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Epidemiological and immunological findings on human hydatidosis

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30 patients infected with hydatidosis from different hospitals in Baghdad were included in this study. All these patients were diagnosed by imaging techniques and surgically proven, immunological study were performed on blood samples. The study showed that the incidence of hydatidosis in females was higher than that of the males. The highest age distribution of hydatidosis patients was between 12 and 40 years. The percentage of liver hydatidosis was higher (64.66%) than any other organs. The results of IgG, IgM, C3 and C4 determination were significantly increased for IgG concentration in males compared with females and highly increased in males at age group 10 - 20 years. There was significant difference in IgM concentration which was significantly increased in females especially at fourth age group comparative with significant decrease in IgM concentration in males at different ages. Also, there was significant increase in C3 and C4 concentrations in females compared with males.

Key words: Hydatidosis, immunology, epidemiology.

INTRODUCTION

Hydatidosis till now is considered as major public health problem in the world. Immunological relationship between immune host reaction against the parasite and the inhibition of host defense by the parasite (McManus et al., 2003).

Compared with events occurring during early infection, the immune response to establish cysts has received much more attention (Daeki et al., 2000). Despite years of research and useful development there is no any standard found that is highly sensitive and specific sero-logic test for cystic echinococcosis antibody detection. The most abundant detectable antibodies in the serum of patients are of IgG, variable IgM and IgE (including specific IgE against hydatid cyst) levels (Safioleas et al., 2005).

The aim of this study is to determine the levels of immunoglobulins IgG and IgM and components of complements: C3 and C4 and the study of relationship between the demographical parameters with these elements.

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MATERIALS AND METHODS

Hydatid cyst patients

Patients for this study were selected from 4 hospitals in Baghdad city namely Baghdad teaching hospital, Al-Shaheed Adnan teaching hospital, liver and digestive disease teaching hospital and Ibn - Al-Nafees teaching hospital from January - July 2009.

Patients clinically diagnosed and surgically confirmed with cysts in the liver, lung, spleen and ovary. The viability of hydatid protoscolices was determined by viable stain (5% aqueous eosin) red protoscolices: dead and green protoscolices: live.

Patient age groups

Hydatidosis patients were subdivided into 4 groups: Group 1 between 10 - 20 years, group 2 between 20 - 30 years, group 3 between 30 - 40 years and group 4 > 40 years.

Blood samples collection

Blood sample collected was transferred immediately into plain plastic tubes and the serum was obtained by (cooling centrifuge), centrifugation at 4° , 4000 rpm/15 min. The serum was dispensed in plastic appendrof tubes, 0.5 ml in each tube and stored in (-20°) until used for serological testing.

Table 1. Distribution of hydatidosis patients according to age and sex of 30 patients.

Age(years)	Males No. (% Total)	Females No. (% Total)	Total No. (% Total)
10 - 20	4 (40%)	6 (60%)	10 (100%)
20 - 30	1 (11.11%)	8 (88.88%)	9 (100%)
30 - 40	3 (37.5%)	5 (62.5%)	8 (100%)
> 40	0	3 (100%)	3 (100%)
Total	8 (26.66%)	22 (73.33%)	30 (100%)

Table 2. Sex distribution of 30 patients infected with hydatid disease.

Females	Males	Ratio F : M	Total number
22 (73.33%)	8 (26.66%)	2.75 : 1	30

Immunological study

Immuno-precipitation kits were used in this study according to the manufacturer recommendations supplied by (Bussero, Milano / Italy):

Agarose gel containing monoclonal IgG antisera
Agarose gel containing monoclonal IgM antisera
Agarose gel containing monoclonal C3 antisera
Agarose gel containing monoclonal C4 antisera

By removing the plates from their envelopes and the were leave to stand at room temperature for few minutes so that any condensed water in the wells will evaporate, the wells was fill with 5 µl of sample (patient's serum) and wait for 5 min to be completely adsorbing, before handling the plates, it was close and put in moist champer, after that the plate was incubate the for 72 h in incubator at 37° (Woollard et al., 2000) and reading plates by measuring the diameter of the precipitating ring and correspond it with concentration values in enclosed references tables (WHO reading, mg/dl) .

RESULTS AND DISCUSSION

Epidemiology of hydatid disease

Hydatid disease is characterized by cystic space - occupying lesions in the liver, lungs and rarely in other parts of the body (Lone et al., 2002). All evidences provided ensure that hydatid disease till now major health problem in Iraq in spite of modern equipments available for diagnosis and treatment. The surgically confirmed cases are the only reliable source of data on human hydatidosis, since hydatid infection is a notifiable disease, and its difficult to determine the specific source of infection and its usually impossible to know when the infection was acquired this may be due to the fact that cysts are usually slowly growing and the development of symptoms or the ability to diagnose the conditions may require from 6 months to several years after exposure to the infections (Dziri, 2001) .

Distribution of disease according to age

The ages of patients in present study varied between 12 - 57 years, the maximum incidence recorded was among patients between 12 - 40 year, mean was 22 (73.33%) showed in Table 1 also reported by Yang et al. (2006) while, Al-Sanafi and Farjou (2001) and Mongha et al. (2008) showed high rate of infection was between 20 - 30 years. Also this study showed that cases less than 10 years of age are rare, this may be due to variation of interval times that required for hydatid disease to manifest clinically.

Distribution of disease according to sex

The present study showed that the predominance of hydatidosis was in females 22 (73.33%) than in males 8 (26.66%) Table 2, in rate of infection female : male 2.75:1. Highly infection rate in females is in agreement with most of other studies which have shown a high frequency in females (Abdul-Karim, 2001; Al-Qadhi, 2005). The highest risk group in our country specifically and in Arab Gulf region - in general- are women and children. Traditionally, rural women still bear the biggest burden of tending animals - whether breeding, milking, or wool -shearing- and domestic or stray dogs are never faraway. The added chore of women preparing and cooking contaminated food and vegetables with little clean water at hand increases considerably the risk of infection. In many parts of middle east during springtime, it is common practice together berries and various wild plants which are eaten unwashed and geophagia among children and pregnant women is well known.

Not surprisingly, infection rates among women are shown to be the highest and children who acquire the disease in early life may not present with symptoms until adulthood (Nakao, 2007). Also estrogen hormone plays an important role in dissolve egg shells and facilitating hatched oncosphere to penetrate host tissues in females of mice (Brunetti et al., 2005). However this result doesn't agree with the findings of Torgerson et al. (2003) who observed high rates of infection in males. From this findings we cannot draw a conclusion on human infected with hydatidosis because of sample size is not large enough (Safioleas et al., 2005).

Table 3. Distribution of hydatidosis patients according to site of infection and sex.

Site of infection	Males No. (%+ve)	Females No. (%+ve)	Total No. (%+ve)
Liver	4 (13.33%)	10 (33.34%)	14 (46.67%)
Lung	4 (13.33%)	3 (10%)	7 (23.33)
Ovary	0	6 (20%)	6 (20%)
Multi organs	0	3 (10%)	3 (10%)
Total	8 (26.66%)	22(73.34%)	30(100%)

Table 4. Explain the effect of age on fertility of hydatid cysts.

Group	Male (age group)				Female (age group)				
Type of hydatid cyst	1 (No. %)	2 (No. %)	3 (No. %)	4 (No. %)	1 (No. %)	2 (No. %)	3 (No. %)	4 (No. %)	Total %
Sterile	1 9.09%	0	2 18.18%	1 9.09%	0	6 54.54%	1 9.09%	0	11 36.66%
Fertile	2 10.52%	1 5.26%	0	1 5.26%	1 5.26%	4 21.05%	4 21.05%	6 31.57%	19 63.33%
Total	3 10%	1 3.33%	1 3.33%	2 6.66%	1 3.33%	10 33.33%	5 16.66%	6 20%	30 100%

Distribution of disease in various organs

The liver act as the first filter for larval infection and the lung acts as the second filter. Distribution of infection in different organs showed that the liver was the most freq-ently involved (64.66%) when compared ith ung 7(23.33%) and ovary 6 (20%) and other multiple infected organs such as spleen, peritoneum, bone and brain showed in Table 3, generally these proportions approxi-mately in agreement with most of previously recorded data by Ahmadi and Al-Dalimi (2006) and others.

Fertility of hydatid cysts

Fertile hydatid cysts are formed in intermediate hosts (human and herbivores) producing protoscolices, the infective form to canines, at their germinal layers. Infertile cysts are also formed, but they are unable to produce protoscolices. The molecular mechanisms involved in hydatid cysts fertility/infertility are unknown. Neverthe-less, previous work has suggested that apoptosis is involved in hydatid cyst infertility and death. On the other hand, fertile hydatid cysts can resist oxidative damage due to reactive oxygen and nitrogen species. On these foundations, we have hypothesized that when oxidative damage of DNA in the germinal layers exceeds the capability of DNA repair mechanisms, apoptosis is triggered and hydatid cysts infertility occurs. Fertility of hydatid cyst is important factor in stimulation of immune response in patients with hydatidosis as observed in case of increasing of IgG and IgM concentrations when there's daughter vesicles within cyst and this factor directly proportional with advanced ages showed in Table 4 as we found there was significance difference at level ($P < 0.05$) between 2^{nd} and 4^{th} age groups.

IMMUNOLOGICAL STUDY

Measuring of Immunoglobulins IgG, IgM and components of complement C3 and C4

Echinococcus infections are among the more dangerous helminthic diseases in human. This disease is usually diagnosed by clinical examinations using different imaging techniques, which are supported by the demon-stration of specific serum antibodies. The serological diagnosis is a routine laboratory test depends mainly on the detection of immunoglobulin class G (IgG) antibodies directed against different antigens of *E. granulosus* (Bardonnet et al., 2003). In this study sera of 30 patients with hydatidosis were taken and the results of analysis of immunoglobulins IgG and IgM and components of com-plement C3 and C4 concentrations explained in Table 5. Statistically highly significant difference ($P < 0.001$) to the IgG concentration in 1^{st} age group of the males compared with another age groups observed in Table 6 and evi-denced by Figure 1) as we see significant increase in IgG concentration among age group between (10 - 20) years and this result supported by Carmena et al. (2006) that found greatly increasing in IgG concentration through analysis sera of infected younger ages of males with hydatidosis in study included 560 patients with

Table 5. Means of immunoglobulins IgG and IgM and components of complement C3 and C4 mg/dl in sera of patients with hydatidosis in different age groups.

Age groups / Means	IgG (mg/dl)	IgM (mg/dl)	C3 (mg/dl)	C4 (mg/dl)
1 (10 - 20)	305	170.62	114.8	19.43
2 (20 - 30)	154.22	193.62	140.3	27.96
3 (30 - 40)	146.5	174.63	148.52	29.41
4 (more than 40)	116	204.3	133.2	21.06

P < 0.001

Table 6. Mean concentration of IgG mg/dl in sera of patients with hydatidosis with statistically comparison of various age groups.

Age (I)	Age (II)	Mean	Standard error	Significant
1.00	2.00	150.77*	24.41	0.000
	3.00	158.50*	25.20	0.000
	4.00	189.00*	34.97	0.000
2.00	1.00	-150.77*	24.41	0.000
	3.00	7.72	25.81	0.768
	4.00	38.22	35.42	0.292
3.00	1.00	-158.50*	25.20	0.000
	2.00	-7.72	25.81	0.768
	4.00	-30.50	35.97	0.405
4.00	1.00	-189.00*	31.97	0.000
	2.00	-38.22	35.42	0.292
	3.00	-30.50	35.97	0.405

P < 0.05

hydatidosis in case-control study at 2005 -2006. While didn't see any considerable difference between second and third age groups, also there isn't any significant difference to the IgG concentration among female at all age groups (P < 0.001).

Also, IgM concentration measured in this study appear to be significantly difference among 2nd age group of males compared with 1st and 3rd groups (P < 0.001) Table 7, there was an increase in IgM concentration in second age group (20 - 30) years, also there was highly increase in IgM concentration in ages between (40 > more) as evidenced by Figures 2 and 3. There was a significant difference (P < 0.05) in the IgM concentration. Between 1st age group in both sexes, highly increasing in IgM in case of female hydatidosis, female liver hydatidosis (m ± s.e.m 212.36 ± 21.38), lung hydatidosis (m ± s.e.m 125. 30±45.31), in many cases of lung hydatidosis show high levels of IgM which related with recently infections or cysts with many daughter cysts, ovarian hydatidosis (m ± s.e.m 227.90 ± 23.41) in compared with male liver and

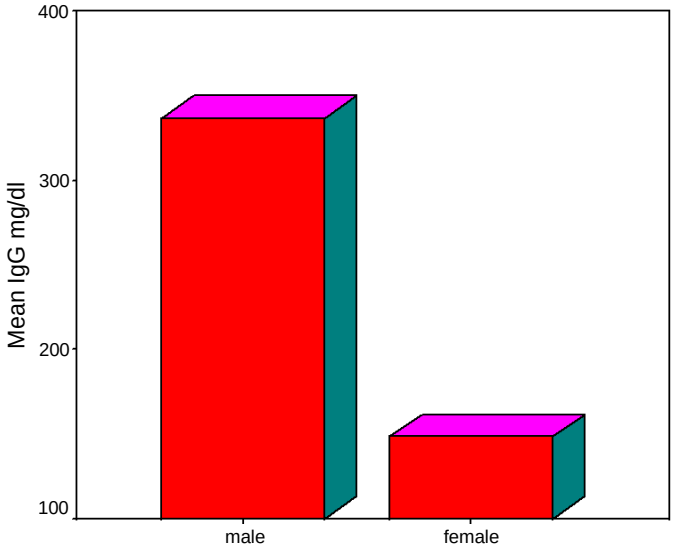

Figure 1. Explain the effect of sex on IgG in patient with hydatidosis

Table 7. Mean concentration of IgM mg/dl in sera of patients with hydatidosis with statistically comparison of various age groups.

Age (I)	Age (II)	Mean	Standard error	Significant
1.00	2.00	-23.00	35.37	0.522
	3.00	-3.42	36.51	0.926
	4.00	-69.01	50.67	0.186
2.00	1.00	23.00	35.37	0.522
	3.00	19.58	37.40	0.606
	4.00	-46.01	51.32	0.379
3.00	1.00	3.42	36.51	0.926
	2.00	-19.58	37.40	0.906
	4.00	-65.60	52.11	0.221
4.00	1.00	69.01	50.67	0.186
	2.00	46.01	51.32	0.379
	3.00	65.60	52.11	0.221

P < 0.05

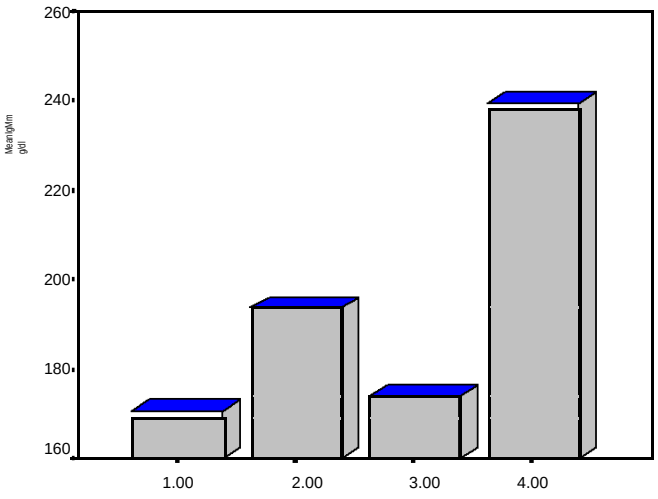

Figure 2. Explain the effect of age on lgM in patient with hydatidosis.

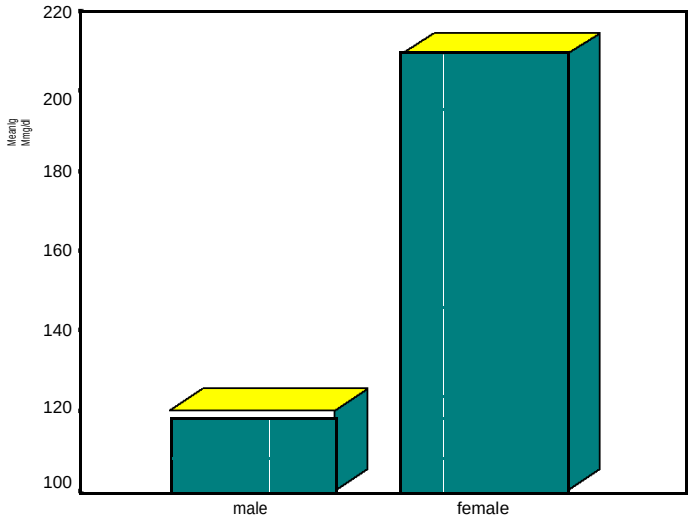

Figure 3. Explain the effect of sex on lgM in patient with hydatidosis.

lung hydatidosis (m ± s.e.m 146.87 ± 50.91) (m ± s.e.m 93.05 ± 20.90) respectively, other ages don't have significant differences also all female age groups. For instance, few cases of human lung hydatidosis tend to be associated with lower serum antibody levels or not detected in others this also reported by (Unsal et al., 2001). The immunological mechanisms underlying undetectable or absent humoral response remain undefined. Among the possible causes of negative serological response are the number, site, integrity and morphology of hydatid cyst, high concentration of circulating immune complexes in hydatid disease, has been documented by previous work (Pavlov et al., 2006). Thus rendering antibodies unavailable for detection, also the possibility of antigen induced specific immunological tolerance has also been raised. Such complexes in the serum of hydatid cyst patients may cause false negative reactions in serological tests with clinically and surgically confirmed disease. This result may be due to the fact, that the immune response in large cyst is weak or completely absent because it has a thick fibrous capsule, which may prevent the release of antigens (Petrov et al., 2001).

Elements of complement system C3 and C4, there's significant differences at level (P < 0.05) in concentration of C3 between male 1st age group (10 - 20) years old with

Table 8. Mean concentration of C3 mg/dl in sera of patients with hydatidosis with statistically comparison of various age groups.

Age (I)	Age (II)	Mean	Standard error	Significant
1.00	2.00	-25.50	21.28	0.243
	3.00	-33.72	21.97	0.138
	400	-18.40	30.49	0.552
2.00	1.00	25.50	21.28	0.243
	3.00	-8.22	22.51	0.718
	4.00	7.10	30.88	0.820
3.00	1.00	33.72	21.97	0.138
	2.00	8.22	22.51	0.718
	4.00	15.32	31.36	0.630
4.00	1.00	18.40	30.46	0.552
	2.00	-7.10	30.88	0.820
	3.00	-15.32	31.36	0.630

P < 0.05

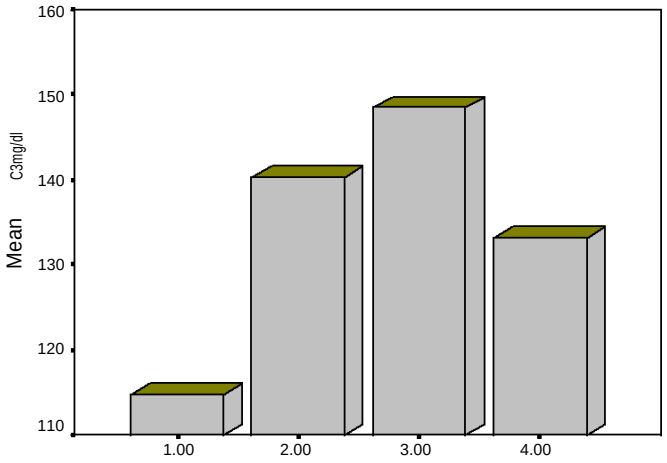

Figure 4. Explain the effect of age on C3 in patient with hydatidosis.

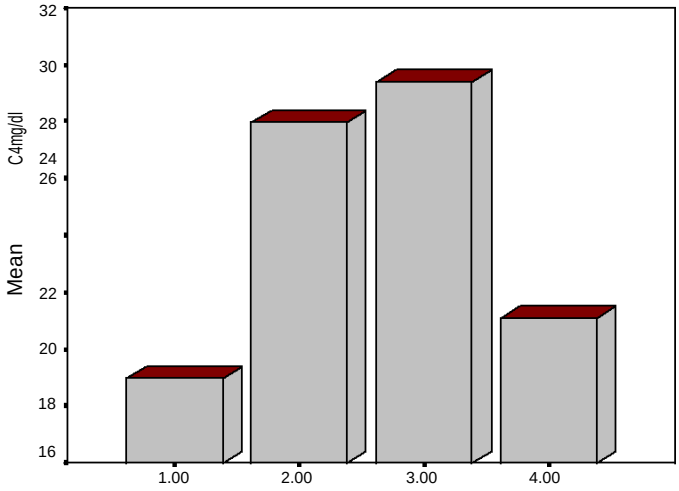

Figure 6. Explain the effect of age on C4 in patient with hydatidosis.

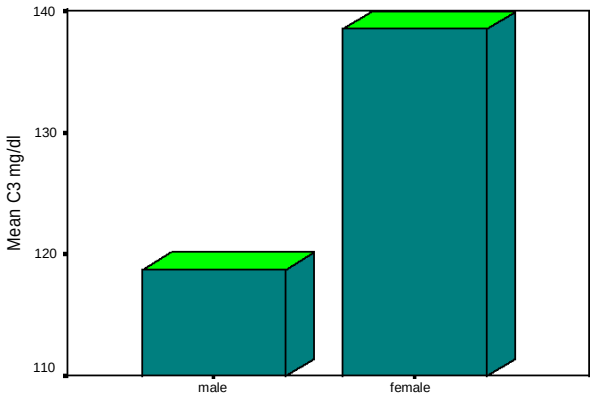

Figure 5. Explain the effect of sex on C3 in patient with hydatidosis.

2nd (20 - 30) and 3rd (30 - 40) groups shown in Table 8 and evidenced by Figure 4, also there was significant importance at level (P < 0.05) to the C3 concentration to the 1st group to both sexes. No significance relation to C3 concentration among female age groups, increasing in concentration of C3 in female is explained in Figure 5).

Results obtained in this experiment indicate to significant difference of C4 concentration at level (P < 0.05) to the 1st and 3rd age groups of males and 1st group to both sexes Table 9, that is, there was increasing of C4 in ages between (30 - 40) years old Figure 6 and any significance is not observe in concentration between female age groups and in case of age sex interference, increasing in concentration of C4 in female was explained in Figure 7.

Table 9. Mean concentration of C4 mg/dl in sera of patients with hydatidosis with statistically comparison of various age groups.

Age (I)	Age (II)	Mean	Standard error	Significant
1.00	2.00	-8.97	5.62	0.124
	3.00	-10.42	5.80	0.086
	4.00	-2.07	8.05	0.799
2.00	1.00	8.97	5.62	0.124
	3.00	-1.44	5.94	0.810
	4.00	6.90	8.15	0.406
3.00	1.00	10.42	5.80	0.086
	2.00	1.44	5.94	0.810
	4.00	8.34	8.28	0.324
4.00	1.00	2.07	8.05	0.799
	2.00	-6.90	8.15	0.406
	3.00	-8.34	8.28	0.324

P < 0.05.

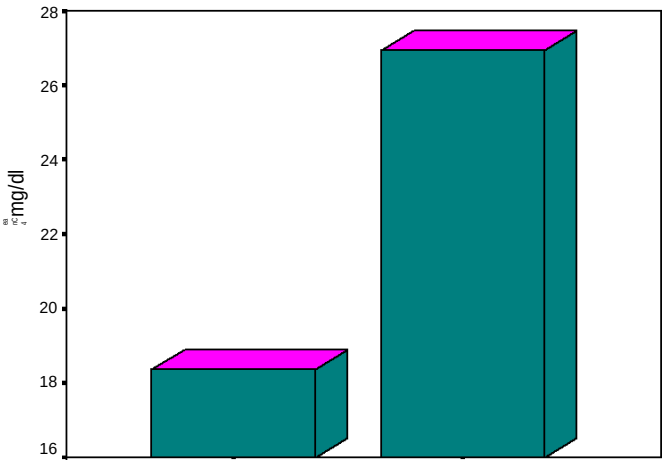

Figure 7. Explain the effect of sex on C4 in patient with hydatidosis.

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