

## Original Article

# Diagnostic Role of Anti-Lectin Antibodies for the Detection of Amoebic Liver Abscess

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### Abstract

In this prospective study, among 47 clinically suspected cases of Liver abscess, 46 were diagnosed as of Amoebic origin. Confirmation of Amoebic Liver abscess (ALA) was done by ribosomal RNA (rRNA) gene detection from Liver abscess pus using real-time Polymerase Chain Reaction (PCR). In addition, microscopy for detection of the parasite in Liver abscess pus, and Lectin antigen as well as anti-Lectin antibody were detected by Enzyme Linked Immunosorbent Assay (ELISA) from both abscess pus and sera samples. Microscopically, 05 (10.89%) samples were found positive for motile *Entamoeba histolytica* and *E. histolytica* rRNA gene were detected in 46 (97.8%) cases by real-time PCR. Among the 47 Liver abscess pus, 12 (25.54%) were *E. histolytica* Lectin antigen positive and 4/47 (8.51%) of the sera samples were positive for *E. histolytica* Lectin antigen by ELISA. Anti-Lectin IgG was found positive in 91% (43/47) of the sera investigated. Reviewing the test results, it appears that detection of anti-Lectin IgG by ELISA may be considered as a useful, rapid and convenient immunological tool for the diagnosis of ALA cases.

**Keywords:** *Entamoeba histolytica*, Amoebic Liver abscess, Lectin antigen, Anti-Lectin antibody, ELISA, real-time PCR

### Introduction

Amoebic Liver abscess (ALA) is a worldwide disease but incidence is very high in the tropical countries.<sup>1</sup> Approximately 70,000 deaths occur annually from Amoebiasis. It is the fourth leading cause of death due to protozoan infection after Malaria, Chagas' disease and Leishmaniasis according to the recent World Health Organization estimates.<sup>2</sup> Incidence of ALA in Bangladesh is yet to be estimated.

Clinical and laboratory diagnosis of ALA is a challenging task since clinical manifestations of the disease are very similar to pyogenic (bacterial) Liver abscess. Moreover, biochemical and haematological values of both the types are almost similar and overlapping. Advance imaging techniques like Ultrasonogram, Computed scan, Magnetic Resonance Imaging (MRI) have excellent sensitivity for Liver abscess arising from any cause but none of the techniques can distinguish pyogenic from Amoebic type.<sup>3</sup> For the laboratory diagnosis of ALA, direct microscopical detection of the trophozoites of *E. histolytica* in wet preparation from abscess pus is a rapid and definitive evidence but have a very unsatisfactory sensitivity.<sup>4</sup> Detection of *E. histolytica* antigen from Liver abscess pus have been done by some investigators with a detection rate ranging from

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30-40% after antiprotozoal therapy, but prior to therapy the rate was about 50%.<sup>5</sup>

Recently, detection of *E. histolytica* ribosomal RNA (rRNA) gene from Liver abscess pus by real-time polymerase chain reaction (PCR) has been shown to be very promising in the diagnosis of ALA with a sensitivity of >97%<sup>6,7</sup> but it is very costly tool for routine laboratory use.

In this study, determination of Lectin antigens (Gal-Gal NAC lectin) of *E. histolytica* from Liver abscess pus and serum anti-Lectin IgG have been done. Confirmation of ALA cases has been done by microscopy and *E. histolytica* rRNA gene detection in Liver abscess pus specimens by real-time PCR.

## Methods

### Study population

Included 47 Liver abscess patients admitted into Rajshahi Medical College Hospital, Rajshahi, during the period of January to December, 2006. Liver abscess pus (5.0 ml) and serum (2.0 ml) were collected from each of the patients. Blood samples from 20 voluntary healthy controls with comparable age, sex and ethnicity were also taken. The study was approved by the ethical review committee of Rajshahi Medical College.

Clinical diagnosis of Liver abscess was based on high fever with right hypochondriac pain, and a space occupying lesion in the Liver suggestive of abscess by Ultrasonogram. Clinically diagnosed cases were confirmed as of Amoebic Liver abscess by any two of the following criteria: (a) detection of trophozoite of *E. histolytica* from Liver abscess pus by microscopy; (b) detection of *E. histolytica* Lectin antigen from pus and serum by ELISA; (c) detection of anti-Lectin IgG from serum; and (d) detection of small subunit of rRNA gene of *E. histolytica* from Liver abscess pus by real-time PCR.

### Laboratory procedures

Wet mount preparation of Liver abscess pus for trophozoite(s) of *E. histolytica* was done within half an hour of collection of the sample.

The Techlab's (Black's Burg, Virginia, USA) *E. histolytica*

test II was used to detect Lectin antigen in pus specimens according to the manufacturer's instructions. Specimens were vortexed and centrifuged at 10,000 g for 10 minutes and 100 µl of undiluted supernatant was added into the microtitre well containing immobilized polyclonal antibodies. One drop of conjugate (monoclonal antibody peroxidase) was added, followed by 5-6 times washing with diluted soap solution for removal of unbound materials. Two drops of the substrate was then added and incubated at room temperature for 10 minutes, followed by addition of 1 drop stop solution (1 N Sulphuric acid) and finally reading was taken after 2 minutes. The absorbance was measured on ELISA reader (BIO-RAD, USA) at 450 nm and a test was considered positive when the optical density (OD) of a sample was scored >0.15. Antigen detection from serum was also done like that of supernatant of pus and the test was considered positive when OD value was greater than 0.5.

For antibody (anti-Lectin IgG) detection, 1µg/ml Lectin antigen for anti-Lectin IgG was prepared with 1× Phosphate Buffered Solution (PBS). After overnight incubation at 4°C, washing with 0.05% Tween 20 in PBS and blocking with 1% Bovine Serum Albumin (BSA) in PBS was done. The sample (100 µl) was added in each well, incubated for 2 hours at room temperature and washed with the buffer. Rabbit anti-Human IgG conjugate (100 µl) was added to each well. Thereafter, 100 µl TMB-substrate was added, incubated for 10 minutes at room temperature, and finally 100 µl stop solution (1N H<sub>2</sub>SO<sub>4</sub>) was added to stop the reaction. The absorbance was and considered positive when OD value was greater than 0.5.

### Detection of *E. histolytica* ribosomal RNA gene (DNA) by real-time PCR

The real-time PCR for detection of *E. histolytica* infection in Liver abscess pus was carried out according to protocol mentioned by Roy *et al.*<sup>7</sup> The oligonucleotide primers and Taqman probes were designed to specifically amplify a 135-bp fragment inside the 16S-like small-subunit rRNA gene of *E. histolytica* (Gene Bank accession number X64142). The primers and the probe set were purchased from Eurogentec, United Kingdom which consisted of the forward primer (Ehf) 5'-AAC AGT AAT AGT TTC TTT GGT TAG TAA AA-3', the reverse primer (Ehr) 5'-CTT AGA ATG TCA TTT CTC AAT TCA T-3' and the probe-Eh-YYT sequence-ATT AGT ACA AAA TGG CCA ATT CAT TCA-Dark.

Statistics: Positive test results by real-time PCR was considered true positive or gold standard for determination of sensitivity of different test methods used.

## Result

Out of 47 clinically suspected cases of Liver abscess, 46 were diagnosed as Amoebic Liver abscess (ALA) following case confirmation criteria. Among the suspected cases, active motile trophozoite of *E. histolytica* were detected in only 05 (10.89%) and remaining 42 (89.11%) cases were found negative.

Among all the study cases, 12 (25.54%) were found positive for *E. histolytica* Lectin Ag by ELISA done on the abscess pus and remaining 35 (74.46%) were negative. Only 4 (8.52%) sera samples were found Lectin antigen positive by ELISA. Significant high-titres of serum IgG were found in 43 (91.5%) cases and remaining 04 (8.5%) were negative by ELISA with a calculated sensitivity of 93%. *E. histolytica* rRNA genes on DNA were detected in 46 (97.9%) cases by real-time PCR. (Table I) None of the control subjects showed significant titre of serum anti-Lectin IgG. (Figure 1)

Table I: Comparison of different diagnostic tools for the detection of Amoebic Liver abscess cases

Test	Positive	Negative	Sensitivity
Microscopy	05 (10.89%)	42 (89.11%)	11%
Lectin Antigen detection by ELISA	04 (8.52%)	43 (91.48%)	09%
From pus	12 (25.54%)	35 (74.46%)	27%
From serum	04 (8.52%)	43 (91.48%)	09%
Anti-lectin IgG from serum by ELISA	43 (93.4%)	04 (8.5%)	93.4%
Real Time PCR	46 (97.9%)	01 (02.1%)	98%

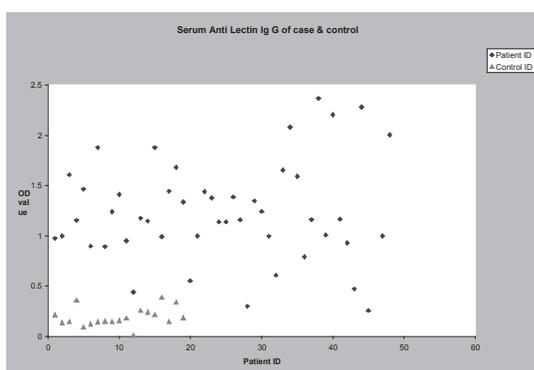


Figure 1: OD values of anti-Lectin IgG from ALA patients and controls

## Discussion

Investigators are currently trying to establish immunological tools for the diagnosis of different parasitic infections in humans for many years. Because an Amoebic Liver abscess (ALA) may be the cause of death of a patient if adequate therapy is delayed, early diagnosis is very much essential. Although the most definitive method for diagnosis of ALA is to demonstrate the organism in abscess fluid but there are many limitations in direct detection of Amoeba by microscopy. In this study, conventional microscopy (wet preparation) as well as immunological and molecular tests were performed to confirm the clinically suspected cases of ALA.

Microscopy revealed only 11% (5/47) active motile trophozoite of *E. histolytica* in abscess pus specimens. This finding is similar to previous studies published earlier.<sup>5,8</sup> Microscopical detection also needs rapid processing of the samples, is often misleading with macrophages and many living cells of Liver abscess pus. For these reasons, skilled microscopist is required for correct identification of the parasite.

Diagnosis of ALA by immunological means also has been trying for long time. It has recently been identified that an antigen named Galactose-N-acetyl-D-galactosamine Lectin, which is an integral component of *E. histolytica* cell wall, can be detected in stool and liver abscess pus.<sup>1,9</sup> Detection of this antigen from any sample of a patient with ALA can confirm the presence of *E. histolytica*. It brought new hope for the diagnosis of the disease but result was disappointing since the antigen disappears quickly from blood and Liver abscess pus specimens soon after treatment with Metronidazole. In this prospective study, Lectin antigen detection from the Liver abscess pus showed 27% sensitivity, which is similar to many previous studies. Ahmed *et al* performed antigen detection by ELISA from Liver abscess pus and found positive in 12 cases out of 29 with a calculated sensitivity of about 40%.<sup>8</sup> Another study by Haque *et al* showed antigen detection rate of about 41% (11/27).<sup>5</sup> Variation of sensitivity to Amoebic antigen (Lectin) probably relates to sample collection prior or after therapy of anti-protozoal drugs. Detection of Lectin antigen from serum also showed an unsatisfactory result with only 8.52% (4/47) positive results and sensitivity of about 9%, showing a less sensitive result than microscopy. In this study, most of the samples were taken from hospitalized patients who consumed initial anti-protozoal therapy and a therapy prior to sample collection may be the most probable reason for low sensitivity of Lectin antigen determination.<sup>8</sup> In addition, anti-Amoebic antibody

(anti-Lectin IgG) was also detected in this study and found a very good sensitivity of approximately 93% (43/46).

real-time PCR was also used for the detection of rRNA gene. It has been proven as the most sensitive and specific tool for the detection of *E. histolytica* in abscess pus of the ALA compared to traditional PCR and ELISA. However, it requires expensive equipments and a setup with specialized personnel for analysis of the result.

The present study demonstrated that Amoebic Liver abscess can be diagnosed by different diagnostic techniques using conventional and molecular tools but determination of serum anti-Lectin IgG by ELISA is most promising. It showed superiority to traditional microscopy both in sensitivity and specificity and also provided superior results to Lectin antigen detection. Results of this study has clearly shown that titres (OD values) of anti-Lectin IgG by ELISA in cases and controls significantly differ and clearly distinguish endemic controls from patients, which would be useful in identifying patients in endemic area.

Determination of serum anti-Lectin antibody may be a new hope for rapid and non-invasive diagnostic tool for ALA cases. But more extensive studies are required. Quantitative estimation of the same antibody response from saliva and urine in a large population both in cases and controls may help to identify more non-invasive and patient-friendly devices for diagnosis of ALA.

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[Conflict of interest: None declared]