IgG ELISA for invasive amoebiasis in endemic areas

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ABSTRACT
An IgG ELISA was evaluated as a serological test for the diagnosis of invasive amoebic disease in an endemic area. Sixteen patients with proven amoebic dysentery, 22 with suspected amoebic dysentery and 21 with amoebic liver abscesses were studied. Control subjects were 26 asymptomatic amoebic cyst passers, 13 patients with giardiasis, 26 with enteric fever and 31 individuals with no evidence of parasitic infestation.

The mean optical density (OD) value of the negative controls + 2 standard deviations (SD) was taken as the cut-off point to differentiate seropositive from seronegative individuals. It was found that all patients with amoebic liver abscesses had diagnostically raised IgG titres with specificity and sensitivity rates of 60.5% and 100% respectively; 25% of the patients with proven and 50% with suspected amoebiasis had positive antibody values with specificity and sensitivity rates of 50% and 25% respectively. Among the control subjects only 4 (15%) asymptomatic cyst passers, 4 (31%) patients with giardiasis, 3 (11%) of those with enteric fever and 5 (16%) of individuals without parasite infestation were seropositive.

The results of this study indicate that the IgG ELISA often provides equivocal results in cases of amoebic dysentery in endemic areas. However, specific IgG titres are significantly raised and of value in the diagnosis of patients clinically suspected to have an amoebic liver abscess.

INTRODUCTION
Detection of IgG antibodies against Entamoeba histolytica antigens is routinely used to diagnose amoebiasis, particularly invasive amoebic diseases. Of the various serological methods used, enzyme-linked immunosorbent assay (ELISA) is the most sensitive and specific. However, its value in the diagnosis of amoebic disease has only been reported from non-endemic areas. As IgG antibodies tend to persist in the circulation for long periods, their detection in patients with symptoms in an endemic zone may not provide evidence of active infestation. The present study evaluates the diagnostic efficacy of the IgG ELISA test in patients suffering from invasive amoebic disease in two large hospitals in an endemic area.

MATERIALS AND METHODS
Patients
Patients suspected to have both invasive and non-invasive amoebic disease admitted to the Victoria Hospital and the St. John's Medical College Hospital, Bangalore were included in the study. The patients who had amoebic dysentery (n=16) and amoebic liver abscess (n=21) were considered to have invasive amoebic disease while asymptomatic E. histolytica cyst passers (n=26) were considered to have non-invasive amoebiasis. Other groups of patients included diseased controls, namely, patients who had cysts of Giardia lamblia detected in their stools (n=13), and patients who had enteric fever (n=26). In addition, 31 persons without any overt intestinal disease and without any evidence of helminthic infestation (confirmed by microscopic examination of their stools) were also included. Twenty-two patients with clinically suspected amoebic dysentery, which could not be substantiated by repeated microscopic examination of the stool, were also included in the study.

Diagnostic criteria
Amoebic liver abscess (ALA): Patients were diagnosed to have ALA if they presented with tender hepatomegaly with or without fever, with ultrasonographic demonstration of a space-occupying lesion in the liver from which sterile pus was aspirated and who responded to standard antiamoebic drugs such as metronidazole.

Amoebic dysentery: Patients with amoebic dysentery were those who had large bowel diarrhoea, with sigmoidoscopic evidence of colonic or rectal mucosal inflammation and demonstration of haematophagous E. histolytica in the stool.

Amoebic cyst passers: Asymptomatic cyst passers were those who had E. histolytica cysts in their stools but no intestinal symptoms.

The other patient groups were those in whose stools, cysts or trophozoites of Giardia lamblia were demonstrated, and patients with enteric fever who had a positive Widal test with or without positive blood cultures for Salmonella typhi.

Methods
Stool examination for amoebic trophozoites: Faecal samples from patients suspected to have amoebic dysentery were subjected to staining techniques to determine the presence of haematophagous trophozoites as indicators of invasive disease. All specimens were initially subjected to wet mount screening techniques using 0.85% sodium...
RESULTS
Results of the IgG ELISA in different patient and control groups are shown in Fig. 1 and Table I. Four out of 16 (25%) patients with proven amoebic dysentery and 11 out of 22 (50%) with suspected amoebic dysentery but without *E. histolytica* trophozoites in their stool had positive antibody titres. An analysis of the duration of symptoms in these two groups showed that seropositive groups were out of the IgG ELISA test in patients with amoebic dysentery antibody titres. An analysis of the duration of symptoms of 49%. Positive antibody titres were obtained in all cases (100%) of ALA. The mean OD recorded among these patients was 0.6 which is considerably higher than the cut-off between seropositive from seronegative results which is 0.4. The sensitivity and specificity of IgG ELISA in patients with ALA were 100% and 60.5% respectively with a negative predictive value of 100% and positive predictive value of 58%.

Low levels of anti amoebic antibody titre were recorded in 4 out of 13 (31%) proven cases of giardiasis, while in the other control groups 4% to 16% of the patients were positive for IgG antiboody.

DISCUSSION
The present study indicates that in an endemic area such as South India it is possible to detect IgG antibody against *E. histolytica* antigens in 31% of the patients with giardiasis...
and 16% of individuals without any intestinal disease. Further, even in patients with frank amoebic dysentery the specificity and sensitivity of the IgG ELISA is unacceptably low. However, in patients with ALA the test is sensitive and specific with a high negative predictive value although the positive predictive value is low. Thus IgG ELISA can be used to help in the diagnosis of patients with amoebic liver abscess but cannot be used as a confirmatory test because of its low specificity rate (60%) and positive predictive value (58%). Similar conclusions have been reported previously from other countries.

The diagnostic efficacy of IgG ELISA in amoebic dysentery in the present series remains extremely poor in contrast to the experience of others in non-endemic areas. However, other reports from endemic areas have also shown a persistent antibody response from previous E. histolytica infection which interferes with its diagnostic efficacy. Contrary to the pattern of antibody response previously described, positive antibody levels in the present series were detected in only a few (4/16) proven patients with amoebic dysentery in spite of demonstrable tissue invasion by the E. histolytica trophozoites. On the other hand 50% (11/22) of patients with non-amoebic dysentery had a positive antibody titre. It is possible that the antibody response may vary according to the E. histolytica strain and its degree of invasion. This has been suggested previously by Healy et al. The particular strain causing amoebic dysentery or the inadequate host immune system might be responsible for such a poor antibody response. It is possible that a greater degree of invasion is necessary for an adequate antibody response to occur. This has been uniformly seen in patients with ALA in endemic as well as non-endemic areas.

In the patients in whom E. histolytica trophozoites were demonstrated the presence of amoebic antibody in 50% might be explained by E. histolytica trophozoites being missed in their stools or because they had a previous subclinical infection. Under these circumstances the detection of amoebic antigen in the stool described recently will be of considerable value. We therefore conclude that the IgG ELISA is of little diagnostic value in amoebic dysentery, but is more useful in patients with amoebic liver abscesses.

Serological investigation of the patients with amoebic liver abscess in the present study indicates that the IgG ELISA is of particular diagnostic value in endemic areas. If a serological diagnosis is not available, clinical diagnosis is usually achieved by recovery of typical anchovy sauce fluid which is bacteriologically sterile. Bacterial infection of the abscess, however, could lead to aspiration of foul smelling, contaminated pus which might originally have been sterile. In other cases where overt abscess formation has not occurred or micro-abscesses cannot be detected, and there are no immediate indications for drainage, hepatic amoebiasis is purely an unproven but well-recognized clinical entity. Serological tests are therefore of immense value in these cases.

It is apparent that a stool examination remains the first line of investigation for intestinal amoebiasis. While detection of IgM antibodies and faecal antigen assays require further evaluation, the IgG ELISA is to be recommended as a useful adjunct to the diagnosis of suspected cases of hepatic amoebiasis particularly in endemic areas.

ACKNOWLEDGEMENTS

We are grateful to Dr S. C. Pal and Dr P. Das of the National Institute of Cholera and Enteric Diseases, Calcutta, for supplying us the antigen and providing us training facilities. We also thank Dr M. J. G. Farthing, Consultant Gastroenterologist, St. Bartholomew's Hospital, London for advice and assistance, the Karnataka State Council for Science and Technology for funding this project and Mrs. Lakshmi Menon for secretarial help.

REFERENCES