Detection of *Entamoeba histolytica* using polymerase chain reaction in pus samples from amebic liver abscess

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**Background and objective:** Direct demonstration of *Entamoeba histolytica* by conventional microscopy and *in vitro* culture in pus obtained from amebic liver abscess (ALA) is often unsuccessful. We evaluated polymerase chain reaction (PCR) for detection of *E. histolytica* DNA in such pus. **Methods:** Species-specific primers were used for the amplification of *E. histolytica* DNA from liver pus obtained from 30 patients with ALA. Patients with pyogenic liver abscess and sterile (autoclaved) pus spiked with *Entamoeba dispar* and bacteria (*Escherichia coli*, *Klebsiella spp.* and *Bacteroides spp.* ) were used as negative controls. **Results:** PCR was positive in 83% of pus specimens from patients with ALA, and was negative in all 25 pus specimens obtained from pyogenic abscess and autoclaved pus spiked with known bacteria. Sensitivity and specificity of PCR were 83% and 100%, respectively. The overall positivity of PCR was higher compared to serological tests. **Conclusion:** PCR may be a more reliable and better alternative diagnostic modality for ALA. [Indian J Gastroenterol 2006;25:55-57]

The diagnosis of amebic liver abscess (ALA) may be difficult since direct demonstration of trophozoites of *Entamoeba histolytica*, the causative organism of this disease, in clinical specimens is difficult. In *in vitro* culture of *E. histolytica* from stool or liver pus and isoenzyme analysis also have a low sensitivity and are not routinely used. Further, microscopy and culture can detect trophozoites of *E. histolytica* in only 0%-10% of patients with ALA. Serum antibodies to *E. histolytica* can be detected in 75%-85% of symptomatic individuals; these, however, may not distinguish past infection from current infections, especially in endemic regions. Detection of circulating amebic antigen in serum is promising but remains experimental.

We describe the utility of the polymerase chain reaction (PCR) for detecting *E. histolytica* DNA in pus obtained from patients with ALA.

**Methods**

Thirty patients who were admitted in the Gastroenterology department of our tertiary-care hospital with a diagnosis of ALA were studied. The diagnosis of ALA was based on clinical data (pain in right upper quadrant of abdomen, tenderness in right hypochondrium, hepatomegaly and/or fever), corroborative radiological findings, and supportive serological and microbiological findings. In each patient, 3-5 mL of liver pus was aspirated under sterile conditions and under ultrasound guidance. In addition, 5 mL of blood was collected for serum separation. Informed consent was obtained from all patients or their legal guardians. Our institution’s Ethical Committee approved the study.

**Microscopy and culture of pus**

Pus specimens were examined for *E. histolytica* using microscopy and culture. Microscopy included examination using Gram’s and Ziehl-Neelsen stains. For culture, 200-300 μL of pus was inoculated into fresh National Institute of Health (NIH) medium, and the culture bottles examined at 24, 48 and 72 hours for presence of motile *E. histolytica* trophozoites. Anaerobic and aerobic bacterial cultures were also done using standard media. Specimens that were positive for bacteria either at microscopy or culture were excluded from further analysis.

**Serology**

Serum anti-amebic IgG antibodies were detected using a commercial ELISA kit (r-biopharm, Amoebiasis, Germany), and were used as gold standard for the diagnosis of ALA.

**Stool examination**

Three consecutive stool specimens were obtained from each patient and examined for *E. histolytica* trophozoites or cysts by microscopy (direct and concentrated methods) and for trophozoites by *in vitro* culture.

**PCR**

For DNA extraction, 200 μL of pus was diluted with an equal volume of TE buffer [10 m N-Tris-HCl (pH 7.5), 1 mM EDTA], then mixed with 400 μL of lysis buffer (200 mM NaCl, 20 mM EDTA, 20 mM Tris-HCl [pH 8.0], 4% w/v sodium dodecyl sulphate, 1 mg/mL proteinase K), and incubated at
60°C for one hour. DNA was extracted twice with an equal volume of phenol-chloroform/isoamyl alcohol (25:24:1). Extracted DNA was precipitated using ethanol with sodium chloride and was further pelleted by centrifugation at 10,000 g at 4°C for 10 min. The DNA pellet was washed in 70% ethanol, centrifuged, dried and re-suspended in 30 μL of TE buffer; it was further diluted (1:10) with distilled water before use in PCR. Similar procedure was followed for positive and negative controls.

A 125-basepair region of extra-chromosomal circular DNA of *E. histolytica* was amplified using primers 5′-TCAAAATGCTGCTCTAGGC-3′ and 5′-CAGTTAGAAATTATTGACTTGTGTA-3′. Amplification was done using an initial denaturation at 94°C for 2 min, 35 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 60 s, and extension at 72°C for two min, and final extension at 72°C for 5 minutes. Template DNA extracted from pus samples was dissolved in 30 μL of TBE and diluted 1:10 times. Five μL of the diluted template DNA was used in a 25-μL PCR reaction. Ten microliter of the PCR amplified products were subjected to electrophoresis on a 2% agarose gel containing 0.5 μg/mL of ethidium bromide. The gels were photographed under ultraviolet light. All PCR assays were performed twice. In specimens testing negative or showing a smear after electrophoresis, PCR was repeated with undiluted or more diluted (1:30) template DNA, respectively. With these modifications, four and one specimens that initially showed inconclusive results became positive, respectively.

To determine the minimum number of organisms in liver pus samples that can be picked by our PCR system, the following experiment was performed. Trophozoites harvested from the culture were pelleted down by centrifugation and the cell pellet was suspended in 1 mL of PBS, and one drop of fluid was charged in Neubauer’s chamber and cells were counted under microscope the low power magnification (100×). The cell pellet containing 10⁵ cells was selected and diluted 10 times in PBS to obtain different concentration of cells, such as 10⁴, 10³, 10², 50 and 10 cells/mL. To each of these dilutions 1 mL ALA pus (autoclaved) was added and mixed thoroughly. DNA extraction and PCR was done following the aforementioned protocol. PCR gave a positive signal in pus containing up to 100 organisms or more but not in pus sample containing 50 and 10 organisms, respectively.

DNA extracts from *E. histolytica* strains HK-9 and HM-1:IMSS were used as positive controls and *E. dispar* CDC 0784 (provided by Dr S Bhattacharya, Department of Environmental Sciences, JNU, New Delhi) as negative control in each PCR run. Specificity of PCR was assessed using ten pus specimens from patients with pyogenic liver abscess and sterile (autoclaved) pus spiked with *E. dispar* and known bacteria.

**Results**

Of the 30 patients with ALA (age 1-64 years [median 35]; 23 men), 27 had solitary lesions and 3 had multiple lesions. Lesions were 2 cm to 15 cm in diameter and were most frequently localized in the right liver lobe (24 patients). Microscopy and culture showed *E. histolytica* in none and two specimens, respectively. No pus specimen showed bacteria on microscopy and culture. Anti-amebic IgG antibody was detected in 23 (76%) patients.

Amplification of *E. histolytica* by PCR was detected in 25 patients with ALA. Thus, the sensitivity of PCR was 83%. The minimum detectable parasite number was 100 (Fig). Most pus specimens gave positive result when 5 mL of template DNA (equivalent to 3-4 mL of aspirated pus) was used for PCR reaction. All pus specimens obtained from patients with pyogenic liver abscess and the autoclaved pus specimens spiked with *E. dispar* or bacteria tested negative in PCR, yielding a specificity rate of 100%.

**Discussion**

Direct microscopy and in vitro culture are simple and cheap tests for diagnosis of *E. histolytica* in-

![Fig: PCR results for PCR from liver abscess pus. Lane 1: 100-bp molecular weight marker (M0321); lane 2: positive control showing a specific 125-base pair; lane 3: negative control (*E. dispar* DNA) showing no specific band; lanes 4, 5, 6: clinical samples showing specific bands of 125 bp; lane 7: clinical samples (initially negative with diluted DNA [1:10]) showing specific band of 125 bp with undiluted DNA](image)
PCR for detection of E. histolytica from amebic liver abscess

In conclusion, PCR may provide a more sensitive method of demonstrating presence of E. histolytica infection in patients with liver abscess.

References


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