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Diagnosis of Amebiasis in Bangladesh

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Diagnosis of amebiasis by microscopic identification of the parasite in stool and liver abscess pus is insensitive and unable to distinguish the invasive parasite *E. histolytica* from the commensal parasites such as *E. dispar* and *E. moshkovskii*. New approaches to the detection of *E. histolytica* are based on detection of *E. histolytica*-specific antigen and DNA in stool and other clinical samples. Several molecular diagnostic tests for diagnosis of amebiasis have been developed and used to diagnose *E. histolytica* in Bangladesh. We have compared the TechLab *E. histolytica*-specific antigen detection test with PCR assays and with isoenzyme analysis of cultured amebas. The PCR assays are based on amplification of the multi-copy small subunit ribosomal RNA gene of *E. histolytica* and *E. dispar*. PCR assays and antigen detection test had comparable sensitivities when performed directly on fresh stool specimens. The correlation of antigen detection with PCR assays for identification of *E. histolytica* was excellent. TechLab's *E. histolytica*-specific antigen detection test was both rapid and simple to perform, making it appropriate for use in the developing world, where amebiasis is most prevalent.

Key Words: Amebiasis, Diagnosis, *E. histolytica*, Antigen detection, PCR.

Introduction

Amebiasis, a disease caused by the protozoan parasite *Entamoeba histolytica*, is the third leading leading parasitic cause of deaths worldwide in humans (1,2). There are at least eight other amebas that can be found in human and are generally accepted as nonpathogenic organisms, but some of them are morphologically identical to *E. histolytic* as seen by traditional light microscopy. Although *E. histolytica* differs from *E. dispar* biochemically, immunologically and genetically, they cannot be differentiated from each other morphologically by normal light microscopy (3). Amebiasis is distributed worldwide, although the majority of cases are found in developing countries including Bangladesh.

Individuals become infected with *E. histolytica* when they ingest cysts in fecally contaminated food or water. When those cysts reach the intestine, they swell and release the motile, symptom-inducing form of *E. histolytica* called the trophozoites that can remain there and even form new cysts without causing disease symptoms. However, trophozoites can also invade epithelial cells killing host cells and causing amebic colitis, acute dysentery, or chronic diarrhea. In addition, trophozoites can be carried through the blood to other organs, most commonly the liver, where they form life-threatening abscesses. Historically, diagnosis of amebiasis was complicated and often unreliable for various reasons. Signs and symptoms of amebiasis can provide the means to obtain clinical diagnosis. However, the confirmation of an amebic infection rests with laboratory identification. Over the last 25 years various molecular diagnostic tests have been developed to diagnose *E. histolytica*.

Diagnosis of Amebiasis

The traditional microscopic examination cannot differentiate *E. histolytica* from the nonpathogenic but identical-appearing parasite *E. dispar*. Identification and differentiation of *E. histolytica* from *E. dispar* in stool sample, liver abscess pus and biopsy by microscopy is imprecise. While *E. histolytica* trophozites are more likely than *E. dispar* to

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contain ingested erythrocytes, the organisms are identical in appearance (4,5). Not only is microscopy unable to differentiate *E. histolytica* from *E. dispar*, it is at best only 10–60% sensitive and confounded with false-positive results due to misidentification of macrophages and nonpathogenic species of *Entamoeba,* especially *E. dispar* and *E. moshkovskii* (5–7). Colonization with these nonpathogenic species of *Entamoeba* is more frequent than with *E. histolytica* in countries where amebiasis is endemic. Isoenzyme (zymodeme) analysis of cultured ameba enables differentiation of *E. histolytica* from *E. dispar* and is considered the gold standard for diagnosing amebic infection. However, amebic cultures and isoenzyme analysis require a week to complete and are negative in many microscopy-positive stool samples, in some cases due to delay in sample processing or due to the institution of anti-amebic therapy prior to stool collection (8,9). A WHO/PAN American Health Organization/ UNESCO Expert Panel recently recommended the development of improved method for the specific diagnosis of *E. histolytica* infection using technologies appropriate for developing countries (1). To address the need for a specific diagnostic test for amebiasis, a substantial amount of work has been carried out involving University of Virginia, International Center for Diarrheal Disease Research, Bangladesh (ICDDR,B), Dhaka, Bangladesh and TechLab, Blacksburg, Virginia over the last decade.

Molecular Diagnostic Tests for Diagnosis of Amebiasis

New approaches to the detection of *E. histolytica* are based on detection of *E. histolytica*-specific antigen and DNA in stool and other clinical samples. PCR tests are capable of distinguishing *E. histolytica* and *E. dispar*. These tests are now quite sensitive and specific for detection of *E. histolytica* and are increasingly being used for both clinical and research purposes worldwide, including Bangladesh. In addition to antigen detection and PCR-based tests, detection of antibodies to *E. histolytica* (serological test) is also being used in different studies. A major drawback of current serological tests that detects antibodies against total amebic antigens is that patients remain positive for years after infection (10,11).

Use of Molecular Diagnostic Tests for Diagnosis of Amebiasis in Bangladesh

Antigen Detection Test

This antigen detection test captures and detects the parasite's Gal/GalNAc lectin from stool or other clinical samples. Antigenic differences in the lectins of *E. histolytica* and *E. dispar* amebas enable specific identification of the disease-causing amebas, *E. histolytica* (12).

E. histolytica-specific antigen detection test was developed by our group in 1993 using anti-lectin monoclonal antibodies (8,13). Antigen detection tests show good sensitivity and specificity for detection of *E. histolytica* antigen in stool specimens of people suffering from amebic colitis, asymptomatic intestinal infection and serum of amebic liver abscess patients prior to anti-amebic therapy. The sensitivity of this method for the detection of antigens in the stool of patients with amebic colitis is $>85\%$, and its specificity when compared with zymodeme analysis is $>90\%$ (9,14). When used to screen stool samples from children in the Mirpur area of Dhaka, Bangladesh, the TechLab *E. histolytica* II test was three times more sensitive than stool microscopy and culture for the identification of *E. histolytica* (15). Antigen detection test has been used to test diarrheal stool specimens of children from an urban hospital and non-diarrheal stool samples from an urban slum and normal stool samples from children of a rural village in Bangladesh and showed prevalence of *E. histolytica* of 4.2, 4.3, and 1%, respectively (6,16). *E. histolytica*-specific antigen detection test has also been used in a prospective study on amebiasis in Bangladesh and has demonstrated its usefulness in this study during the first year of follow-up and 39% (105/269) of children had at least one new *E. histolytica* infection as determined by the antigen detection test (16). ELISA-based antigen detection test can also be used for the detection of *E. histolytica* lectin antigen in the serum and liver abscess pus of patients with liver abscess. In Dhaka, Bangladesh, 96% (22/23) and 100% (3/3) of patients with amebic liver abscess had detectable levels of lectin antigen in their serum and liver abscess pus samples, respectively, before treatment with metronidazole. However, sensitivity of this method was only 33% (32/98) and 41% (11/27) for detection of lectin antigen in serum and liver abscess pus, respectively, after a few days of treatment with metronidazole (15). ELISA-based antigen detection kit is now commercially available. TechLab's *Entamoeba histolytica* II kit is the only FDA-approved test that is specific and sensitive for the pathogenic parasite *E. histolytica*. Detection of lectin antigen in stool and serum using rapid diagnostic test is now under development in TechLab (personal communication).

PCR Tests

Work on the diagnosis of *E. histolytica* by PCR test also started in the early 1990s. Differentiation of *E. histolytica* from *E. dispar* by restriction fragment analysis of a single gene amplified *in vitro* was first reported in 1991 (17). The sensitivity and specificity of PCR-based methods for diagnosis of *E. histolytica* infection in stool samples is comparable to those of zymodeme analysis and antigen detection tests. Field studies conducted in Bangladesh compared the PCR test with the stool culture and isoenzyme analysis or antigen detection test for the diagnosis