The significance of free and immune-complexed hydatid-specific antigen(s) as an immunodiagnostic tool for human hydatidosis

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Summary. Micro-enzyme-linked immunosorbent assay (Micro-ELISA) systems were developed and evaluated for the detection of circulating (free or immune-complexed) hydatid antigens in the sera of patients with hydatidosis, by employing monospecific antibodies to hydatid-specific antigens of 8-kDa and 116-kDa. Fifteen (75%) of 20 sera from patients with hydatidosis had both 8-kDa and 116-kDa antigens freely circulating in their sera while three and two samples, respectively, had only 8-kDa or 116-kDa antigen. All the surgically confirmed cases of hydatidosis had detectable levels of both 8-kDa and 116-kDa circulating immune complexes in glycine HCl-treated sera. However, none of the sera from control subjects (patients with cysticercosis, ascariasis, ancylostomiasis, hymenolepiasis, amoebic liver abscess or viral hepatitis) had any detectable level of either type of circulating specific antigen. These results suggest that the demonstration of either 8- or 116-kDa antigen(s) in free or immune-complex form could confirm the diagnosis of hydatidosis.

Introduction

Echinococcosis (hydatidosis) is one of the world's major zoonotic diseases. The developing metacestode stage of *Echinococcus granulosus* in man results in fluid-filled hydatid cysts in several organs, especially the liver and lungs.^{1,2} Early detection of hydatid cyst disease in patients is important, to initiate appropriate anti-parasitic chemotherapy, such as mebendazole^{3,4} or albendazole,^{5–7} or for early surgical removal of the cysts. Roentgenography, radioisotopy, magnetic resonance imaging, ultrasonic scanning and computerised axial tomography provide clues to the size, physical appearance and location of a fluid-filled or calcified mass suspected to be a hydatid cyst.^{1,2} Such diagnostic procedures may not be practicable in centres lacking appropriate facilities or expertise, especially in developing or underdeveloped countries. Alternatively, several assays for anti-hydatid antibodies have been developed and evaluated as diagnostic tools.^{2,8–12} Most antibody detection assays are neither very sensitive nor very specific,^{10,11} and there is no correlation between the levels of anti-hydatid antibodies and the size or location of the hydatid cyst.^{2,13} Moreover, anti-hydatid antibodies persist for years after surgical removal of the cyst.¹⁴ The non-specificity of the antibody detection assays is thought to be due to antigenic sharing with other hel-

minths.^{1,2,15–17} Antigens used routinely in serological diagnosis of echinococcosis are obtained from the hydatid cyst fluid. Recently, two major antigenic components of hydatid cyst fluid, antigen 5 and antigen B, have been employed to improve the specificity of antibody detection assays.^{1,2} Antigen 5 is a thermolabile lipoprotein and has been shown to be specific for the formation of arc '5' in immunoelectrophoresis.¹⁰ Antigen B is a thermostable lipoprotein. Recent investigations revealed that antigen '5' or antigen B also cross-react with sera from subjects with other helminth infections, especially cysticercosis.^{1,2,15–17} Recently, circulating immune complexes (CICs) have been demonstrated in 50–60% of clinical subjects with hydatidosis.^{13,18–20} In most cases, the CICs contained the IgA class of immunoglobulins.¹⁹ Hydatid-specific CICs or circulating free antigen have been demonstrated in 85% of cases of hydatidosis^{13,21} and circulating anti-hydatid antibodies have been shown to bind and form 67-kDa hydatid antigen CICs.¹³ The 67-kDa hydatid antigen is one subunit of antigen '5'. This subunit has been shown to cross-react either with other helminths^{2,15–17} or with human albumin.^{2,22} Thus, an assay system to detect either the 67-kDa antigen or antibodies to it may not be specific for hydatidosis.^{16,17} Therefore, it is pertinent to identify hydatid-specific antigen(s), in order to develop a specific and sensitive immunodiagnostic tool. Recently we have reported that 8-kDa and 116-kDa hydatid antigens were always recognised by sera of hydatid patients and never by sera from patients with other

helminth infections or health control subjects, suggesting that the 8-kDa and 116-kDa antigens are hydatid-specific.²³ The present study was designed to detect 8-kDa and 116-kDa antigen(s), either free or in immune-complex form, in the circulation by an enzyme-linked immunosorbent assay system (ELISA) employing monospecific antibodies.

Materials and methods

Antigens

Fertile hydatid cysts were obtained from sheep slaughtered at a local abattoir. Cyst fluid was aspirated aseptically, centrifuged at 2000 *g* for 30 min at 4°C, dialysed extensively against distilled water, lyophilised and stored at -20°C.²³ The protein content of hydatid cyst fluid was estimated.²⁴

Anti-hydatid hyperimmune serum (AH-HIS)

Anti-hydatid serum was raised in a rabbit by four subcutaneous injections at weekly intervals of hydatid cyst fluid. Each dose contained 1 mg of hydatid cyst fluid protein. The first dose was emulsified in Freund's complete adjuvant and three other doses were given after emulsification of the hydatid cyst fluid in incomplete Freund's adjuvant. A week after the last immunising dose, the rabbit was bled, and serum was collected and labelled as anti-hydatid hyperimmune serum (AH-HIS).

Antibodies to hydatid-specific antigen(s)

Two hydatid-specific antigens of 8-kDa and 116-kDa had been identified previously by means of Western immunoblot assay.²³ Antibodies to these antigens were raised in rabbits. Briefly, 2 mg of hydatid fluid protein was subjected to electrophoresis under reducing and denaturing conditions. Gel containing the antigenic bands at the 8- or 116-kDa position was cut and the antigens were eluted separately in normal saline at 37°C. Several runs of hydatid cyst fluid were made by SDS-PAGE to obtain the required amounts of 8- and 116-kDa hydatid-specific antigens. The antibodies to these antigens were raised in rabbits by a procedure identical to that described above, except that each dose contained 250 µg of the particular hydatid-specific antigen. The monospecificity of each antibody preparation to either 8- or 116-kDa antigen was assessed by a Western immunoblot assay (figure). The mol. wt standards used were: carbonic anhydrase (29 kDa), ovalbumin (45 kDa) and bovine serum albumin (66 kDa). The *M_r* of hydatid cyst fluid antigens recognised was estimated by using a logarithmic plot of the migration of standards. The IgG fraction of the hyperimmune serum to each antigen was obtained by DEAE cellulose chromatography,²⁵

concentrated by lyophilisation and labelled as either anti-8- or anti-116-kDa antibody.

Clinical samples

Serum samples were obtained from the following patients and controls:

Hydatidosis (20). These were patients with hydatidosis, confirmed both radiologically and surgically. Microscopy of hydatid cyst fluid revealed the presence of protoscolices of *E. granulosus*. Sera from these patients had anti-hydatid antibodies (OD > 0.53), as assessed by micro-enzyme-linked immunosorbent assay (ELISA) employing crude hydatid cyst fluid as antigen.

Cysticercosis (15). In 13 patients, a diagnosis of neurocysticercosis was based on clinical symptoms—headache, nausea, impaired vision, confusion, dizziness etc. In addition, the computerised tomography result was compatible with cysticercosis. Sera from these cases contained anti-cysticercus antibodies (OD > 0.43) as assessed by the micro-ELISA test. In addition, sera from two cases of epidermal cysticercosis, confirmed by histopathology, were also investigated. These sera had anti-cysticercus antibodies (OD > 0.43).

Other helminthic infections (27). This group comprised 12 cases of ascariasis (microscopy of stools revealed eggs of *Ascaris lumbricoides*), five cases of ancylostomiasis (stools contained eggs of *Ancylostoma duodenale*) and 10 cases of hymenolepiasis (*Hymenolepis nana* eggs were seen in stools).

Amoebic liver abscess (ALA) (10). The 10 ALA cases had clinically enlarged tender livers with palpable abscesses and associated systemic toxæmia. The aspirated "anchovy-sauce" pus was either sterile on bacteriological examination or contained *Entamoeba histolytica* trophozoites. Sera from these subjects had anti-amoebic antibodies (indirect haemagglutination titre > 64). The ALA patients each made good clinical recovery with metronidazole treatment.

Viral hepatitis (13). These cases were confirmed by demonstration of hepatitis markers such as hepatitis B surface antigen (HBsAg), hepatitis *e* antigen of B virus (HBeAg), IgM or IgG antibodies to hepatitis B core antigen and hepatitis A virus, and antibodies to HBsAg and HBeAg, as assessed in micro-ELISA assays.²⁶

Healthy control subjects (25). These were adults, aged 17–32 years, residents in India since birth and classified as normal by physical and medical examinations. Their sera contained no antibodies to *E. granulosus* or *Taenia solium* antigens. Repeated (three) stool examinations of these subjects revealed no ova or cysts of any parasites.

Detection of anti-hydatid antibodies

Anti-hydatid antibodies were assayed in sera by a peroxidase micro-ELISA test as described previously.²³ An absorbance value of ≥ 0.53 (mean + 2SD of

absorbance values of sera from healthy subjects) was considered to indicate the presence of anti-hydatid antibodies.

Detection of hydatid-specific antigen(s)

The hydatid-specific antigens (8-kDa and 116-kDa) were detected in untreated or acid-treated (for CICs) serum samples, by utilising the respective monospecific antibodies in ELISA systems. For acid-treatment, serum samples were mixed with 0.2 M glycine HCl, pH 3.0, at a ratio of 1:3, for 1 h at room temperature.²¹ Optimal dilutions of sera (untreated or acid-treated) and of respective monospecific antibodies required to detect the respective antigen(s) were determined by a chess-board titration in micro-ELISA. Either the free 8- or 116-kDa antigen (in untreated sera) or the 8- or 116-kDa antigen in immune-complex form (in acid-treated sera) was detected in an ELISA system with monospecific antibodies.

Optimally diluted untreated sera (1 in 320) or acid-treated sera (1 in 3200) were coated on the wells of micro-ELISA plates. After being washed three times with phosphate-buffered saline (PBS, pH 7.2)-Tween 20, the micro-ELISA wells were treated with one of the monospecific antibodies and then incubated with horseradish peroxidase-conjugated anti-rabbit immunoglobulins (IgG + M + A) (Jackson Immuno-Research Laboratories, Baltimore Pike, USA); the reaction was developed with orthophenylene diamine (Sigma) as substrate. The absorbance was read at 492 nm with an ELISA reader (Labsystems Uniskan-I, Helsinki, Finland).

An untreated serum sample from a patient with an absorbance value of ≥ 0.15 (mean value + 2SD of sera from healthy subjects) with anti-8-kDa monospecific antibody as capturing antibody was considered to contain free hydatid-specific 8-kDa antigen. An untreated serum sample with an absorbance value of ≥ 0.14 (mean + 2SD of absorbance values of sera from healthy subjects) with anti-116-kDa monospecific antibody as capturing antibody was considered to contain free circulating hydatid-specific 116-kDa antigen. Similarly, acid-treated serum samples with absorbance values of ≥ 0.13 (i.e., mean + 2SD of absorbance values of sera from healthy subjects) with anti-8-kDa monospecific antibody as capturing antibody were considered to contain immune-complexed 8-kDa antigen. An untreated serum sample with an absorbance value of ≥ 0.10 (i.e., mean + 2SD of absorbance values of sera from healthy subjects) with anti-116 kDa monospecific antibody as capturing antibody was considered to have immune-complexed 116-kDa antigen.

Diagnostic evaluation

The predictive value for positive results, predictive value for negative results, diagnostic efficacy, specificity and sensitivity of micro-ELISA systems to detect anti-hydatid antibodies and hydatid-specific antigens

in untreated or acid-treated sera were calculated, as percentages, as described by Galen and Gambino.²⁷ In brief:

$$\text{Predictive value of a positive result} = \frac{\text{Number of true positive results}}{\text{Total number of positive results}} \times 100$$

$$\text{Predictive value of a negative result} = \frac{\text{Number of true negative results}}{\text{Total number of negative results}} \times 100$$

$$\text{Diagnostic efficiency} = \frac{\text{Number of true positive} + \text{true negative results}}{\text{Number of true positive} + \text{false positive} + \text{true negative} + \text{false negative results}} \times 100$$

$$\text{Sensitivity} = \frac{\text{Number of true positive results}}{\text{Number of true positive} + \text{false negative results}} \times 100$$

$$\text{Specificity} = \frac{\text{Number of true negative results}}{\text{Number of true negative} + \text{false positive results}} \times 100$$

Results

Detection of anti-hydatid antibodies

Sera from all the 20 confirmed cases of hydatidosis gave positive results in tests for anti-hydatid antibodies (table I). Eleven (73%) of 15 sera from cysticercosis patients, eight (30%) of 27 sera from patients with other helminth infections, six (60%) of 10 sera from cases of amoebic liver abscess and one (4%) of 25 sera from apparently healthy subjects also contained anti-hydatid antibodies (table I).

Circulating hydatid-specific antigen(s) in untreated serum samples

The monospecificity of anti-8- and anti-116-kDa antibodies was indicated by obtaining a single band at the 8- or 116-kDa position in Western immunoblots of crude hydatid antigen (figure, lanes B and C); normal rabbit serum failed to recognise either hydatid-specific antigen (figure, lane D).

In tests employing monospecific anti-8-kDa antibody, sera from 17 (85%) of the 20 surgically confirmed cases of hydatidosis had detectable levels of free circulating 8-kDa antigen. None of the sera from cases of cysticercosis, ascariasis, ancylostomiasis, hymenolepiasis, amoebic liver abscess or viral hepatitis had any detectable free circulating hydatid-specific 8-kDa antigen (table II). Similarly, in tests employing monospecific antibody to 116-kDa, 18 (90%) of 20 cases of hydatidosis had free circulating 116-kDa antigen; none of the sera from other control subjects had free circulating 116-kDa antigen (table II).

Table I. Detection of antibodies to hydatid cyst fluid by ELISA

Clinical condition	Number of cases tested	Range of OD ₄₉₂ (mean; SD)	Number (%) positive*
Hydatidosis	20	0.76–1.99 (1.26; 0.37)	20 (100)
Cysticercosis	15	0.04–1.50 (0.85; 0.47)	11 (73)
Other helminthic infections:			
ascariasis	12	0.05–0.52 (0.30; 0.10)	0
ancylostomiasis	5	0.03–0.55 (0.23; 0.08)	1 (20)
hymenolepiasis	10	0.15–1.60 (0.28; 0.20)	1 (10)
Amoebic liver abscess	10	0.33–1.08 (0.62; 0.26)	6 (60)
Viral hepatitis	13	0.08–0.42 (0.22; 0.12)	0 (0)
Healthy controls	25	0.02–0.65 (0.25; 0.14)	1 (4)

*OD₄₉₂ > 0.53. **Figure.** Western immunoblot of hydatid cyst fluid with: A, anti-hydatid serum; B, monospecific antibodies to 8-kDa hydatid-specific antigen; C, monospecific antibodies to 116-kDa hydatid-specific antigen; and D, normal rabbit serum.

Fifteen (75%) of 20 sera from patients with hydatidosis had both 8-kDa and 116-kDa free circulating antigens; three and two serum samples, respectively, from patients with hydatidosis had either 8-kDa or 116-kDa free circulating antigen (table III).

Hydatid-specific circulating antigen(s) in acid-treated serum samples

All the acid-treated serum samples from hydatidosis cases had 8-kDa hydatid-specific antigen in immune complexes. None of the acid-treated sera from patients with cysticercosis, other helminth infections, amoebic liver abscess or viral hepatitis patients had any detectable 8-kDa hydatid antigen (table II). Similarly, acid-treated sera from all the 20 surgically confirmed cases of hydatidosis also had 116-kDa hydatid-specific antigen in immune complexes; none of the acid-treated

sera from control subjects had 116-kDa antigen in immune complexes (table II).

All the 20 acid-treated sera from cases of hydatidosis contained both 8-kDa and 116-kDa antigens in immune complexes (table III).

Comparative efficacy of ELISA systems for detecting hydatid specific 8- or 116-kDa antigen in acid-treated or untreated serum samples

Although sera from 17 (85%) of the 20 hydatidosis cases had detectable levels of 8-kDa antigen in both acid-treated and untreated samples, there were three (15%) with a detectable level of 8-kDa antigen only in acid-treated samples (table IV). Eleven (73%) of the serum samples from cysticercosis patients had anti-hydatid antibodies, but none had detectable levels of 8-kDa hydatid-specific antigen in acid-treated or untreated samples (table IV).

Sera from 18 (90%) of hydatidosis patients had hydatid-specific 116-kDa antigen in acid-treated and untreated samples. Two (10%) cases of confirmed hydatidosis had a detectable level of circulating specific 116-kDa antigen in acid-treated serum only. Although sera from 11 (73%) of 15 cases of cysticercosis had antibodies to hydatid antigen, none of these serum samples contained 116-kDa antigen (table V).

Diagnostic evaluation of different ELISA systems

The sensitivities for detection of free 8-kDa and 116-kDa circulating antigens was 85% and 90%, respectively. The specificities for both the 8-kDa and 116-kDa antigens in untreated sera were 100%. The diagnostic efficiency of detectability of 8-kDa and 116-kDa hydatid-specific free antigen(s) in circulation was 97% and 100%, respectively. However, the sensitivity, specificity and diagnostic efficiency for detection of either antigen in acid-treated serum samples (CICs) was 100% (table VI).

Discussion

We²³ and others,^{1, 2, 8–10, 28} have reported that anti-hydatid antibody detection assay systems, including ELISA, with either hydatid cyst fluid as antigen or

Table II. Detection of circulating hydatid-specific antigens in untreated or acid-treated serum samples

Disease (Number of cases)	Sera of patients tested by micro-ELISA employing anti-8 kDa rabbit IgG antibodies			
	Free circulating antigen		Circulating antigen in immune complexes	
	Range of OD (Mean, SD)	Number (%)* positive	Range of OD (Mean, SD)	Number (%)† positive
Hydatidosis (20)	0.08–0.57 (0.29, 0.12)	17 (85)	0.26–0.80 (0.51, 0.21)	20 (100)
Cysticercosis (15)	0.02–0.07 (0.05, 0.02)	0	0.01–0.09 (0.05, 0.02)	0
Other helminthic infections (27)	0.01–0.12 (0.05, 0.03)	0	0.02–0.12 (0.06, 0.03)	0
Amoebic liver abscess (10)	0.05–0.10 (0.09, 0.02)	0	0.05–0.10 (0.08, 0.02)	0
Viral hepatitis (13)	0.03–0.10 (0.07, 0.02)	0	0.01–0.11 (0.06, 0.03)	0
Healthy control subjects (25)	0.03–0.14 (0.09, 0.03)	0	0.03–0.12 (0.07, 0.03)	0

	Sera of patients tested by micro-ELISA employing anti-116 kDa rabbit IgG antibodies			
	Free circulating antigen		Circulating antigen in immune complexes	
	Range of OD (Mean, SD)	Number (%)‡ positive	Range of OD (Mean, SD)	Number (%)§ positive
Hydatidosis (20)	0.08–0.53 (0.32, 0.11)	18 (90)	0.14–0.57 (0.47, 0.12)	20 (100)
Cysticercosis (15)	0.03–0.09 (0.07, 0.02)	0	0.01–0.07 (0.05, 0.02)	0
Other helminthic infections (27)	0.01–0.12 (0.07, 0.03)	0	0.01–0.11 (0.06, 0.03)	0
Amoebic liver abscess (10)	0.04–0.07 (0.06, 0.02)	0	0.06–0.09 (0.08, 0.01)	0
Viral hepatitis (13)	0.03–0.12 (0.07, 0.03)	0	0.02–0.09 (0.05, 0.02)	0
Healthy control subjects (25)	0.04–0.13 (0.08, 0.03)	0	0.03–0.09 (0.06, 0.02)	0

*OD > 0.15.

†OD > 0.13.

‡OD > 0.14.

§OD > 0.10.

Table III. Proportion of patients and control subjects whose untreated or acid-treated sera contained either of the hydatid-specific antigens

Circulating antigen(s)	Untreated sera from hydatid cases (20)	Acid-treated sera from hydatid cases (20)	Untreated sera from controls (90)*	Acid-treated sera from control (90)*
Both 8- and 116-kDa antigens	15	20	0	0
8-kDa antigen only	3†	0	0	0
116-kDa antigen only	2†	0	0	0
Neither 8- nor 116-kDa antigen	0	0	90	90

*Cysticercosis (15), other helminth infections (27), amoebic liver abscess (10), viral hepatitis (13) and from healthy controls (25).

†On albendazole for a period of not more than 3 months before surgical removal of hydatid cyst.

antigens '5' or B from hydatid cyst fluid, are sensitive diagnostic tools. However, because of variable degrees of specificity of antibody detection systems, the demonstration of anti-hydatid antibodies is considered to

be of little or no value in confirming the diagnosis of hydatidosis. The poor specificity has been attributed to cross-reactions between hydatid cyst fluid antigens and antigens of other related helminths^{15–17} or with

Table IV. Anti-hydatid antibodies and hydatid-specific 8-kDa antigen in acid-treated or untreated serum samples

Clinical condition	Number of serum samples			
	tested	Ab+ FCAg+ CAgCom+	Ab+ FCAg- CAgCom-	Ab- FCAg- CAgCom-
Hydatidosis	20	17	0	0
Cysticercosis	15	0	11	4
Other helminth infections	27	0	2	25
Amoebic liver abscess	10	0	6	4
Viral hepatitis	13	0	0	13
Healthy controls	25	0	1	24

Ab, anti-hydatid antibodies; FCAg, free circulating antigen; CAgCom, circulating antigen immune-complexed.

Table V. Anti-hydatid antibodies and 116-kDa hydatid-specific antigen in acid-treated or untreated serum samples

Clinical condition	Number of serum samples			
	tested	Ab+ FCAg+ CAgCom+	Ab+ FCAg- CAgCom-	Ab- FCAg- CAgCom-
Hydatidosis	20	18	0	0
Cysticercosis	15	0	11	4
Other helminth infections	27	0	2	25
Amoebic liver abscess	10	0	6	4
Viral hepatitis	13	0	0	13
Healthy controls	25	0	1	24

Ab, anti-hydatid antibodies; FCAg, free circulating antigen; CAgCom, circulating antigen immune-complexed.

Table VI. Diagnostic evaluation of ELISA for diagnosis of hydatidosis

Diagnostic evaluation criterion	Circulating anti-hydatid antibodies (percentage value)	Percentage value of the ELISA test for detection of specific antigens			
		8-kDa antigen		116-kDa antigen	
		free antigen*	immune complexes†	free antigen*	immune complexes†
Sensitivity	100	85	100	90	100
Specificity	77	100	100	100	100
Predictive positive	49	100	100	100	100
Predictive negative	78	97	100	98	100
Diagnostic efficiency	81	97	100	98	100

*In untreated sera.

†In acid-treated sera.

contaminant host proteins.^{20,22} Currently, efforts are being made to identify hydatid-specific antigen(s). In a recent investigation, we identified two hydatid-specific antigen(s) of 8 and 116 kDa.²³ These were always recognised by sera from the surgically confirmed cases of hydatidosis in Western immunoblot assays of crude hydatid cyst fluid and were never recognised by sera from other control subjects or patients with non-hydatid disease. As shown in the present study, we

have now developed antibodies to hydatid-specific 8- and 116-kDa antigens eluted from SDS-PAGE preparations. The antibodies were specific for these antigens as assessed in Western immunoblots of crude hydatid cyst fluid antigen (figure). With these monospecific antibodies, an ELISA system has been developed for the detection of free circulating 8- or 116-kDa antigen(s) in untreated sera, or circulating 8- and 116-kDa-related immune complexes in acid-treated

sera. The results indicated that both were detectable in free or immune-complexed form in sera from 15 (75%) or 20 (100%), respectively, of 20 cases of hydatidosis.

The use of monospecific anti-8-kDa or anti-116-kDa antibodies was 10% more efficient in detecting hydatid-specific antigen in acid-treated sera than in untreated sera. This may be due to the availability of relatively more free antigen after dissociation of immune complexes by glycine-HCl.²¹ It has been reported earlier that the treatment of serum with polyethylene-glycol (PEG) preferentially precipitates larger immune complexes.²⁹ Therefore, in a system in which the serum has been treated with PEG to precipitate CICs, the smaller immune complexes would be missed. This was the reason for not using PEG in this study. Acid-treated sera are likely to contain a relatively greater quantity of antigen after dissociation of CICs. This may explain the higher detection efficiency with sera treated with glycine-HCl than with untreated sera. The system that was developed to detect specific antigens in the CICs of acid-treated sera was more efficient than the system reported previously with PEG precipitation of CICs.^{13, 21} Furthermore, the use of acid-treated serum samples provides information on both free and immune-complex antigens. None of the sera from patients with helminth infections other than hydatidosis had any

detectable level of the antigens, either in free or immune-complex form.

The sera from five of the 20 hydatidosis patients had either 8- or 116-kDa free antigen. However, all had both 8- and 116-kDa antigen in CICs. The reasons for the variation in detectability of either antigen in untreated or treated sera from these five subjects are not clear. Since they had been treated with albendazole before surgery for not more than 3 months, it is probable that the hydatid cyst wall may have become more permeable,⁴ leading to release of antigens that, in turn, may have resulted in exaggerated humoral immune responses. Such responses are likely to form complexes with any antigen free in the circulation, leaving no free antigen in circulation. This could be the reason why only one of the antigens was detected in a free state in five cases of hydatidosis but both were present in CICs.

The results of this study indicate that the detection of hydatid-specific antigens (8- or 116-kDa), especially in CICs, could provide a hydatid-specific immunodiagnostic system. However, the persistence of either antigen after effective chemotherapy or surgical removal of the hydatid cyst needs to be assessed. Such an investigation could provide clues to the effectiveness of chemotherapy or complete surgical removal of cysts in clinical cases of hydatidosis.

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