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Evaluation of Various Serodiagnostic Tests for Human Hydatidosis

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Abstract

A total of 216 serum samples from 27 surgically confirmed hydatid patients, 19 patients with parasitic infections other than hydatidosis, 69 university students, and 101 outpatients were serodiagnosed for the presence of antibodies against hydatid fluid. The tests employed for serodiagnosis were double diffusion (DD), countercurrent immunoelectrophoresis (CIE), indirect haemagglutination (IHA) and the enzyme linked immunosorbent assay (ELISA). The ELISA and the IHA tests were found to be most sensitive, each detecting 85.2% of the hydatid cases while the CIE and DD detected 66.7% and 40.7% respectively. Out of 27 cases, 25 (92.6%) were detected by a combined use of ELISA and IHA. None of the sera from university students, outpatients or patients with parasitic diseases other than hydatidosis showed any cross reactivity with hydatid fluid antigens. Both IHA and ELISA especially their combined use, are sensitive enough to be suitable for seroepidemiological surveys of hydatidosis in man.

Key words: Serodiagnosis, Hydatidosis, Human.

Introduction

Hydatid disease is caused by the development of the matacestode of *E. granulosus.* Our recent studies on disease prevalence among domestic animals in North Jordan showed a high prevalence rate especially among sheep and cattle (Al-Yaman *et al.* 1985 and Abdel Hafez *et al.*, 1986). Stray dogs were also found to have a high infection rate with the adult worm (Ajluoni *et al.*, 1984). Thus, human infections are not uncommon (Dajani and Shihabi, 1979; El-Muhtaseb, 1984; Shennak *et al.*, 1985).

The clinical symptoms of hydatid disease are often non specific and are associated with the pressure effects of the developing cyst. Several visual methods are available for cyst detection. However, these techniques usually detect well developed cysts during the late phase of the disease or any space-occupying lesion (Braithwaite *et al.*, 1983, Kune *et al.*, 1982). Consequently, immunodiagnostic tests have been employed for the detection of anti *Echinococcus* antibodies (Farag *et al.*, 1975; Matossian, 1977; Felgner, 1978; Varela-Diaz *et al.*, 1983; Dottorini *et al.*, 1985).

In the present paper, we report on the comparative assessment of four serological tests used for the diagnosis of surgically confirmed hydatid cases in Jordan.

Materials and Methods

Hydatid fluid antigen

A pool of sheep hydatid fluid (SHF) was prepared by extracting fluid from fertile liver cysts recovered from indigeneous sheep slaughtered at Irbid or Ramtha abattoirs in North Jordan. The fluid was clarified by centrifugation at 1000g for 30 min. at 4°C and then lyophilized. The SHF was reconstituted by dissolving 200 mg dry weight of the material in 1 ml deionized distilled water at 4°C overnight, then centrifuged at 30,000g for one hour. The supernatant fluid was dialysed against phosphate buffered saline (PBS), pH 7.2, and its

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protein content was determined using the Lowery method (Lowery *et al.*, 1951).

Other antigens

For specificity studies several other parasitic antigens were used. These included *Fasciola hepatica* adult worm lyophilized antigen (FAA) donated by Dr. V. Theodorides (Smithkline and French Co., USA); *Ascaris* adult worm lyophilized antigen (AAA) obtained from Professor J. Schad (University of Pennsylvania, USA); and *Leishmania donovani* promastigote antigen (LPA) obtained through the courtesy of Dr. J. Farrell (University of Pennsylvania, USA). *Schistosoma mansoni* egg antigen (SEA) and cercarial antigen preparation (CAP) were those described by Zodda *et al.*, (1983). Bacilli Calmette Guerin (BCG) antigen was purchased commercially (BCG Institute, Merieux, France). A *cysticercus taenicollus* larva antigen (CLA) was prepared from pooled fluid using a protocol similar to that described for SHF antigen preparation. Moreover, *Monezia* adult worm antigen (MAA) was prepared according to the method described by Conrath and Coupe (1978).

Sera

Human serum samples were collected from 216 subjects which included 27 surgically proven cases of hydatid patients (Al-Bashir Hospital in Amman; Princess Basma Hospital and Irbid Military Hospital in Irbid). Other sera comprised 69 samples from university students, 101 samples from outpatients attending Jerash Hospital and 19 samples of patients with tumors or infected with parasites including *Schistosoma haematobium, Toxoplasma gondii*, cutaneous leishmaniasis, pinworm, *Ancylostoma duodenale* and *Hymenolepis nana*. Many of the control serum samples were obtained from people with no signs or symptoms suggestive of hydatid disease.

Serological methods

Four different serological techniques were employed on all serum samples. These included double diffusion (DD), countercurrent immunoelectrophoresis (CIE), indirect haemagglutination (IHA) and the enzyme linked immunorsorbent assay (ELISA).

Immunodiffusion and countercurrent electrophoresis

Agar gel diffusion was performed on microscopic 5x7.5 cm slides. Each slide received 12 ml of 1% agarose in phosphate buffered saline (pH 7.2). Wells, 3 mm in diameter and 4 mm apart were cut. Slides for countercurrent electrophoresis were performed in the same way except that the agarose was dissolved in barbital buffer (pH 8.2). Electrophoresis was carried out for 1 hr at 110V (Johnson and Thorpe 1982).

Indirect haemagglutination test (IHA)

IHA tests were performed according to the method of Dada *et al.*, (1981). Sheep red blood cells were treated with a 1:40000 dilution of tannic acid for 10 min at 37°C and subsequently coated with 150 μg/ml SHF antigen. This concentration was found to be optimum in checker-board titration. All the serum samples were absorbed with sheep red blood cells and a 5 fold serial dilution was performed in PBS containing 1% normal rabbit serum. After preliminary studies using IHA to test sera of patients with other parasitic infections in this area (specificity studies), IHA titres below 1:125 were considered to be negative.

Enzyme linked immunosorbent assay (ELISA)

The ELISA was carried out as described by Zodda *et al.*, (1983). Human serum samples diluted 1:400 using 1% BSA (γ globulin free Sigma chemical company, USA) in PBS. The optimal concentration of SHF used for coating the microtitre plates was found to be 10 μg/ml. The enzyme used was horseraddish peroxidase with O-phenylenediamine as its substrate. Control wells consisted of those without antigen added. The results are expressed in terms of optical density (OD) at 495 nm. The OD values for sera of patients with parasitic infections other than hydatidosis and of outpatients at Jerash Hospital as well as those of University students ranged between 0.01 ~ 0.39. Thus, an OD value of less than 0.4 was considered to be negative for hydatidosis.

Results

Table 1 lists all the 27 human hydatid cases as diagnosed by various serological and non-serological techniques. There were 8 males and 19 females with ages ranging from 12–70 years. Cysts in the liver alone were found in 15 patients (55.6%) while the simultaneous involvement of the liver and of other organs (lungs, spleen, kidney, peritoneal cavity, diaphragm was seen in six out of the 27 patients (22.2%). Thus, the total involvement of the liver was seen in 77.8% of the patients. Of interest in these series was the high involvement of the kidneys in 4 patients (14.8%). Hydatid cysts were found in the lung of 3 patients, two of which had cysts in the liver as well. In 2 cases, cysts involving the peritoneal cavity were

Table 1 Comparative evaluation of DD, CIE, IHA, and the ELISA using 27 serum samples obtained from human subjects with hydatid disease

Sex	Age (Years)	Cyst Locality*	Cyst Type†	Serodiagnostic tests and results			
				DD	CIE	IHA Log 5	ELISA O. D.
M	36	Li	ND	–	+	3	1.17
	50	Li	ND	–	–	3	0.89
	65	Li	F	+	+	6	1.14
	60	Li & K	C	–	+	4	1.16
	27	K	F	–	–	4	0.82
	70	K	C	–	+	4	1.17
	30	Lu	F	–	–	0	0.27
	28	Thigh	F	–	–	2	0.39
F	12	Li	F	+	+	9	0.84
	19	Li	F	–	–	3	1.16
	22	Li	ND	–	+	5	0.36
	23	Li	F	+	+	6	0.90
	40	Li	F	+	+	11	0.69
	50	Li	CC	–	–	0	0.85
	50	Li	F	–	–	1	1.20
	53	Li	F	+	+	8	1.37
	55	Li	C	+	+	10	1.19
	60	Li	CC	–	–	4	0.68
	60	Li	F	–	+	4	0.26
	65	Li	ND	–	–	4	1.07
	17	Li & Lu	F	+	+	7	1.13
	38	Li & Lu	F	+	+	12	1.42
	40	Li & Sp	F	+	+	6	0.90
	21	Li & D	F	–	+	6	1.26
	28	Li & Pc	F	+	+	6	0.90
	14	K	F	+	+	6	1.42
	50	P	ND	–	+	4	1.26

* Abbreviations for cyst locality are as follows:
D, diaphragm; K, kidney; Li, liver; Lu, lung; Pc, peritoneal cavity; Sp, spleen.

† Abbreviations for cyst type are as follows:
C, calcified; CC, calcified with bacterial contamination; F, fertile; ND, not determined.

Table 2 Number and percentage of hydatid cases which were serologically positive using DD, CIE, IHA and ELISA

Patient's Sex	No. Tested	No. and Percentage of Seropositive Cases							
		DD		CIE		IHA		ELISA	
		No.	%	No.	%	No.	%	No.	%
Males	8	1	12.5	4	50	6	75	6	75
Females	19	10	52.6	14	73.7	17	89.5	17	89.5
Total	27	11	40.7	18	66.7	23	85.2	23	85.2

found.

The results of various serodiagnostic tests employed in this study for each individual are shown in Table 1 and the sensitivity of each serological test is shown in Table 2. All cases which were positive in double diffusion were also positive in the 3 other tests. However, the sensitivity of this test was the least. Eleven out of the 27 samples (40.7%) were positive in this test. Countercurrent immunoelectrophoresis detected 18 cases (66.7%), while IHA and ELISA were equally sensitive each detecting 23 (85.2%). A combined use of IHA and ELISA detected 25 cases (92.6%).

Figure 1 shows analysis of IHA titers (log 5) and ELISA results as determined by the OD value of each sample. Obviously, there were 4 cases which were seronegative in either test. Two of these were negative in both tests. One of the latter cases was for a patient with lung hydatidosis, the other for a case involving hydatid cysts in the thigh muscle (Table 1). These two cases gave also negative results utilizing DD and CIE. In 11 cases the 4 serological tests were positive (Table 1). All of these cases except one had fertile cysts. That exception was for a 55 year old female who had a single large (13x7 cm) calcified cysts in the liver.

None of the control serum samples from university students, outpatients at Jerash Hospital or patients infected with other parasites yielded any precipitin lines against sheep hydatid fluid antigen in DD and CIE. Moreover, all these samples gave titers of 1:25 or lower in the IHA and an OD value ranging between 0.01

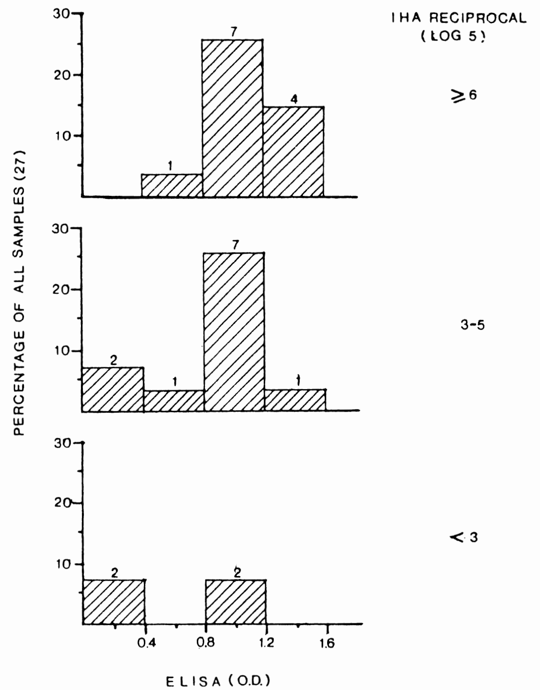

Fig. 1 Correlation between ELISA OD values and IHA antibody (Log 5) for 27 hydatid patient serum samples

~ 0.38 in ELISA. Both of these levels are regarded as limits for distinguishing serologically positive from negative sample.

Specificity studies of serum samples for hydatid patients against a panel of antigens including FAA, AAA, LPA, SEA, CAP, BCG and MAA all showed a lack of reactivity. However, two of the 18 hydatid cases which were positive in CIE yielded a positive reaction against *cysticercus* antigen as well.

Discussion

Immunodiagnosis using hydatid fluid antigens appears to be very useful in the diagnosis of human hydatidosis in Jordan. It would appear advisable for a suspected case of hydatid disease which gave a negative result in one test to be retested using a different serological method before any definite conclusion is reached. IHA and the ELISA have been employed for the diagnosis of hydatidosis in various parts of the world (Farag *et al.*, 1975; Felgner, 1978; Kune *et al.*, 1983; Hossain *et al.*, 1985). The results presented in this paper regarding the ELISA and IHA, especially their combined use, fit within the sensitivity levels reported by these authors. Moreover, the comparable sensitivity of these two tests is agreeable with that reported by Felgner (1978). When specificity studies were analyzed, cross reactivity of the serum samples from the confirmed hydatid cases was detected against a battery of antigens prepared from other parasites in the ELISA or IHA tests. However, serum samples of 2 patients reacted positively with *Cysticercus tenuicollis* antigen in CIE. None of the serum obtained from university students, outpatients or patients with parasitic infections other than hydatidosis gave a positive reaction in IHA and/or ELISA. These results are consistent with those reported by Yarzabal *et al.*, (1974), Farag *et al.*, (1975) and Hossain *et al.*, (1985). However, Farag *et al.*, (1975) reported cross reactivity with fasciolasis patients. No such patients were available for testing in the present study. The present number of serum samples from patients with other parasitic infections is too small to draw any conclusion regarding specificity particularly in light of the repeated findings of false positive reactivity with sera from patients infected with schistasomiasis (Rickard *et al.*, 1984) and taeniasis (Schantz *et al.*, 1980; Rickard and Lightowlers, 1986).

An attempt to correlate the serological reactivity with the clinical status of the patient was made in this study. It was found that the two cases which were not detected by any of the four tests employed were of patients having lung and thigh cysts. It has been shown by other investigators that the location of the cyst influences the serological reactivity of the patient (Todorov *et al.*, 1979a). Thus, false negative results are less likely to occur with cysts found in the liver or peritoneal cavity or when multiple cysts are involved, than with cysts located in the lung or the spleen. Hyaline cysts seem to stimulate less reaction than damaged cysts which could leak antigens. Cysts in the lung or spleen seem to have a healthy appearance which could possibly explain seronegativity of such cases (Yarzabal *et al.*, 1974). However, it has been proposed recently that the antibody response may be related to the thickness of the fibrous capsule of the cyst as well as the number and size of the cyst (Todorov *et al.*, 1979b). Alternatively, the lack of detection of any serological reactivity in these two patients in the present study may result from the formation of immunocomplexes which interfere with the detection of free antibody in the serum (Richard-Lenoble *et al.*, 1978; Ibarrola *et al.*, 198 Craig *et al.*, 1986).

The present study contrasts that reported by Craig *et al.*, (1986) in the Turkana region in which a high number of false negatives were detected. A possible explanation might be related to difference in the antigen load in people from the two localities. Indeed, a high percentage (75%) of the negative cases contained circulating immunocomplexes in the Turkana region.

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