Laboratory Tests in Hydatid Disease: a Comparison of the Indirect Haemagglutination, Complement-fixation and Intradermal Tests

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The laboratory diagnosis of echinococcosis has been based chiefly on the results of the Casoni intradermal (ID) or the complement-fixation (CF) test. The CF test has a limited sensitivity, while the ID test may be unreliable since, once acquired, skin sensitivity may persist for life. A positive test may, therefore, indicate only a previous exposure to echinococcus antigen. The recent application of Boyden's tannic acid indirect haemagglutination (IHA) test [a] to the diagnosis of hydatid disease by Garabedian and co-workers [b, c, d] has awakened a new interest in the serology of this disease.

The purpose of this note is to evaluate the sensitivity and reliability of the IHA test and to compare the results with those of CF and ID tests.

Materials and methods

Serum samples. 270 sera were obtained from patients admitted to the medical and surgical departments of different hospitals in Athens. Of these sera, 120 were from patients confirmed by surgical intervention as having hydatid disease (*Echinococcus granulosus*) of the lung, liver or other organs. The remaining 150 sera were from persons suffering from other illnesses. In the latter category, the diagnoses were established by surgical intervention or by clinical and laboratory studies. In each case the sample of blood was obtained early in the morning, before breakfast, to avoid accumulation of lipids in the serum. After separation from the clot, the serum was stored at —20°C until use. Prior to the test each sample was thawed and inactivated at 56°C for 20 minutes.

Antigen and antigen titration. The antigen employed in the IHA and CF tests was hydatid fluid aspirated from hydatid cysts removed by operation from patients with echinococcosis of the liver and lungs. The aspirated fluid from each cyst was clear and contained many scolices. The bacteriologically sterile hydatid fluids were pooled and stored at −20°C. The necessary amount was removed and thawed prior to each experiment. The same batch of antigen was used throughout this investigation. The antigen was titrated against a known positive serum of high titre. In the IHA test the antigen was diluted 1 : 3; in the CF test it was used undiluted.

Indirect haemagglutination test. The tannic acid haemagglutination test of Boyden,[a] as applied by Garabedian and co-workers [b, c, d] to the diagnosis of echinococcus disease, was followed with slight modifications. Sheep erythrocytes were collected in Alsever's solution and stored for 3 days at +4°C before use. Just prior to use, the erythrocytes were washed three times in phosphate-saline buffer (pH 7.2). The packed erythrocytes from the last washing were used in the test.

To 9.5 ml of a 1 : 20 000 dilution of freshly prepared tannic acid in buffered saline (pH 7.2), 0.5 ml of packed erythrocytes was added and the mixture incubated at 37°C for 10 minutes, with frequent shaking. The cells were washed free of excess tannic acid with buffered saline (pH 7.2). To the packed, tannic-acid-treated erythrocytes, 9.5 ml of a 1 : 3 dilution of hydatid fluid in buffered saline was added and the suspension was left at room temperature for 30 minutes. The cells-antigen mixture was washed once with inactivated normal human serum diluted 1 : 250 in buffered saline (pH 7.2). A 0.5% suspension of the packed cells was made in the above buffered saline.

For control purposes, a 0.5% suspension of normal sheep erythrocytes was prepared in buffered saline (pH 7.2).

Twofold serial dilutions of sera were made in buffered saline (pH 6.4) containing 4% inactivated normal human serum, starting at 1 : 20 dilution. In both the IHA and the CF tests, the tubes were replaced by Perspex plates.

[a] Boyden, S. V. (1951) *J. exp. Med.*, **93**, 107.

[b] Garabedian, G. A., Matossian, R. M. & Djanian, A. Y. (1957) *J. Immunol.*, **78**, 269.

[c] Garabedian, G. A., Matossian, R. M. & Djanian, A. Y. (1957) *J. méd. Liban*, **10**, 275.

[d] Garabedian, G. A., Matossian, R. M. & Suidan, F. G. (1959) *Amer. J. trop. Med. Hyg.*, **8**, 67.

TABLE 1
RESULTS OF INDIRECT HAEMAGGLUTINATION, INTRADERMAL AND COMPLEMENT-FIXATION TESTS ON PATIENTS WITH HYDATID DISEASE

Site of hydatid disease	Number of patients	IHA test		ID test		CF test	
		Positive reactors	Percentage positive	Positive reactors	Percentage positive	Positive reactors	Percentage positive
Lung	57	47	82	43	75	27	47
Liver	59	57	97	43	73	44	75
Other organs	4	4		3		3	
Total	120	108	90	89	74	74	62

To 0.25 ml of diluted serum, 0.25 ml of antigen-treated red cells was added and the reading was made after the plates had stood at +4°C overnight.

Controls of serum and sensitized erythrocytes were included, and the tannic acid was tested for ability to sensitize the cells without agglutinating them automatically.

In positive tests, the erythrocytes showed agglutinating masses sedimented uniformly across the bottom of the Perspex plate cavities. In negative tests, the erythrocytes settled at the bottom and attained a button-like appearance.

Complement-fixation test. The CF tests were also performed in Perspex plates. Veronal buffer saline was used as diluent. The sera under examination were inactivated at 56°C for 30 minutes. The antigen employed in the test was undiluted hydatid fluid. The volume used in the test was 0.5 ml per cup of the Perspex plate.

An amount of 0.1 ml each of antigen, serum dilution 1 : 4 [e] and complement (2 units) was allowed to react for 90 minutes at 37°C. Then 0.2 ml of a sensitized 1% suspension of sheep erythrocytes was added to each cup, and the plates were incubated for 30 minutes at 37°C. All necessary controls were included. The test was read after the plates had stood overnight at +4°C. Fixation greater than 2+ (less than 50% haemolysis) was considered as a positive reaction.

Intradermal test. The ID test was performed and read by the attending physicians of the hospitals, a commercially available antigen being used. The results of the ID tests were obtained from the patients' case-histories at the time of the visit to hospital for the collection of blood samples.

[e] Only one serum dilution (1:4) was used in the complement-fixation tests.

Results

Control sera. In the IHA test, only 3 (2%) of the 150 control sera obtained from patients suffering from illnesses other than hydatid disease gave a measurable titre (ranging from 1 : 160 to 1 : 320); the other 147 sera did not react even in the lowest dilution (1 : 20). Consequently, it was decided to consider as true positives sera with a titre $\geqslant$1 : 640.

In the CF test, 4 control sera were positive (2.7%).

The ID test was performed on only 3 out of the 150 control patients suffering from illness other than hydatid disease. The test was positive in these 3 patients, who when operated upon were found to have carcinoma of the liver. In the same patients the IHA and CF tests were negative.

Sera from verified cases of hydatid disease. In Table 1 the results of IHA, ID and CF tests on the sera from 120 patients with hydatid disease confirmed by surgical intervention are tabulated.

Of the 57 patients with hydatid disease of the lung, 47 (82%) gave positive results in the IHA test, 43 (75%) in the ID test and 27 (47%) in the CF test.

Of the 59 patients with hydatid disease of the liver, 57 (97%) gave positive results in the IHA test, 43 (73%) in the ID test and 44 (75%) in the CF test. The mean percentages for the total of 120 cases tested by the IHA, ID and CF tests were 90, 74 and 62, respectively.

The titres of the positive sera in the IHA tests ranged from 1 : 640 to 1 : 160 000 (Table 2). It can be seen from Tables 1 and 2 that the sera from

TABLE 2

TITRE RANGE OBTAINED IN INDIRECT HAEMAGGLUTINATION TESTS ON PATIENTS WITH HYDATID DISEASE OF DIFFERENT ORGANS

Titre range	Lung	Liver	Other organs	Total
1 : 640	9	3	1	13
1 : 1 250-1 : 2 500	23	26	1	50
1 : 5 000-1 : 20 000	13	15	1	29
1 : 40 000-1 : 80 000	2	5	1	8
1 : 160 000	—	8	—	8
Total	47	57	4	108

patients with hydatid disease of the liver gave slightly higher percentages of positive results, as well as higher titres, than those from patients with hydatid disease of the lung. In Table 3 the results of the IHA, ID and CF tests on the 120 sera from patients with hydatid disease are correlated with one another. From this table it can be seen that of the 120 patients, 59 (49%) were positive reactors to all three tests, while 8 (7%) were negative in all of them.

From Table 3 it can also be seen that the IHA and the ID tests taken together gave a high percentage of positive reactions (92%). Thus only 9 sera out of 120 (8%) were negative in both tests.

TABLE 3

CORRELATION BETWEEN THE RESULTS OF INDIRECT HAEMAGGLUTINATION, INTRADERMAL AND COMPLEMENT-FIXATION TESTS ON PATIENTS WITH HYDATID DISEASE

Number of patients	IHA test	ID test	CF test	Percentage positive
59	+	+	+	49
29	+	+	−	24
12	+	−	+	10
8	+	−	−	7
8	−	−	−	7
1	−	+	+	1
2	−	+	−	2
1	−	−	+	1

Discussion

The results of the present investigation confirm the findings of Garabedian et al.[d] and Kagan et al.[f] who reported that the indirect haemagglutination test was more sensitive than the complement-fixation or the intradermal test for the diagnosis of hydatid disease. Thus 90% of the sera from confirmed cases of hydatid disease gave a positive reaction in the IHA test. The percentage of false positive reactions in the same test was found to be very low (2%). In none of the false positive reactions was the titre obtained higher than 1 : 320. In view of this, we think that in the IHA test only titres $\geqslant$1 : 640 should be regarded as positive.

In our own series, the ID test was positive in only 74% of the confirmed cases of hydatid disease and the CF test in 62%. In the published reports the positivity of these tests has been found to vary greatly.

In our opinion, the higher percentage of positive sera from patients with hydatid disease of the liver than from those with hydatid disease of the lung, as well as the higher titres in the same category of patients, can be explained by the difference in the permeability of the cyst wall. It seems that cysts of the liver supply more antigenic stimuli to the host tissues.

The technique of the performance of the IHA test does not present difficulties. The important points are the selection of the antigen and the quality of the tannic acid used for the sensitization of the red cells. The antigen should be titrated before use in the IHA test and the tannic acid should be tested for ability to sensitize the cells without agglutinating them automatically.

The performance of the CF test is more difficult and the reactivity of the sera from echinococcus patients is low. For the routine diagnosis of hydatid disease we suggest that both the IHA and the ID tests should be applied. From Table 3 it can be seen that these two tests taken together give positive reactions in 92% of the known cases of hydatid disease examined.

[f] Kagan, I. G., Allain, D. S. & Norman, L. (1959) *Amer. J. trop. Med. Hyg.*, **8**, 51.