organization (WHO), patients infected with even a single *E.histolytica* cyst/trophozoite should be treated with antiamebic therapy even if the patients are not showing manifestation of amoebiasis because such patients are potential carriers and may spread the infection in community,[11] whereas those infected with *E.moshkovskii* or *E.dispar* should not be treated. The antiamebic drugs have side effects on humans and add up to the cost of treatment so; its use should be limited.

To make the diagnosis of amoebiasis simpler, cost effective and time saving, many laboratories world wide including ours are evaluating different strategies which could detect and differentiate all the three morphologically similar species *E.histolytica*, *E.dispar* and *E.moshkovskii* in stool samples. We believe these newer techniques will soon be available for accurate diagnosis of *E.histolytica*, *E.dispar* and *E.moshkovskii* in stool samples for better patient care.

References

1 Parija SC (2004). Text book of Medical Parasitology Protozoology and Helminthology), 2nd edn. All India Publishers & Distributors, Chennai (India), pp. 26-61.

2 Kean BH, Mott KE, Russell AJ (1978). Lösch F A Massive development of amoebae in the large intestine (Massenhafte Entwickelung von Amöben im Dickdarm) (Translation); in *Tropical medicine and parasitology,* classic investigations (New York: Cornell University Press) vol. 1, pp. 71–79.

3 Tanyuksel M, Petri WA (2003). Laboratory Diagnosis of amoebiasis. *Clin. Microbiol.*16, 713–729.

4 Clark CG, Diamond LS (1991). The Laredo strain and other *Entamoeba histolytica*– like amoebae are *Entamoeba moshkovskii*. *Mol Biochem Parasitol* 46, 11–18.

5 Haque R, Ali IKM, Clark CG, Petri WA Jr (1998). A case report of *Entamoeba moshkovskii* infection in a Bangladeshi child. *Parasitol Int* 47, 201–202.

6 Gonzalez-Ruiz A, Haque R, Aguirre A, Castanon G, Hall A, Guhl F, Ruiz-Palacios Gonzalez-Ruiz A, Haque R, Aguirre A, Castanon G, Hall A, Guhl F, Ruiz-Palacios G, Miles MA, Warhurst DC (1994). Value of microscopy in the diagnosis of dysentery associated with invasive *Entamoeba histolytica*. *J Clin Pathol* 47, 236-239.

7 Parija SC, Khairnar K (2005). *Entamoeba moshkovskii* and *Entamoeba dispar*-associated Infections in Pondicherry, India. *J Health Popul Nutr* .23, 292-295.

8 Mohandas, Sehgal R, Sud A, Malla N (2002). Prevalence of intestinal parasitic pathogens in HIV-seropositive individuals in Northern India. *Jpn J Infect Dis* 55, 83-84.

9 Mukhopadhyay A, Chakraborti A, Mahajan RC, Ganguly NK (2002). *Entamoeba histolytica*: rapid identification and differentiation of Indian isolates by riboprinting. *Exp Parasitol* 102, 109-112.

10 Bansal D, Sehgal R, Chawla Y, Mahajan RC, Malla N (2004). In vitro activity of antiamoebic drugs against clinical isolates of *Entamoeba histolytica* and *Entamoeba dispar*. *Ann Clin Microbiol Antimicrob* 3, 27.

11 World Health Organization (1997). Amoebiasis. *Wkly Epidemiol Rec* 72, 97-10