SHORT ARTICLE

Detection of circulating antigen in amoebic liver abscess by counter-current immunoelectrophoresis

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Fifty serum samples from patients with amoebic liver abscess and 50 from subjects who had not suffered from the disease (25 from patients with other, chiefly parasitic, infections and 25 from healthy blood donors, staff or students) were tested for circulating amoebic antigen by counter-current immuno-electrophoresis (CIEP). Amoebic antigen was detected in 38 sera (76%) from cases of amoebic liver abscess, but in none of the other sera. Although CIEP is only moderately sensitive, the high specificity suggests that this simple test may be useful in the diagnosis of amoebic liver abscess.

Introduction

Clinical manifestations of amoebic liver abscess are variable, and diagnosis depends upon radiography, ultrasonography, computed tomography, magnetic resonance imaging and serodiagnostic methods [1].

Serodiagnosis is based mainly on the demonstration of circulating amoebic antibodies [2]. Although antibody-based serological tests are specific, false negative results commonly occur and these tests do not discriminate between active and past infection, as amoebic antibody titres persist for months or years even after treatment. Serological tests for the detection of circulating amoebic antigens provide a better way of discriminating between current or recent and past infection, as the presence of antigen in serum indicates an active infection [3].

The objective of the present study was to evaluate counter-current immuno-electrophoresis (CIEP) for the detection of amoebic antigen in serum for the diagnosis of amoebic liver abscess. CIEP has been used for the detection of specific antigens in various diseases [4–7], including hydatid disease [8, 9]. Although the test has been used for the detection of antibodies in serum and amoebic antigen in the liver pus of patients with amoebic liver abscess [6], to the

best of our knowledge there is no report of the use of CIEP for the detection of circulating amoebic antigen in serum.

Materials and methods

Serum

A total of 100 serum samples was collected from patients and control subjects attending the Jawaharlal Institute of Postgraduate Medical Education and Research Hospital, Pondicherry, India. Fifty of these sera were from patients with amoebic liver abscess, selected on the basis of three or more criteria modified from those recommended by Chuttani *et al.* [10], and described in an earlier study [11], as follows: (a) needle aspiration from an enlarged and tender liver of bacteriologically sterile 'anchovy sauce' pus, with or without demonstration of *Entamoeba histolytica* trophozoites by direct microscopy, or culture, or both; (b) radiological evidence (raised and fixed right cupola of the diaphragm) and ultrasonography findings indicative of an abscess (no significant wall echoes; round or oval shape; less echogenicity than normal liver parenchyma, with fine homogeneous low level echoes throughout at high gain with particulate motion and a location contiguous to the liver capsule and distal sonic enhancement) [12]; (c) amoebic serum antibody titre ≥ 128 ; (d) positive response to anti-amoebic therapy.

Sera were also collected from 10 cases of hydatid disease, 10 cases of filariasis, three cases of hookworm infection, two cases of lower respiratory tract infection and 25 apparently healthy subjects (staff, students and

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blood donors) who had not suffered from any symptoms of amoebiasis in the previous 6 months.

All sera were inactivated at 56°C for 30 min, preserved with sodium azide 0.015 mol/L and stored at -20°C until used.

Antigen

A sonicated extract of axenically grown *E. histolytica* (NIH: 200) prepared by the method described by Sawhney *et al.* [13] was used. Briefly, amoebae from a 48-h culture were washed three times in sterile physiological saline, concentrated by centrifugation at 500 *g* for 30 min and sonicated in the cold for 5 min in an ultrasonic disintegrator (MSE Scientific Instruments) with 1-min bursts and 2-min breaks between bursts. The sonicate was centrifuged at 10 000 *g* for 30 min and the supernate was used as axenic amoebic antigen.

Hyperimmune antiserum

Axenic amoebic antigen was emulsified with an equal volume of Freund's complete adjuvant. Adult rabbits (3–4 kg) were given 0.5 ml of this emulsion by intramuscular injection in all four limbs. Injection was repeated after 6 weeks, and 10 days later serum was collected and tested for antibodies to amoebic antigen by indirect haemagglutination assay (IHA) [14]. The amoebic antibody titre of the serum was 1024.

CIEP

Gels prepared on 50 × 75 mm glass slides with Difco Bacto agar 1% in veronal buffer (pH 8.4), were allowed to set at room temperature and stored at 4°C overnight. Six pairs of wells, 4 mm in diameter and 3 mm apart, were punched out on the slides with a template. Each well was filled with 10 µl of the appropriate reactant. Wells containing hyperimmune antiserum (titre 1024) were placed on the anodic side of the electrophoretic chamber and patients' sera on the cathodic side. The chambers were filled with veronal buffer (0.05 M, pH 8.4) and a current of 8 V/cm was applied for 30 min. Immediately after completion of electrophoresis the slides were read unstained with oblique lighting against a black background. The slides were then stained and re-read.

Unprecipitated protein was washed out of the agar by immersion of the slide for 24 h in veronal buffer. The slide was then washed for 15 min in acetic acid 1% to remove excess salt. Staining was with a solution of amidoblack 1% made in a solvent containing glacial acetic acid 1 ml, distilled water 49 ml and methylated spirit 50 ml for 30 min. Excess stain was washed with the solvent to give a preparation with dark bluish precipitation bands on a clear background. The slide was finally soaked in acetic acid 1% containing

glycerol 1% for 15 min and dried at 37°C in an incubator. Positive reactions were defined by lines of precipitation between antigen and hyperimmune serum. Positive and negative controls were included in each test.

IHA

Circulating antibodies in serum were measured by IHA as described by Parija *et al.* [14]. An antibody titre of ≥128 was considered to be diagnostic of amoebiasis.

Analysis of data

Data were analysed with the Epi-info package (World Health Organization). Sensitivity and specificity of the assays were calculated by the method of Park [15].

Results and discussion

The results are summarised in Table 1, which shows the comparative sensitivity and specificity of CIEP and IHA for the detection of serum antigen and antibodies, respectively, in the diagnosis of amoebic liver abscess. Serum specimens collected from healthy controls and from patients with other parasitic and infective conditions were uniformly negative when tested by CIEP and mostly negative by IHA. The positive and negative predictive values of CIEP were 95.7% and 90.5%, respectively; those of IHA were 93.1% and 83.9%, respectively.

The observed sensitivity of CIEP (76%) compares well with that of an enzyme-linked immunosorbent assay (ELISA) (93%) for the detection of antigen in sera of patients with amoebic liver abscess [16], but is lower than the 100% sensitivity of solid phase radio-immunoassay reported by Pillai *et al.* [17]. In the present study, 41 sera (82%) from cases of amoebic liver abscess were positive for amoebic antibodies by IHA. CIEP detected antigen in sera of 9 cases (18%) which were negative for circulating antibodies by IHA. Conversely, CIEP failed to detect 12 cases (24%) of amoebic liver abscess which were positive by IHA. The failure of CIEP to detect antigen in these cases may be due to the formation of immune complexes of amoebic antigen and antibodies, leading to the absence of free amoebic antigen in the serum.

Table 1. Evaluation of CIEP and IHA in the diagnosis of amoebic liver abscess

Subject groups	Number (%) positive by CIEP	Number (%) positive by IHA
Amoebic liver abscess (n = 50)	38 (76)	41 (82)
Infected controls* (n = 25)	0	3 (12)
Healthy controls (n = 25)	0	0

* Hydatid disease (10); filariasis (10); hookworm infection (3); lower respiratory tract infection (2).

Alternatively, the volume of free antigen may be too low to be detected by CIEP, which is only moderately sensitive.

CIEP has been widely used for the detection of antigen in serum, or other body fluids in the diagnosis of various parasitic and other infections [4–9]. It has also been used previously for the detection of amoebic antibodies in serum in the diagnosis of amoebic liver abscess, but not for the detection of circulating amoebic antigen.

ELISA [16] and solid phase radio-immunoassay [17] have been used to detect circulating amoebic antigen in the diagnosis of amoebiasis. These tests, although sensitive, are expensive and require technical expertise and thermolabile reagents. They are difficult to adopt in poorly equipped laboratories and there is need for a simple and economical, yet reliable test which permits the spot diagnosis of amoebic liver abscess in the field and rural health centres with inadequate laboratory facilities. CIEP, which requires simple instrumentation and inexpensive reagents, fits these criteria well.

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