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STUDIES ON EXTRAINTESTINAL AMEBIASIS IN IRAN BY SEROLOGIC METHODS

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Abstract

Sera from ninty one patients clinically suspected to extraintestinal amebiasis (ex-am) were collected and examined for presence of anit Entamoeba histolytica (E.h) antibody. The tests were Indirect Immunofluorescence antibody, (IFA or IIF) Gel Diffusion (GD) and counter Immuno ELectrophoresis (CL EP). Thirty one patients (34%) had anti E.h. antibody titers of >1:80. Among them eight patients (9%) had acute infection. IFA titers of > 1:640 were found to be significant for differentiation of present infection from past. The sensivity specificity, negative and positive predictive value of this test was 100%, 98% 100% and 89%. In GD or CIEP tests 13 positive cases (12%) were detected.

Introduction

Amebiasis is a world-wide disease (16). It is endemic in tropical regions of the world including Iran (11). Epidemiological studies in Iran using stool examination have shown 8-30% intestinal infection in different parts of the country (13). It has been reported that 5% of intestinal amebic patients may develops extraintestinal form. For diagnosis of ex-am, several methods such as aspiration, serological tests and sonography have been used (10). The most appliable and available one is serological tests (7). The problems with these tests some times acute infection could not be differentiate from chronic (6). Although antibody titer remains at high level for several months after recovery. The IFA titer of > 1/64 with GD positive confirm the infection was reported (14). On the other hand in acute cases of ex-am titer of >1/800 by IFA have been

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reported (2). It seems, antibody titer should be seprately evaluate in each country. Among serologic test, IFA is one of the easy and sensitive test for ex-am (15). With consideration of this facts and availability of IFA test we decide use IFA test in this investigation.

Materials and methods

Antigen preparation: a) Crude antigen : This antigen prepared from 48h, culture of E.h in Hsre + s (Horse serum + ringer + egg albumin + starch) (80) and Hsrp + s (horse serum + ringer + panmed 5% + starch) media (2,9). b) Soluble antigen : Monobacterial cultures in above media were applied using the following method :

1. suitable cultures containing 4×10^6 parasities were selected and chilled on ice for 10 min then shaked and centrifuged at 3500 rpm for 10 min.
2. super natant was discarded and the pellet was washed three times using P.B.S,PH. 7.4.
3. The sediment were suspended in P.B.S. and centrifuged at 1000 rpm for 10 min.
4. Sediment was transfered in strile test tube. In order to disrupt the parasite, freeze and thaw technique was used until there were no amoeba detected microscopicaly.
5. The suspension was centrifuged at 3000 rpm for 3 min and supernatant fluid was chosen as soluble antigen.

c: collection of sera: Sera from patient clinically suspected of ex-am were obtained from different public and private hospitals, history of illness and various clinical and paraclinical data were recorded. Sera were obtained from 1987-1990 and maintained at-20^{oc} , until examination.

d: IFA test: A dilution of 1/10, 1/20, 1/40 ... of sera were used and routine step for preparation of test have been done (15).

e: GD and CLEP: The sam method as vinayak, Halpern and starm were used (9) reaction for GD have been read after 48h and for CLEP after 24h.

Results

Sera of 91 patients with different clinical pictures were tested by IFA, GD and CLEP as shown in Table 1. In IFA test 32 of these patients had titer of >1/80 indicating as infected (Table 2), eight of these patients had titers of >1/640 indicating as acute infection (Table 3). Theses paraclinical finding were

confirmed by response to metronidazol and hydroemetine treatment. In the , meantime one patient with titer of 1/640 had liver adenocarcinoma. All of control sera had titer of 1/80 which was considerd to be negative (Table 1). Twelve of IFA positive (> 1/160) cases also were positive with GD and CIEP tests.

Discussion

This study has showed IFA test with titers of >1/640 indicated of acute ex-am infection. Two patients who had titers of 1/640 one responded to drug treatment and were cured, but the second one showed liver adenocarcinoma. There were some reports on association of intestinal anal carcinoma with invasive intestinal amebiasis (4) but there were no reports on liver carcinoma associated with intestinal amebiasis one care with titers of >1/1280 demonstrated an amebic abcess which responded to drug treatment and recoverd. Most of the patients were from western part of the country. Recently 8% *Entamoeba histolitica* infection in the same area was reported, too (13). In this study it was identified that IFA test was more sensitive and can be used as a screening test all over the country, it is cheeper, easy to handle than the other techniques. In the present study twelve of the patients with titers of 1/160 (by IFA) were positive using either GD or CIEP precipitation tests (1-3 bands) were seen with no corrolation to high or low IFA titers and acut infection. Inspit of some studies (1,3) there were no relation between number of bands and acut infection but there is reports on low sensivity of this comparing with other tests such as IFA (5).

Table 1- Result of suspected ex-am sera patients examined by IFA, GD, CIEP and negative controls

Result	Tests			
Negative <1.80 in IFA	IFA	GD and CIEP	Negative control by IFA test	Negative control by GD & CIEP
	59(65%)	79(86%)	30(100%)	30(100%)
Positive	32(35%)	12(13%)	-	-
Total	91(100%)	91(100%)	30(100%)	30(100%)

Table 2- IFA titers in suspected ex-am patients, with different clinical pictures

IFA titer	Clinical findings		
	Hepatitis	Hepatic absoss	Lung absoss
Negative and			
1.80	22	46	2
1.80	-	-	-
1.160	1	6	1
1.320	1	3	-
1.640	-	2	-
1.280	1	6	-
Total	25	63	3

Table 3- Sensitivity specificite and predictive value of IFA test

Sensitivity	Specificity	Negative P.	Positive P.
100%	98%	100%	89%

Table 4- Information of ex-am patients were founded by IFA test (recently had been infected)

Sex	Age	Job	Place of living in county	Clinical symptoms	IFA titer
Male	20	soldier	west	haptic abscess right and left lob diarrhea + fever	> 1.1280
Male	20	Student	north	hepatic abscess in right lob+ fever and diarrhea	1.640
Male	24	soldier	west	haptic abscess in right lob+fever	>1.1280
Male	30	labour	west	haptic abscess in right and left lob+fever and diarrhea	>1.1280
Male	36	labour	north	hapatitis+fever and diarrhea	>1.1280
Male	38	farmer	west	haptic abscess in righ +fever and diarrhea	>1.1280
Male	39	farmer	Karaj	hepatic abscess in right	>1.1280
Male	40	houswife	north	hepatic abscess in right lob+fever and diarrhea	>1.1280

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