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Perturbation of liver function markers and serum electrolytes associated with *Echinococcus granulosus* infection in sheep

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Abstract

Histopathological alterations in the liver of intermediated hosts infected with *Echinococcus granulosus* parasite can be occurred by necrotic expansion as a result of an increase in growth of the parasite larva stage (metacestode), which may cause perturbation in production of liver function markers. Thus, this study aims to determinate the liver biochemical profile and some serum electrolytes of *E. granulosus*-infected sheep at different hydatid cyst infection ratios (IRs). fifty livers from naturally *E. granulosus*-infected sheep with five livers from uninfected-sheep as a control group were enrolled in this study. Cardiac blood samples under sterilized conditions were gently collected and isolated sera were biochemically assayed for determination of liver function markers including; Aspartate transaminase (AST), Alanine transaminase (ALT), alkaline phosphatase (ALP), Albumin (ALB) and total protein (TP), as well as the level of some serum electrolytes including Ca, K, Na and Cl using fully automatic biochemical analyzer, FUJI-Film. The current data indicated a progressive increase in the level of AST, ALT, ALP, Ca and K. The level of ALB and TP were gradually declined with a rise of liver infection ratio with hydatid cysts. In conclusion, the current findings indicated perturbation of liver function markers and the level of some serum electrolytes, mainly Ca and K in *E. granulosus*-infected sheep based on the infection ratio of liver. Additionally, acute and chronic infection of *E. granulosus* parasites in sheep can be determined based on the level of liver function markers in serum.

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Introduction

Echinococcosis is a parasitic disease caused by the *E. granulosus* parasite's larva stage in intermediate hosts, including humans and other warm-blooded vertebrates. Morphologically, the disease can be recognized as multiple hydatid cysts within different internal organs of infected host particularly in the liver (1). The disease represents a real global challenge against health and economic development, especially in the third world states, due to lack of critical health care in humans and dogs, as well as large numbers of wild dogs that live near to the human communities which increase the infection risk. Thus, direct and indirect depletion of manpower and livestock can threat the economy in many countries around the world by echinococcosis disease (2). Delay or accidental diagnosis of expanded hydatid cysts can increase disease complications and reduce the probability of the complete healing. Asymptomatic growth and distribution of hydatid cysts in the host body increase disease fight challenges (3). The necrotic cell death that occurred around the *E. granulosus* larva stage (metacestode) by host cell lysis is an action of parasite development (4), by which provides enough space for expanding of hydatid cysts (5). Moreover, histopathological changes that occur from the mass expansion of hydatid cysts cause pressure on the surrounding host tissues, in which may have

pathophysiological effects on the infected organs. Although, liver is the mean organ that produce AST, ALT and ALP, many organs including; pancreas and kidney as well as different cells such as red blood cells and muscular cells, can produce these enzymes but at lower levels (6). Recently, level of AST and ALT, known as glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT), respectively, in the serum were reported to be early specific markers of liver damage even before symptoms onset (7). Histopathologically, the slight hemorrhage, white blood cells (WBCs) infiltration and denaturation of hepatocytes are the most frequent features of livers infected with the metacestode stage of *E. granulosus* (8). The liver is an essential organ that produce a range of enzymes and participates in detoxification (9).

Thus, this study aimed to evaluate the alteration of liver function markers and some serum electrolytes in *E. granulosus*-infected sheep, which may reflect the level of liver damage at different infection ratios of the liver and may support the radiological diagnosis. Additionally, this may also allow to biochemical tracing of parasite infection and determination intensity of parasites.

Materials and methods

Blood samples and biochemical analysis

For cardiac blood collection, hearts and livers were removed from *E. granulosus*-infected and uninfected sheep slaughtered by butchers in the open sheep market in Mosul city, north of Iraq. Followed by sterilization of heart surface using 70% ethanol and direct gently aspiration of blood from the heart to avoid blood agglutination. The cardiac blood samples were kept in 10 mL test tubes and transferred to the research laboratory building in the College of Education for Pure Science/ Mosul University to be assayed for liver biochemical profile and serum electrolytes determination. The liver biochemical markers including AST, ALT, ALP, ALB and TP as well as some serum electrolytes including Ca, K, Na and Cl of *E. granulosus*-infected sheep at different liver infection ratios were determined versus the control group using a fully automatic biochemical analyzer, FUJI-Film, (DRI-CHEM NX500 – Fujifilm, Tokyo, Japan).

Calculation of hydatid cyst infection ratio (IR) of liver

The weight of hydatid cysts and liver were precisely measured using a digital milligram scale. The hydatid infection ratio of livers was measured by dividing the weights of a single liver's hydatid cysts of on whole weight of infected liver and times by hundred. Hydatid cyst infection ratio of liver = Weights of Single liver's hydatid cysts/ Whole weight of infected liver *100.

Statistical analysis

The data of liver biochemical markers in the serum including AST, ALT, ALP, ALB, TP and some serum electrolytes of *E. granulosus*-infected and uninfected sheep were statistically analyzed by One-Way ANOVA, with Dunnett post-test formulation using GraphPad Prism 5.0 software (GraphPad Software, Inc., San Diego, California, USA). The probability value (P-value) of variation between variables were calculated and considered to be significant *, **, *** at $P < 0.05$, 0.01 and 0.001, respectively.

Results

This study is originally established to investigate the impact of transmission and mass growth of the metacestode stage (hydatid cysts) of *E. granulosus* parasite in the liver on the liver biochemical profile and some serum electrolytes. The main liver biochemical markers including AST, ALT, ALP, ALB and TP, a well as some serum electrolytes including; Ca, K, Na and Cl levels were investigated. The data of liver enzyme profile indicated a significant increase ($p< 0.01$) in the level of AST, ALT and ALP which started at 10-19% IR (371.2±9.3), 30-39% IR (209.66±8.38) and 40-49% IR (406.66±11.59), compared to the control group which reported (91.2±9.8), (23.2±9.2), (80.2±12.6), respectively (Table 1).

Table 1: The level (mean±SD) of AST, ALT, ALP, ALB and TP in serum of *E. granulosus*-infected and uninfected sheep (control) at different hydatid cyst infection ratios (IR) of liver

Infection ratio (IR)	AST (U/L)	ALT (U/L)	ALP (U/L)	ALB (g/dl)	TP (g/dl)
Control (n=5)	91.2±9.8	23.2±9.2	80.2±12.6	2.98±0.51	6.56±0.65
10-19% IR (n=8)	371.2±9.3**	68.5±6.24	224.5±10.9	1.6±0.6**	4.45±1.08**
20-29% IR (n=8)	448.2±19.7***	91.75±9.11	292.5±6.35	2.5±0.49*	5.8±0.64*
30-39% IR (n=5)	598.3±7.63 ***	209.66±8.38**	256.66±9.6	1.5±0.34**	4.63±0.51**
40-49% IR (n=6)	922.33±9.45***	153.6±7.63*	406.66±11.59**	2.43±0.41*	4.83±0.75*
50-59% IR (n=10)	826.4±18.29***	374.8±16.6***	434.4±6.02***	1.8±0.31**	2.7±0.69***
60-69% IR (n=6)	961.6±10.4***	625.1±11.8***	670.33±10.6***	1.96±0.35*	2.36±0.37***
70-79% IR (n=7)	996.33±6.3***	522±10.5***	563.3±8.96***	1.25±0.07***	2.7±0.81***

* = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$, n is the number of liver samples

Although, the serum level of ALB and TP in *E. granulosus*-infected sheep reported a significant decrease, starting at the early stage of infection in infected sheep comparing to uninfected sheep, but this decrease was fluctuated at different hydatid cyst infection ratios. The lowest level of ALB and TP were reported at 70-79% IR and at 60-69% IR, respectively (Table 1). The serum electrolytic analysis data indicated noticeable and graduated increase in the levels of Ca and K in infected sheep compared to the control group. The significant increase in the levels of Ca and K were 7.57±2.7 and 8.95±1.84, respectively, where both started at 20-29% IR. Additionally, both Ca and K levels were reached to the highest significant values 12.86±0.91 and 13.4±1.01 at 60-69% IR and 70-79% IR, in comparison to the control group which reported 4.84±0.57 and 6.12±0.74, respectively. The difference in the levels of Na and Cl between infected and uninfected sheep was not statistically taken into consideration, both electrolyte's level was relatively lower in infected sheep compared to uninfected group (Table 2).

Table 2: The level of some serum electrolytes including; Ca, K, Na and Cl of *E. granulosus*-infected and uninfected sheep (control) at different hydatid cyst infection ratios (IR) of the liver

Infection ratio (IR)	Ca (mg/dl)	K (mEq/L)	Na (mEq/L)	Cl (mEq/L)
Control (n=5)	4.84±0.57	6.12±0.74	137.40±8.73	104.40±5.77
10-19% IR (n=8)	6.25±1.91	8.50±1.63	130.01±12.35	99.75±11.75
20-29% IR (n=8)	7.57±2.7*	8.95±1.84*	134.04±14.16	101.03±8.16
30-39% IR (n=5)	8.16±1.05*	10.16±0.61**	127.66±19.13	95.53±13.92
40-49% IR (n=6)	9.60±1.21**	13.10±0.88***	132.02±11.7	100.33±7.02
50-59% IR (n=10)	10.72±0.73***	11.90±1.59***	107.01±9.51*	92.80±9.54
60-69% IR (n=6)	12.86±0.91***	11.46±1.20***	118.66±9.01	90.66±11.05
70-79% IR (n=7)	12.13±0.75***	13.40±1.01***	125.33±7.02	94.67±7.01

*= $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, n is the number of liver samples

Discussion

Liver metastasis occurs in the chronic stage of various liver diseases such as cancer, hepatitis (10) cirrhosis (11) and fasciolosis which may impact on the liver functions (12). The liver function markers including, AST and ALT which shown in the current study are comparable to data that obtained previously in the liver fibrosis cases (Ahmed&Ahmed-13). In clinical study of cystic echinococcosis, biochemical analysis of 49 individuals showed an increase in the serum level of AST, ALT and ALP in 26.4%, 24.5% and 35.8% of the cases, respectively (14). The serum level of AST and ALT can be considered as biochemical markers of liver function, where an increase of their levels refers to damage occurring in the liver cells (15-16). An increase of AST, ALT, ALP, and GGT level in the report case of *Fasciola hepatica*-infected woman was identified (17). This data is agrees with the data that obtained in the current study, where ALT level dramatically increased with an increase in the level of liver's level of hydatid cyst infection ratio. The oxidative damage in the mice liver tissues induced by glyphosate was accompanied by an increase in the serum level of AST and ALT (18). The abnormality of liver enzyme levels may indicate liver damage at different stages of infection, where in chronic stage of viral hepatitis, the liver enzymes, in particular AST and ALT levels were increased (19). Moreover, in case of colestatic hepatitis of Cytomegalovirus-infected infants, the level of AST and ALT concentrations were dramatically higher than the serum normal range of healthy individuals (20). In another related study on parasitic tapeworms, no obvious correlation was reported between liver AST level and infection with tapeworm *Khawia armeniaca* in fish host (21).

In partial matching to the current data, production of circulating proteins that are synthetized by the liver, such as ALB and clotting factors, were dramatically reduced in chronic liver diseases (22). Alteration in serum levels of ALB and TP during various liver diseases were previously determined, where low serum level of ALB and TP in infected sheep may be due to concentrating these elements in hydatid cyst fluid and protoscoleces (23) or may be related to the effect of expanded hydatid cysts on protein secretion from the liver (24). Thus, the graduated reduction in ALB and TP levels that are indicated in the current study seems to be associated with an increase in liver's hydatid cyst infection ratio, which may be caused by expand growing of hydatid cysts in the liver. In agreement with the current data, during endoparasitic infections such as hydatid cysts and *Dicrocoelium dendriticum* infection, high plasma levels of liver enzymes such as AST and ALT with low level of TP and vitamin-A were determined (25).

Generally, successful infection of a pathogen is based on the immune status of the host and nutrition available, including the main elements and minerals (26). The importance of minerals in the maintenance of normal physiological functions of organs and protection the organisms from diseases were previously reported, where some of the minerals are involved in the structure of enzymes or serve as cofactors that participate in enzymes'

activity (27). In the current study, a significant increase in the level of some serum electrolytes such as Ca and K may indicate the importance of these elements in host-parasite interaction, where Ca ions involve in the activation of calmodulin protein (Ca-binding protein) which is highly expressed in protoscoleces and the germinal layer of *E. granulosus* (28). The importance of calmodulin is reported to be as a critical protein in the Ca signaling pathway, mitochondria events, gene expression and it involves in calmodulin-dependent kinase activities (29). As shown previously, the high concentration of Na and Cl in hydatid cyst fluid may interpret decrease their levels in the serum of infected sheep as shown in the current study (30). It has been indicated that Na level in hydatid cyst fluid higher than the level of Ca and K by more than 12 and 6 times, respectively, in which can be suggested that accumulation of Na in hydatid cyst fluid at high concentration lead to reduce its level in the serum as shown in the current study (31).

Conclusion

Serum biochemical parameters including; liver enzymes (AST, ALT and ALP) and liver-secreted proteins (ALB and TP), as well as some serum electrolytes such as Ca and K can be used as biochemical indicators of liver infection ratio or liver damage caused by *E. granulosus* parasites. This can be dependent as complementary indicators which may support the radiological diagnosis of hydatid disease. Additionally, early and chronic infection of the liver with *E. granulosus* can be determined based on the level of liver function markers in particular AST, ALT and ALP.

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Conflicts of interests

The authors declare no conflict of interests.

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اضطراب مؤشرات وظائف الكبد والكتروليتات المصل المرتبطة بعدوى المشوكة الحبيبية في الأغنام

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الخلاصة

يمكن أن تحدث التغيرات النسجية المرضية في كبد المضائف الوسطية المصابة بطفيل المشوكة الحبيبية نتيجة التمدد النخري بسبب ازدياد نمو الطور اليرقي (الشريطية البعدية) للطفيل والتي قد يؤدي إلى اضطراب في إنتاج مؤشرات وظائف الكبد. لذلك، هذه الدراسة تهدف إلى تحديد الصورة الكيموحيوية للكبد وبعض الالكتروليتات للأغنام المصابة بالمشوكات الحبيبية عند مستويات مختلفة من خمج الكبد. استخدمت في الدراسة الحالية ٥٠ كبد للأغنام المصابة بصورة طبيعية بالمشوكات الحبيبية مع ٥ أكباد من أغنام غير مصابة استخدمت كمجموعة سيطرة. تم جمع عينات دم القلب تحت ظروف معقمة والأمصال المعزولة تم فحصها باختبارات الكيمياء الحياتية لتحديد مؤشرات وظائف الكبد والتي تشمل ناقلة أمين الاسبارتيت وناقلة أمين الالنين والفوسفتاز القاعدي والألبومين والبروتين الكلي، بالإضافة إلى مستوى بعض الكتروليتات الموجودة في المصل وتشمل الكالسيوم والبوتاسيوم والصوديوم والكلور باستخدام جهاز فوجي فلم. أظهرت النتائج الحالية زيادة تدريجية في مستوى ناقلة أمين الاسبارتيت وناقلة امجن الالنين والفوسفتاز القاعدي والكالسيوم والبوتاسيوم بالإضافة إلى انخفاض تدريجي في مستوى الألبومين والبروتين الكلي مع زيادة نسبة خمج الكبد بالأكياس العدرية. يستنتج من الدراسة الحالية حدوث اضطراب في مؤشرات وظائف الكبد ومستوى بعض الالكتروليتات في المصل وخاصة الكالسيوم والبوتاسيوم في الأغنام المصابة بالمشوكات الحبيبية اعتمادا على نسبة خمج الكبد. بالإضافة إلى ذلك، يمكن تحديد الإصابة الحادة والإصابة المزمنة بطفيل المشوكات الحبيبية في الأغنام بالاعتماد على مستوى مؤشرات وظائف الكبد في المصل.

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