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Diagnostic parameters of serological ELISA for invasive amoebiasis, using antigens preserved without enzymatic inhibitors

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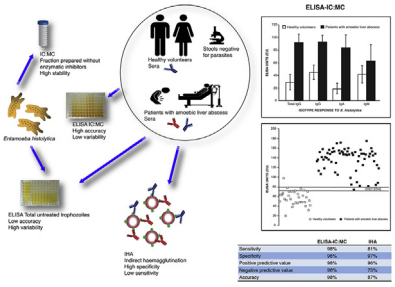
HIGHLIGHTS

- ELISA-IC:MC has important serodiagnostic value in patients with invasive amoebiasis.
- ELISA-IC:MC is useful in populations from endemic zones carrying antibodies.
- ELISA-IC:MC presented better diagnostic parameters than IHA.
- The amoebic antigens are preserved for years without using enzymatic inhibitors.
- A negative serologic test does not rule out acute invasive amoebiasis.

GRAPHICAL ABSTRACT



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ABSTRACT

Amoebiasis is the third cause of death due to parasites in the world. Although, numerous serodiagnostic and salivary tests have been developed, the majority of these assays lack sensitivity in endemic zones to detect acute amoebic liver abscess. The two main limiting factors to develop reliable assays are the high levels of anti-amoeba antibodies in populations living in endemic zones, and the proteolysis of amoebic extracts even treated with inhibitors. Our group reported a method to preserve amoebic antigens without using enzymatic inhibitors (IC:MC fraction) that shows stability for years. Here we describe the development of a serologic ELISA to diagnose amoebiasis made with IC: MC antigens, and its validation for clinical use in endemic areas. In our study, we included sera from 66 patients diagnosed with acute amoebic liver abscess and 33 volunteers living in an endemic area for amoebiasis. Our assay was compared with an indirect haemaglutination assay (IHA) an ELISA elaborated with antigens derived from untreated trophozoites. The ELISA made with IC: MC antigens presented more reproducibility compared to other assays. Sera from 95% ALA patients showed a positive value. The ELISA (IC: MC) detected 97% of patients with ALA compared to an 81% using IHA. The parameters of ELISA (vs. IHA) were Sensitivity 98% (81%), Specificity 96% (97%), Positive predictive value 98% (96%), Negative predictive value 96% (73%) and Accuracy 98% (87%). A negative serologic test does not rule out the diagnosis of invasive amoebiasis. The ELISA made with antigens preserved without using enzymatic inhibitors has valuable serodiagnostic

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value to diagnose acute amoebic liver abscess, even in populations living in endemic zones of amoebiasis carrying antibodies against amoebas. In conclusion, ELISA-IC:MC presented better diagnostic parameters than IHA although a negative serologic test does not rule out acute invasive amoebiasis.

1. Introduction

Amoebiasis is the third cause of death due to parasites in the world. It is estimated that one-tenth of the world's population is infected with *Entamoeba histolytica* and this infection results in up to 100,000 deaths each year. Amoebiasis infections are endemic in most moderate and tropical climates in the world. The amoebiasis is prevalent in South and Central America, Asia and Africa. In some tropical countries, antibody prevalence rates reflecting past or recent infection exceed 50% (Gonzalez et al., 1995; Caballero-Salcedo et al., 1994; Haque et al., 2003; Ximénez et al., 2009; Walsh, 1986).

Amoebiasis with nonspecific symptoms can mislead the clinical diagnosis. Certain colonoscopic findings predict amoebic colitis while others suggest different diagnoses (Lee et al., 2015). Intestinal amoebiasis is usually diagnosed by the identification of cysts or trophozoites in stools; the invasive pathogenic *E. histolytica* protozoan must be differentiated from *E. dispar* and *Entamoeba moshkovskii*, both are morphologically identical nonpathogenic commensal parasites. *E. histolytica* stool culture followed by PCR analysis is the gold standard for identification of *E. histolytica*, however outside the scientific aspect of identification of the *Entamoeba* strain, in the daily practice, PCR is not used as diagnostic test because costs makes it unfeasible. Mortality from amoebiasis is mainly due to extra intestinal invasion; amoebic liver abscess ALA is the most common. The noninvasive diagnosis of amebic liver abscess is challenging, as most patients at the time of diagnosis do not have a concurrent intestinal infection with *E. histolytica*. Unfortunately, stools analysis is not useful to diagnose extra intestinal amoebiasis because it is reported that less than 10% of patients suffering from amoebic liver abscess eliminate parasites in feces. Then, fecal testing for *E. histolytica* parasite antigen or DNA is negative in most patients with ALA (Haque et al., 2010; Haque and Petri, 2006; Katzenstein et al., 1982).

Diverse serodiagnostic and salivary tests have been developed to detect extraintestinal amoebiasis, however many of these tests do not have enough sensitivity in acute cases or it decay after a few days of treatment with metronidazole before total resolution of abscess. Some studies have reported the detection of *E. histolytica* DNA in blood, urine, and saliva simultaneously to improve diagnostic sensitivity for amebic liver abscess, but the cost of these assays is unfeasible for application in poor countries where this parasitosis is endemic (Abd-Alla et al., 2000; Agundis Mata et al., 1996; Fotedar et al., 2007; Merens et al., 2005). The utility of serologic tests is controversial considering the life-long persistence of antibodies following the first infection and the permanent antibodies against amoebas among populations living in endemic zones, even if they have never suffered invasive amoebiasis, nevertheless these populations were exposed to *E. histolytica* (Caballero-Salcedo et al., 1994; Ximénez et al., 2009). However, serology is the method of choice for diagnosis of extra intestinal amoebiasis in poor countries where amoebiasis is endemic. The indirect haemmaglutination assay (IHA) is yet the assay frequently used by clinical laboratories in endemic countries. In clinical practice, the diagnosis of ALA is based on the positive serological detection of antibodies against *E. histolytica* and possibly, the demonstration of a hepatic lesion by imaging techniques.

There are limitations to develop good serological assays. One of them is the high serum level of antibodies against amoebas among populations living in endemic zones. These antibodies involve high background noise in serodiagnostic tests, implicating lost of efficacy. Proteolysis of amoebic extracts constitutes another restriction to standardize the assays. Amebas express high levels of enzymes as a molecular mechanism to invade the human host. (Serrano-Luna et al., 2013; Araiza–Orozco et al., 1999; Vila et al., 1985; Muñoz et al., 1990, 1984). Worldwide, enzymatic inhibitors are used to reduce the activity of amoebic proteases. Nevertheless, such inhibitors are not completely effective to prevent the amoebic molecules degradation during antigen preparation (Lopez-Revilla et al., 1992; Lopez-Revilla and Baez-Camargo, 1992; Perez-Montfort et al., 1987; Zamudio-Prieto et al., 2014). Since proteolysis causes differences in antigenic composition preventing reproducible diagnostic data, our group developed a method to preserve amoebic antigens from trophozoites without using enzymatic inhibitors. The amoebic fraction (IC: MC) obtained by this procedure shows long antigenic stability and the same antigen lot can be used for several years (Flores-de-Castañeda, 1995; Flores-de-Castañeda, 1999; Flores-de-Castañeda, 2002a; Flores-de-Castañeda, 2002b; Flores et al., 2005; Tamez- Treviño et al., 2000). A real challenge of poor countries remains: to have reliable diagnostic methods, at low cost and applicable in endemic areas for amoebiasis. The aim of this study was to develop an ELISA to diagnose invasive amoebiasis using stable antigens with long shelf-time prepared without enzymatic inhibitors, and validate its clinical use in an area of amoebic endemicity.

2. Material and methods

2.1. Patients and volunteers

2.1.1. Patients with amoebic liver abscess

Group of patients with acute amoebic liver abscess Sixty-six patients admitted to the University Hospital "Jose Eleuterio Gonzalez" at Monterrey Nuevo Leon, Mexico. Patients were diagnosed with amoebic liver abscess (ALA) by clinical symptoms, ultrasound image and/or axial tomography (CT) and positive response to antiamoebic drug treatment. Fifty percent of patients required percutaneous drainage. The drained fluid was microbiologic cultured to discard bacterial infection. Stool samples were not examined due to previous reports indicating that only 10% of patients with liver abscess release amebas in stool. (Katzenstein et al., 1982) Serum from each patient was tested by the Cellognost Amoebiasis Indirect Hemagglutination Assay (Behring Diagnostics GmbH, Germany). All patients in this group were positive by Western Blot to diagnose invasive amoebiasis (Flores-de-Castañeda, 1999; Flores-de-Castañeda, 2002b).

2.1.2. Healthy volunteers

*Group of healthy volunteers*Thirty-three volunteer students of the Universidad Autónoma de Nuevo León. Participants in this group had neither previous invasive amoebiasis, nor any episode of bloody diarrhea within the previous year. Participants were