Short Report: Two Different Chitinase Genotypes in a Patient with an Amebic Liver Abscess: A Case Report

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Abstract. The present work deals with the identification of a patient with two liver abscesses containing two different strains of Entamoeba histolytica, as defined by chitinase gene polymorphisms.

INTRODUCTION

Entamoeba histolytica is an important cause of morbidity and mortality in Latin America, Asia, and Africa. The three major clinical forms of E. histolytica infection are asymptomatic colonization of the intestinal mucosa (asymptomatic cyst passers), amebic colitis or dysentery in which the trophozoites invade the intestinal mucosa, and amebic liver abscesses (ALAs) where trophozoites invade the intestinal mucosa and reach the hepatic parenchyma through the portal circulation system.

In Mexico, ALA is associated with significant morbidity and low but significant mortality.1 Although ALA grows inexorably in humans and is always fatal without appropriate treatment, current anti-amebic therapy2 can treat even large amebic abscesses.

The high rate of intestinal re-infection in highly endemic areas is mainly the result of repetitive exposure to Entamoeba parasites.3,4 The development of repeated ALA is more frequent than previously thought.5,11 Furthermore, it has been reported that roughly 50% of ALA cases develop more than one ALA at the same time.12,13

In the present work, we report an adult patient who developed two simultaneous and independent ALAs. The size of both abscesses necessitated drainage. The drained material was submitted to DNA extraction and subsequent polymerase chain reaction (PCR) amplification of polymorphic regions in the chitinase gene of E. histolytica species (Ehcht). The Ehcht gene encodes a protein with a repeating structure at the amino terminus.14 This degenerate heptapeptide repeat region exhibits a polymorphism among isolates of Entamoeba species with regard to the number and type of repeat copies. Results of the polymorphism study of the Ehcht gene from both of the patient’s aspirated ALAs suggest that the two independent liver abscesses were caused by two genetic variants of E. histolytica.

CASE REPORT

The patient was a 48-year-old male with a history of diabetes, alcoholism, and smoking for the last 20 years. The patient was admitted to the Hospital General del Estado in Hermosillo, Sonora, on September 28, 2007. For 10 days before hospital admission, the patient had been complaining of right upper quadrant abdominal pain, fever (> 38°C) and an unexplained weight loss of 10 kg (22 lbs). The clinical diagnosis was ALA as confirmed by x-rays, a computed tomography (CT) scan, and enzyme-linked immunosorbent assay (ELISA), which indicated high levels (OD, 1.803) of serum anti-E. histolytica antibodies (IgG).15 An electrocardiogram showed no abnormalities and the thoracic x-ray showed a slight elevation of the right hemi-diaphragm (Figure 1A). The abdominal ultrasonography performed during admission showed a 13.1 cm long, 14.6 cm trans, and 9.3-cm AP hypo-echoic area in the left hepatic lobe, which was indicative of a liver abscess. The right hepatic lobe showed only a slight increase in size (data not shown).

To improve the view of the right hepatic lobule, an abdominal CT scan was performed on day eight after admission. A large left lobe abscess was clearly observed, and a second independent abscess was detected in the right lobule (Figure 1B). Anti-amebic treatment with intravenous (IV) metronidazole was established (500 mg every 8 hours) and “intravenously” cefotaxime (1 g every 8 hours) was given for antimicrobial coverage. Cultures of the aspirated material yielded negative results.

The clinical course after a 72-hour treatment was poor because of the persistence of fever (up to 38°C) and abdominal pain. The physician in charge decided to drain both abscesses because of the risk of rupture. The procedure was guided by ultrasound. A multi-purpose catheter was placed in each abscess.

Both catheters were removed after 15 days because of significant clinical improvement and the patient was discharged 3 days later. The patient remained under outpatient management with an oral regimen of metronidazole (500 mg every 8 hours) and ciprofloxacine (500 mg every 12 hours) for another 5 days.

The ELISA method used to detect and quantify E. histolytica antibody was previously reported.16 The cut-off point for IgG (OD of 0.525) was defined as the best optical density value obtained from the receiver operating characteristic (ROC) curve for serum samples and for detection of anti-amebic antibodies.15

The DNA was extracted from independent aspirate samples of each ALA (right and left hepatic lobules) using the QIAamp DNA Mini Kit (Qiagen Inc., Valencia, CA) in accordance with the provider’s protocol.

Amplification of the highly polymorphic region of the chitinase gene was done by PCR using primers described in Table 1. The PCR conditions were previously described.17

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After electrophoresis, the gels were photographed for our records. The PCR products were purified using the GFX kit (Amersham Pharmacia Biotech, Sao Paulo, Brazil) and then sequenced at the University of Arizona (Genomic Analysis & Technology Core, Tucson, AZ). The sequences were aligned with the reported sequences of \textit{E. histolytica} present in the Gen Bank data base (Figure 2A and B).

**RESULTS AND DISCUSSION**

In the present work, we report a patient with ALA who developed two independent abscesses located in the right and left hepatic lobules (Figure 1A and B). The clinical findings were coincident with the presence of high levels of circulating anti-amebic antibodies, as detected by ELISA. Together, these results point to an amebic etiology for the liver abscess (OD at 490 nm of 1.8). \textsuperscript{15,16}

\textit{Entamoeba histolytica} was confirmed as the causative agent by PCR analyses of the drainage material from both abscesses. Samples from the two abscesses contained fragments of the \textit{Ehcht} gene, the amplified region was located between the signal sequence and the catalytic site of the protein, which has heptapeptide repetitive sequences rich in hydrophilic amino acids (Ser, Glu, Ssp, Hys, and His). However, some hydrophobic amino acids (Pro, Val, and Ile) were also present in the sequences. \textsuperscript{18} The alignment of the respective nucleotide sequences of the PCR products obtained from material of both left and right hepatic lobules and the reported sequences in the Gen Bank database (accession no. XM 647113.1) for \textit{E. histolytica} HM1:IMSS is shown in Figure 2A. Sequences of both left and right lobules abscesses are shorter than the \textit{E. histolytica} HM1:IMSS strain, there is also a nucleotide change (G by A) in position 109 on the HM1:IMSS strain (Figure 2B). The sequence of \textit{Ehcht} gene in the left hepatic lobule abscess is identical to genotype H reported previously in ALA cases in Mexico (Gen Bank accession no. EF-445962). However, the \textit{Ehcht} gene sequence obtained in the right hepatic lobule abscess is identical to the one on NIH-200 and K1 \textit{E. histolytica} strains reported by Ghosh and others.\textsuperscript{4}

Translation of the nucleotide sequences into amino acid sequences and the corresponding alignment is shown in Figure 2B and correlates with the nucleotide sequences mentioned earlier.

Even though simultaneous intestinal infections by \textit{E. histolytica} and \textit{E. dispar} have been previously reported,\textsuperscript{17,19–21} this was the first report that showed amebic infection by more than one genetic variant of the \textit{E. histolytica} species in the same patient. This phenomenon has been described for other parasitic infections, such as trypanosomiasis, leishmaniasis, and malaria.\textsuperscript{22,23}

It is known that ALA occurs without any colitis symptoms and without any detectable parasites in the colon. On the basis of these clinical observations, Charmot\textsuperscript{24} emphasized the possibility that \textit{E. histolytica} hepatotropic strains may exist by mentioning that “the localization, heterogeneity, and the experimental data associated with clinical expression suggest the existence inside the species \textit{E. histolytica} of various strains: non-pathogenic one, invasive for the intestine mucous membrane ones, and invasive for liver tissue ones.” Recently, Ali and others\textsuperscript{25} genotyped \textit{E. histolytica} strains from fecal and aspirated ALA samples of the same patient and demonstrated the existence of co-infection by different types of strains. Their results, together with our finding, have opened a series of possible explanations for this phenomenon. Ali’s group used polymorphic intergenic regions (short tandem repeats) associated with tRNA genes as the target for genotyping the strains. The authors mentioned that their results were compatible with a possible new infection during the lapse between the first inoculum and the development of ALA symptoms, an account that is reasonable in endemic regions. Another possible explanation is the existence of ecologic niches in endemic environments in
which genetically heterogeneous Entamoeba species could exist (i.e., E. histolytica and E. dispar species, as was previously mentioned, or intraspecies genetic variants). 6,19–21

Another explanation mentioned by Ali and others is that a recombination event may have occurred on the parasite DNA during the migration process from the intestine to the liver. 25 These events are relatively frequent in multiple copy genes, such as the STR sequences in tRNA genes. 26,27 These types of chromosomal structures are less stable than the sequences located in single copy genes, such as the chitinase gene.

In fact, we tested three loci of the intergenic region associated with tRNA genes in the abscess material (loci D-A, STR S^{D-S,D} S-Q) (data not shown). The same genotype was obtained in the two abscess samples. Genotype was also detected in ALA cases elsewhere. 25,26,29

Our results and those reported by Ali and others underscore the complexity of the host-parasite relationship of Entamoeba and humans. This report furthers our understanding of the genetic heterogeneity of E. histolytica species in endemic environments.

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