

Detection of specific IgE antibodies in sera from patients with hydatidosis

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SUMMARY

A simple and sensitive immunoenzymatic assay for detection of specific IgE in hydatid disease is presented. Sera from 43 patients with hydatidosis, eight with schistosomiasis, two with taeniasis, two with cancer and 16 healthy blood donors were tested by both the immunoenzymatic technique and the classical radioallergosorbent test. Data were analysed in terms of different sensitivity and specificity of the two methods. The results indicate that the enzymatic method performed in microplates is as sensitive as the radioimmunological one (77% of positivity for both the tests), but the latter suffers from an higher rate of false positive reactions with sera from patients with other helminthic diseases (80% *vs* 20%). When the specific IgE evaluation is compared with other classical serological techniques, such as indirect haemagglutination and double diffusion in terms of different sensitivity in revealing an anti-Echinococcus immunological reactivity, the results suggest that specific IgE evaluation represents a useful addition to the classical serodiagnostic tests.

Keywords Echinococcosis hydatidosis IgE RAST ELISA

INTRODUCTION

The serodiagnosis of hydatid disease is generally performed by well established serological techniques such as indirect haemagglutination (IHA), immunoelectrophoresis, immunofluorescence, double diffusion in gel (DD), latex agglutination and complement fixation (reviewed in Kagan, 1968, 1976).

In recent years, more sensitive techniques have been introduced to further improve the serodiagnosis of hydatid disease, such as radioimmunoelectrophoresis (Musiani *et al.*, 1974; Matossian, 1981) and enzyme linked immunosorbent assay (ELISA) (Farag, Bout & Capron, 1975; Felgner, 1978; Iacona, Pini & Vicari, 1980; Speiser, 1980).

The current status of serodiagnosis of hydatid disease in man has been recently reviewed (Schantz & Kagan, 1980; FAO/WHO/UNEP, 1981).

All the abovementioned techniques measure antibodies mainly of the IgM and/or IgG classes and none of them can detect reaginic or IgE antibodies. This antibody can be detected by the intradermal test (ID) extensively used in the past for the clinical diagnosis of hydatid infection. However, skin testing in hydatidosis patients presents two disadvantages: (1) it shows a high rate of false positives and (2) it may stimulate the production of circulating antibodies. Therefore, it has been suggested (WHO, 1979) that the ID test should be replaced by serological procedures aimed at the detection and measurement of specific reaginic antibodies. Results obtained by the radioallergo-

sorbent test (RAST) have shown that the percentage of hydatidosis patients with increased specific IgE levels varies between 30 and 90% (Huldt *et al.*, 1973; Dessaint *et al.*, 1975; Vervloet *et al.*, 1976; Bout *et al.*, 1977; Richard-Lenoble, Smith & Loisy, 1978; Sorice *et al.*, 1979). In one of these studies patients infected with a silvatic strain of *Echinococcus granulosus* were found to develop IgE antibodies (Huldt *et al.*, 1973) without demonstrable increase in other immunoglobulin classes. Cross-reactions with sera from other parasitic diseases were also reported by the above authors.

The present paper reports on the detection of specific IgE antibodies in human hydatidosis; besides the RAST, an enzyme linked immunosorbent assay (ELISA) adapted to the measurement of IgE anti-*E. granulosus* antibodies was also employed. The sensitivity and specificity of the two procedures were compared and the relative merits of specific IgE determination were evaluated in comparison with other immunodiagnostic tests, such as IHA and DD.

MATERIALS AND METHODS

Sera. The following samples of human serum were used: (1) blood samples obtained in the Sardinian area from 43 patients with clinically diagnosed hydatidosis, confirmed in all cases by surgery; (2) 16 sera from healthy blood donors; (3) eight sera from humans infected with *Schistosoma mansoni*; (4) two sera from proven cases of taeniasis and (5) two sera from patients with cancer.

Antigens. Sheep hydatid fluid was collected in the Sardinian area from fertile cysts. The following antigen preparations were used: (1) a crude concentrated hydatid fluid prepared by the method of Bombardieri *et al.* (1974); (2) a purified hydatid fluid fraction obtained by the method of Oriol *et al.* (1971) containing antigen 5 (A, as designated by Oriol *et al.*, 1971) and antigen B. The purified fraction, containing antigen 5 and B, was employed in all tests. The crude concentrated hydatid fluid was used in the DD test only.

Serological methods

Indirect hemagglutination test. IHA test was performed as previously described (Bombardieri *et al.*, 1974; Pini, Pastore & Valesini, 1983). Tanned blood cells were coated with the purified fraction, containing antigen 5 and B (see above).

Double diffusion. DD test was performed as previously described (Bombardieri *et al.*, 1974). Sera were tested against the concentrated hydatid fluid and the purified fraction.

Determination of IgE levels in serum. Total serum IgE were determined by an enzyme immunoassay based on the sandwich principle (Enzygnost-IgE, Behring, Germany). Results were expressed in iu/ml by comparison with an IgE reference serum calibrated against the WHO reference preparation.

Determination of specific IgE. IgE specific for *E. granulosus* antigens were detected by the classical RAST and by indirect ELISA.

RAST was carried out according to Ceska, Eriksson & Varga (1972). Cyanogen bromide activated discs were coupled with the purified hydatid fluid fraction containing antigens 5 and B; the antigen solution was adjusted to 65 µg/ml. Two hundred microlitres of serum samples, diluted 1:10, were incubated with the discs for 3 h at room temperature under shaking and then, after washing, 50 µl of ¹²⁵I-labelled anti-IgE (Pharmacia Fine Chemicals, Uppsala, Sweden) (40,000 ct/min/50 µl) were added and left overnight as above. After washing, discs were counted in a LKB Ultrogamma counter Mod. 1280 for 5 min each. Results were expressed as a percentage of total counts added and values above 1% (mean + 2 standard deviations of the binding activity of sera from 16 healthy blood donors) were considered positive.

ELISA was performed by a microtitration procedure. Disposable polyvinyl plates (Falcon 3912) were used. Wells were filled with 200 µl of hydatid fluid fraction, containing antigen 5 and B, diluted in 0.1 M carbonate-bicarbonate buffer, pH 9.6, to a content of 65 µg/ml. Plates were covered with plastic foil, left for 3 h at 37°C in a water bath and overnight at 4°C. Before assay the plates were washed three times with 0.015 M phosphate-buffered saline-Tween 20 (PBS-Tween). Two hundred microlitres of serum samples, diluted 1:10, were added to each well and plates were

incubated for 2 h at 20°C in a waterbath. As a control, a supernatant from a human IgE myeloma cell culture containing 20,000 iu/ml of IgE was used in the evaluation of non-specific IgE adsorption on hydatid antigens. After further washing, 200 μ l of rabbit anti-human IgE, peroxidase conjugated, (Behring, OSMS, 10/11) were added and the plates were incubated for another 2 h at 20°C. Excess conjugate was then washed out and the amount of peroxidase bound to the wells was determined by adding 200 μ l of the chromogen buffer substrate solution (Enzygnost IgE, Behring Institute). The enzymatic reaction was stopped after 30 min at room temperature by the addition of 50 μ l of 0.5 N sulphuric acid to each well. Absorbance of the developed colour was measured at 492 nm in a Gilford Model 240 spectrophotometer calibrated for use with microcuvettes. Values higher than 0.057 OD (mean + 2 standard deviations of the absorbance readings of 16 healthy blood donors) were considered positive.

Statistical analysis. The McNemar test for significance of changes and linear regression analysis were employed to compare the different serological techniques (Sokal & Rohlf, 1969).

RESULTS

Specific IgE antibodies were detectable in most sera from patients with hydatid disease, the rate of positivity being 77% in both the ELISA and RAST procedures (Table 1). Moreover, a correlation was found between the ELISA and RAST values ($r=0.7$, $P<0.01$), thus indicating that the two methods are equivalent. However, ELISA appears to be a more specific method as compared to RAST, since a higher number of sera from patients with schistosomiasis and taeniasis showed some activity by the latter method. The rate of non-specificity for other helminthic cases was 20% in ELISA and 80% in RAST procedure; cancer sera resulted negative by both procedures. Comparison of the sensitivity and specificity of the two procedures is given in Table 1.

Table 2 reports the results obtained when specific IgE evaluation, IHA and DD are compared. Statistical analysis of the data showed a significant higher sensitivity of the IgE ELISA as compared to the IHA test ($\chi^2=5.1$, $0.05>P>0.01$). No difference was observed between the two antigenic preparations employed in DD test.

Table 3 shows the distribution of sera from hydatid patients positive or negative for specific IgE according to their reactivity in DD and IHA. It is evident that sera positive in DD and IHA were also usually positive for specific IgE. Only one serum was found to be positive in DD and negative for specific IgE; no serum was found to be IHA positive and specific IgE negative. On the other hand, detectable levels of specific IgE were present in five patients out of the 14 with no demonstrable increase in other immunoglobulin classes as detected by DD and IHA.

The total serum IgE levels were also measured for all patients with hydatid disease. The distribution of sera positive or negative for specific IgE according to three different arbitrary classes

Table 1. Sensitivity and specificity of ELISA and RAST in detecting anti-*E. granulosus* IgE antibodies

Source of sera	No. of sera tested	ELISA	RAST
		No. (%) positive	No. (%) positive
Patients with hydatid disease	43	33 (76.7)	33 (76.7)
Patients with schistosomiasis	8	2 (25.0)	7 (87.5)
Patients with taeniasis	2	0	1 (50.0)
Patients with cancer	2	0	0
Normal subjects	16	0	0

Table 2. Sensitivity and specificity of different serological techniques in detecting antibodies in hydatid diseases

Source of sera	No. of sera tested	DD	IHA	IgE-ELISA
		No. (%) positive	No. (%) positive	No. (%) positive
Patients with hydatid disease:				
hepatic localization	23	16 (69.6)	14 (60.9)	17 (73.9)
pulmonary localization	16	10 (62.5)	8 (50.0)	12 (75.0)
multiple localization	4	3 (75.0)	4 (100)	4 (100)
total	43	29 (67.4)	26 (60.5)	33 (76.7)
Patients with other helminthic diseases	10	0	0	2 (20.0)
Patients with cancer	2	0	0	0
Normal subjects	16	0	0	0

Table 3. Specific IgE reactivity of sera from hydatid patients in comparison with DD and IHA

	Number of sera	Number of sera positive for specific IgE	%
DD positive	29	28	96.5
DD negative	14	5	35.7
IHA positive	26	26	100
IHA negative	17	7	41.4

Table 4. Distribution of sera positive or negative for specific IgE in relation to their levels of total IgE grouped in three arbitrary classes

	Number of sera	Distribution of sera according to their level of total IgE (iu/ml)		
		< 100	100–1,000	> 1,000
Patients with hydatid disease				
positive for specific IgE	33	5	16	12
negative for specific IgE	10	5	5	
Normal subjects	16	9	7	

of total IgE is reported in Table 4. The 10 sera from patients with hydatid disease which were negative for specific IgE were equally distributed between the lower and the middle classes (< 100 iu/ml and 100–1,000 iu/ml, respectively), whereas 28 (85%) out of the 33 sera positive for specific IgE were distributed in the middle and the upper classes (> 1,000 iu/ml) with 12 sera (36%) in the latter. A good correlation between total and specific IgE ($r = 0.5$, $P < 0.01$) was found when the data were analysed by linear regression test.

DISCUSSION

The results of the present study show that anti-*E. granulosus* IgE antibodies are detectable in a high percentage (77%) of patients with hydatid disease. The specific IgE response in our series of 43 patients could be evaluated by both the RAST procedure and an ELISA, specifically adapted to the detection of anti-*E. granulosus* IgE antibodies. The same rate of positivity obtained by the two procedures and the correlation found between the corresponding values show that ELISA and RAST are equivalent methods in terms of sensitivity for the detection of specific IgE in hydatidosis.

This is an interesting finding since a lower sensitivity of ELISA versus RAST in detecting IgE in the course of parasitic disease in man has been reported (Hamilton *et al.*, 1981). However specific IgE have been successfully detected by ELISA in patients infected with *Schistosoma mansoni* (Lunde & Ottesen, 1980) or in serum from mice immunized with allergens (Giallongo, Kochoumian & King, 1982).

The rate of non-specific reactions is lower for the ELISA (20%) than for the RAST (80%). High rates of non-specific reactions for the latter procedure (up to 90%) have been reported by others (Sorice *et al.*, 1979). Since the two methods have the same sensitivity, the higher specificity of ELISA could be due to the two different types of association between the antigen and the solid phase, represented by absorption for ELISA and by a covalent linkage for RAST, the latter possibly giving rise to the immobilization of additional parasite antigens and/or host antigens present as impurities in the hydatid fraction employed in the reaction.

The 77% of hydatid patients with increased specific IgE levels found is quite in agreement with most of the results reported in the literature. Indeed, percentages of positivity of 59, 80 and 90% have been reported for patients with active hydatidosis by Dessaint *et al.* (1975), Sorice *et al.* (1979) and Vervloet *et al.* (1976) respectively, using a RAST procedure and comparable antigen preparations. Comparison of the specific IgE levels with total IgE concentrations shows a highly significant correlation which is also in agreement with the results reported in the above mentioned studies.

The comparison between specific IgE evaluation and classical serological techniques in the diagnosis of hydatid disease yields interesting information. Indeed, for the 43 sera from patients with hydatid disease the percentage of positivity using the classical serological techniques (IHA and DD) is lower than that obtained by specific IgE measurement. Moreover, in the case of IgE determination no significant differences in terms of sensitivity are present when the sera are analysed according to cyst localization, whereas sera from subjects with hepatic cyst are confirmed to be better reactors in DD and IHA than pulmonary ones.

From the above mentioned data, it is evident that, providing the coating of the plate and the antigen preparation are suitable, ELISA is a very sensitive method for detecting specific IgE in hydatidosis, which does not suffer from interference due to other classes of specific antibodies.

Even though a comparison with the ID test was not made in this study, the advantages of ELISA as an *in vitro* method for detecting reaginic antibodies are quite obvious. Moreover, the presence of hydatid patients with detectable levels of specific IgE and no demonstrable increase in other immunoglobulins classes renders the ELISA for specific IgE antibodies a useful addition to the range of immunodiagnostic tests available in the serology of hydatid disease.

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REFERENCES

- BOMBARDIERI, S., GIORDANO, F., INGRAO, F., IOP-
POLO, S., SIRACUSANO, A. & VICARI, G. (1974) An
evaluation of an agar gel diffusion test with crude
and purified antigens in the diagnosis of hydatid
disease. *Bull. WHO.* **51**, 525.
BOUT, D., DESSAINT, J.P., DUPAS, H., YARZABAL, L. &

- CAPRON, A. (1977) Characterization of allergens in *Schistosoma mansoni*, *Fasciola hepatica* and *Echinococcus granulosus*. *Ann. Immunol. Ist. Pasteur*, **128C**, 687.
- CESKA, M., ERIKSSON, R. & VARGA, J.M. (1972) Radioimmunosorbent assay of allergens. *J. Allergy clin. Immunol.* **49**, 1.
- DESSAINT, J.P., BOUT, D., WATTRE, P. & CAPRON, A. (1975) Quantitative determination of specific IgE antibodies to *Echinococcus granulosus* and IgE levels in sera from patients with hydatid disease. *Immunology*, **29**, 813.
- FAO/UNEP/WHO guidelines for surveillance, prevention and control of echinococcosis/hydatidosis. (1981) WHO, Geneva.
- FARAG, H., BOUT, D. & CAPRON, A. (1975) Specific immunodiagnosis of human hydatidosis by the enzyme-linked immunosorbent assay (ELISA). *Biomedicine*, **23**, 276.
- FELGNER, P. (1978) Antibody activity in stick-ELISA as compared to other quantitative immunological tests in sera of echinococcosis cases. *Tropenmed. Parasitol.* **29**, 417.
- GIALLONGO, A., KOCHOUMIAN, L. & KING T.P. (1982) Enzyme and radioimmunoassay for specific murine IgE and IgG with different solid phase immunosorbents. *J. Immunol. Meth.* **52**, 379.
- HAMILTON R.G., HUSSAIN, R., OTTESEN, E.A. & ADKINSON, F. (1981) The quantitation of parasite-specific human IgG and IgE in sera: evaluation of solid-phase RIA and ELISA methodology. *J. Immunol. Meth.* **44**, 101.
- HULDT, G., GUNNAR, S., JOHANSSON, O. & LANTTO, S. (1973) Echinococcosis in Northern Scandinavia. *Archiv. Environ. Health*, **26**, 36.
- IACONA, A., PINI, C. & VICARI, G. (1980) Enzyme-linked immunosorbent assay (ELISA) in the serodiagnosis of hydatid disease. *Am. J. Trop. Med. Hyg.* **29**, 95.
- KAGAN, I.G. (1968) A review of serological tests for the diagnosis of hydatid disease. *Bull. WHO* **39**, 25.
- KAGAN, I.G. (1976) Serodiagnosis of hydatid disease. In *Immunology of parasite infections* (ed. by S. Cohen & E.H. Sadun) pp. 130-142. Blackwell Scientific Publications, Oxford.
- LUNDE, M.N. & OTTESEN, E.A. (1980) Enzyme-linked immunosorbent assay (ELISA) for detecting IgM and IgE antibodies in human schistosomiasis. *Am. J. Trop. Med. Hyg.* **29**, 82.
- MATOSSIAN, R.M. (1981) A simplified radioimmunoassay technique for hydatid disease and human trichinosis. *J. Helminth.* **55**, 49.
- MUSIANI, P., PIANTELLI, M., ARRU, E. & POZZUOLI, R. (1974) A solid-phase radioimmunoassay for the diagnosis of human hydatidosis. *J. Immunol.* **112**, 1674.
- ORIOLE, R., WILLIAMS, J.F., PEREZ-ESANDI, M.V. & ORIOLE, C. (1971) Purification of lipoprotein antigens of *Echinococcus granulosus* from sheep hydatid fluid. *Am. J. Trop. Med. Hyg.* **20**, 569.
- PINI, C., PASTORE, R. & VALESINI, G. (1983) Circulating immune complexes in sera of patients infected with *Echinococcus granulosus*. *Clin. exp. Immunol.* **51**, 572.
- RICHARD-LENOBLE, D., SMITH, M.D. & LOISY, M. (1978) Human hydatidosis: evaluation of three serodiagnostic methods, the principal subclass of specific immunoglobulin and the detection of circulating immune complexes. *Am. Trop. Med. Parasitol.* **72**, 553.
- SCHANTZ, P.M. & KAGAN, I.G. (1980) Immunological investigation of echinococcosis (hydatidosis), in *Immunologic Investigations of Tropical Parasitic Diseases* (ed. by V. Houbert) p. 104. Churchill Livingstone, Edinburgh.
- SOKAL, R.R. & ROHLF, J.F. (1969) *Biometry* p. 413. W.H. Freeman & Co., San Francisco.
- SORICE, F., DELIA, S., VULLO, V., ACETI, A. & FERONE, U. (1979) Valore e limiti del RAST (radioallergo-sorbent test) nella diagnosi biologica della idati-dosi. *Ann. Sclavo*, **21**, 800.
- SPEISER, F. (1980) Application of the enzyme-linked immunosorbent assay (ELISA) for the diagnosis of filariasis and echinococcosis. *Tropenmed. Parasitol.* **31**, 450.
- VERVLOET, D., DUMON, H., QUILICI, M. & CHARPIN, J. (1976) Les IgE spécifiques dans l'hydatidose. *Rev. Franc. Allergol.* **16**, 73.
- WORLD HEALTH ORGANIZATION (1979) Parasitic Zoonoses. *WHO Technical Report Series*, No. 637.