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The Serological Diagnosis of Amoebiasis in Ceylon

Part I. The Indirect Haemagglutination Test (IHAT)

by

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SUMMARY Sera from patients with clinical evidence of amoebiasis were tested by the Indirect Haemagglutination Test (IHAT) for the presence of antibodies to *Entamoeba histolytica*.

The results show that diagnosis levels of antibody are present in cases of amoebic hepatitis and amoebic liver abscess. The test is of value in confirming the diagnosis of amoebic hepatitis and amoebic liver abscess. Its value in the diagnosis of intestinal amoebiasis requires further study, though a positive result is indicative of amoebic infection.

INTRODUCTION

Amoebiasis is known to affect 10% of the world's population. Till comparatively recent times, the diagnosis depended on the finding of *Entamoeba histolytica* in the stool, or, more rarely, in aspirates. With the application of serological tests to the diagnosis of parasitic diseases, the usefulness of these tests in the diagnosis of amoebiasis engaged the attention of many workers.

The IHAT was first reported by Kessel, Lewis, Ma and Kim (1961). This was in the nature of a preliminary report. It was subsequently reported by Maddison (1965), Kessel, Lewis, Molina-Pasqual and Turner (1965), Milgram, Healy and Kagan (1966) and Healy (1968). Minor variations in the performance of the test, such as the type and concentration of red cells and the concentration of tannic acid, were employed by these different workers. Since amoebiasis is a common disease in our country, it was decided that we carry out this test on sera from patients in our hospitals.

MATERIALS AND METHODS

Test sera were collected from three groups of patients and a control group.

Group 1, consisted of sera from patients who had symptoms and signs of intestinal amoebiasis with *E. histolytica* in their stools.

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Group 2, were sera obtained from patients with symptoms and signs of extra-intestinal amoebiasis, diagnosed as amoebic hepatitis.

Group 3, were sera from clinically confirmed cases of liver abscess where pus had been withdrawn from the liver.

Group 4, consisted of control sera from a group of Peace Corps volunteers. Their stools were negative for amoebae, ova and cysts by direct saline smear and merthiolate iodine formaldehyde concentration test.

Negative and positive control sera and antigen were provided by Professor Healy from the CDC Laboratories, Atlanta, Georgia. The negative control serum (No. 36) had been used in several series of tests which had been reported at the WHO Expert Committee on Amoebiasis, at Teheran in 1968 (personal communication, Prof. Healy.) The positive control serum was K-4036. The antigen was Lot 9-69 *E. histolytica* HK 9.

The antigen was reconstituted in phosphate buffered saline (PbS), pH 6.4 and stored at 17°C. The antigen was titrated against positive and negative control sera using local sheep red cells, to determine the optimum dilution. It was found that a 1/40 dilution was the most suitable working dilution and all tests in this series were carried out at this dilution (CDC Laboratories working dilution 1/50. personal communication, Professor Healy).

The cells used were sheep red cells not more than 2 to 3 days old. Citrated cells were washed 3 times in PbS, pH 7.2. To an aliquot of cells an equal volume of 1/30,000 solution of tannic acid in PbS 7.2 was added. The cells were then incubated in a water bath at 37°C for 15 minutes, with frequent shaking. The tanned cells were washed once with PbS and re-suspended to give a concentration of 1.5% in physiological saline. An equal volume of antigen (1/40) was added to the tanned cells and incubated in a water bath for 15 minutes with frequent shaking to sensitise the cells. The tanned sensitised cells were then washed twice in PbS 7.2 and re-suspended in 1% normal rabbit serum, to a 1.5% suspension. Tests were run in micro-titre plates. Each well received 0.05 ml of PbS-NRS (normal rabbit serum). Twofold dilutions were made with 0.05 micro-titre loops going from 1 : 2 to 1 : 4096. The sensitised cells were dropped with an 0.025 micro-titre dropper to all wells. Each test series included a positive and negative control from the CDC Laboratories. After the first run negative and strongly positive sera from local serum groups were included. Each test also included tanned unsensitised cells and serum, diluted at 1 : 2 and unsensitised cells with buffer, in order to determine non specific reactions. The protocol was as recommended by the CDC Laboratories, with two variations. Since the available horse serum proved unsatisfactory in use, normal rabbit serum instead of normal horse serum was used. The test serum was added to the sensitised cells in all test wells and a separate set of wells was run to test the non-specific reactions between non-sensitised cells and sera. The serum dilutions were from 1 : 2 to 1 : 4096 with twelve wells for each test serum.

RESULTS

Table 1 shows the results of titres obtained with the four different groups of sera. Column 1 shows the serum dilutions. The other columns show the number of each which gave the respective titres indicated.

TABLE 1

Shows the serum dilutions and the results obtained with the IHAT, in the 4 groups tested.

Serum Dilutions	*Group* 1 Intestinal Amoebiasis	*Group* 2 Amoebic Hepatitis	*Group* 3 Aspirated Liver Abscess	*Group* 4 Controls
1:2 Negative	0	1	0	3
1:2	1	2	1	5
1:4	0	1	1	10
1:8	0	3	0	21
1:16	1	1	1	9
1:32	0	2	1	4
1:64	0	2	0	0
1:128*	3	4	3	0
1:256	0	4	2	0
1:512	1	6	3	0
11024	0	6	1	0
1:2048	0	2	1	0
1:4096	2	22	27	0
TOTAL	8	56	41	52
Percentage with 1:128 or	75	78	90	0

* *Diagnostic titre*

The diagnostic titre was taken as 1 : 128 (Healy, 1968). In Group 1, six of a total of eight cases (75%) showed diagnostic titres. Two had titres of 1 : 2 and 1 : 16 respectively. Both patients had *E. histolytica* in their stools and had been diagnosed as cases of intestinal amoebiasis warranting institutional treatment.

In Group 2 (amoebic hepatitis), 44 of 56 (78%) showed a diagnostic titre of 1 : 128 or more ; 12 showed titres of 1 : 64 or less and one did not show any reaction. Clinically, all twelve had been diagnosed as amoebic hepatitis and had responded to treatment with emetine and chloroquine. Seven had typical histories and in the others the history was incomplete.

Group 3. A total of 41 sera were examined, and 37, (90%) had a titre of 1 : 128 or more. Two cases had titres of 1 : 2 and 1 : 4 respectively ; pus had been aspirated from the liver of both patients though their stools had been negative for amoebae. One case had a titre of 1 : 16. This patient had a history of fever of 10 days duration, with pain in the abdomen. A ruptured liver abscess had been diagnosed, and the patient responded to treatment with emetine and chloroquine. This was the only case in the group in which pus had not been aspirated. One other case where pus had been aspirated gave a titre of 1 : 32.

Group 4. In this group, which was the control group, all titres were below 1 : 64.

DISCUSSSION

In this investigation the number of tests that could be performed was necessarily limited by the availability of antigen. This, together with our anxiety to do as many tests as possible once the antigen was diluted, led us to use sera collected from cases in which the clinical histories were not well documented, though the clinical diagnosis was available to us.

In Group 1, of which our series is very small, two sera showed low titres of antibody. Milgram *et al.* (1966) reported that 25 of 68 cases were negative in this group, in spite of having gastro-intestinal symptoms. They do not advocate this test for routine use in intestinal amoebiasis, as it is not known how long it takes for significant levels of antibody to appear. Kessel *et al.* (1966) found 273 of 280 (97%) positive. These conflicting results make it essential for further examination of sera from clinically positive and normal persons from a similar environment.

In amoebic hepatitis (Group 2), the diagnosis is made on the presence of fever, pain and tenderness over the liver ; the diagnosis is generally confirmed by a quick response to treatment (approximately 3 days) with emetine and chloroquin or metronidazole. In this group 78% had a positive titre. In the 12 cases with less than diagnostic levels of antibody, 4 had well documented histories with symptoms and signs of amoebiasis. The others were clinically diagnosed as such and are recorded as having responded to treatment. Twenty two of 56 had titres of 1 : 4096. End titres were not determined due to the need to conserve antigen. Though it is not possible to determine the minimum duration of disease before which significant antibody levels appear in the different groups, it does appear that in this group (at the time of clinical presentation) such levels are generally present and the IHAT could be a valuable confirmatory aid in the diagnosis of amoebic hepatitis.

Group 3 contained the highest percentage of positives, 90%, (37 of 41 cases). Twenty seven (65%) had titres of 1 : 4096. It is regretted that though all cases in this group (bar one) had pus aspirated from the liver, we did not examine the aspirate for amoebae. According to the classification of Kagan (1968), ours would fall into the group of unconfirmed liver abscess. It is necessary to mention that our physicians aver that all cases of liver abscess in this country are of amoebic origin and the clinical entity of a pyogenic liver abscess such a rarity, it is considered virtually non-existant. It appears that the IHAT is a valuable confirmatory test in the diagnosis of this group also.

Group 4. All persons in this group were from a socially and economically different stratum to those in other groups. Their sera were collected soon after arrival (one week) in this country. Unless they were infected prior to arrival, it is extremely unlikely that sufficient time could have elapsed between arrival and taking of serum for antibody to develop following a recent infection. We examined all sera from this group, as these could provide a good background for any control for later work as well, as it it not always possible to obtain suitable sera in large numbers. All sera gave less than diagnostic titres with the IHAT.

CONCLUSIONS

The results obtained with the IHAT are without ambiguity as there are no positive results with the controls.

In all three clinical groups sera examined by the IHAT showed the presence of diagnostically high levels of antibody. The largest number positive was found in sera from cases of amoebic liver abscess. The next highest levels were in those with amoebic hepatitis. In intestinal amoebiasis also the percentage positive was high though our series was small, and therefore needs further study.

It appears that where tissue destruction is extensive a greater number show a positive reaction to the IHAT together with a high titre of antigen antibody reaction.

A positive result provides confirmatory evidence of amoebic infection irrespective of clinical type.

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