Original Article

Serovalue of hydatid disease in Baghdad

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Summary:

Background: Echinococcus granulosus is a one major species of medical and public health importance which causes cystic echinococcosis.Hydatid disease is able to modulate antiparasite immune responses, persist and flourish in humans.
Objective: The main objective of this study is to diagnostic value for hydatidosis and to identify the prevalence in human in two different areas of Baghdad city which include urban and rural areas. Evaluating hydatid fluid antigen and hydatid cyst wall antigen for diagnosis of cystic echinococcosis(CE). preparation ELISA kit for detecting specific antibodies in patients and relative sera is considered as an important step in determining the recurrent case after surgical operation.
Patients and Methods: Fifty patients infected with hydatidosis , One-hundred and seven relatives' patients and Thirty patients' follow-up. NAJAT-enzyme-linked immunosorbent assay(ELISA) and NAJAT-latex test for the Immunodiagnosis of cystic echinococcosis of human hydatid disease by detecting the specific antibodies in patients.
Results: hydatidosis in females was the same as in the males. One-hundred and seven relatives' patients. In rural area the seroprevalence were 12 male cases and 4 female cases ,While in urban area 5male cases,5 female cases,respectively. Thirty patients' follow-up four patients developed recurrences at 3-7 months postoperatively.
Conclusion: In relatives' patients no statistically significant differences in the frequency between both sexes. But a highly statistically significant differences (p=0.05) were shown between hydatidosis infected persons in relation to area(urban area,rural area). There were no significant differences in the rates seropositive case for different occupations(p=0.05). NAJAT-enzyme-linked immunosorbent assay(ELISA) and NAJAT-latex test appeared to be a useful confirmatory tests with specific antigens represent good candidates for the Immunodiagnosis of cystic echinococcosis of human hydatid disease by detecting the specific antibodies in patients.
Keywords: Hydatid desease;serovalue; NAJAT- ELISA assay ;NAJAT-latex test;Baghdad

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Introduction:

Hydatidosis due to *Echinococcus granulosus* (E.g.) is an endemic zoonosis characterized by worldwide distribution particularly in Mediterranean countries(1). one of the most serious and life-threatening zoonoses in the world,is caused by the metacestode larval stage of *Echinococcus*(2). Echinococcosis,which is sometimes referred to as hydatid disease or echinococcal disease,is a disease that affects both humans and other mammals (3). Cystic echinococcosis is an important public health problem worldwide (4).
The hydatid cyst is normally well tolerated in humans until its development results in pressure on adjacent tissue or organs (6).The cysts are most commonly found in the liver and lungs,although they may also occur in other organs (7).

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Depending on the location of the cyst in the body ,the patient could be asymptomatic even Though the cysts have grown to very large size or be symptomatic even if the cysts are absolutely Tiny (8). The most commonly involved anatomical locations are the liver and lung. Occasionally the cyst may progressively increase in size,mimicking gross ascites or intrabdominal tumor(9).

Materials and Methods:

Fifty patients included in this study ,(25) females and (25) males,their age range from(4 – 67) years.Those patients were collected and studied from three teaching hospitals in Baghdad; AL-Kadhmiya teaching hospital,Medical city teaching hospital and AL-Yarmok teaching hospital. The diagnosis of each case was established by the radiological tests in addition to the clinical examination by the specialists. One-hundred and seven relatives of patients,(38) females and (69) males,their age range from(8 - 62) years.Those were selected for living in the same houses of the patients.All relatives were apperantly

healthy persons without previous history of hydatid disease. Blood samples were collecting from each of these relatives.Relapsed CE was defined as a recurrence of active CE following a period of quiescent disease as shown by the radiologic enlargement of the existing cysts and/or the development of new daughter cysts . Thirty patients (15 males and 15 females)were provided by retrospective post-surgical follow-up . The blood samples were analysed by ELISA for antibody (Ab) detection. Sera were obtained between three and seven months after surgery and the eating with anthelminthic drugs . Thirty blood samples were collected from post-surgical follow-up patients. Three to five ml of venous blood were collected from the individuals.The blood samples were transferred into caped plastic tubes and transported to the laboratory of AL-Nahrain college of medicine.Diagnosis of hydatid disease in humans is based on the detection of specific antibodies against antigens by using either crude antigen preparations for total hydatid fluid or the germinal layer of hydatid cyst(enriched in antigens 5 and B and glucoprotein).A Primary human pulmonary hydatid cyst from female (age 5 years) (right side of the lung) was 8 cm(length) and7 cm(width) in diameter.NAJAT-Enzyme immunoassay for detection of IgG antibodies to human hydatid disease active cyst (Fertile cyst) in serum or plasma was used. NAJAT-ELISA was performed essentially as described with slight modification (8). NAJAT-ELISA incorporating crude *E. granulosus* human hydatid cyst antigen is the main serologic test used to measure total CE-specific IgG antibody. Using cyst material from a human. Many antigens tend to bind to polystyrene.The wells of a microtitre tray are first filled with a dilute solution of the appropriate antigen and time is allowed for the antigen to bind to the polystyrene before any excess is washed away.NAJAT-ELISA standardization included determination of the optimal working dilution (and concentration) for the antigen, sera and anti-species immunoglobulins or conjugates using checkerboard titration from serial dilutions with known positive and negative serum samples. Dilution of the conjugate and antigen concentration at which positive and negative controls yielded the maximum optical density (OD) value were chosen for the subsequent testing of the samples.The reference serum samples used in this standardization were from humans confirmed to have heavy infection with CE and identified to have high antibody titre. In addition, negative control serum samples,essentially parasite free, were purchased from a supplier.A NAJAT-ELISA cut-off value for the antigen was based on the mean OD values of all serum samples from the negative control plus 2 standard deviations above the mean. The specificity and sensitivity of NAJAT- ELISA were calculated using this positive–negative cut-off value.Data above this threshold were considered positive and those below as negative. The optimized NAJAT- ELISA procedures were as follows (8). The NAJAT-ELISAs were all optimized by checkerboard titration using defined positive and negative sera and standard laboratory methods. Serum samples for the present study were collected from 30 surgically confirmed human cases with hydatidosis(15 males and 15 females),and from 30 clinically healthy subjects(15 males and 15 females) with no history with hydatidosis of living in endemic areas and free from parasitic infections. The OD cut-off for each of the five assays was determined as the mean+ 3SD of the control sera (n = 20). In these assays, 100 µL volumes were used throughout unless otherwise stated. microtiter plates (Sterilin limited,Feltham,England) were coated overnight at room temperature with centrifuged crude hydatid cyst fluid (diluted to 9 µg/mL). Plates were washed four times with phosphate-buffered saline, pH 7.2, 0.05% Tween (PBS-Tween) and the wells were then blocked with 1% V/V of bovine serum albumin PBS-Tween for 60 minutes at room temperature (11).The circulating antibody titer in the serum was estimated by performing a quantitative test. It was performed by testing serum at several dilutions.The highest dilution of the serum showing agglutination was the latex titer (12). Fifty microlitres PBS (pH 7.2) were distributed in a v-shaped microplate, apart from the second column.Tested sera, including positive and negative controls,were diluted in a twofold dilution series in PBS (1:8, 1:16,1:32 . . . 1:2048). Of each diluted serum sample 50 µl were added to microplate wells. To the second column, 100 µl of test sera diluted at 1:8 in PBS (pH 7.2) were added.Serial twofold dilution was made by transferring 50 µl serum to the next wells.Following these steps, 50 µl of 0.2% sensitized and coated latex particles were added to the respective wells and kept on the bench undisturbed. After incubation from 2 h to overnight at room temperature, the total antibody titre in the test serum was recorded as one dilution before that which yielded a clear, sharp, dark spot similar to those in the negative control wells. A sensitized and coated latex particles titre less than or equal to 32 was considered negative as determined by several pooled negative serum samples. Moreover, the sensitized and coated latex particles results were also evaluated with different sets of cut-off titres (1:16, 1:32, and 1:64) (8).

Results:

Fifty hydatid disease patients were included in this study 25 females and 25 males. Patients age between 24-33 years represent the highest percentage(32%, 16 patients)than the other younger or older ones.The percentage of the males infected with the hydatid

disease according to their residency is higher in urban than rural areas,17(68%)males in the urban area while it was 8(32%)males in the rural area .the females infected with the hydatid disease according to their residency is higher in urban than rural areas,15(60%)females in the urban area while it was 10(40%)females in the rural area . A high percent (64%) of patients with hydatid cyst lived in areas(urban)while A low percent (36%) of patients with hydatid cyst lived in areas (rural) . The patients were of different occupations living in urban and rural areas.fifty (50) surgically confirmed cases of hydatidosis patients.The number of males was 25 (50%) and of the females was 25 (50%). The aged of patients between 4 to 67 years were collected and studied from three teaching hospitals in Baghdad (28 patients at the AL-Kadhmiya teaching hospital,10 patients at the Medical city teaching hospital and 12 patients at the AL-Yarmok teaching hospital) during the period from July 2010 till March 2011.Details concerning these patients are presented in Table (4.1).One-hundred and seven relatives of patients,(38) females and (69) males,their age range from(6 - 65)years old.Those were selected according to living in the same houses of the patients. All relatives apperantly healthy persons without previous history of hydatid disease.The number of samples were obtained from relatives (107 blood samples).One-hundred and seven volunteer subjects aged 6 to 65 years were served as controls.their sex and aged are elucidated in Table (4.2).The number of females follow-up was 15(50%) and the number of males follow-up was 15 (50%) provided by retrospective post-surgical follow-up . patients follow-up were analysed by NAJAT-ELISA for Ab detection. The follow-up duration ranged from 3to 7months.Four patients developed recurrences at 3-7 months postoperatively.Although the recurrence rate was reduced significantly in the radical surgery group. The results showed that more males(13.3%) were found to be infected than females(0%).The ages of patients follow-up studied were between 4 and 50 years old.The majority of cases which were found to be recurrent 9(69.2%)were the age group between 4 and 25 years .Blood samples of patients follow-up were collected after the operation(post operative).The majority of samples 23 patients(76.66%)were collected three months after the operation, 6 patients (20%) were collected four to six months after the operation,while only one patient (3.33%) was collected seven months after the operation .Serological findings by NAJAT- ELISA From the absorbance values of the 30 serum samples from a non-infected humans, means and standard deviation (SD) were calculated. OD cut-off was calculated by addition of 2SD to mean of OD values of the 30 negative sera. Values above or equal this cut-off were recorded as positive and below as negative. Positive (30 positive serum samples) and negative (30 negative serum samples) serum controls samples were included in each plate. Samples were tested in duplicate and the experiments were repeated twice.Table(4.3) and Table(4.4).Serological findings by NAJAT- Latex titre less than or equal to1: 32 was considered negative as determined by several pooled negative serum samples.Moreover, the sensitized and coated latex particles results were also evaluated at cut-off point titre 1:64 as determined by pooled negative sera.

Discussion:

The present work is one of the first studies in the world to evaluate the antigenic ability of crude hydatid cyst fluid and crude hydatid cyst wall collected from infected human and for the diagnosis of hydatidosis in human using NAJAT-enzyme-linked immunosorbent assay(ELISA) and NAJAT Latex test. The source of the antigenic fractions to identify the diagnostic values was fertile crude cyst fluids and crude hydatid cyst wall collected from naturally infected human (female) at the Baghdad hospital. Sera samples from surgically confirmed hydatidosis cases were used for identifying antigenic determinants in hydatid cyst fluid and hydatid cyst wall which are shared with control sera samples (patients and clinically healthy individuals).In the present study, NAJAT- ELISA was used that incorporated crude E. granulosus hydatid cyst fluid rather than purified antigen (antigen B or antigen 5) as used in previous studies (11). Our rationale was that the broad diversity of antigens present in crude hydatid cyst fluid might enhance detection of subclass antibodies with different antigen specificities. Using crude hydatid cyst fluid in the NAJAT- ELISAs, background OD values among the negative controls were high. Furthermore, a wide range of ODs was observed in all five assays used and were in general higher .The sensitivity,specificity,for NAJAT- ELISA were 100%,100% respectively and for NAJAT-latex test were 100%,100% respectively.These results showed that although both NAJAT- ELISA and NAJAT-latex test had similar results,but NAJAT-ELISA was a better diagnostic performance test for initial screening of suspected cases of human hydatidosis and was more acceptable due to it is higher sensitivity, specificity.using antigens from human hydatid cyst in NAJAT-ELISA. These are higher sensitivity and specificity values than the present findings. According to sensitivity, specificity and efficiency of NAJAT-latex test and NAJAT- ELISA were high and hydatidosis was seen in 30 patients . The diagnostic sensitivity and specificity of serological tests may also be related to technical factors, such as the source and quality of both crude hydatid cyst fluid antigen and crude hydatid cyst wall antigen, and laboratory techniques being used. In this study NAJAT- ELISA showed 0% ‘false-negative’ and 0% ‘false-positive’ values (30 patients and 30 healthy controls), while the corresponding rates in

NAJAT-latex test were 0% and 0%, respectively. The seropositive patients in urban area were significantly higher(64%) than rural area(36%);this can be explained by many factors that can play important role in the spreading hydatidosis in that urban area like: In Baghdad, hydatidosis is endemic,with the magnitude of records ranging from medium tohigh rates of occurrence. Serological tests were done for 107 serum samples obtained from relatives of hydatidosis patients.The results of NAJAT- ELISA and NAJAT-latex test were In rural area the seroprevalence were 12 male cases and 4 female cases ,While in urban area 5male cases,5 female cases, respectively; this can be explained by many factors that can play important role in the spreading hydatidosis in that rural area like: poor hygienic,lower in come,limited education,live stock owner ship,home slaughter house and stray dogs. There were no statistically significant differences in the frequency between both sexes (p=0.05)this may give no relation or independent between gander (male,female) and serum (positive,negative). But a highly statistically significant differences (p=0.05) were shown between hydatidosis infected persons(serum positive,serum negative) in relation to area(urban area,rural area).On the other hand the Chi-square revealed no statistically significant difference in the frequency between ages (p=0.05)this may give no relation or independent between gander (male,female) and serum (positive,negative) in rural area.Also chi-square revealed no statistically significant differences (p=0.05) were shown between ages and serum (serum positive,serum negative) in urban area. The detailed clinical and serologic follow-up of these patients longitudinally over a 3–7 months period provided the opportunity to correlate IgG subclass antibody responses with sur outcome, whereas previous studies ha sectional in design. We found that a NAJAT-ELISA OD was significantly greater proportion of patients with the IgG2 IgG3and IgG4) assay .Postoper was carried out by NAJAT- ELISA te latex test in order for there not to be a to whether there had been any rec disease in the future,all patients under were started at the end of the thre months.The follow-up period was c patients at 7 months.During this per suspected of recurrence were ex frequently.Recurrence is one of the ma the management of pulmonary Recurrence disease is defined as the new active cysts after therapy extrapulmonary disease.The failure permanent control of the primary considered to be the cause of local re recurrence occurs after surgical c intervention and manifests as the reapp cysts at the site of a previously trea appearance of new extrapulmonary d from procedure-related spillage.The recurrence after lung cyst hydatid surg to 27.27% in new study.In our stu occurred in none of the patients trea surgery .In our own study,four ca recurrence developed were treated wi surgery.Recurrence may occur mar ,however,and longer follow-up is reco possible.

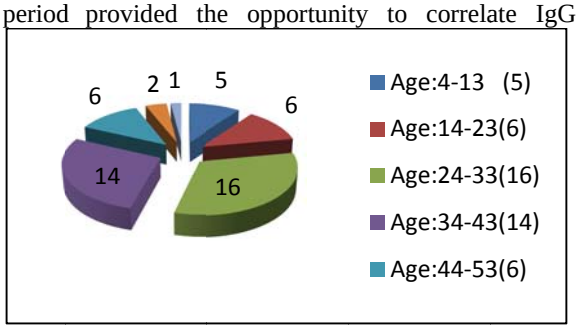

Figure (1-1): The age distribution between the patients with hydatid disease.

Table (1-1): sex and cyst location of patients with hydatid disease.

Sex	Spinal cord	heart	kidney	ovary		liver			lung			muscles under skin	
				R.	L.	R.	L.	R.&L.	R.	L.	R.&L.	R.lower limb	R.&L.lo limb
F.	1	0	1	1	1	13	0	1	4	1	0	2	0
M.	0	1	0	0	0	6	2	0	7	5	1	1	1
T.	1	1	1	1	1	19	2	1	11	6	1	3	

Table (1-2): Sex distribution and number of recurrent case in 30 patients follow-up with hydati different hospitals in Baghdad.

Sex	Number of patients follow-up	Recurrence	Recurrence r
Females(F) follow-up	15	0	0%
Males(M) follow-up	15	4	13.3%
Total	30	4	13.3%