THE USE OF THE INDIRECT HEMAGGLUTINATION TEST FOR THE DIAGNOSIS OF EXTRA-INTESTINAL AMEBIASIS IN JAKARTA

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In clinical amebiasis, the most reliable method for diagnosis is the recovery of the tissue-form of the Entamoeba histolytica from pathological specimens of patients. In amebiasis of the colon this traditional method gives almost good results; however in extra-intestinal amebiasis it is not often successful. The immunological approach of the problem on the other hand has given quite satisfying results and since the early investigations of Terry and Bozicevich (1949) and Hussey and Brown (1950) with the complement fixation test numerous serological studies had been made on amebiasis. From the various serological techniques developed during the course of time, such as the double diffusion principle of Ouchterlony (1948; 1958), the passive hemagglutination reaction (Boyden, 1951), the coated inert particle aggregation test (Ager et al, 1959; Christian et al, 1958), the immuno-fluorescent indirect method (Coons et al, 1941; Coons & Kaplan, 1950; Beutner et al, 1965), and immuno-electrophoresis (Grabar and Williams, 1955), two had found practical application for the routin serodiagnosis of amebiasis: The double diffusion test (Maddison, 1965; Maddison et al, 1965 (1) & (2); Powell et al, 1965; Powel et al, 1966) and the indirect hemagglutination test (Kessel et al, 1961; Kessel et al, 1965; Milgram et al, 1966; Maddison et al, 1968; Kasliwal et al, 1970). Both tests have been compared and evaluated by Maddison et al, (1965), (1) and Kagan & Norman (1970) and were found of equal sensitivity. The agar-gel-precipitin test has the advantage that it is simpler to perform, however it requires a concentrated antigen and an incubation period of at least 19-72 hrs and the test cannot be quantitated.

In this presentation the indirect hemagglutination test (IHA) is used for the serological studies of human amebiasis, in particular of the extra-intestinal amebiasis in Jakarta.

MATERIALS AND METHODS

Indirect hemagglutination test. The indirect hemagglutination test described by Kessel and Lewis (1961) and Lewis and Kessel (1961) was employed with minor modifications. A 2.5 per cent suspension of human red blood cells type 0 was tanned with tannic acid (Perla Merba, R.P.) at 1: 20.000 dilution in a 37°C waterbath for 15 minutes. The tanned cells were sensitized with antigen at the proper dilution at a pH of 6.4 ± 0.2 in a 37°C waterbath for 15 minutes and used immediately after washing. The tests were performed with the microtiter equipment purchased from Flow Laboratories, Rockville, Maryland. Sera were diluted serially from 1 : 2 to 1 : 4096 dilution and the tests were read after an incubation of 30 minutes at room temperature and then 18 hr at 5 - 10°C. A negative reaction showed

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a tiny button or a smooth ring of sedimented cells at the bottom of the well, a positive reaction showed a carpet of agglutinated cells with irregular edges. Only strong positive reactions were read. Sera which gave titers lower than 1:128 were recorded as negative. The antigen employed was a lyophilized antigen obtained from Parke & Davis and from the Parasitological Unit of C.D.C. Atlanta, Georgia. In the hemagglutination test the Parke & Davis antigen was used at 1:8 dilution and the C.D.C. antigen at 1:10 dilution. The dilution of each brand of antigen was determined against serial dilutions of two high positive (sera with titers greater than 1:256), two low positive (sera with titers 1:128 or 1:256), and two negative reference sera. These reference sera were obtained from C.D.C. Atlanta.

A. Sera. 78 patients from Dr. Tjipto Mangunkusumo, Persahabatan, Cikini hospitals and from a few patients treated ambulatory at the Department of Parasitology were studied. The age of the patients ranged from 18 to 60 years. These patients were grouped according with their clinical records into the following:

- Confirmed amebic liver abscess. Fifteen patients with amebic liver abscess, confirmed by aspiration of typical pus or demonstrating *E. histolytica* trophozoites in pus.
- Unconfirmed amebic liver abscess. Four patients in whom a clinical diagnosis of liver abscess was made without aspiration of pus.

*B. Sera from 43 blood donors which were obtained from the Indonesian Red Cross Clinic at Dr. Tjipto Mangunkusumo hospital. The age of the donors ranged from 18 to 50 years and most of them were males. All sera were handled with a-septic precautions and were stored at 20°C. They were examined for the indirect hemagglutination test in groups of 25 sera at a time.*

**RESULTS**

Table 1 shows the results of the IHA with 78 sera from various disease groups and with 43 sera from blood donors.

<table>
<thead>
<tr>
<th>Disease group and blood donors</th>
<th>Total number examined</th>
<th>Negative</th>
<th>Positive</th>
<th>percentage No. positive/total No. examined</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>no agglutination or agglutination with titer lower than 1:128</td>
</tr>
<tr>
<td>1. Confirmed amebic liver abscess</td>
<td>15</td>
<td>0</td>
<td>2</td>
<td>13</td>
</tr>
<tr>
<td>2. Unconfirmed amebic liver abscess</td>
<td>4</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>

*Chronic intestinal amebiasis.* Six patients in whom amebic colitis developed several months following amebic diarrhea.

*Asymptomatic carrier.* One person who came to the Department for health certificate harbouring *E. histolytica* cysts in the stools.

*Other diseases.* Thirty-nine patients suffering from other diseases such as: 9 liver diseases, carcinoma of liver, cirrhosis of liver, pyogenic liver abscess, hepato-splenomegaly, 23 lung diseases such as pulmonary tuberculosis, tuberculous pleural effusion, bacterial empyema, pyogenic lung abscess, carcinoma of lung, 3 cases of nephritis, 1 Hodgkin’s disease, 1 typhoid fever, 2 cases of undetermined dysentery.
THE INDIRECT HEMAGGLUTINATION TEST

3. acute intestinal amebiasis
   Number of cases: 13
   Hemagglutination titer: 11
   2: 0
   1: 15

4. chronic intestinal amebiasis
   Number of cases: 6
   Hemagglutination titer: 3
   3: 0
   1: 50

5. asymptomatic carrier
   Number of cases: 1
   Hemagglutination titer: 1
   0: 0
   0: 0

6. Other diseases
   Number of cases: 39
   Hemagglutination titer: 39
   0: 0
   0: 0

7. Blood donors
   Number of cases: 43
   Hemagglutination titer: 43
   0: 0
   0: 0

Liver abscesses. All sera from 17 cases of confirmed amebic liver abscesses and two sera from unconfirmed amebic liver abscesses were positive for amebic antibodies. Most of these sera titers were high, up to 1:4096 and only two sera had 1:128 titer.

Intestinal amebiasis. The results of the IHA on 20 selected cases of intestinal amebiasis showed a definite higher frequency of amebic antibodies with high titer (1:128) in the chronic stage than in the acute cases whilst one asymptomatic carrier had no antibodies. Low antibody titers were found in 3 cases of acute intestinal amebiasis (table 2).

Table 2. Low titer amebic antibodies found in the indirect Hemagglutination test.

<table>
<thead>
<tr>
<th>Clinical state</th>
<th>Number of cases</th>
<th>1:16</th>
<th>1:32</th>
<th>1:64</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute intestinal amebiasis</td>
<td>3</td>
<td>2</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Lung abscesses with &amp; without hepatitis</td>
<td>4</td>
<td>1</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>Blood donors</td>
<td>11</td>
<td>2</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>

Other diseases and blood donors. In these groups no serum has antibodies up to 1:128 dilution of serum. Amebic antibodies of low titers were found in four out of 39 persons suffering from other diseases and in 11 blood donors. The frequency distribution of these low titer serums are shown in table 2.

DISCUSSION

The finding of 100 per cent frequency for amebic antibodies with high titers in amebic liver abscess proves a high sensitivity of the IHA for the diagnosis of extra-intestinal amebiasis and emphasizes its value in detecting occult invasive amebiasis. These results are in conformity with those reported by various workers such as Kessel et al. (1961), Kasliwal et al. (1970), who found a 100 per cent frequency of positive test in liver amebiasis and Milgram et al. (1966) who found a 99 per cent frequency.

The results of the test on intestinal amebiasis however are markedly different from those of Kessel et al. (1961), Milgram et al. (1966), Healy (1968) and Krupp (1970). Kessel et al. (1961) found a 98 per cent frequency of amebic antibodies in 37 sera from intestinal amebiasis whereas Milgram et al. (1966), Healy (1968) and Krupp (1970) found respectively 82, 85 and 81 per cent frequency in their material of respectively 83, 63 and 168 cases of amebic dysentery. In this study a 26 per cent positive test was found in 19 cases of symptomatic intestinal amebiasis. In a similar small number of material (18 cases) Kasliwal and
Associates (1970) found a higher (44.4) percentage of positive test. It should be mentioned, however, that these last authors used the 1 : 16 titer as the diagnostic titer for advanced amebiasis. Many factors play a role in inducing antibodies in intestinal amebiasis, such as the extend of the lesions in the intestinal mucosa caused by the invasion of the amebia, the response of the host as well as the time at which the blood sample is drawn. When the serum sample is drawn only a few days after the elicit of symptoms, the change of getting a positive result is meagre. This fact can be shown from the frequency of the positive test found in the chronic forms of intestinal amebiasis. Other factors which may cause discrepancies in results are the use of different techniques, such as differences in the kind of red blood cells, differences in the use of positive reference sera, and differences in interpretation of positive tests from the titers given by the sera. It is known that sheep red blood cells are more sensitive for tanning procedures than human red blood cells and thus it might be possible that the liver abscess sera in this study gave much lower titers than those in the studies of Milgram et al (1966) who reported to find an average titer of 1 : 12000 in sera from liver abscesses.

The interpretation of a positive IHA from a given titer has been of much dispute in the past. Kessel et al (1961) compared the hemagglutination test with the complement fixation test and found no agreement of positive hemagglutination test between 1 : 8 – 1 : 32 and partial agreement from 1 : 128 and higher. Maddison (1965) and many others were of the opinion that the hemagglutination test is a more sensitive test than the agar-gel-precipitin test whilst the complement fixation test is the least sensitive test for amebiasis. Because of its sensitivity the recognition of a positive test from the titer is important for the hemagglutination test. Milgram et all (1966) regarded a titer of 1 : 128 as the lowest positive titer. Investigating a large number of selected groups of cases of amebiasis and using several selected groups of controls, they found good correlation between a positive hemagglutination test and clinical amebiasis. Similarly, Healy (1968; 1970) using the same technique as Milgram et all had found high specificity and sensitivity of the hemagglutination test. A method for evaluation of the hemagglutination test for clinical amebiasis had been presented by Krupp (1970). The sera and clinical state of 392 persons living in an endemic country were studied and the titers obtained in the IHA were plotted in a graph against frequency of distribution. Thus doing, a bimodal curve was obtained, showing one sharp (negative population) and one low (positive population) peak. By interpolating at the point of sharp decrease in percentage of persons at the negative curve a titer of 1 : 40 was found which then was taken as the highest negative titer. Thus the IHA applied to that particular population was established as being positive starting at 1 : 80 titer. Kasliwal and Associates (1970) in their studies of the significance of the IHA for the diagnosis of amebiasis in Jaipur, India, approached the problem of determination of the diagnostic titer of the IHA, by investigating sera from persons with clinical amebic hepatitis. They found in 16 sera (64%) antibodies with titers varying between 1 : 16 and 1 : 64. In two cases of more advanced invasive amebiasis with involvement of the lungs and pleura the antibody titer found was 1 : 16. Based upon above information the 1 : 16 titer was regarded as the lowest positive titer. In this preliminary study, the interpretation of the titer of a positive IHA was made by the study of the test on 15 sera from patients with frank amebic liver abscess. As shown in table 1 most of the sera had high titers up to 1 : 4096, but two had 1 : 128 titer. Consequently I have regarded the 1 : 128 titer as the lowest positive titer. Thus regarding the test as positive when hemagglutination was observed at 1 : 128 serum dilution, the majority (85%) of the sera from acute intestinal amebiasis were found negative whilst 50 per cent of the persons with a history of chronic amebic dysentery showed a positive test. A thorough clinical follow up study on these patients unfortunately had not been made so that it is not possible at this time to make any speculation as to whether these high anti-
body titers came from advanced processes in
the intestinal wall or from other organs.
The significance of antibodies in serum dilutions
lower than 1:128 cannot be evaluated in this
study since the medical records on the patients
with acute amebic dysentery did not include
thorough clinical history, physical examination
and routine laboratory investigations. As regards
to the 4 patients with low titer antibodies in the
group with other diseases, one had a hydro-
thorax and amebic antibody titer 1:16, 3 other
patients with 1:64 titer had ulcerative lung
abscesses of tuberculous origin. The clinical
state of the blood donors from which blood
samples were taken several months before the
test was done, was unknown.

SUMMARY
The incidence of amebic antibodies as reve-
aled by the indirect hemagglutination test
was determined in 5 different groups of ameb-
biasis, other patients with other diseases and in
blood donors.
A 100 per cent correlation was found between
the prevalence of antibodies of high titer
(1:128 - 1:4096) and incidence of amebic
liver abscesses. A higher incidence of antibodies
of titer 1:128 was found in chronic than in
acute amebiasis of the colon.
None of the persons in the control groups had
antibodies of significant titer. The above obser-
vations indicate that the indirect hemaggluti-
nation test is useful for the diagnosis of occult
invasive amebiasis when the usual methods fail
to show the organism.

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