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THE CLINICAL SIGNIFICANCE OF THE INDIRECT HAEMAGGLUTINATION TEST FOR AMEBIASIS IN TAIWAN

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The indirect haemagglutination (IHA) test for amebiasis using antigen from axenic culture of *Entamoeba histolytica* was evaluated in patients with confirmed amebiasis and in patient without evidence of amebiasis. The test was found to be highly specific in patients with amebic liver abscess in which 100% of 41 patients demonstrated titers of 1:128 or greater. In 18 patients with proven pyogenic liver abscess and 31 with hepatoma, 94.4% and 96.8% respectively demonstrated negative results. The test therefore has shown to be excellent for differentiating amebic liver abscess from pyogenic liver abscess and hepatoma. The test was found to be less specific in cases of intestinal amebiasis since only 67.8% of those with confirmed amebic colitis demonstrated significanly high IHA antibodies. On the other hand, 92.5% of the patients without evidence of amebiasis had negative antibody titers. In follow up studies it was shown that IHA antibodies for *Entamoeba histolytica* might be present for one or more years with a gradual decrease in titer after effective antiamebic therapy. Therefore, a positive titers may be the result of a current acute infection or a previous exposure to *Entamoeba histolytica*.

INTRODUCTION

The definitive diagnosis of amebiasis is the recovery of cysts or trophozoites of *Entamoeba histolytica*. In cases of intestinal amebiasis, the parasite may be easily recovered from the stools but with extraintestinal amebiasis, the parasite can not always be demonstrated. In recent years, serological methods have been improved whereby specific antibodies are readily demonstrated in the sera of patients with extraintestinal amebiasis.[1-6] This adjunct to the diagnosis of amebiasis will be of great value to the clinicians in Taiwan.

A great number of serologic techniques have been developed for the study of amebiasis; the complement fixation test[1,7,8] immobilization test[9,10] agar gel diffusion test[4,11,12] fluorescent antibody technique[13-16] the indirect hemagglutination test[1,2,3,17,18] the bentonite flocculation test[5] and phagocytosis test[19]. Among these the indirect hemag glutination test using the micro-titration system has been found to be very promising and offering high specificity[3,20].

During the past 3 years, sera were obtained from patients seen in the Department of Internal Medicine at the National Taiwan University Hospital and Taipei Municipal Ho-ping Hospital. The sera were tested by the indirect hemagglutination test using an antigen provided by the Medical Ecology Department of the U.S. Naval Medical Research Unit No. 2, in Taipei. The results of this study are presented in this report.

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MATERIALS AND METHODS

Sera: The sera tested were obtained from patients with the following illness:

(1) Amebic liver abscess: The diagnosis was made by finding either amebic trophozoite in aspirated pus, or bacteriologically sterile pus with characteristic chocolate like color, or space occupying lesion detected by liver scanning or by liver angiography with the lesion disappearing following specific antiamebic therapy.

(2) Amebic dysentery: All had demonstrable motile hematophagic amoebic trophozoite in stool.

(3) Pyogenic liver abscess: Demonstration of pus and isolation of bacteria in cultures.

(4) Hepatoma: Confirmed by biopsy or necropsy.

(5) Colitis: Mucoid or bloody mucoid stool without demonstrable amoebae after repeated examinations.

Antigen: The antigen was prepared from axenic cultures of the HK-9 strain of *E. histolytica* maintained at NAMRU-2. The procedure for making the antigen has been described by Thompson *et al.*[3]

Indirect hemagglutination test:

The indirect HA test used was a modification of the method described by Kessel and Lewis[1]. The sheep red blood cells used in the test were prepared as follows:

Fresh sheep red blood cells (SRBC) in Alsever's solution were used. One ml of 10 per cent SRBC (0.1 ml packed cell) was washed, 4 times with 9 ml phosphate buffered saline (PBS) pH. 7.2. After the final wash, 10 ml of 1:20,000 tannic acid in PBS pH 7.2 was added to the packed cells (0.1 ml) and incubated in a 37°C water bath for 15 minutes with frequent shaking. The mixture was centrifuged at 2000 r. p. m. for 5 minutes at room temperature. The tanned cells were washed with 40 ml PBS pH 7.2 then suspended in 10 ml normal saline and coated with 10 ml of optimal diluted antigen in PBS pH 6.4 (i.e. about 1:40 of original antigen which contained 3 mg protein/ml). This mixture was incubated in a 37°C water bath for 15 munutes and shaken frequently, then washed with 20 ml 1% rabbit serum in PBS pH 7.2. One precent rabbit serum in PBS pH 7.2 was added to the sediment. (Final concentration being a 1% sensitized SRBC suspensions).

The microtiter method for performance of the IHA test was used and has been described elsewhere[1].

Absorption of the sera to be tested was done before each test and four controls used for each test: 1. a positive serum with known titer, 2. a negative serum, 3. normal SRBC plus positive serum, 4. sensitized SRBC plus rabbit serum.

RESULTS

Fig. 1 shows the results of the IHA test with sera from patients with amebiasis and other diseases.

Amebic liver abscess: Forty one patients with confirmed amebic liver abscess had titers from 128 to 8192 and over with the majority of the cases with titers of 1024 or greater. The IHA tests was 100% positive in cases of amebic liver abscess, if the significant titer is considered to be 128.

Symptomatic intestinal amebiasis: Sera from 28 patients with proved intestinal amebiasis revealed a wide distribution of IHA titers, however, most of sera had titers of 64 or more, and some had titers as high as 2048. Only one serum from a confirmed case of intestinal amebiasis failed to demonstrate any IHA antibody.

Pyogenic liver abscess: Seventeen out of the 18 patients with proven pyognic liver abscess demonstrated zero to low antibody titers for amebiasis. Most of the patients completely lacked antibodies.

Hepatoma: Thirty cases of pathologically proven hepatoma were negative and one patient had a titer of 256.

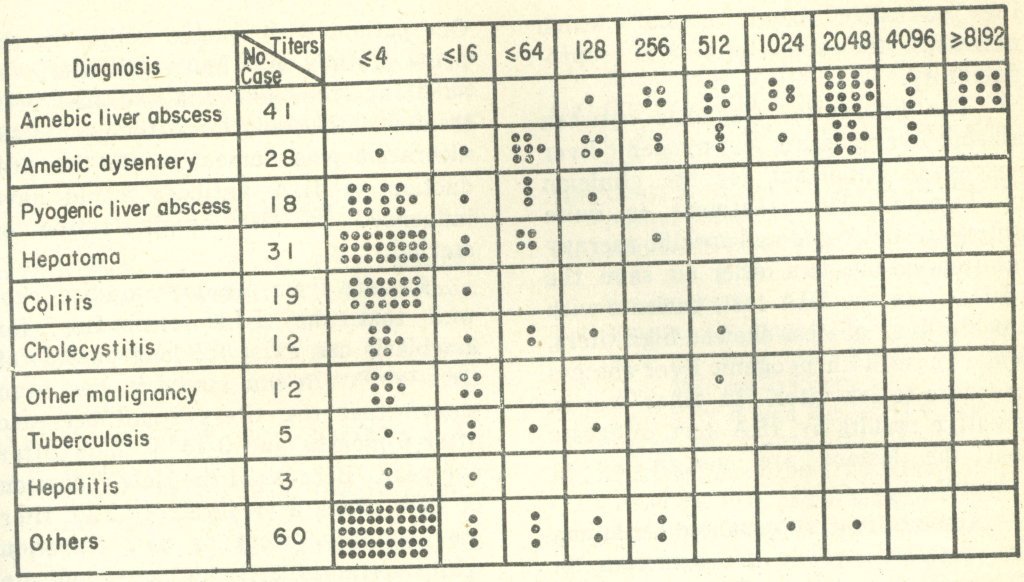

Fig. 1. Indirect Haemagglution Test for Amebiasis

Acute and chronic colitis: The sera from 19 patients with colitis, were negative. These diagnoses included ulcerative colitis (7 cases) shigellosis (3 cases) and other non-specific colitis (9 cases).

Cholecystitis: The sera from 10 patients with acute cholecystitis were negative by the IHA test for amebiasis and only one patient had a titer of 512.

Among 80 patients with diseases other then those above, 58 demonstrated zero titers. Some of the patients had low titers which were considered negative. Three patients had moderate to high titers, they were one with rectosigmoid carcinoma (×512), one with fever of unknown origin (×1024), and one with pulmonary infiltration and eosinophilia with undetermined etiology (×2048). Neither the trophozoites nor cystic stages of *E. histolytica* could be found from these 3 patients.

The IHA antibodies seemed to be a transient rise in titer during the early phase of treatment, but a gradual decrease 3 months after treatment. In 5 patients in which follow-up IHA antibody titers were determined over a period of more than 6 months (Fig. 2). The titers exhibited a gradual decrease with time. Some titers persisted at a low to moderately high level even after one or two years.

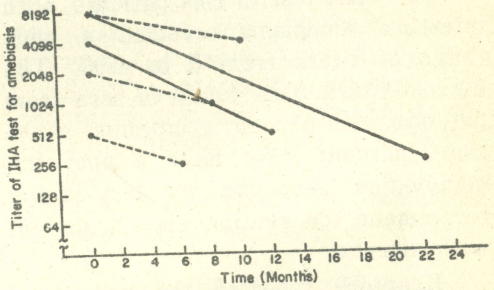

Fig. 2. Follow-up titers of IHA test in 5 patients with amebic liver abscess

DISCUSSION

The results of this study demonstrates the high specificity of the IHA test for extra-intestinal amebiasis, and emphasizes the values of this test in differentiating amebic liver abscess from pyogenic abscess or liver malignacy. Cross reported[6] that the IHA test was specific, sensitive and reproducible in the diagnosis of amebic liver abscess. This high specificity may be attributed to the use of pure ameba antigen from

the "so called axenic strain" which increases the sensitivity and reproducibility.

The differential diagnosis between amebic liver abscess and pyogenic liver abscess is important for the clinician in Taiwan. The treatment is quite different, and rapid and specific therapy is often essential in order to save the patient. In the IHA test, patients with amebic liver abscess showed high titers, while those with pyogenic liver abscess had zero to low titer. In other words, negative results by IHA test suggests that the lesions are non-amebic in origin[1].

Amebic liver abscess and hepatoma are diseases which are common in Taiwan[20,22] and it is often difficult to differentiate between the two if neither fever nor leukcytosis are seen in patients. By using the IHA test, however, most cases with hepatoma would be negative, while cases of amebic liver abscess have moderate to high titers.

The IHA test in the patients with intestinal amebiasis revealed a wide range of titers from 0 to 4096. The highest titers were found in sera from patients with chronic symptoms. Since some patients may have a poor immunological response, the test is not recommend for routine use in patient with diarrha[2].

Krupp[23] reported the results of IHA testing on 274 persons in Cali, Colombia, and the test gave positive results for 9% of 75 asymptomatic persons who passed cysts and 81% of 168 persons with amebic colitis. In another report, Neal[24] studied the virulence of *E. histolytica* in rats and concluded that the serological response was associated with the virulence of the strain of *E. histolytica*. Therefore, the greater degree of tissue invasiveness by the parasite, the greater the amount of antigen made available to the host. On the other hand, poor antibody response of the host may also affect the titer. One patient with amebic colitis in the present study who had a zero titer, was suffering from leukemia and had been treated with steroid. Although rectal ulceration was demonstrated, the production of IHA antibody could have influenced by the administration of steriod.

Results from previous studies showed that the IHA antibodies for amebiasis can exist for long periods of time[1,20,23]. In this study, it has been shown that the titers could decrease after 6 months and 3 to 4 folds after one year. Because of the slow reduction of IHA titers, a moderatedly high titer may suggest recent or past infection while extraordinarily high titer indicate recent infection or persistant chronic infection.

Seroepidemiology of amebiasis among apparently healthy individual in Taiwan has not been reported and therefore the percentage of IHA seropositivity which can be expected in "normal" populations remains to be determined. A 6% seropositivity was noted in Argentine recruits, 33% in Columbia army recruits, 96% in Yanamamo Indians (US), 3% in Cherkee Indian school children (US), 0% in Calion, Arkansas (US)[25], and 7% in Cali, Columbia[23]. Tsai[26] reported 11 of 28 cases (39%) of healthy persons living in endemic area Peikang, Taiwan, with antibody titers ranging from 64 to 512, while in healthy persons living in non-endemic area were negative. This may explain why a small percentage of low to moderate IHA titers existed in our selected groups. The seroepidemiologic curves in a population will depend on personal hygiene, sanitary facilities, as well as the presence or absence of invasive strains of the parasite[25].

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阿米巴間接血球凝集試驗在臨床上之意義

王　麗　都

從阿米巴疾病和非阿米巴疾病患者，用 axenic 阿米巴株爲抗原做間接血球凝集試驗之結果，知對阿米巴性肝膿瘍有很高的特異性。即41例阿米巴性肝膿瘍患者有100%之陽性率，其 titer 在等於或高於 128 倍。對18例化膿性肝膿瘍患者，94.4%爲陰性。而31例肝癌患者，96.8% 爲陰性。這種現象對阿米巴性，化膿性，或肝癌之鑑別診斷頗有助益。但是間接血球凝集試驗對阿米巴大腸炎，只67.8%病人之血清，有明顯增加 titer。又此試驗對非阿米巴性病人，92.5%呈陰性反應。

從阿米巴間接血球凝集抗體 titer 之追踪，知此抗體自治療後慢慢的降下，歷時一年或以上才能恢復正常。故此特殊抗體之異常增加，表示患者現在，或過去有阿米巴之感染。

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