Effects of Hydatid cyst infection on some biochemical and haematological parameters in experimental mice Balb\c strain

Dr. Amal Kh. Khalaf.

Microbiology department, college of medicine , university of Thi-qar

Abstract:

Hydatid cyst disease is endemic infection in Iraq , so the aim of the present study is to determine the negative effects of disease on some haematological and biochemical parameters , the results found that the number of white blood cell has increase (16.37) and increased in the number of lymphocyte (68) and eosiphil (4) after counting the differential WBC of positive group while the number of red blood cell , hemoglobin, and packed cell volume has not affected with infection of hydatid cyst .

Blood urea and glucose (33.5 mg\ml and 88.33 mg\dl respectively) were decrease while aspartate aminotransferase (21.0 mg\dl) has increase after checking of biochemical parameters of infected group and compared with uninfected negative control group.

The present study has explained that the infection with hydatid disaese affect on the liver function after examination of some biochemical parameters that related to liver function.

Introduction:

Hydatid disease, hydatidosis, cystic echinococcosis, unilocular hydatid disease , *E. granulosus* Echinococcosis , and Al - akyas al-mai'yah' and 'al atash' (in Arabic) all terms describing infections which are caused by cestodes of the genus *Echinococcus* particularly *E. granulosus* (Dar and Alkarami , 1997 and Akhan *etal*., 2002).

The hydatid cyst remains a significant public health hazard in endemic areas such as Iraq , Turkey, the Middle East, South America, New Zealand , Africa , China, northern Kenya, Australia, and other sheep-raising areas (Tiaoying *etal*., 2005) . As an endemic disease, it causes social and economic losses for countries. WHO reports stated that approximately 100,000 people in the world are infected with this disease every year (Roming , 2003) which is common in rural populations of underdeveloped countries because of their close association with domestic and wild animals (Parija and Sheela, 1999) .

Effects of Hydatid cyst infection on biochemical and haematological parameters were checked in the world and in Iraq by Al – Nasiri (2006) ; Al- Mobarak (2006) ; Abdulla (2007) ; Moraitaki *etal*.(2010) and Al – Humairy (2010)

Materials and methods:

1. Parasite Materials & Protoscolices Preparation

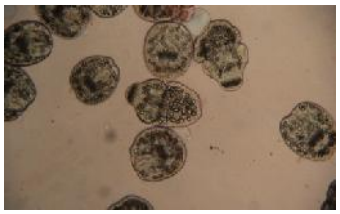
Fresh hydatid cysts were obtained from livers and lungs of naturally-infected sheep, which had been slaughtered at local abattoirs in Basrah city, human hydatid cysts were obtained from Al- Sadir teaching hospital in Basrah city. They were wrapped carefully in clean plastic bags, placed in an ice box, and transported to the Department of Biology, College of Education, Basrah University, where protoscolices were isolated according to Smyth (1964) method . Protoscolices were counted according to method cited by Al- Humairy (2010). The viable protoscolices were counted in 1ml based on the formula :

Viability in 1 ml = number of protoscolices in (10 µl) × 100

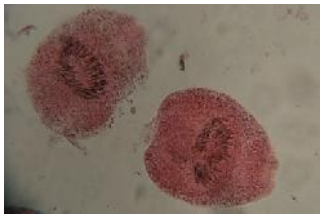
Eight male of *Mus musculus* mice Balb\C strain were injected with 0.2 ml 480/ ml (2400/5ml rate of viability) of protoscolices intraperitoneally and consider as positive group and left for six months. also, negative control group were included in the study and involve eight of uninfected male mice.

Hydatid cysts after removal from liver

Germinal layer containing protoscolices removed from hydatid cyst



Viable protoscolices



Non - viable protoscolices

2. The Study of the effect of infection on haematological and Biochemical Parameters

Blood samples were obtained from the heart of each animal after anesthesia using 1ml volume syringe. The samples were collected in two types of vials: 0.2 ml of blood was placed into a vial containing the ethylene diamine tetra acetic acid (EDTA) for the determination of hemoglobin (Hb) concentrations , total WBC count , RBC count , differential WBC count and packed cell volume (PCV). 0.8 ml of blood was placed in a vial without any anticoagulant for the determination of the aspartate aminotransferase (AST) , blood urea nitrogen (BUN) and serum glucose determination .

Blood parameters were counted by the use of the hematological analyzer system (coulter differential analyzer) which includes WBC, RBC, PCV , Hb and differential WBC count while the biochemical parameters were tested by the spectrophotometer using a suitable kit for each of BUN, the serum glucose concentration , and the aspartate aminotransferase based on Schalm *et al.*(1975) and Jain(1986) methods as follows:

Blood Urea Nitrogen Test (BUN)

BUN was used to determine the functional status of the kidney and it was measured by using a special kit (Biomerieux \ France) as follow :

Test Principle :

$$\text{Urea} + H_2O \xrightarrow{\text{urease}} 2NH_3 + CO_2$$

$$NH_4 + \text{Salycilate} + NaClO \xrightarrow{\text{Nitroprusside}} \text{indophenol} + NaCl$$

Test Procedure

Solution	Blank	Standard	Sample
Working reagent	1000 µl	1000 µl	1000 µl
Sample	-	-	10 µl
Standard	-	10 µl	-
The tubes were mixed and incubated 5 minutes at 37.			
NaoH	1000 µl	1000 µl	µl1000

The tubes were mixed and incubated for 5 minutes at 37°C. Within 60 minutes the absorbance of sample was read against the reagent blank by the spectrophotometer at a 600 nm wave length.

Calculation

$$\frac{\Delta A \text{ Sample}}{\Delta A \text{ Standard}} \times \text{Standard concentration} = \text{urea concentration}$$

Standard concentration: 50 mg/dl.

Aspartate aminotransferase Test (AST)

The AST activity was used to determine the functional status of the liver and a special kit (Biomerieux \ France) was used to measure it :

Test principle :

$$\text{L- Aspartate} + \text{2-Oxoglutarate} \xrightarrow{\text{AST}} \text{Oxaloacetate}$$

Test procedure

Solution	Blank	Sample
R1	0.5 ml	0.5 ml
Sample	- - - -	100 µl
D.W.	100 µl	- - - -
The tubes were mixed and incubated 30 minutes at 37°C.		
R2	0.5 ml	0.5 ml
The tubes were mixed and incubated 20 minutes at room temperature .		
NaOH	0.5 ml	0.5 ml

The tubes were mixed and left for 5 minutes at room temperature . The absorbance of sample was read against the reagent blank by the spectrophotometer at a 546 nm wave length and then compared with the standard curve .

Serum Glucose Determination

The serum glucose was measured by using a special kit (BIOCON/GOD – PAP, Germany).

Test Principle :

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{Glucose oxidase}} \text{Gluconic acid} + H_2O_2$$

$$2H_2O_2 + \text{Phenol} + 4 - \text{aminoantipyrine} \xrightarrow{\text{Peroxidase}} \text{Red Chinonimin} + 4H_2O$$

Test procedure

Solution	Blank	Standard	Sample
Working reagent	1000 µl	1000 µl	1000 µl
Sample	-	-	10 µl
Standard	-	10 µl	-

The tubes were mixed and incubated for 5 minutes at 37°C. The absorbance of sample was read against the reagent blank by spectrophotometer at a 505 nm wave length.

Calculation

$$\frac{\Delta A \text{ Sample}}{\Delta A \text{ Standard}} \times \text{Standard concentration}$$

= Glucose concentration

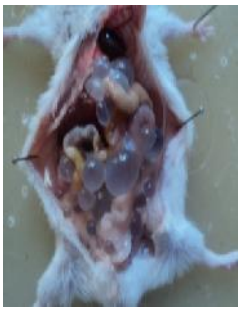
Standard concentration: 100 mg/dl.

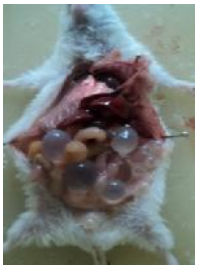
Statistical analysis:

ANOVA or analysis of variant was used in the present study and detected by Revised Least Significant Difference (R.L.S.D) with the help of SPSS programs .

Results :

The examination of experimentally infected males Balb/c mice with protoscolices at 6 months – post infection revealed that the presence of hydatid cysts in liver, spleen, mesenteries, kidneys and lungs .





Male of infected mice with hydatid cysts after six month- post infection

The results of blood parameters were obtained from positive and negative control groups. It is found that the number of WBC had increased in the positive group (16.37) compared with 10 of WBC in the negative control group as listed in Table (1).

The results of the present study showed that the means of RBC, PCV, and Hb were at normal levels where it is 9.68 for RBC, 34.8 for PCV, and 13.8 for Hb in the negative control group and 8.12 for RBC, 31.4 of PCV, and 12.2 for Hb in the positive control group .The results of differential WBC showed an increase in the number of lymphocytes (68) and eosinophiles (4) and a decrease in the number of neutrophil (41), while the number of monocyte was (1) in the positive control group . The negative control group showed slight differences in the mean number of differential WBC (Table 1).

Table(1): Blood parameters of experimentally infected mice with hydatid cyst

Groups	Mean of WBC, RBC, PCV, Hb and dWBC of infected mice							
	WBC	RBC	PCV	Hb	Differential WBC			
					L	N	E	M
Negative control group	10.0	9.68	34.8	13.8	56	49	2	1
Positive group	16.37	8.12	31.4	12.2	68	41	4	1
R.L.S.D.	1.125	0.83	1.34	0.89	4.3	4.08	-	-
Significant differences , $P \leq 0.05$, n=8								

L:lymphocyte; N: neutrophile ; M: Monocyte ; E: eosinophile

The biochemical parameters of each of the negative and the positive group were investigated. They included the blood urea test (BUN), the serum glucose concentration, and the aspartate aminotransferase (AST). The results showed a decrease in the concentration of blood urea (33. 5) in the positive control

group compared to 37.37 in the negative control group .

The glucose concentration slightly decreased in the positive group compared with the negative control group . Table (2) showed that the concentrations of AST in blood were increased after the infection with hydatid disease recording 21.0 of AST level in the positive group compared with 14.0 in the negative control group

Table (2): Biochemical parameters of mice infected with hydatid cyst

Groups		Mean of biochemical parameters		
		BUN mg\ml	Glucose mg\dl	AST mg\dl
Negative control group		37.37	103.38	14.0
Positive group		33.5	88.33	21.0
R.L.S.D.		1.011	4.61	0.92

Significant differences , $P \leq 0.05$, n=8

Discussion :

Blood and biochemical parameters were checked in the present study to examine the overall health of experimental animals before and after infection and it was found that the numbers of WBC, lymphocytes, eosinophils have increased while the number of neutrophils decreased in the positive group compared with the negative control group as presented in table (1) . These results agreed with the study of Al – Nasiri (2006) and Moraitaki *etal*.(2010) who found an increase in the number of leukocytes, lymphocytes and eosinophils and a decrease in the number of nuetrophils. They, however, disagree with Al- Mobarak (2006) who found an increase in the number of nuetrophils. Increases in numbers of WBC, lymphocytes and eosinophils were observed in the present study which may be considered as a defense mechanism against the inflammatory processes in the body especially in the liver, spleen and kidneys where the inflammation stimulates the bone marrow to produce a large

number of WBC. The increase of the eosinophil count could be attributed to the long period of the disease. Nguyen and Diamond (2000) explained that eosinophilia was produced

due to the ability of parasites to infect the tissue and this agreed with Al – Humairy (2010) study . RBC, PCV, and Hb were measured and slight differences were found between positive and negative control groups (Table, 1), therefore, that parameters were kept under normal levels and these results were similar to Al-Mobarak (2006) and Moraitaki *etal*.(2010) studies .

The results of biochemical parameters of each positive control group , negative control group are listed in table (2) and it was found a decrease in urea values in the positive group compared with the negative control group . Low serum urea concentrations have been recognized previously in association with liver failure and have been suggested to indicate reduced hepatic synthesis of urea from ammonia. The decreased serum urea is associated with more severe hepatopathies and has prognostic relevance . on the other hand, it may be absorbed by the hydatid cyst since Ozen *etal*.(1992) detected a large number of carbohydrate molecules such as glycogen , glucose and polysaccharides in the hydatid cyst fluid. Other researchers also detected urea and uric acid (Sharif *etal*., 2004).

The serum glucose concentration had slightly decreased in the experimentally infected animals as shown in table (2). This may be due to the effect of the infection on the liver which played an important role in the glucose metabolism as it increased glucose excretion and decreased blood glucose. Ozen *etal*. (1992) and Sharif *etal*. (2004) found the carbohydrates of the protoscolices were glycogen, , glucose and alkali stable carbohydrates. Further, Rafik *etal*.(2002) recorded 47.2 and 35.8 mg\l of glucose in the fluid of the liver and lung hydatid cyst composition..

Aspartate aminotransferase (AST) was also measured showed an increase among the positive group compared to the negative control group; a result agreed with Abdulla (2007) who found that the liver infection with cestoda tapeworms led to hepatocye destruction and enzyme release , therefore , the concentration of AST increased . Some studies advised checking aminotransferase enzyme continuously during the infection and treatment with albendazole (Yarsan *etal*.,2003 and Shindala *etal*., 2007) .

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تأثير الاصابة بمرض الاكياس العدرية على بعض المعايير البايوكيمياوية والدموية لدى الفئران سلالة Balb\c

د. أمل خضير خلف

فرع الاحياء المجهرية / كلية الطب / جامعة ذي قار

الملخص :

يعتبر مرض الاكياس العدرية من الامراض المتوطنة في العراق ، لذلك استهدفت الدراسة الحالية التأثيرات السلبية للمرض على بعض المعايير الدموية والبايوكيمياوية اثناء الاصابة بالكيس العدري أذ وجد ت النتائج زيادة في اعداد كريات الدم البيض وزيادة الخلايا اللمفاوية والحمضة ونقصان الخلايا العدلة بالنسبة للعد التفريقي لخلايا الدم البيض بالنسبة لمجموعة السيطرة الموجبة المصابة بالمرض اما الخلايا الدموية الحمراء وخضاب الدم وحجم الخلايا المرصوص فلن تتأثر بالاصابة بالكيس العدري.

انخفض تركيز كل من اليوريا وسكر الدم وازداد تركيز انزيم الاسبارتيت امينوترانزفيريز بالنسبة للمعاييرالبايوكيمياوية مقارنة بمجموعة السيطرة السالبة الغير مصابة بالمرض .

تبين من الدراسة الحالية ان الاصابة بمرض الاكياس العدرية يؤثرسلباً في وظائف الكبد من خلال فحص المعايير البايوكيمياوية المتعلقة بوظائف الكبد .