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Evaluation of Some Enzymes of Iraqi Patients Infected with Hydatidosis

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ABSTRACT

This study was carried out to evaluate some enzymes activity in hydatidosis infection which were considered as a biomarkers to monitor their levels and prognostic values. They were performed on 50 blood samples of hydatidosis surgically proved and 20 samples as control group which were collected between October, 2015 and April, 2016 from different hospitals in Al-Furat Al-Awsat district. The mean levels of alkaline phosphatase (ALP) and lactate dehydrogenase (LD) exhibited a significant elevation ($P < 0.05$) in hydatidosis patients compared to the healthy control group. Also the same findings were seen in pre-operative patients compared to post-operative patients. The levels of both enzymes increased in liver and multi-organs hydatidosis than any other site. The patients with large size cyst showed a significant increase ($P < 0.05$) in ALP and LD levels compared to small size cyst. There was a significant decrease($P < 0.05$) in the levels of adenosine deaminase (ADA) and glutathione reductase (GHR) enzymes in hydatidosis patients compared to healthy control. The ADA levels in large cyst were significantly increased ($P < 0.05$) compared to patients with small cyst. The (GHR) enzyme level was decreased in patients with small size cyst compared to the large ones. Also the multi-organs and liver hydatidosis showed significant decrease ($P < 0.05$) in the levels of GHR compared to lung or kidney.

Key words: hydatidosis and enzymes (ALP, LD, ADA, and GHR) .

INTRODUCTION

Echinococcosis or hydatid disease in man is caused by the larval stage of the dog tapeworm, *Echinococcus granulosus* [1] . Human infection occurs as zoonoses primarily in sheep-raising areas where domestic dogs are used in herding [2] . The hydatid cyst is normally well tolerated in humans until its development results in pressure on adjacent tissues or organs [3] . Lung cysts are usually asymptomatic until there is a cough, shortness of breath or chest pain. Hepatic cysts result in pressure on the expanding hydatid cysts cause necrosis of the surrounding tissues [4]. Some studies [5] suggest that the response of the liver to any form of biliary-tree obstruction is to synthesize more ALP, some of the additional enzyme enters the circulation to raise the enzyme levels in serum. The role of ALP in host parasite relationship emphasize its potential importance as a diagnostic and prognostic antigen in the monitoring of hydatid infection [6]. Higher level of ALP enzyme activity in cystic liver in comparison with healthy liver could be considered as a pathological biomarker for diagnosis of hydatid disease parameters [7] . An elevated of ALP activity is strongly suggestive of a hepatic space-occupying lesion [8] . Parasitic diseases also showed elevated levels of ALP enzyme, such as leishmaniasis [9] and schistosomiasis [10] . Elevation of LD enzyme activity are seen in patients with liver disease [11] . In parasitic disease, elevated serum LD levels have been reported in patients with sarcocytosis [12], toxoplasmosis and leishmaniasis [9] . There are some studies to investigateADA enzyme activity in parasitic diseases, and showed that the enzyme level was decreased, in human schistosomiasis and in mice which were infected experimentally with secondary hydatid disease [10][13], while women actually infected with *Trichomonas vaginalis* were showed an elevation of ADA [14], however, in visceral leishmaniasis, the enzyme level was significantly increase ($P< 0.05$)compared with control group [9] . In

erythrocytes, the pathway has a major function in preventing hemolysis by providing NADPH to maintain glutathione in the reduced state as the substrate for glutathione peroxidase [15]. There was a significant increase in GHR and superoxide dismutase (SOD) enzyme activity in patients with leishmaniasis [9]. In general, the investigators were concluded that parasitic disease could be considered as an important cause of liver enlargement with an increase in enzyme activities [16]. So many researches have been done on different aspects of hydatid disease. In Iraq very little information is available about biochemical changes associated with this disease, and also to provide further biochemical evidences on the involvement of *E. granulosus* in evasion strategies and to clarify some of the factors that may eventually lead to establishment of cystic echinococcosis . Therefore, this study aims at carrying a biochemical aspects to monitor the level and prognostic values of some enzymes in hydatidosis as follow :

1. Hepatic enzymes (ALP and LD)
2. Adenosine deaminase (ADA) which may reflect change in the immune response
3. Glutathione reductase (GHR) which is important in antioxidant defense

MATERIALS AND METHODS

Between October 2015 and April 2016, 5 ml of blood samples were collected from patients, their ages ranging from 10-75 year, 50 patients were clinically diagnosed (pre-operation) and surgically confirmed (post-operation) as they attended hospitals in Al-Furat Al-Awsat district of Iraq . The cysts were excised from liver, lung, heart, spleen and kidney . None of the subjects studies had a history of any diseases other than hydatidosis or under any type of therapy . Indirect haemagglutination test (Bio Merieux, France) was used for diagnosis. Control samples were collected from 20 apparently health patients who had no known illness or under any type of therapy at the time taking blood samples. All blood samples and other subjects were collected with permission of the Iraqi Ministry of Health and the informed consent of the parents / guardians of each patients investigated. Each blood samples obtained in a plain tube centrifuge as soon as possible and serum separated, liquated into portions to avoid separate freezing and thawing then stored at – 20 ℃ until used . Some information was taken for each patients such as name, gender, age, address, and other subjects.

Biochemical Test

Alkaline phosphatase (ALP) activity

The activity was determined in the serum and the assay was carried out using BioMerieux alkaline phosphatase – kits .

Lactate dehydrogenase (LD) activity

The activity was determined in the serum; the assay was carried out using Randox – kit for lactate dehydrogenase.

Adenosine deaminase (ADA) activity

The activity was determined in serum according to method of [17] .

Glutathione reducatase (GHR) activity

The activity of this enzyme was determined in the serum according to [18] .

RESULTS

Enzymes

Alkaline phosphatase (ALP)

A highly significant increase ($P < 0.05$) in the level of ALP of hydatidosis patients in comparison with healthy control (tab.1) . There was a significant decrease in the level of enzyme of the post-operative sera compared to pre-operative sera ($P < 0.05$) (tab. 2) . The levels of enzyme in both, liver and multiorgans sites showed a significant increase ($P < 0.05$) in comparison to lung and kidney hydatidosis patients (tab. 3) Patients with large size cysts also showed significant elevation ($P< 0.05$) of those with small size cysts (tab. 3).

Lactate dehydrogenase (LD)

There was a significant increase ($P < 0.05$) in serum LD levels of hydatidosis patients compared to the healthy control group (tab. 1) . Also patients with large cyst showed elevated serum LD levels when compared to those with small cyst (tab. 4), this increase was significant ($P< 0.05$) . There was also a significant increase ($P< 0.05$) between pre – operative and those post – operative patient's (tab. 2). The site of infection also represented significant difference ($p < 0.05$) characterized by elevation of serum LD level in both multi – organs and liver hydatidosis patients in comparison to those with lung and renal hydatidosis (tab. 3).

Table 1 : Mean concentration of ALP and LD enzymes in the sera of 50 hydatidosis patients and healthy control

Enzymes (U/l)	Patients Mean ± SE	Healthy control Mean ± SE	P – value
ALP	180.52 ±2.85	80.22 ±4.6	< 0.05
LD	248.44 ± 6.65	146.20 ± 5.82	< 0.05

Table 2 : Mean concentration of ALP and LD enzymes in the sera of 50 hydatidosis patients and healthy control in relation to pre and post operation

Enzymes (U/l)	Pre-operation Mean ± SE	Post-operation Mean ± SE	P – value
ALP	194.32 ± 5.32	174.83 ± 2.62	P < 0.05
LD	273.44 ± 6.65	236.35 ± 2.93	p < 0.05

Table 3 : Mean concentration of ALP and LD enzymes in the sera of 50 patients infected with hydatidosis in relation to site of infection

Enzymes (U/l)	Multi-organ Mean ± SD	Liver Mean ± SD	Lung Mean ± SD	Kidney Mean ± SD	P – value
ALP	204.82±5.2	191.20±4.30	162.20±5.6	162.6±305	P < 0.05
LD	283.62±15.5	266.5±10.3	222.2±11.8	156.0±28.9	P < 0.05

Table 4 : Mean concentration of ALP and LD enzymes in the sera of 50 patients infected with hydatidosis in relation to size of cyst

Enzymes (U/l)	Diameter < 5 cm Mean ± SE	Diameter > 5 cm Mean ± SE	P – value
ALP	170.12 ± 6.00	195.80 ± 4.10	P < 0.05
LD	226.93 ± 14.20	266.80 ± 11.20	p < 0.05

Adenosine deaminase (ADA)

The activity of ADA enzyme in hydatidosis group was lower as compared with its control. The difference between the two groups were significant (P< 0.05) (tab. 5) . The ADA serum level in patients with large cyst was higher than that of small cysts and the differences was significant (P < 0.05) (tab.7).

Glutathione reductase (GHR)

The mean concentration of GHR of healthy control was significantly higher (P< 0.05)than that of patients with hydatidosis (tab. 5) . The site of infection showed significant differences (P< 0.05) in relation to infected organs that the multi – organs and liver hydatidosis showed significant decrease in comparison to lung hydatidosis (tab. 6) . The size of cyst also showed significant difference (P< 0.05) characterized by elevation of serum GHR levels in patients with small size cyst (tab. 7) .

Table 5 : Mean concentration of ADA and GHR enzymes in the sera of 50 patients infected with hydatidosis and healthy control group

Enzymes	Control Mean ± SE	Patients Mean ± SE	P – value
ADA (U/l)	33.24 ± 2.65	27.80 ± 1.24	< 0.05
GHR (µmol/ml)	34.18 ± 2.15	27.92 ± 1.82	< 0.05

Table 6 : Mean concentration of ADA and GHR enzymes in the sera of 50 hydatidosis in relation to site of infection

Enzymes	Multi-organ Mean ± SE	Liver Mean ± SE	Lung Mean ± SE	Kidney Mean ± SE	P – value
ADA (U/l)	26.86 ± 2.04	28.20 ± 2.60	28.26±1.50	23.80±2.80	< 0.05
GHR (µmol/ml)	24.20 ± 2.85	23.68 ± 1.30	28.86±2.00	29.60±0.72	< 0.05

Table 7 : Mean concentration of ADA and GHR enzymes in the sera of 50 patients infected with hydatidosis in relation to the size of cyst

Enzymes	Small cyst (diameter < 5 cm) Mean ± SE	Large cyst (diameter > 5 cm) Mean ± SE	P – value
ADA (U/l)	27.8 ± 1.66	30.30 ± 1.25	< 0.05
GHR (µmol/ml)	22.12 ± 2.80	27.06 ± 2.00	< 0.05

DISCUSSION

The activity of ALP was found to be significantly higher in patients with hydatidosis than in the healthy control. This increase may be from the parasite itself as [19] who demonstrated, that the metacestodes possess a high alkaline phosphatase activity (about 50 fold higher than that of ALP from mammalian liver tissue). We know surgical intervention is always combined with chemotherapy (benzymidazole) pre – and post – operation. This chemotherapy

is accompanied by significant damage of the germinal layer associated tissue, also alterations occurring on most of the outer a cellular and carbohydrate - rich laminated layer of metacestode [20] . This damaged tissues leading out of its enzymes, so this may be probably cause of increase in ALP levels in patients with hydatidosis, and this explain to us the reason of ALP elevation in preoperative patients more than postoperative. Other reports showed that any factor affecting the liver metabolism was found to cause an increase in ALP activity [21] . ADA was found to be affected by liver function [22] and because ADA decreased as recorded in our results ALP elevation may be due to this factor, and it occurs in patients with liver hydatidosis more than in patients with other organs involvement. The elevation of ALP in patients with large cysts, may be due to the heavy cyst pressure on the adjacent organs, while its non-significant elevation in younger more than other age groups may be due to the disease itself as well as physiological conditions as [23] explained, that infancy and childhood showed high ALP values as much as 3-4 times that of adult values. Similar results were obtained with LD enzyme, where there was an increase in its activity in hydatidosis patients in comparison to healthy controls. In general this enzyme is usually used for detection of hepatocellular damage and muscle damage, whenever liver cells are damaged or injured, the liver enzymes leak out into the circulation across the damaged cell membrane [24] . As we mentioned previously ADA play a role in liver function [22], so it deficiency leads to liver disturbance, hence, elevated, levels of LD in hydatid disease patients will be attributed to hepatic involvement by chronic disease [25], as well as the rise in enzyme levels in blood may be due to tissue damage. This will lead us to understand the cause of LD elevation levels in liver hydatidosis patients more other organ involvement patients. The clinical manifestation of this infection depended on the site and size of the cyst, resembling a slow growing tumor that gradually increasing the pressure on adjacent tissue, so pressure effect eventually develops. This fact explains the high level of LD in our patients with large cysts in comparison to those with small cysts. Other reasons can be due to the variation in LD levels among hydatidosis patients, the parasite itself, plays a role, i.e. intraspecific variation within *E. granulosus* strains as [26] stated that carbohydrate metabolism is lactic acid, which can affect the LD levels in hydatidosis patients. ADA enzyme is one of the most essential immune enzymes; its function gives a clear picture of the immune status of the body. It was found to play critical role in the normal development of immune system, and for the proper development of T and B – lymphocytes in mammals. In the present study, there was a significant decrease in ADA levels in patients infection, and we can correlate this result with the reduction in its level in experimental hydatidosis in mice [14] . The only explanation of the decreased level of ADA in hydatidosis patients is that it is probably due to toxic effect of the parasite metabolites. The decrease in ADA specific activity could cause a state of immunosuppression. For ADA plays a critical role in the normal development on the immune system [27] . B – cell dysfunction was also found to be associated with ADA deficiency . This was found to be due to the alteration in antigen receptors [28]. When ADA is not functional, intracellular levels of dAMP to the point that ribonucleoside reductase become inhibited. The inhibition of ribonucleoside reductase result in a shutdown of DNA synthesis because of low levels of the other dNTBs . Thus, cells that must replicate DNA and divide in order to be functional (B-cells and T-cells, for instance) can not function when adenosine deaminase activity is deficient [29] . The block in T – cell maturation is more than in B – cell maturation [30] . These findings correlate with recent results obtained by [31], who showed that there was a decrease in the mitotic index MI and replicative index RI of PBL in hydatidosis patient, such results document the critical role of ADA enzyme in the normal development of immune cells as previously reported by [32]. The increase in ADA levels near to its normal level in patients with large hydatid cysts as recorded in this current study may be due to the well developed laminated layer to an extent that no more antigenic components pass through it. This probably led us to conclude that there is a vital effect of the parasite antigens on the ADA level. The antioxidant defense system is sophisticated and adaptive and GSH is a central constituent of this system [33]. GSH depletion may be the ultimate factor determining vulnerability to oxidant attacks, so intracellular GSH status appears to be a sensitive indicator of the cells overall health [34] . In our study there was a significant decrease in glutathione reeducates GHR enzyme activity in patients with hydatidosis. This enzyme is responsible for maintaining the reduced glutathione GSH in the cell by the glutathione peroxidase – glutathione reducates system as previously mentioned in the literature review. So its depletion leads to depletion of GSH in the body. The consequence of sustained GSH depletion are grim. As cellular GSH is depleted, first individual cells die in those areas most affected. The zones of tissue damage begin to appear, those tissues with highest content of polyunsaturated lipids and/or the most meager antioxidant defenses are generally the most vulnerable [35] . The liver is the organ most involved with the detoxification of xenobiotics(substances foreign to the body), and also is the main storage local for GSH (actually exporting GSH to other organs) [36] . As our result showed there were probably liver metabolism and function disturbances, this explains why there is more GHR depletion in liver hydatidosis than in other organs involvement. Other reports referred that many pharmaceutical products are oxidants capable of depleting GSH from the liver, heart and other tissue [37] . They deplete GSH from the cells of the liver and by so doing render the liver more vulnerable to toxic damage. So we can consider this as another factor for ALP and LD elevation in patients with liver hydatidosis. GSH is considered the free radical scavenger and repairer of radical mediated biological damage [38], so its deficiency leads to accumulation of free radicals [33] who showed that the cumulative damaging effects of oxygen radicals and other oxidant are main contributors to degenerative disease. Probably, the decrease in serum GSH levels in our study may be explained by the fact, that this enzyme is a scavenging enzyme, its release

into circulation decreased, since it is more required during this disease. Finally, we confirm that this parasitic disease could be characterized by a biochemical syndrome.

REFERENCES

[1] Z. A. Elizabeth, Clinical Parasitology, A Practical Approach, W.B. Saunders Company, Philadelphia, **2013,** 2, 258 .
[2] L. Ruth, F. Russel, Medical Parasitology, A Self Instructional Text, helminthes, F. A . Davis Company, Philadelphia, **2012**, 6, 54 .
[3] A. Permin, J. W. Hansen, A Zoonotic parasitic disease**, 2002**, (web site) .
[4] H. TaherKhani, M. Rogan, *Iran J. Med. Sci.* , **2000,** 25 : 95 –104.
[5] M. M. Kaplan, *J. Clin. Investigation*, **1970 ,** 49(3) : 500 – 516 .
[6] P. Lawton, E. Sarciron, A. F. Petavy, *J . Parasitol*, **1994,** 80 (5) : 667– 673.
[7] F.M. Karibozorg, A. Farahanak, M. B. MolaeiRad, T. Glomohammadi, M. R. Eshraghian, *J. Med . Micro . and Infect . Disease*, **2014**, 2(2) , 86 – 70 .
[8] M. M. Kaplan, *Hepatology*, **1986**, 6 :526 – 528 .
[9] J.H. Taher, N.A. Abdulla, S. Muhammad, E. Faris, *Der Pharma Chemica*, **2015** ,**7** (10) : 1 – 5 .
[10] A.S.M. Juma,. Ph. D.Thesis, Al-Nahrain University (Baghdad, Iraq, **1999**).
[11] W. Tietz, Fundamentals of Clinical Chemistry. Enzyme, W. B.Saunders Company Philadelphia, **1982,** 2,653 .
[12] K. W. Prass, R. Fayer, *Vet . Path.* , **1981**, 18 , 358– 367 .
[13] J.J. Sacks, D.G. Delgado, H.O. Lobel, R.L. Parker, *Am. J. Epidemiol*, **1983,** 118 , 832 – 838 .
[14] A.S.M. Juma, T.I. Al-Jeboori, E.K. Shubber , *Dirasat, Med . Bio. Sci* **2000** , 27 : 110 – 116 .
[15] R.S. Al-Shawk, M. Sc Thesis, Al – Mustansiryah University (Baghdad, Iraq, **1999**).
[16] N.A. Hassan, Ph D Thesis, Al-Nahrain University (Baghdad, Iraq, **1996**).
[17] G. Giusti, *Verlage . Chemies International J . Florida*,**1981,** 2,1092 –1099
[18] N. Le– Trang, K. Bhargavan, A. Cerami, *Analy. Biochem. J*., **1983** , 133, 94 – 99 .
[19] P. Lawton, P. Sarciron, M. Sarciron, A. Petavy, *J. Com. Biochem . physol.* **1995** , 112 : 295 –301.
[20] M. Stettler, M. Siles – Lucas, E. Sarciron, Ph.Lawton, B. Gottstien, A. Hemphil, *J. Antimicrobial agents chemotherapy*,**2001,** 45, 1-7 .
[21] M.R. Pincus, J.A. Schaffner, Assessment of liver function. In: Clinical diagnosis and management by laboratory methods. Henry, J.B. (ed.) **1996,** 19**,** 253 –267 .
[22] M.E. Bollinger, F.X. Arrindo–vega, I. Santistebance, K. Schwarz, M.S. Hershfield, H.M. Lederman *N.J. Med*. **1996** , 334, 1367 – 1371 .
[23] A.F. Smith, G.J. Beckett, S.W. Walker, P.W. Rae, Lecture Notes on Clinical Biochemistry, Black well Science, U.K. **2000**, 6, 82 –83,
[24] D.W.Moss, A.R. Henderson, J.F. Kachmar, Enzymes Tietz Textbook of Clinical Chemistry. Tiest, N.W., W.B. Saunders Co. London ,**1986, 2,** 619-677
[25] R.A. Kreisbergs, *Ann . Int . Med.* **1978** , 88 : 681 – 695 .
[26] Ch. Bryant, C. Behm, Biochemical adaptation in parasites, Chapman and Hall, U.S.D. **1989,** 1, 198 – 199 .
[27] L. Thompson, J. Seegmiller, *Adv. Enzymol*, **1980,** 51, 167 – 210 .
[28] L. Gangi – Peterson, D. Srscher, J. Renoldes, T. Kepler, B. Mitchell, *J. Clin. Invest.*; **1999**, 103, 833 – 841 .
[29]R.J. Huskey, **1999** Ribonucleoside reductase(web site) .
[30] IPOPI , (**2002**) Patients with SCID due to ADA deficiency . pp : 1 – 2 . (web site) .
[31] A. Ubaidi, M Sc Thesis, Al-Nahrain University (Baghdad, Iraq,**2002**) .
[32] L. Roitt, J. Brostoff, D. male, Immunology , Mosby, London,2001, 6, 210.
[33] C.E. Cross, B. Halliwell, E.T. Borish, *Ann. Int.. Med*., **1987**, 107,526 - 545 .
[34] R.C. Duck, M. Ojcius , E. Young , *American Science* , **1996,** 9 , 79 – 87 .
[35] P.M. Kidd, Oxidant – antioxidant adaptation : Looking at both sides (conference presentation) . Houston, Texas : American College of advancement in Medicine (ACAM) spring meeting, **1993** .
[36] L.P. Deleve, N. Kaplowitz, *Seminars Liver Dis*., **1990**, 10 , 251 – 266 .
[37] A. Hoyumpa, S. Schenker, Drugs and the liver, WC , Philadelphia : *Curr – Med* ., **1996**, 6 , 6 – 22 .
[38] N.S. Kosower, E.M. Kosower, *Int. Rev. Cytol*., **1978,** 54 , 109 – 156 .