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Pre- and Postsurgical Detection of IgG, IgM, and IgA Specific to Hydatidosis by ELISA With Purified Antigen Enriched With the 5/B Antigen Complex

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An enzyme-linked immunoassay (ELISA) using purified 5/B *Echinococcus* enriched antigen was used to follow IgG, IgM, and IgA antibody levels pre- and posttreatment or surgical removal of hydatid cysts. The sensitivity was 97%, 37.5%, and 54.5%, respectively, and the specificity was 95.7%, 100%, and 98.9%, respectively. All isotypes could be detected 3 years after surgical removal of cysts in patients showing no remaining cyst evidence. This was especially true for IgG, which persisted in 85.2% of the patients. The data indicate that antigen purification improves specificity without affecting sensitivity, although this new antigen offers no advantages in the postsurgical monitoring of the patients. J. Clin. Lab. Anal. 16:295–298, 2002.

Key words: *Echinococcus granulosus*; hydatidosis; antigens; serological follow-up

INTRODUCTION

The cross-reactions between *Echinococcus granulosus* and other infectious agents are well known and affect the specificity of serological tests. Cross-reactivity has been reported in infections by *E. multilocularis*, *E. vogeli* (1), *Taenia saginata* (2–7), *Taenia solium* (5,8–10), *Hymenolepis nana* (10), *Ascaris lumbricoides* (3), *Trichinella* (11), *Fasciola* (8,11), *Giardia lamblia* (5), *Toxoplasma gondii* (5), *Plasmodium falciparum* (5), and *Leishmania* (4). Furthermore, bacterial infections, such as tuberculosis (8), and host antigens, such as seroalbumin (12,13) and red blood cells (14–16), are sources of false-positive results in hydatidosis serological tests.

Several *Echinococcus* antigen fractions have been tested for improved sensitivity and specificity of serological tests to diagnose hydatidosis (1,10). Preparations enriched with 5 and B fractions were most successful (17,18).

In the present work we used an enzyme-linked immunosorbent assay (ELISA) to study the specific IgG, IgM, and IgA responses to purified hydatid antigen, enriched with the 5/B antigen complex. We analysed the sensitivity and specificity of the 5/B antigen complex, as well as its possible application in the diagnosis and posttreatment monitoring of human hydatidosis.

MATERIALS AND METHODS

Patients

Group 1

This group was comprised of 72 patients with hydatidosis (36 males and 36 females, 18–82 years old, average 50.1 years, SD = 15.2) who had been diagnosed and monitored in the Hospital Clínico Universitario of Zaragoza between 1994 and 1999. The inclusion criteria were indirect hemagglutination assay (IHA) (Fumouze, Levallois Perret, France) $\geq 1/160$ in at least two sera,

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and the presence of radiologic, ecographic, or tomographic images compatible with hydatidosis.

The cysts were detected in the following locations: 47 liver, five lung, five peritoneum, one spleen, one pancreas, one gluteus, and 12 multiple. In 38 patients, the cysts were not complicated (whole with a crystalline fluid and hyaline aspect); in 28 patients the cysts were complicated (fissured, ruptured, or infected), according to the classification of Verastegui et al. (10); and in six patients the cysts were calcified. During this time, 45 patients underwent surgery, 20 received medical and surgical treatment, two received medical treatment (mebendazole or albendazole), and five patients were not treated. A total of 233 samples were taken, with an average of 4.7 samples per patient (range 2–14). Thirty-three samples (from 33 patients) were taken prior to treatment, and 200 were taken during the monitoring period. During this monitoring period, asymptomatic patients were considered cured after radiologic, ecographic, and tomographic examinations were performed 1 year after treatment.

Group 2

In group 2 we studied cross-reactions in sera from 93 patients: 30 with rheumatoid factor, 10 with antinuclear antibodies, 12 with toxoplasmosis, six with cysticercosis, 12 with leishmaniasis, and 23 with trichinosis.

All samples from both groups were stored in aliquots and kept at –70°C until they were studied.

Techniques

The samples were analysed using commercial ELISA kits (Hydatidosis ELISA IgG, IgM, and IgA; Vircell, SL, Granada, Spain), following the manufacturer's instructions. These kits incorporate a purified antigen enriched with the 5/B antigen complex, according to the methodology of Rogan et al. (19) (modified by Sbihi et al. (17)), and based on the procedures of Oriol et al. (20) using hydatid fluid from sheep-liver cysts.

Briefly, the samples were added to microtitre plates (Nunc Maxisorb, Denmark) containing the antigen, and incubated for 45 min at 37°C. For the IgG determination, 5 μL of the samples were diluted in 100 μL of dilution buffer. To determine the IgA and IgM, 5 μL of the samples were diluted in 75 μL of dilution buffer and 25 μL of anti-human IgG serum. Next, 100 μL of peroxidase-conjugate goat antibody were added to human IgG, IgA, or IgM and incubated for 30 min at 37°C. Finally, 100 μL of tetramethyl-benzidine were added. After incubation for 20 min at room temperature, the reaction was stopped by the addition of 50 μL of sulphuric acid 0.5 M. Absorbance values were measured at 450/620 nm. The results were expressed as an index (sample absorbance/cut-off). Samples with an index lower than 0.9 were regarded as negative, between 0.9 and 1.1 as a grey-zone result, and higher than 1.1 as positive.

RESULTS

The IgG was positive in 32 of the 33 samples immediately prior to treatment (97%), while the ELISA IgM and IgA were positive in 37.5% and 54.5% of the sera, respectively (Table 1). The only negative sample had an IHA titre of 1/160, a grey-zone result for IgM, and a negative result for IgA. This patient was a 40-year-old female with a 7-cm nonfertile hyaline liver cyst.

The complicated cysts gave the most frequent positive results for all the immunoglobulin isotypes. Sensitivity of IgM and IgA was low in the noncomplicated cysts (37.5% and 43.8%, respectively) but somewhat higher in the complicated ones (50% and 76.9%, respectively). The IgG was positive in all patients with complicated and calcified cysts. No relationship was found between the presence of IgM and the cyst status. In nine of the 65 patients (13.8%) a positive IgM result was detected immediately after the operation, with no significant differences between cured and noncured patients.

In the control group (Table 2), the detection specificity of the different isotypes was 95.7%, 100%, and 98.9% for IgG, IgM, and IgA, respectively. The most frequent sources of positive hydatidosis serology in the absence of *Echinoccocus* parasitism were cysticercosis for IgG and leishmaniasis for IgA. It should be noted that leishmaniasis and trichinosis were sources of ELISA grey-zone results.

Of the 65 surgically-treated patients, 38 were considered cured, but in the other 27 the hydatidosis persisted. Cysts persisted in six of the seven patients not surgically treated. One patient was considered cured after cyst calcification and negative serology, 5 years after diagnosis.

In 38 patients who were considered cured, a progressive decline in the number of seropositive cases was observed in all tests, although the rate of negative results varied. The IgA was the test that became negative

TABLE 1. ELISA results in samples of patients with hydatid disease before surgery

Cyst status	No.	EIA IgG Positive/ analyzed	EIA IgM Positive/ analyzed	EIA IgA Positive/ analyzed
Noncomplicated	16	15/16 (93.8%)	6/16 (37.5%)	7/16 (43.8%)
Complicated	13	13/13 (100%)	6/12 (50%)	10/13 (76.9%)
Calcified	4	4/4 (100%)	0/4 (0%)	1/4 (25%)
Total	33	32/33 (97%)	12/32 (37.5%)	18/33 (54.5%)

TABLE 2. Results of hydatid serology in 93 samples of the control group

	IgG		IgM		IgA	
Patients	Positive (%)	GZR (%)	Positive (%)	GZR (%)	Positive (%)	GZR (%)
Rheumatoid factor	0/30 (0%)	0/30 (0%)	ND	ND	0/30 (0%)	1/30 (3.3%)
Antinuclear antibodies	0/10 (0%)	0/10 (0%)	ND	ND	0/10 (0%)	0/10 (0%)
Toxoplasmosis	1/12 (8.3%)	0/12 (0%)	ND	ND	0/12 (0%)	0/12 (0%)
Cysticercosis	1/6 (16.7%)	0/6 (0%)	ND	ND	0/6 (0%)	0/6 (0%)
Leishmaniasis	0/12 (0%)	2/12 (16.7%)	ND	ND	1/23 (8.3%)	0/12 (0%)
Trichinosis	2/23 (8.7%)	0/23 (0%)	0/23 (0%)	1/23 (4.3%)	0/23 (0%)	0/23 (0%)
Total	4/93 (4.3%)	2/93 (2.2%)	0/23 (0%)	1/23 (4.3%)	1/93 (1.1%)	1/93 (1.1%)

GZR, grey zone of reaction; ND, non-determinate.

TABLE 3. Post-surgical evolution of serology in 38 patients without evidence of hydatidosis

	Months after surgery					
	6	12	18	24	30	36
IgG +	100%	97.1%	90%	89.7%	88.9%	85.2%
IgM +	62.5%	25%	19%	12.5%	11.1%	8.6%
IgA +	52.5%	27.3%	21.7%	16.7%	11.5%	2.9%

TABLE 4. Post-surgical evolution of serology in 27 patients with cyst persistence

	Months after surgery					
	6	12	18	24	30	36
IgG +	100%	100%	100%	100%	100%	100%
IgM +	90.5%	81%	71.4%	61.9%	57.9%	42.9%
IgA +	87%	80%	84.2%	78.9%	72.2%	54.5%

most rapidly (2.9% positive at 3 years; Table 3). In 27 patients with persistent cysts after treatment, the IgG proved positive in 100% of the cases after 3 years (Table 4), while the IgA and IgM remained positive in around 50% of the cases during the same period. In six patients not surgically treated and with persistent cysts, the IgG remained positive in 100% of the tests over the entire observation period, and the percentages of positive IgA and IgM showed no change (Table 5).

TABLE 5. Post-chemotherapy evolution of serology in six patients with cyst persistence

	Months after surgery					
	6	12	18	24	30	36
IgG +	100%	100%	100%	100%	100%	100%
IgM +	75%	25%	25%	25%	25%	20%
IgA +	66.6%	66.6%	66.6%	66.6%	66.6%	20%

DISCUSSION

The sensitivity of the ELISA-IgG with enriched antigen was equal to or higher than that reported by other authors performing ELISA with crude or lipoprotein antigen, ranging from 87% to 100% (3,5,8,11). Nevertheless, it would also be necessary to study the sensitivity of our ELISA in a group of patients not selected for having other serologically positive tests. The sensitivity of the test with enriched antigen for detecting specific IgM and IgA was insufficient. The values for IgM were lower than those reported by Matossian et al. (11) (58%), Orduña et al. (5) (80.4%), and Candolfi et al. (21) (54%) with crude antigen, but higher than those reported by Tassi et al. (1) with antigen fractions and scolex (with percentages of 27–40% and 33–35%, respectively), according to the antigen fraction considered. In the case of IgA, its values were similar to those found by Force et al. (8) (50%) and Candolfi et al. (21) (53%) using crude antigen.

The specificity of the enzyme-immune assay ranged from 95.7% to 100% according to the isotype of the immunoglobulin considered. Only sera of patients with cysticercosis, leishmaniasis, toxoplasmosis, and trichinosis gave false-positive results with the different isotypes of immunoglobulins, with percentages between 8% (toxoplasmosis, leishmaniasis, and trichinosis) and 16% (cysticercosis), these values being lower than those reported by other authors. Orduña et al. (5), using crude and lipoprotein antigens, detected one false-positive result in two patients suffering cysticercosis. Matossian et al. (11) found 29% false-positives for ELISA-IgG and 7% for ELISA-IgM in patients with trichinosis, using crude hydatid fluid as antigen. The behaviour of the isotypes, depending on the state of the cyst, was similar to that described by other authors (3), and showed greater sensitivity in the complicated cysts.

All specific immunoglobulin isotypes from patients considered cured presented prolonged persistence, especially for the IgG. Although the IgA and IgM became negative most rapidly, it should be taken into account that their sensitivity in presurgical sera was insufficient, and that a negative trend was also found (although it was much slower) in patients with persistent cysts after

treatment. Such circumstances limit its usefulness in postsurgical monitoring.

The use of ELISA with enriched antigen fraction did not improve the posttreatment follow-up of the ELISA (22,23) or IHA (22,24,25) described to date. Nor did we find any relationship between the presence of IgM and the cyst status, in contrast to the results of Tassi et al. (1), who used a micro-ELISA with antigen fractions. However, in accordance with Tassi et al. (1), we related the postsurgical presence of this isotype to antigen release during surgery. Therefore, we conclude that following the serological evolution in each patient and verifying the persistence or decrease of specific antibody levels is the most effective serological alternative to treatment monitoring.

The data of the present study indicate that purification of the antigen improves specificity without diminishing sensitivity; however, no advantages are gained in the postsurgical follow-up of the patient.

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