

Serum Antibody Detection in Echinococcosis: Specificity of *Hydatidosis* enzyme-linked immunosorbent assay (ELISA) IgG

Metwally D M^{1&2} and Al-Olayan E M²

¹Parasitology Department, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt.

²Zoology Department, Faculty of Science, King Saud University, Riyadh, KSA.

mdbody7@yahoo.com; eolayan@ksu.edu.sa

Abstract: Hydatidosis is diagnosed by serological together with radiological and ultrasound examinations. but cross-reactivity with other parasites, over evaluating the real prevalence, represents a big limitation. The aim of the present study was to develop a specific and simple antibody detection (IgG)-based ELISA method. The samples in this study included 35 patients in the following groups: IHAT (Indirect Haem Agglutination Test) and abdominal ultrasonography confirmed hydatidosis patients (20 cases), control with other parasitic diseases (5 cases Toxoplasmosis) and healthy controls (10 cases). Antibody detection by indirect ELISA using *Echinococcus granulosus* antigen showed that 65% of hydatid patients (13 cases) have anti-hydatid cyst antibodies in their serum while no cross reaction was detected. A sensitivity of 72.22% and specificity of 75% were found for the antibody detection assay. Findings of this study indicated that antibody detection assay is a highly sensitive and specific approach for diagnosis of hydatid cyst.

[Metwally D M and Al-Olayan E M. Serum Antibody Detection in Echinococcosis: Specificity of *Hydatidosis* enzyme-linked immunosorbent assay (ELISA) IgG. *Life Sci J* 2013;10(1):2107-2110]. (ISSN: 1097-8135). <http://www.lifesciencesite.com>. 298

Keywords: hydatidosis, ELISA IgG diagnosis, sensitivity, specificity.

1. Introduction:

Echinococcus granulosus is the causal agent of human hydatidosis. Hydatid cysts are mainly located in liver (right lobe). The intensity of the immune response depends on the location and integrity of the cyst. Cysts in lung, brain or spleen are less reactive than those in liver or bone.

This parasite has a worldwide distribution and is one of the most important zoonotic diseases prevalent in different parts of the world including the Middle East (Eckert and others, 2001; Sadjjadi and others, 2006). The infection may be asymptomatic, especially in its early stages, many cases go unreported within endemic countries (Craig and Nelson, 1984). Diagnosis of hydatidosis is based on immunodiagnostic methods along with radiological and ultrasound

Examinations (Parija, 1998; Sadjjadi and others, 2001). Large number of immunological assays have been developed for detection of anti-hydatid cyst antibodies (IgG), and recently, hydatid antigens in the serum (Ortona and others, 2003). These include indirect hemagglutination (IHA), indirect immunofluorescence (IFA), immunoelectrophoresis, counter-current immunoelectrophoresis (CIEP), radioimmunoassay (RIA), and ELISA (Siavashi et al., 2005; Sarkari et al., 2007). Moreover, enzyme-linked immunoelectrotransfer blots (EITB), enzyme-linked immunoelectrodiffusion assay (ELIEDA), time-resolved fluoroimmunoassay (TR-FLA), and

immunoblot assay have been developed for detection of anti-hydatid cyst antibodies (Aceti and others, 1991; Ortona et al., 2000). Antibody detection assays cannot distinguish between past and present infections and cannot be used for assessment of the efficacy of treatments but Antigen detection assay may circumvent this problem (Doiz et al., 2001). It has been shown that hydatid cyst antigen can be detected in the serum or urine of hydatidosis patients. Circulating hydatid antigens are present in the serum only during active infection, and the levels of these antigens continue to decrease after surgical removal of the hydatid cyst or successful chemotherapy (Devi and Parija, 2003). ELISA has been used for the detection of specific *E. granulosus* antibodies in serum with variable results (Kaddah and others, 1992; Hernandez and others, 2008). The ELISA, with hydatidic fluid of sheep origin as antigen, is widely used for the diagnosis of the disease, showing a high sensitivity for hepatic cysts, although lower for pulmonary locations. Cross-reactions may appear in patients infected by other parasites. The carbohydrate epitopes in hydatid cyst fluid antigen (Alyam and Knobloch, 1989) cross-react with antigens shared with other pathogens leading to reduced specificity and sensitivity of diagnostic assays.

The present study aimed to test Specificity of the enzyme-linked immunosorbent assay (ELISA) for *Hydatidosis* IgG antibody

2. Materials And Methods

Hydatid sera

Serum samples from patients were collected from Almattaria teaching hospital, Cairo between June 2012 and September 2012 for the diagnosis of hydatidosis. Twenty serum samples of E. granulosus patients confirmed by abdominal ultrasonography, computerized tomography and IHAT (Indirect Heam Agglutination Test). Five serum samples from patients with toxoplasmosis were used as control for hydatid patients(to test the specificity of technique for hydatidosis). Sera were also collected from healthy controls (10 cases)

Enzyme linked immunosorbent assay

Enzyme-linked immunosorbent assay was performed using a specific kit Hydatidosis Elisa IgG(G1006) supplied by the Vircell microbiologist(GRANADA* SPAIN)

The principle of the assay is based upon the reaction of antibodies in the sample tested with the antigen adsorbed on the polystyrene surface. Unbound immunoglobulins are washed off. An enzyme-labelled anti-human globulin binds the antigen-antibody complex in a second step. After a new washing step, bound conjugate is developed with the aid of a substrate solution (TMB) to render a blue coloured soluble product which turns into yellow after adding the acid stopping solution. The absorbance was measured at 450 nm using a microplate ELISA reader (Model IRE96.CompanySFRI).

Calculations

Sensitivity was calculated by dividing the number of true positive cases to total number of positive cases and false negative cases . Specificity

was also calculated by dividing the number of true negative cases to total number of negative cases and false positive cases.

3. Results

The positive predictive value of antibody IgG was detected in 13 out of 20 (35%) of sera among hydatidosis patients, 2 out of 20(10%) were equivocal and 5 of 20 (25%) were not having IgG specific antibody against hydatidosis(Table.1). Therefore, a sensitivity of 72.22% and specificity of 75% was calculated for the antibody detection assay. No antibody was detected in patients with toxoplasmosis (Table.2) so the sensitivity in case of toxoplasmosis is 0% while the specificity is 100%. Antibody IgG was detected in 2 out of 10 healthy control group(Table.3).The equivocal samples were excluded from calculations. The results of Antibody detection in the serum by indirect ELISA (IgG)was illustrated in(Fig.1)

Table1.hydati group

RESULTS	-Ve	Equivocal	+Ve	Total
NO. of Samples	5	2	13	20

Table2.toxoplasmosis group

RESULTS	-Ve	Equivocal	+Ve	Total
No. of Samples	5	0	0	5

Table3.control group

RESULTS	-Ve	Equivocal	+Ve	Total
No. of Samples	6	2	2	10

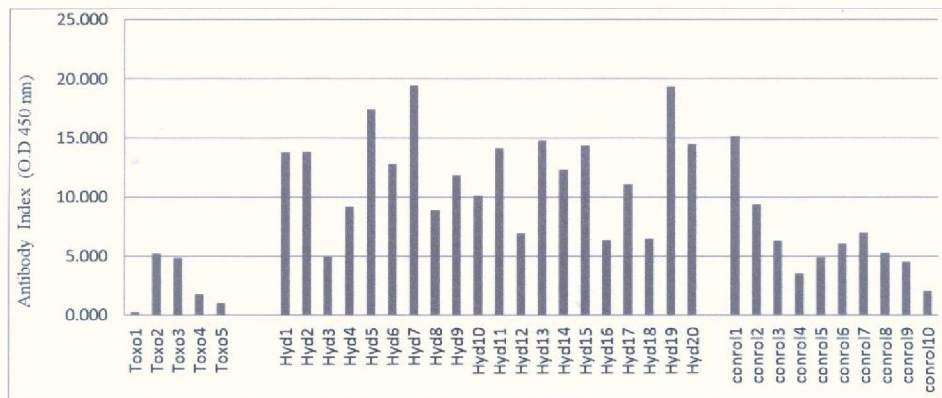


Fig.1 Antibody detection in the serum by indirect ELISA using plate coated with echinococcus granulosus antigen.

Antibody index > 11 positive
 Antibody index 9-11 equivocal
 Antibody index < 9 negative

4. Discussion

Large number of serological tests such as ELISA executed on patients' sera to detect specific antibodies leads to variable results of sensitivity and specificity. False negative results in human hydatidosis make a serious puzzle to get a conclusion, as it ranges between 3-5% of hydatid patients and may up to 35-40% in hyper-endemic areas (Craig and Nelson, 1984; Gottstein, 1984).

Some of the hydatidosis patients not raised the antibody or the titer is low especially in infants and old people. Also the antibody titer is low and cannot be easily detected in cerebral, ocular, and calcified cysts, (Gottstein et al., 1984 ; Ravinder et al., 1997) From another aspect, the long persistence of anti- *E. granulosus* antibodies after surgical removal of the cysts gives rise to unreliable diagnosis of relapse in patients (Todorov and Stojanov, 1979).

The antibody detection in this study was found that 65% of patients (13 out of 20 cases) have IgG specific antibodies against *Echinococcus granulosus* in their serum. The sensitivity of the assay to detect antibody was high (72.22%), its specificity was high too (75%) and no additional cross reactions were found in patients with toxoplasmosis but in 2 healthy control. These results are in agreement with (Sadjjadi et al., 2009) in which he found that 94.2% of patients (33 cases) have anti-CE antibodies in their serum (high sensitivity) and he recorded that no cross reaction in patient with toxoplasmosis too, but in his detection assay, specificity was rather low since cross reaction was noted in sera of patients with ascariasis and strongyloidiasis and one healthy control. In addition, (Younis et al., 2008) reported 90% sensitivity and 75% specificity by the conventional elisa, no cross reaction recorded with toxoplasmosis but with amoebiasis, fascioliasis, schistosomiasis and one healthy control.

Declaration of interest

The authors report no declarations of interest. The authors alone are responsible for the content and writing of the paper

The source(s) of financial support for the study.

Zoology Department, Faculty of Science, King Saud University.

Corresponding author

Dina Mahmoud Ahmed Metwally

¹Parasitology Department, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt.

²Zoology Department, Faculty of Science, King Saud University, Riyadh, KSA.

mdbody7@yahoo.com

Acknowledgments

This research project was supported by a grant from the "Research Center of the Center for Female Scientific and Medical Colleges", Deanship of Scientific Research, King Saud University.

References

1. Aceti A, Pennica A, Teggi A, Grilli A, Caferro M, Celestino D, Leri O, Sebastiani A, DeRosa F, 1991, The serological diagnosis of human hydatid disease by time-resolved fluoroimmunoassay: J Infect, 22, 135-141.
2. Al-Yam F, Knobloch J, 1989, Isolation and partial characterization of species-specific and cross-reactive antigens of *Echinococcus granulosus* cyst fluid: Mol Biochem Parasitol, 37, 101-7.
3. Craig PS, Nelson GS, 1984, The detection of circulating antigen in human hydatid disease: Ann Trop Med Parasitol, 78, 219-227.
4. Devi CS, Parija SC, 2003, A new serum hydatid antigen detection test for diagnosis of cystic echinococcosis: Am J Trop Med Hyg, 69, 525-528.
5. Doiz O, Benito R, Sbihi Y, Osuna A, Clavel A, Gomez-Lus R, 2001, Western blot applied to the diagnosis and post-treatment monitoring of human hydatidosis: Diagn Microbiol Infect Dis, 41, 139-142.
6. Eckert J, Deplazes P, Craig PS, Gemmell MA, Gottstein B, Heath D, Jenkins DJ, Kamiya M, Lightowers M, 2001, Echinococcosis in animals: clinical aspects, diagnosis and treatment. In: Eckert J, Gemmell MA, Meslin FX, Pawlowski ZS, editors. WHO/OIE Manual on Echinococcosis in Humans and Animals: a Public Health Problem of Global Concern. Paris, France: World Organization for Animal Health, pp. 72-79.
7. Gottstein B, 1984, An immunoassay for the detection of circulating antigens in human echinococcosis: Am J Trop Med Hyg, 33, 1185-1191.
8. Hernandez A, Muro A, Barrera I et al., 2008, Usefulness for different *Echinococcus granulosus* recombinant antigens for serodiagnosis of unilocular hydatid disease (UHD) and post surgical follow-up of patients treated for UHD: Clin Vaccine Immunol, 15(1), 147-53.
9. Kaddah MH, Maher KM, Hassanein HI et al., 1992, Evaluation of different immunodiagnostic techniques for diagnosis of hydatidosis in Egypt: J Egypt Soc Parasitol, 22(3), 653-65.
10. Ortona E, Riganò R, Buttari B, Delunardo F, Ioppolo S, Margutti P, Profumo E, Teggi A, Vaccari S, Siracusano A, 2003, An update on

- immunodiagnosis of cystic echinococcosis: *Acta Trop*,85,165–171.
11. Ortona E, Rigano R, Margutti P, Noralgiacono S, Ioppolo S, Vaccari S, Barca S, Buttari B, Profuma, Teggi A, Siracusano A,2000, Native and recombinant antigens in the immunodiagnosis of human cystic Echinococcosis: *Parasite Immunol*,22,553–559.
 12. Parija SC,1998, A review of some simple immunoassays in the serodiagnosis of cystic hydatid disease.:*Acta Trop*,70,17–24.
 13. Ravinder PT, Parija SC, Rao KS,1997, Evaluation of human hydatid disease before and after surgery and chemotherapy by demonstration of hydatid antigens and antibodies in serum: *J Med Microbiol*,47,859–864.
 14. Sadjjadi SM, Ardehali S, Noman-Pour B, Kumar V, Izadpanah A,2001,Diagnosis of cystic echinococcosis: imaging or countercurrent immuno-electrophoresis?: *East Mediterr Health J*,7,907–910.
 15. Sadjjadi SM,2006, Present situation of echinococcosis in the Middle East and Arabic North Africa: *Parasitol Int*,S197–S202.
 16. Sadjjadi SM ,Sedaghat F,Hosseini VH and Sarkari B,2009, Serum Antigen and Antibody Detection in Echinococcosis: Application in Serodiagnosis of Human Hydatidosis:*KJP*,47(2), 153–157.
 17. Sarkari B, Sadjjadi SM, Abidi H, Izadpanah A, Kazemian S, Rafati A,2007, Application of western blotting using native antigen B for serodiagnosis of human cystic echinococcosis: *Iran J Parasitol*,2,7–12.
 18. Siavashi MR, Taherkhani H, Rezaei KR, Razavi Deligani MR, Assmar M,2005, Comparison of dot-ELISA and sandwich ELISA diagnostic tests in detection of human hydatidosis: *Iran Biomed J*,9,91–94.
 19. Todorov T, Stojanov G,1979, Circulating antibodies in human echinococcosis before and after surgical treatment.: *Bull World Health Org*,57,751–758.
 20. Virginio V G, Hernandez A, Rott MB *et al.*,2003, A set of recombinant antigens from *Echinococcus granulosus* with potential for use in the immunodiagnosis of human cystic hydatid disease: *Clin Exp Immunol*: ,132,309- 15.
 21. Younis T,Fahmi I,Hala S E,Nihad M A,Ayman I,2008, Attempt to Improve the Diagnostic Efficacy of Enzyme-Linked Immunosorbent Technique for the Serodiagnosis of Cystic Echinococcosis:*PUJ*,129-136.

2/2/2013