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EVALUATION OF IFA, IHA AND BLA TESTS IN THE SEROLOGICAL DIAGNOSIS OF AMEBIC LIVER ABSCESS

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ABSTRACT

As there was not any previous comprehensive study on the serological tests for hepatic amebiasis in Iran, the indirect hemagglutination (IHA), indirect fluorescent antibody (IFA), and biocolored latex agglutination (BLA) tests were evaluated in the serodiagnosis of amebic liver abscess(ALA). For this purpose a total of 165 serum samples 18 of which were obtained from patients known to have ALA, were examined for *Entamoeba histolytica* antibodies.

E. histolytica antigen used for IFA technique was prepared in the Intestinal Protozoa Laboratory, Protozoology Unit, School of Public Health, Tehran University of Medical Sciences. The results of the survey showed that the IHA and BLA were somewhat more sensitive than IFA in the serodiagnosis of ALA. The sensitivity, specificity, and positive and negative predictive value of these tests were compared. *MJIRI, Vol. 7, No. 3, 161-164, 1993.*

INTRODUCTION

Amebiasis is a common protozoal infection caused by *E. histolytica*, affecting about 10% of the world population. However, only a few of them have clinical signs and symptoms of the disease. In 1981 it was estimated that 34-50 million of the world population suffered from invasive amebiasis (colitis and abscess).[4,10] It is probable that invasive amebiasis accounts annually for 40,000 to 110,000 deaths in the world.[9] The diagnosis of ALA is based on clinical symptoms and signs usually supplemented by serological tests.[6] It is now generally accepted that serology holds a key position in the diagnostic methods available for the patients with ALA.[2] The immunologic tools used in the diagnosis of amebiasis have been carefully reviewed by Healy.[1]

MATERIALS AND METHODS

Sera: 165 serum samples were obtained from different patients. According to medical documents 18 of them had amebic liver abscess (ALA). All of these 18 patients had clinical signs and symptoms of ALA and showed space occupying lesion of liver by CT scanning and ultrasound examinations. Aspirated pus from eight of them was cultured and no bacteria were grown. In most of them by using antiamoebic drugs, some of the clinical signs rapidly regressed.

The rest were as follows: 45 patients had pyogenic abscess, hydatid cyst, tumors and hepatocarcinoma. 43 had colitis, diarrhea and intestinal disorders, 30 were asymptomatic but carriers of *E. histolytica*, 15 with other parasitic protozoa, and finally 14 were apparently healthy individuals without any parasitic infection. We grouped all of them as individuals without ALA.

Antigen and other reagents: Antigen for IFA was prepared from xenic cultivation of *E. histolytica* in horse serum-Ringer-egg + starch (Hsre + s) medium in our laboratory.[3] There was good agreement between our prepared antigen and ameba-spot IF for serodiagnosis of amebiasis (Bio Merieux, France, Ref. 72901, IHA Kits for amebiasis (Cellognost amebiasis) from Behring, Germany, Apr. 1990, OTMOG 9801261, and BLA Kits for amebiasis (Bichro-

Table I. Results of IFA and IHA tests in detecting *E. histolytica* antibodies in 165 serum samples.

Tests / Groups \ Antibody titers		—	10	20	40	80	160	320	640	1280	2560	5120	10240	total
IFA*	ALA⁺	—	—	—	—	—	—	1	1	5	6	3	2	18
	ALA⁻	4	2	21	38	41	30	8	3	—	—	—	—	147
IHA	ALA⁺	—	—	—	—	—	—	—	—	2	3	2	11*	18
	ALA⁻	6	9	12	24	60	19	5	4	4	3	—	1	147

IFA*: IFA tests by using our prepared Ag

*: Ab titers in these 11 individuals were from 10240 to 163840

Table II. Results of BLA test in detecting *E. histolytica* antibodies in 165 serum samples (n= 165).

Tests / Groups \ Intensity of reaction		—	+	++	+++	total
BLA	ALA⁺	—	—	1 (5.6)	17 (94.4)	18 (100)
	ALA⁻	129 (87.8)	5 (3.4)	2 (1.4)	11 (7.4)	147 (100)

Table III. Results of IFA* and IHA tests according to cut off titers (n= 165).

Cut off titers \ Tests / Groups	IFA*		IHA	
	ALA⁺	ALA⁻	ALA⁺	ALA⁻
≥ 320	18 (100)	11 (7.4)	18 (100)	17 (11.6)
< 320	—	136 (92.6)	—	130 (88.4)
≥ 640	17 (94.4)	3 2	18 (100)	12 8.2
< 640	1 (5.6)	144 (98)	—	135 91.8
≥ 1280	16 (88.8)	—	18 (100)	8 5.4
< 1280	2 11.2	147 (100)	—	139 (94.6)

Latex-Amibe) from Fumouze, France, No; 08050-34-09/89).

Table IV. Results of BLA tests according to intensity of reaction (cut off) (n=165).

Cut off titers \ Tests / Groups	BLA	
	ALA⁺	ALA⁻
≥ +	18(100)	18 (12.2)
—	—	129 (87.8)
≥ ++	18(100)	13 (8.8)
< ++	—	134 (91.2)
+++	17 (94.4)	11 (7.5)
< +++	1(5.6)	136 (92.5)

RESULTS

The results of the study are shown in Tables I and II. The range of IFA antibody titers in patients with ALA varied from 1/320 to 1/10240 and 88.8% of them had antibody titers > 1/1280. The range of antibody titers in the subjects without ALA were < 1/640. The range of IHA antibody titers in the patients with ALA varied from 1/1280 to 1/163840 and 88.8% of them had antibody titers > 1/2560. But in 97.2% of the individuals without ALA the antibody titers were < 1/2560 (Table I). In 94.4% of the patients with ALA the sensitivity by BLA were +++ and 87.7% of the individuals without ALA showed no reaction (Table II).

Table V. Comparision of sensitivity, specificity, and positive and negative predictive values of three serological tests in each cut off.

Cut off	≥ 320		≥ +	≥ 640		≥ ++	≥ 1280		+++
test	IFA*	IHA	BLA	IFA*	IHA	BLA	IFA*	IHA	BLA
Sensitivity	100	100	100	94.4	100	100	88.8	100	94.4
Specificity	92.2	88.4	87.7	97.9	91.8	91.1	100	94.5	92.5
Positive P.V.	62	51.4	50	85	60	58.1	100	69.2	70.8
Negative P.V.	100	100	100	99.3	100	100	98.6	100	99.2

Results of three tests based on cut off titers (Table III) or sensitivity (Table IV) were different in positive ALA patients from negative ALA individuals. Finally, the sensitivity, specificity, and positive and negative predictive values of three tests in cut off titers of 320, 640, and 1280 (or +, ++, and +++ in BLA), are shown in Table V.

In the titer more than 1280 the sensitivity of IFA test was less than IHA and BLA, but the specificity of IFA was higher than IHA and BLA, and the positive predictive values were more or less the same in the three tests.

DISCUSSION

The IHA antibody titer to *E. histolytica* in the sera collected from the patients with ALA were higher than IFA antibody titer in the same group (Table I). The cause of this difference seems to be related to the fact that IHA and IFA detect different antibodies, as has been also reported by Stamm and WHO meeting.[7,11] On the other hand, nonspecific reactions observed by IHA and BLA were rather higher than IFA.

It may be related to past infections with *E. histolytica* and persistence of antiamebic antibodies for several years after successful treatment, or to higher sensitivity of these tests.[2,7] Therefore, these tests have more value in seroepidemiological studies, also their negative reactions can emphasise non-amoebic source of disease.

IHA test in cut off titer of >2560 has a good diagnostic value (88.8% sensitivity and 97.2% specificity). BLA test with highest reaction (+++) as cut off, had 94.4% sensitivity and 92.5% specificity.

The present study showed that our prepared antigen by xenic cultivation of *E. histolytica* has enough ability and usefulness in IFA tests. Overall, by having inexpensive antigen, IHA and BLA are more suitable tests for field work and seroepidemiological studies, because these tests have higher sensitivity and negative predictive value and simplicity and do not need expensive tools like fluorescent microscope. On the other hand IFA due to its good sensitivity, high specificity
producing antigen by xenic cultivation, and relatively more rapid drop in antibody titers due to previous exposure to *E. histolytica*, is more accessible and a better test for serodiagnosis.[8,5] Finally, we must emphasize that there is no relation between the antibody titers and severity of disease.[9] Also, the presence of antibodies in high titers, without clinical signs and symptoms of ALA, may not have any diagnostic value.[11]

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