

Full Length Research paper

Epidemiological and immunological findings on human hydatidosis

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Thirty 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 was 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: Patients, hydatidosis, concentrations, infected.

INTRODUCTION

Hydatidosis till now is considering 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 doesn't found any standard, highly sensitive and specific serologic 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 study of relationship between the demographical parameters

with these elements.

MATERIALS AND METHODS

Hydatid cyst patients

Patients for this study were selected from four 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 four groups:

1. Group 1 between 10 - 20 years.
2. Group 2 between 20 - 30 years.
3. Group 3 between 30 - 40 years.
4. Group 4 > 40 years.

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

Age (years)	Males	Females	Total
	No. (% Total)	No. (% 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

Blood samples collection

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

Immunological study

Immunoprecipitation kits were used in this study according to the manufacturer recommendations:

- (i) Agarose gel containing monoclonal IgG antisera,
- (ii) Agarose gel containing monoclonal IgM antisera,
- (iii) Agarose gel containing monoclonal C3 antisera,
- (iv) Agarose gel containing monoclonal C4 antisera.

By removing the plates from their envelopes and leave them to stand at room temperature for few minutes so that any condensed water in the wells will evaporate, fill the wells with 5 µl of sample (patient's serum) and wait for 5 min. to be completely adsorbing, before handling the plates close them and put in moist chamber, after that incubate the plates for 72 h. in incubator at 37°C (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 it is 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%) as shown 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 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 shows the rate of infection of 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 the country is specifically in Arab Gulf region -in general- which 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 does not agree with the findings of Torgerson et al. (2003) who observed high rates of infection in males. From this findings they cannot draw a conclusion on human infected with hydatidosis because of sample size is not large enough (Safioleas et al., 2005).

Distribution of disease in various organs

The liver act as the first filter for larval infection and the

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

Site of infection	Males	Females	Total
	No. (%+ve)	No. (%+ve)	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.

Total	Fertile	Sterile	Type of hydatid cyst (No. %)	
3(10)	2(10.52)	1(9.09)	1	
1(3.33)	1(5.26)	0	2	Male
2(6.66)	0	2(18.18)	3	(age group)
2(6.66)	1(5.26)	1(9.09)	4	
(3.33)	1(5.26)	0	1	
10(33.33)	4(21.0)	6(54.54)	2	Female
5(16.66)	4(21.0)	19.09	3	(age group)
6(20)	6(31.57)	0	4	
30(100)	19(63.33)	11(36.66)	Total%	

lung acts as the second filter. Distribution of infection in different organs showed that the liver was the most frequently involved (64.66%) when compared with lung 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 approximately 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. Nevertheless, 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, they have hypothesized that when oxidative damage of DNA in the germinal layers exceeds the capability of DNA repair mechanisms, apoptosis is triggered

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	IgG	IgM	C3	C4
Means	mg/dl	mg/dl	mg/dl	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

$p < 0.001$

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 2nd and 4th 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 demonstration 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 complement C3 and C4 concentrations (con.) explained in Table 5. Statistically, highly significant difference ($p < 0.001$) to the IgG con. in 1st age group of the males compared with another age groups observed in Table 6 and evidenced by Figures 1 - 3 as we see significant increase in IgG con. among age group between (10 - 20) years and this result supported by Carmena et al. (2006) that found greatly increasing in IgG con. through analysis sera of infected younger ages of males with hydatidosis in study included 560 patients with hydatidosis in case-control study at 2005 - 2006. While they did not see any considerable difference between second and third age groups; also, there is not any significant difference to the IgG con. among female at all age groups ($p < 0.001$).

Also IgM con. also measured in this study, which appear significant difference among 2nd age group of males compared with 1st and 3rd groups ($p < 0.001$)

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	Sig.
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

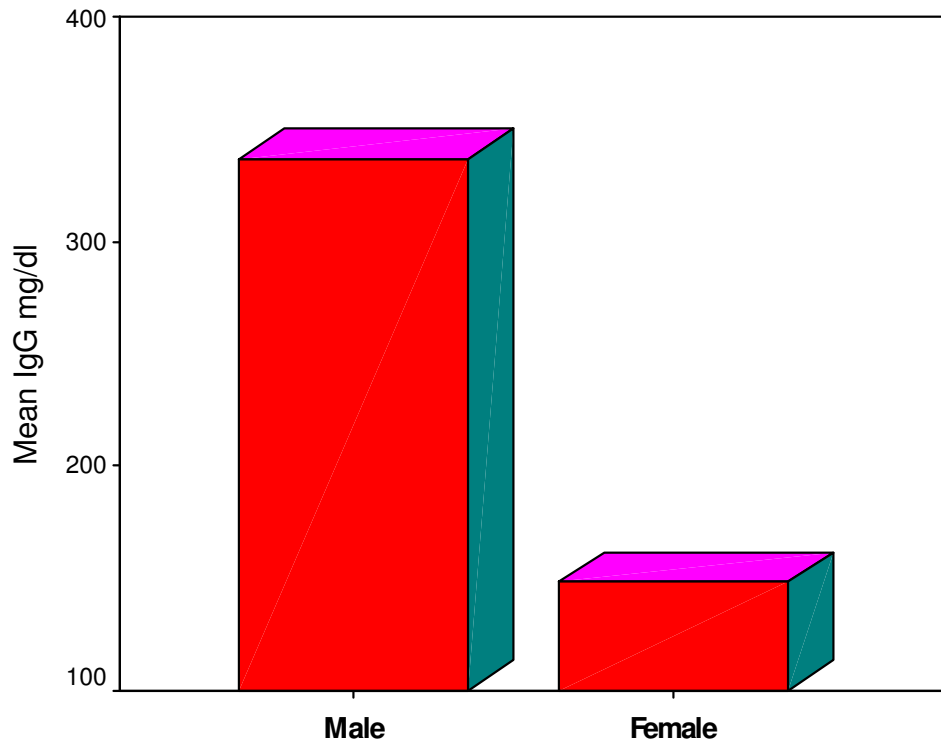


Figure 1. Explain the effect of sex on IgG in patients with hydatidosis.

Table 7, there was increasing in IgM con. in second age group (20 - 30) years also there was highly increasing in IgM con. in ages between (40 > more) as evidenced by Figure 3 - 3 and 3 - 4. There were significant differences ($P < 0.05$) in the IgM con. Between 1st age group in both sexes, highly increasing in IgM in case of female hydatidosis, female liver hydatidosis ($m \pm S.E.M$ 212.36 \pm

21.38), lung hydatidosis ($m \pm S.E.M$ 125.30 \pm 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 ($m \pm S.E.M$ 227.90 \pm 23.41) in compared with male liver and lung hydatidosis ($m \pm S.E.M$ 146.87 \pm 50.91) ($m \pm S.E.M$ 93.05 \pm 20.90), respectively; other ages do not have significant

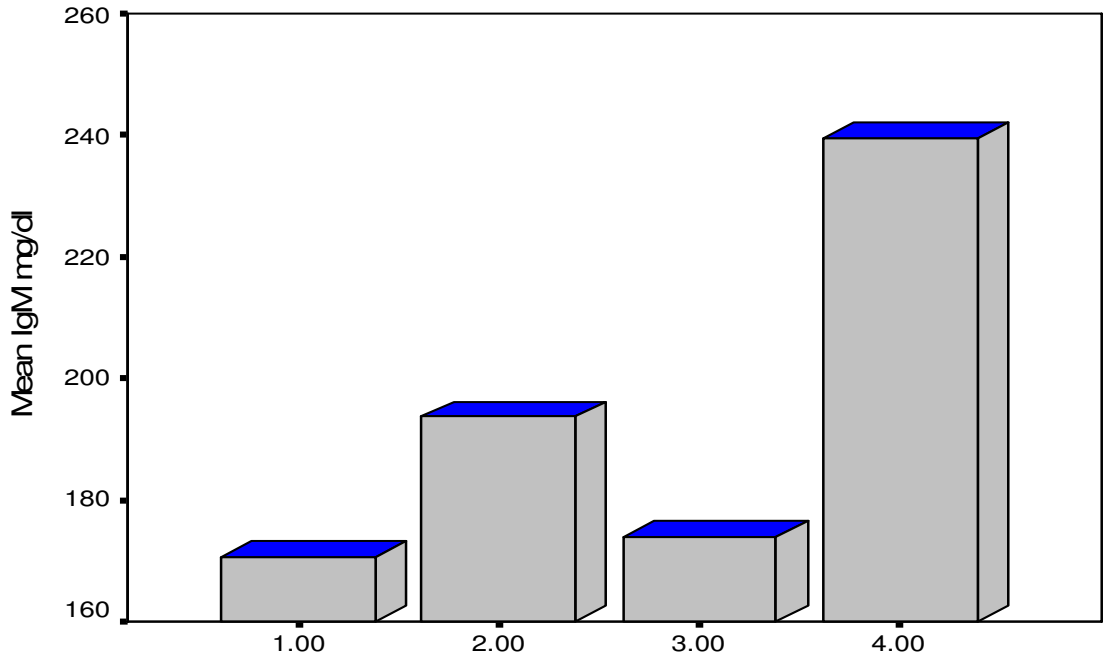


Figure 2. Explain the effect of age on in patients with hydatidosis.

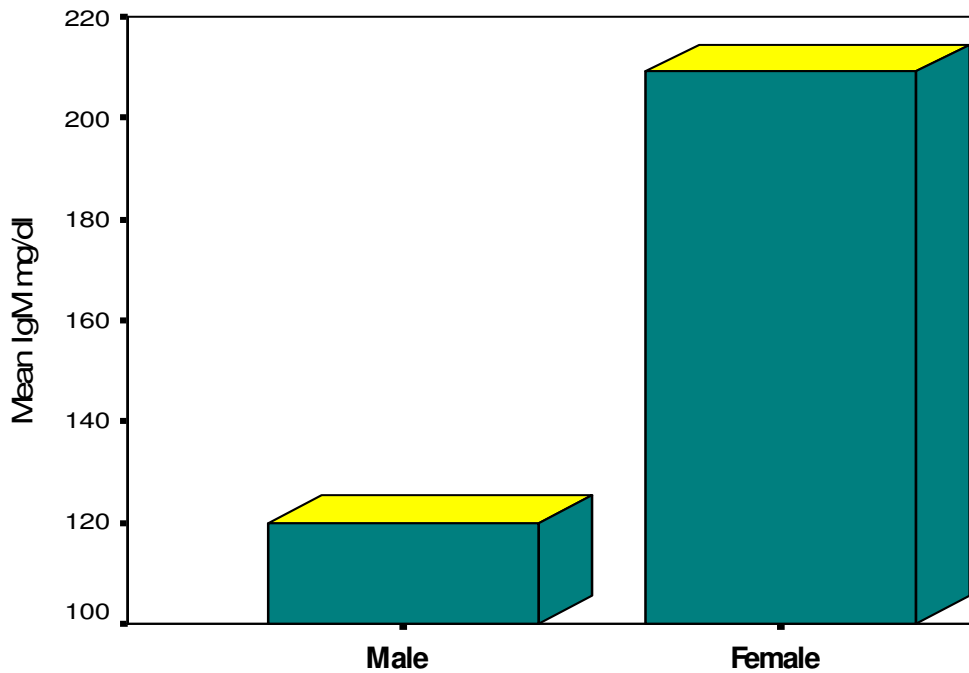


Figure 3. Explain the effect of sex on IgM in patients with hydatidosis.

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

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	Sig.
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

$p < 0.05$.

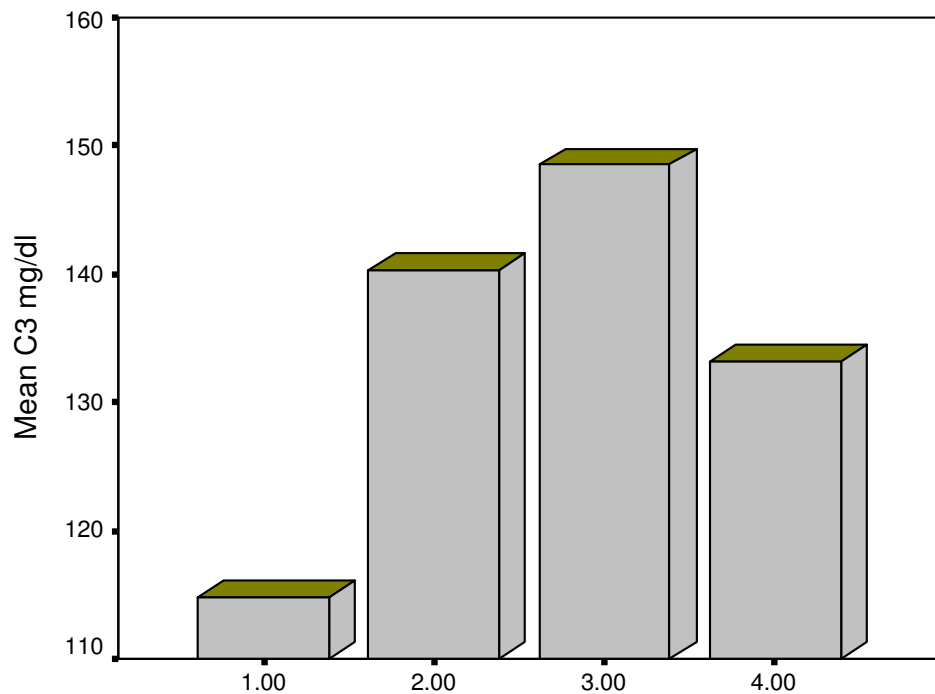


Figure 4. Explain of effect of age on C₃ in patients with hydatidosis.

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 con. of C3 between male 1st age group (10 - 20) years old with 2nd (20 - 30) and 3rd (30 - 40) groups shown in Table 8 and evidenced by Figure (3 - 5), also there was significant importance at level ($p < 0.05$) to the C3 con. to the 1st

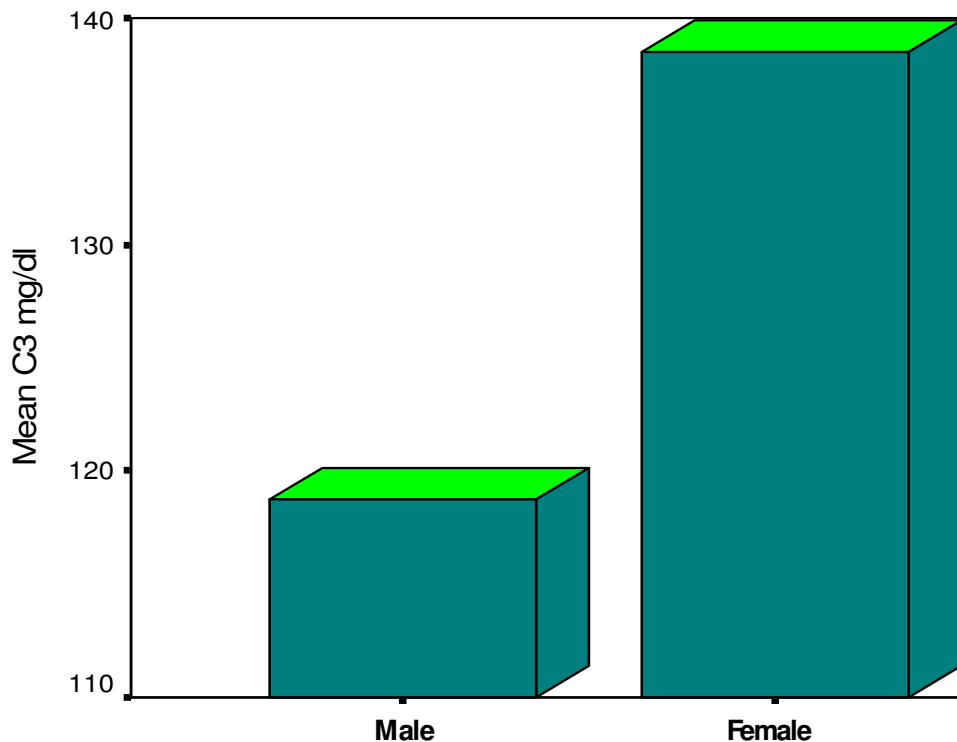


Figure 5. Explain the effect of sex on C₃ in patients with hydatidosis.

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	Sig.
1.00	2.00	-25.50	21.28	0.243
	3.00	-33.72	21.97	0.138
	4.00	-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

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	Sig.
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

group to both sexes. No significance relation to C3 con. among female age groups, increasing in concentration of C3 in female is explained in Figure 3 - 6. Results obtained in this experiment indicate to significant difference of C4 con. 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 3 - 7 and do not observe any significance importance in concentration between female age groups and in case of age sex interference, increasing in

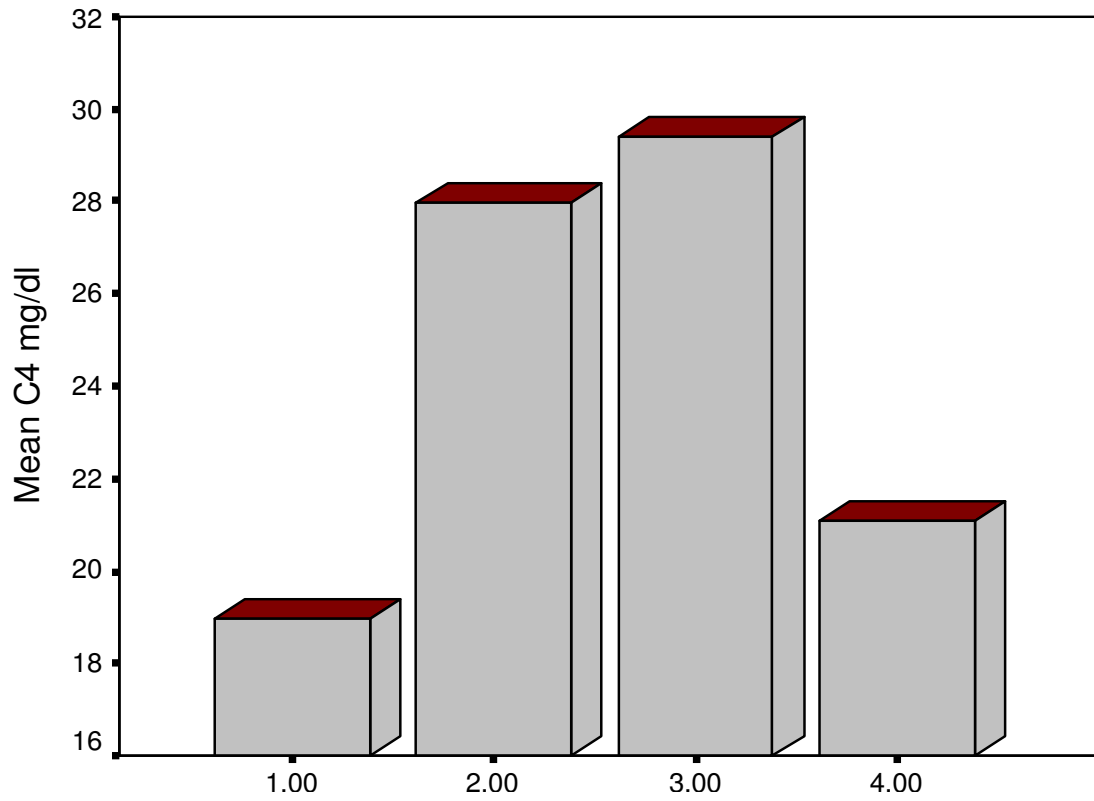


Figure 6. Explain the effect of age on C₄ in patients with hydatidosis.

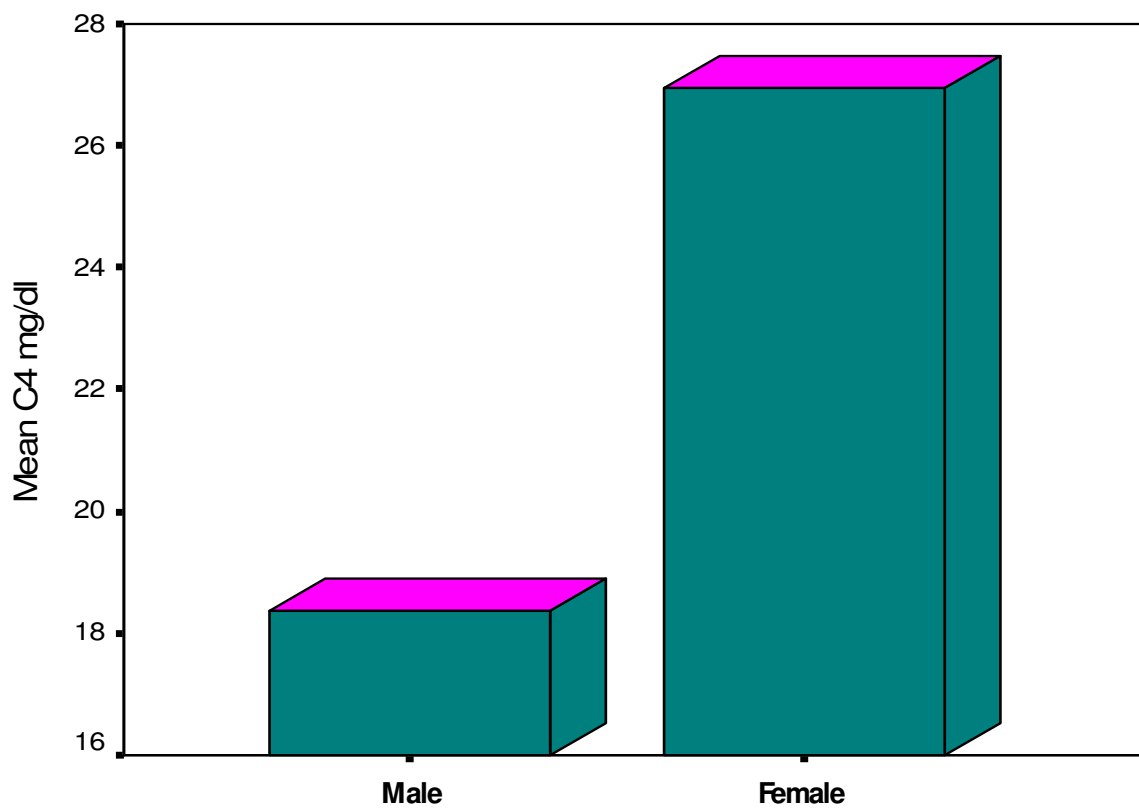


Figure 7. Explain the effect of sex on C₄ in patients with hydatidosis.

concentration of C4 in female was explained in Figure 3 - 7.

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