

Original papers

Evaluation of human cystic echinococcosis before and after surgery and chemotherapy by demonstration of antibodies in serum[1]

Rajesh Kumar Tenguria, Mohd Irfan Naik

Department of Microbiology, Govt. Motilal Vigyan Mahavidyalya, Bhopal-Madhya Pradesh, 462 008 India

Corresponding author: Mohd Irfan Naik; e-mail: irfan_967@yahoo.co.in

ABSTRACT. Human cystic echinococcosis (CE), caused by *Echinococcus granulosu*s, is one of the most important and widespread parasitic zoonoses. As one of the problems that can be encountered after treating CE patients is the risk of postsurgical relapses or treatment failure, a long-term clinical and serological follow-up is required to evaluate the success and failure of therapy. Therefore, the aim of the present study was to identify the best diagnostic and prognostic ELISA markers in patients with CE. The cohort comprised 50 patients with symptomatic CE treated with antihelminthic drugs and surgery, who were followed up clinically and radiologically for a mean of 6 years (range 4–8 years). The results clearly indicate that the hydatid specific antibodies of IgE, IgG1 and IgG4 are the most important antibodies for the serological diagnosis of cystic echinococcosis during the active stage of the disease. None of the serum samples from healthy controls gave a non-specific reaction with IgE, IgG1 or IgG4, and a considerably reduced cross-reaction was observed with these antibodies. During post-operative follow-up, the IgM, IgE, IgG1, IgG2 and IgG4 antibody response provided the best correlate of disease activity. The detection of total IgG and IgG3 subclass antibody response for the assessment of post-treatment disease activity among CE patients was insensitive. All patients responded to treatment except 2 women (32 and 36 years old), in whom multiple cysts (12 and 7 cysts) were detected in the liver and lung two years after the first operation. Hence, it can be concluded that the CE-specific antibodies of IgE, IgG1 and IgG4 are the best immunological markers for diagnosis and prognosis of CE patients.

Key words: immunodiagnosis, cystic echinococcosis, ELISA

Introduction

Cystic echinococcosis (CE) is caused by infection with the larval stage (hydatid) of the cestode *Echinococcus granulosus* and is one of the world's major zoonotic infections. Humans acquire infection by accidental ingestion of *E. granulosus* eggs voided in the feces of infected dogs. CE in humans usually presents with symptoms associated with the presence of fluid-filled cysts in the liver, lungs, or other viscera and diagnosis is usually established by a combination of radiology and serology [1]. Treatment modalities for CE include chemotherapy and surgery [2]. Although surgery still remains the most common approach for treatment of CE throughout the world, long-term albendazole therapy has significant efficacy in approximately 70% of cases [3].

One of the problems that can be encountered after treating cystic echinococcosis patients is the risk of post-surgical relapses or treatment failure due to non-radical surgical procedures or perisurgical spillage of parasite material, especially protoscoleces. Relapses in the form of newly developing cysts have been reported and may affect between 2% and 25% of cases after therapy, according to previous studies [4]. Therefore, patients with cystic echinococcosis need to be carefully monitored after surgery or drug treatment to ensure that they remain free from relapse or treatment failure.

[1] This research received no specific grant from any funding agency, commercial or not-for-profit sectors.The authors declare that there is no conflict of interests.

A post-treatment follow-up method to prognostically determine the efficacy of treatment should therefore include markers that allow the detection of newly growing or relapsing cysts and the tracking of previously undetected but still viable cysts. Conventional tools used to follow-up cystic echinococcosis patients include imaging techniques such as X-ray, ultrasonography, computed tomography, and magnetic resonance imaging [4]. In some cases, however, the prognostic efficiency of imaging tools is significantly hampered when small changes cannot be visualized or cannot be interpreted with regard to a concise discrimination between dead and still viable metacestodes [5]. Therefore, the availability of an immunodiagnostic test to support this discrimination may be of clinical value. The aim of the present study is to identify the best diagnostic and prognostic markers in human CE patients.

Materials and Methods

Patients/Subjects. Fifty patients (16 males and 34 females; mean±SD age was 31.1±11.2 years, range 9–69 years) with clinically and radiologically diagnosed hydatidosis were enrolled for the study after obtaining due informed consent. A detailed clinical history of all hydatid patients was recorded on pretested proforma: clinical complications, demographic details, results of radiologic investigations, anatomical location of cysts, anthelminthic treatment, and histopathology of surgically resected specimens.

Twenty (20) normal, healthy adult individuals with no past history of dog contact, travelling to highly endemic areas or treatment for previous parasitic infections were included as negative controls and an additional group of 40 patients with other parasitic diseases, amoebiasis (10), toxoplasmosis (10), ascariasis (10) or a malignancy (10), were included to check the cross reactivity of antibodies of these patients with the hydatid antigen. The malignancies included gastrointestinal (GI) tract neoplasm (3 cases), lymphoma (2 cases) and lung cancer (5 cases).

Blood samples were collected from all subjects and centrifuged at 2000×g for 10 minutes at 4°C to obtain the serum. The lipaemic or haemolysed sera were discarded. The sera was divided in to 3 tubes for each subject and stored immediately at -70°C until specific antibody detection. All 50 patients received antihelminthic treatment of albendazole, 400 mg twice a day for 3 months, plus praziquantel, 40 mg/kg/day for two weeks as per standard guidelines [6] and underwent surgical procedures.

The most common surgical procedure performed was cystectomy using a freezing cone to prevent intra-operative spillage of cyst contents. Seven patients were treated surgically by the puncture, aspiration, injection, reaspiration (PAIR) procedure. Even after surgery, all CE patients received 3– 6 month cycles of albendazole 10–12 mg/kg per day according to the World Health Organization protocol [6].

The effectiveness of pharmacological and surgical treatment was evaluated by objective criteria mainly based on imaging techniques and antibody determination at six months, one year, two years, three years and four years following pharmacological and surgical treatment. The long-term outcome of therapy was assessed by the presence or the lack of relapse 4 years after the pharmacological and surgical treatment. All procedures were approved by the local Ethical Committee and all subjects gave their informed consent for the study.

Preparation of hydatid antigen. Hydatid cyst fluid antigen (HCF) was prepared according to procedure described earlier [7]. Briefly, the hydatid cyst fluid (HCF) was aspirated from fertile hydatid cysts obtained from livers of naturally infected sheep slaughtered at the local abattoir. The aspirated fluid was centrifuged at 2000×g for 20 min at 4°C to remove the protoscolices. The supernatant was then filtered through a Whatman WCN type membrane filter (cellulose nitrate, 47 mm diameter, 0.45 µm pore size) and dialyzed against distilled water overnight at 4°C using dialysis tubing (Sigma Aldrich, USA) with a 2000 molecular weight cut off. The antigen protein concentration was estimated by the Lowry method with bovine serum albumin (BSA) as a reference standard [8].

Antibody detection. Antibody detection in serum samples of hydatid patients was performed by indirect enzyme linked immunosorbent assay (ELISA) as described earlier with a few modifications [7]. Briefly, microtitration plates were coated with 2µg/100µl/well of crude hydatid sheep antigen (optimum concentration of antigen predetermined by the checker board titration method) diluted in 0.1M carbonate/bicarbonate buffer (pH 9.6). Patient sera was diluted 1:400 for IgG, IgM, IgE and 1:200, 1:100, 1:50, 1:50 for IgG1, IgG2, IgG3 and IgG4 respectively. The

Table 1. ELISA detection of antibodies in pre and post-treatment serum samples

Immunoglobulin Type	Preoperative positive samples with percentage	Post-operative positive samples with percentage	P-value
IgG	46 (92%)	46 (92%)	1.000
IgM	35 (70%)	15 (30%)	0.001
IgE	43 (86%)	21 (42%)	0.021
IgG1	41 (82%)	23 (46%)	0.019
IgG2	37 (74%)	17 (34%)	0.005
IgG3	26 (52%)	18 (36%)	0.646
IgG4	43 (86%)	14 (28%)	<0.0001

different assays were then continued as follows:

Total IgG, IgM and IgE assays: Goat anti-human IgG, IgM and IgE were conjugated to horseradish peroxidase (Sigma Aldrich, USA), diluted 1:4000, 1:8000 and 1:4000 respectively were used as the secondary antibody. Tetramethylbenzidine (TMB) and H_2O_2 were used to visualize the antigen-antibody reaction. Optical density (OD) was registered at 450 nm after the addition of stop solution (2·5N H_2SO_4). Mean OD±3 standard deviations (SD) of the OD values obtained for the healthy sera was used to establish a cut-off value. Values greater than the cut-off value, was considered positive for anti-hydatid antibodies.

IgG subclass assays: The assays for IgG subclasses were performed as described previously with some modifications [9]. For the detection of specific IgG-subclass antibodies in serum monoclonal mouse antihuman IgG1, IgG2, IgG3 and IgG4 (Sigma Aldrich) were diluted 1:500, 1:2500, 1:1,000 and 1:2500 respectively in PBS. Peroxidase conjugated goat anti-mouse Fc-specific IgG was used as secondary antibody diluted 1:24000, 1:10,000, 1:4000 and 1:24000 for IgG1, IgG2, IgG3 and IgG4 assays respectively. Substrate solution was then added and the plate was developed and read as described for the IgG, IgM and IgE assays.

Statistical analysis. The criterion for true positives was based on surgical and radiological findings as no gold standard parameter was available for the diagnosis of human hydatidosis. The results were analyzed statistically by Pearson's chi-square test.

Results

The age of the patients in the present study varied between 9 and 69 years. The mean ±SD age of the patients was 31.12±11.24 years. The highest incidence of disease was recorded among patients between 20 and 49 years of age. A greater occurrence of hydatidosis was noted in females (68% of all patients) than in males (32%). Most patients with cystic echinococcosis (76%) were from rural areas, with only 24% coming from urban areas. Thirty four (34) of the radiologically and surgically confirmed cases were found to have hepatic cysts, while 16 had extrahepatic cysts (12-lung cysts, 3-liver and lung and 1-thigh cyst). All 50 patients received antihelminthic treatment (minimum treatment of albendazole, 400 mg twice a day for 3 months, plus praziquantel, 40 mg/kg/day for two weeks) and underwent surgical procedures. The mean ±SD duration of follow-up after diagnosis was 6±2 years (range 4–8 years). Relapse occurred in two patients.

Table 2. Mean ±SD ELISA OD values of *Echinococcus*-specific antibodies at diagnosis and following cure

Time	Mean ODs ±SD						
	IgG	IgM	IgE	IgG1	IgG2	IgG3	IgG4
Peak OD	0.687±0.275	0.667±0.318	0.508±0.114	0.575±0.263	0.401±0.114	0.390±0.169	0.429±0.111
Cured OD	0.288±0.099	0.169±0.090	0.201±0.057	0.200±0.098	0.183±0.095	0.204±0.123	0.165±0.111
P-value	0.005	<0.0001	0.004	0.004	0.003	0.0157	<0.0001

Serology at diagnosis. Among the cases of cystic echinococcosis studied, the number with positive serology (>Cutoff) at diagnosis was 46 (IgG), 35(IgM), 43(IgE), 41(IgG1), 37(IgG2), 26(IgG3) and 43(IgG4). The highest percentage of positivity was found by IgG with 92%, followed by IgE/IgG4, IgG1, IgG2, IgM and IgG3 with 86%, 82%, 74%, 70% and 52% respectively (Table 1). The diagnostic sensitivity of total IgG, IgE, IgG4 and IgG1 was comparatively greater than IgG2, IgM and IgG3. The mean±SD optical densities (ODs) of sera samples for total IgG, IgM, IgE, IgG1, IgG2, IgG3 and IgG4 were 0.687±0.275, 0.667±0.318, 0.508±0.114, 0.575±0.263, 0.401±0.114, 0.390± 0.169, 0.429±0.111 respectively. The mean ODs of IgG, IgM, IgG1 and IgE were greater than IgG4, IgG2 and IgG3 (Table 2).

Serology in patients with cured CE. Patients with cured disease (n=48) were followed up for a mean±SD of 4±1.8 years. There was a significant decrease in the *Echinococcus*-specific antibody response of IgM, IgE, IgG1, IgG2 and IgG4 in patients with cured disease compared with those demonstrating active disease, but all cured patients were found positive to IgG total antibody (Table 1). The mean ± SD ODs of total IgG, IgM, IgE, IgG1, IgG2, IgG3 and IgG4 assays were 0.288±0.099, 0.169±0.090, 0.201±0.057, 0.200±0.098, 0.183± 0.095, 0.204±0.123, 0.165±0.111, respectively. The mean OD obtained with each of the seven ELISAs decreased substantially between the peak values during active disease and values when cured (Table 2). The greatest mean decrease in OD was observed with the IgM, IgG4 assay. The majority of the patients responded to treatment, but only 2 patients whose hydatid cysts relapsed were found positive in all the specific antibodies except IgG3, and their ODs were also high.

Cross-reactivity, sensitivity and specificity. The highest proportion of cross-reactivity in the sera of patients with other parasitic infections and malignancy was found in patients with ascariasis infection (8) followed by amoebiasis (4), malignancy (4) and toxoplasmosis (1). The maximum percentage of cross-reactions were observed with IgG (17.5%) followed by IgM (7.5%) and IgE or IgG2 (5% each) respectively. The percentage of non-specific reactions was reduced to 2.5% when specific IgG1, IgG3 and IgG4 antibodies were analyzed. Non-specific reactions in healthy controls were observed with IgG, IgM and IgG2. For IgG, a total of 2 healthy controls showed a non-specific reaction, whereas only one healthy control serum showed non-specificity for IgM and IgG2. No non-specific reaction was observed with IgE, IgG1, IgG4 and IgG3 (Table 3).

Considering the surgically confirmed cases as gold standard, the sensitivity for hydatid-specific IgG, IgM, IgE, IgG1, IgG2, IgG3 and IgG4 antibody detection was 92%, 70%, 86%, 82%, 74%, 52% and 86% respectively. Therefore IgG, IgE, IgG4 and IgG1 showed higher diagnostic sensitivity for human hydatidosis. The evaluation of the specificity of IgG, IgM, IgE and IgG subclass antibody revealed various degrees of false positivity with sera from subjects with other parasitic infections (Table 3). Specificity was highest in IgG1, IgG3 and IgG4 (98.33%), followed by IgE (96.66%), IgM (93.33%), IgG2 (95%) and IgG (85%). Due to the high specificity of IgE, IgG1, IgG2, IgG3 and IgG4, the positive predictive value (PPV) and negative predictive value (NPV) was greater in these specific antibodies. The diagnostic accuracy was also comparatively greater in IgE, IgG1 and IgG4 (Table 4).

Table 3. Cross reactivity of other parasitic infections, malignancy and healthy controls against crude antigen

Patients with other parasitic infections	Positive Sera						
	IgG	IgM	IgE	IgG1	IgG2	IgG3	IgG4
Amoebiasis (n=10)	2	–	1	1	–	–	–
Ascariasis (n=10)	3	1	1	–	1	1	1
Toxoplasmosis (n=10)	–	1	–	–	–	–	–
Malignancy (n=10)	2	1	–	–	1	–	–
Cross-reaction percentage	17.5%	7.5%	5%	2.5%	5%	2.5%	2.5%
Healthy controls (n=20)	2	1	–	–	1	–	–

Table 4. Sensitivity and specificity of antibodies against crude hydatid antigen

Immunoglobulin Type	Sensitivity	Specificity	Positive predictive value	Negative predictive value	Diagnostic accuracy
IgG	92	85	83.63	92.72	88.18
IgM	70	93.33	89.74	78.87	82.72
IgE	86	96.66	95.55	89.23	91.81
IgG1	82	98.33	97.61	86.76	90.90
IgG2	74	95	92.5	81.42	85.45
IgG3	52	98.33	96.29	71.08	77.27
IgG4	86	98.33	97.72	89.39	92.72

Data in the table is in percentages; sensitivity was calculated from the hydatid patient (n=50); specificity was calculated from healthy controls and other parasitic control patients (n=60).

Discussion

This study was designed to identify the immunological markers by ELISA for the diagnosis and prognosis of human cystic echinococcosis in surgically confirmed patients. ELISA has been compared to various techniques for diagnosis and post-therapeutic follow-up, using different antigens, either crude hydatid cyst fluid, purified, recombinant or synthetic peptide antigens [10]. However, ELISA has received most attention as an immunodiagnostic method for various parasitic infections.

The results of the pre-operative study clearly demonstrate that the specific antibodies of total IgG, IgE, IgG4 and IgG1 are the most important antibodies for serological diagnosis of cystic echinococcosis using crude hydatid cyst fluid antigen. Our results confirm those of other studies, who also report a predominance of IgE, IgG1 and IgG4 antibody response against cyst fluid antigen in hydatid patients [11,12]. Human hydatid patients demonstrate frequent occurrences of elevated antibody levels, particularly of immunoglobulin G (IgG), IgM, IgE, IgG1 and IgG4 [13,14]. IgG1 and particularly IgG4 are the most predominant isotypes observed among IgG subclass responses in human cystic echinococcosis [15,16]. The low IgG3 seropositivity observed in a significant proportion of patients effectively excluded this antibody subclass as useful serological marker for post-treatment follow-up. The mean ODs were comparatively low in IgG3, moderate in IgG4 and IgG2 and greater in IgG, IgM, IgE, IgG1 assays. These results are in agreement with previous studies, which also report greater mean optical densities in IgG total and IgG1, moderately elevated in IgG2, IgE and IgG4 assays and lower in IgG3 assay [17,18].

The body of research regarding the measurement of hydatid specific antibodies in the post-operative follow-up samples is limited [9]. The longitudinal assessment of changes in *Echinococcus*-specific total IgG, IgM, IgE and IgG subclass (1-4) antibody responses in patients with cystic echinococcosis who received treatment for symptomatic disease clearly demonstrates that the IgM, IgE, IgG1, IgG2 and IgG4 antibody responses provide the best correlates of disease activity. A significant decrease in IgM ($p=0.001$), IgE ($p=0.012$), IgG1 ($p=0.019$), IgG2 ($p=0.005$) and IgG4 ($p<0.0001$) antibody response was noted in patients after surgery. These findings clearly demonstrate that IgM, IgE, IgG1, IgG2 and IgG4 are the best prognostic markers for this disease. The detection of anti-*Echinococcus* specific total IgG antibody response for the assessment of post-treatment disease activity among hydatid patients was insensitive ($P=1.000$).

Our results confirm those of previous investigations which note that total IgG antibody persists in the human host for many years but IgE and IgM decreases rapidly in serum of patients after surgery or successful chemotherapy [14,19–21]. Zarzosa et al. [22], observed progressively decreasing titres of specific IgM and IgE antibodies after surgery in patients who do not demonstrate a relapse, and concluded that specific IgM and IgE antibodies are important for hydatidosis follow-up.

In patients with degenerating and calcifying cysts, IgG4 antibody response declines [23]. Furthermore, patients treated with albendazole who also exhibited a good therapeutic and clinical response to treatment had significantly lower levels of serum IgG4 antibodies than poor responders or non-responders [24]. Guerri et al. [25], and Elsebaie et al. [26], independently detected that the number of IgG4 antibodies at the time of diagnosis decreased gradually after surgery in a significant number of patients and reached normal values after 18 months post-operation, They conclude that the IgG4 subclass is a good prognostic marker in hydatidosis follow-up.

The mean optical densities especially in IgM, IgE, IgG1, IgG2 and IgG4 were significantly decreased in the patients with cured disease. Our results are well supported by earlier reports [9,20]. Michael et al. [27] report a decline in mean optical densities over a period of time in many patients who were cured by surgery. However, they observe that titers remain positive for over four years in a number of patients. Wang et al. [28], observe that specific IgG antibody levels remained positive in most cases of cystic echinococcosis, but exhibited a decreasing tendency in those which were effectively treated by surgery.

In the present study, high false positivity with the serum samples collected from patients of ascariasis (80%), amoebiasis and malignancy (40% each) was observed. Such false positive reactions were observed to the greatest degree with IgG total (17.5%) and reduced with other antibodies. The probable cause of such high false positives could be the use of crude antigen in the study. Our results confirm the observations of previous studies, which also record the highest number of cross-reactions in IgG and reduced values in IgG1, IgG2, IgG3 and IgG4 [17,18]. An earlier study reported frequent cross-reactions in the sera of patients with ascariasis, cysticercosis, amoebiasis and malignancy [29]. The false positive reactions in hydatid cyst fluid antigen of *E. granulosus* with human antibodies of other cestodes and helminths have also been reported in a previous investigation [30].

In conclusion, based on the use of ELISAs incorporating crude *E. granulosus* sheep hydatid cyst fluid antigen, CE-specific IgE, IgG1, and IgG4 were found to be the most specific antibodies for this parasite. Furthermore, during the post-treatment follow-up, CE specific IgM, IgE, IgG1, IgG2 and IgG4 antibodies correlate better with disease activity than total IgG and IgG3 subclass. However, the IgE, IgG1 and IgG4 antibody response was the best correlate of disease activity during post-treatment follow-up.

References

[1] Babba H., Messedi A., Masmoudi S., Zribi M., Grillot R., Ambriose-Thomas P., Beyrouti I., Sahnoun Y. 1994. Diagnosis of human hydatidosis: comparison between imagery and six serologic techniques. *The American Journal of Tropical Medicine and Hygiene* 50: 64-68.

[2] Gottstein B., Reichen J. 2003. Echinococcosis/ hydatidosis. In: *Manson's Tropical Diseases.* (Eds. G.C. Cook, A. Zumla). London, W.B. Saunders, Elsevier Science Ltd: 1561-1582.

[3] Eckert J., Schantz P.M., Gasser R.P., Torgerson P.R., Bessonov A.S., Movsessian S.O., Thakur A., Grimm F., Nikogossian M.A. 2001. Geographic distribution and prevalence. In: *Manual on Echinococcosis in humans and animals: a public health problem of global concern*. (Eds. J. Eckert, M.A. Gemmell, F.X. Meslin, Z.S. Pawłowski). WHO/OIE, Paris, France: 100-142.

[4] World Health Organization (W.H.O) Informal Working Group. 2003. International classification of ultrasound images in cystic echinococcosis for application in clinical and field epidemiological settings. *Acta Tropica* 85: 253-261.

[5] Wen H., New R.R., Craig P.S. 1993. Diagnosis and treatment of human hydatidosis. *British Journal of Clinical Pharmacology* 35: 565-574.

[6] WHO-IWGE 1996. Guidelines for treatment of cystic and alveolar echinococcosis in humans. *Bulletin WHO* 76: 231-242.

[7] Wattal C., Malla N., Khan I.A., Agarwal S.C. 1986. Comparative evaluation of enzyme-linked immunosorbent assay for the diagnosis of pulmonary echinococcosis. *Journal of Clinical Microbiology* 21: 41-46.

[8] Lowry O.H., Rosenbrough N.J., Farr A.L., Randall R.J. 1951. Protein measurement with the folin phenol reagent. *Journal of Biological Chemistry* 193: 265-275.

[9] Lawn S.D., Bligh J., Craig P.S., Chiodini P.L. 2004. Human cystic echinococcosis: Evaluation of post treatment serologic follow-up by IgG subclass antibody detection. *The American Journal of Tropical Medicine and Hygiene* 70: 329-335.

[10] Zhang W., Li J., McManus D.P. 2003. Concepts in immunology and diagnosis of hydatid disease. *Clinical Microbiology Reviews* 16: 18-36.

[11] Chirag S., Fomda B.A., Khan A., Malik A.A., Lone G.N., Khan B.A., Zahoor D. 2014. Detection of hydatid specific antibodies in the serum and urine for

the diagnosis of cystic echinococcosis in patients from the Kashmir Valley, India. *Helminthology* 16: 1-6.

[12] Shambesh M.K., Craig P.S., Wen H., Rogan M.T., Paolillo E. 1997. IgG1 and IgG4 serum antibody responses in asymptomatic and clinically expressed cystic echinococcosis patients. *Acta Tropica* 64: 53-63.

[13] Sterla S., Sato H., Nieto A. 1999. *E. granulosus* human infection stimulates low avidity anticarbohydrate IgG2 and high avidity antipeptide IgG4 antibodies. *Parasite Immunology* 21: 27-34.

[14] Zhang W., Ross A.G., McManus D.P. 2008. Mechanisms of immunity in hydatid disease: implications for vaccine development. *Journal of Immunology* 181: 6679-6685.

[15] Wen H.,Craig P.S.1994. Immunoglobulin G subclass responses in human cystic and alveolar echinococcosis. *The American Journal of Tropical Medicine and Hygiene* 51: 741-748.

[16] Virginio V.G., Hernández A., Rott M.B., Monteiro K.M., Zandonai A.F., Nieto A., Zaha A., Ferreira H.B. 2003. A set of recombinant antigens from *Echinococcus granulosus* with potential for use in the immunodiagnosis of human cystic hydatid disease. *Clinical and Experimental Immunology* 132: 309-315.

[17] Felix G., Friedrich E.M., Jian L., Roberto L. 1998. Analysis of specific immunoglobulin G subclass antibodies for serological diagnosis of echinococcosis by a standard enzyme-linked immunosorbent Assay. *Clinical and Diagnostic Laboratory Immunology* 5: 613-616.

[18] Khabiri A.R., Bagheri F., Assmar M., Siavashi M.R. 2006. Analysis of specific IgE and IgG subclass antibodies for diagnosis of *Echinococcus granulosus*. *Parasite Immunology* 28: 357-362.

[19] Rigano R., Profumo E., Ioppolo S., Notargiacomo S., Ortona E., Teggi A., Siracusano A. 1995. Immunological markers indicating the effectiveness of pharmacological treatment in human hydatid disease. *Clinical and Experimental Immunology* 102: 281-285.

[20] Vedat B., Falya I., Ahmet Yasar Y., Suleyman O., Yavuz I., Ahmet G. 2001. Immunological follow up of hydatid cyst cases. *Memórias do Instituto Oswaldo Cruz* 96: 669-671.

[21] Doiz B.R., Benito R., Gil J., Rojar A., Rubio M.C., Ossuma A. 2002. Pre and postsurgical detection of IgG, IgM and IgA specific to hydatidosis by ELISA with purified antigen enriched with 5/B antigen complex. *Journal of Clinical Laboratory Analysis* 16: 295-298.

[22] Zarzosa M.P., Orduna A., Gutierrez P., Alonso P., Cuervo M., Prado A.M., Bratos A., Garcia-Yuste M., Ramos G., Torres A. 1999. Evaluation of six serological tests in diagnosis and postoperative control of pulmonary hydatid disease patients. *Diagnostic Microbiology and Infectious Disease* 35: 255-262.

[23] Daeki A.O., Craig P.S., Shambash M.K. 2000. IgG-subclass antibody responses and the natural history of hepatic Cystic Echinococcosis in asymptomatic patients. *Annals of Tropical Medicine and Parasitology* 94: 319-328.

[24] Rigano R., Buttari B., Profumo E., Ortona E., Margutti P., Teggi A., Siracusano A. 2002. Apoptosis modulation in PMBC from patients with cystic echinococcosis. *The American Journal of Tropical Medicine and Hygiene* 67: 298-299.

[25] Guerri M.L., Davila M., Rodriguez M., Javier Nieto F., Ladron de Guevara C. 2000. Utility of IgG subclasses in the diagnosis and follow-up of hydatidosis. *Enfermedades Infecciosas y Microbiologia Clinica* 18: 262-266.

[26] Elsebaie S.B., El-Sebae M.M., Esmat M.E., Nasr M.M., Kamel M.M. 2006. Modified endocystectomy versus pericystectomy in *Echinococcus granulosus* liver cysts: a randomized controlled study, and the role of specific antihydatid IgG4 in detection of early recurrence. *Journal of the Egyptian Society of Parasitology* 36: 993-1006.

[27] Michael D., Rickard R.D., Honey J.L., Brumley L., Mitchell G.F. 1984. Serological diagnosis and post operative surveillance of human hydatid disease II. The Enzyme Linked Immunosorbent Assay (ELISA) using various antigens. *Pathology* 16: 211-215.

[28] Wang Y., He T., Wen X., Li T., Waili A., Zhang W., Xu X., Vuitton D.A., Rogan M.T., Wen H., Craig P.S. 2006. Post survey follow up for human cystic echinococcosis in Northwest China. *Acta Tropica* 98: 43-51.

[29] Poretti D., Felleisen E., Grimm F., Pfister M., Teuscher F., Zuercher C., Reichen J. Gottstein B. 1999. Differential Immunodiagnosis between cystic hydatid disease and other cross reactive pathologies. *The American Journal of Tropical Medicine and Hygiene* 60: 193-198.

[30] Sunita T., Dubey M.L., Khurana S., Malla N. 2007. Specific antibody detection in urine, serum and saliva samples for the diagnosis of cystic echinococcosis. *Acta Tropica* 101: 187-191.

Received 25 July 2014
Accepted 11 October 2014