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Evaluation of some enzymes levels in Iraqi children infected with visceral leishmaniasis

Jasim Hameed Taher[1], Noor Al-huda Abdullah[2], Sura Mohammad[2] and Eman Faris[2]

[1]Department of Medical Laboratory Techniques, College of Health and Medical Techniques / Kufa – Iraq
[2]Department of Medical Laboratory Techniques, Al-Furat Al-Awsat Technical University, Iraq

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ABSTRACT

The study was carried out to evaluate some biochemical aspects which were performed on blood samples of visceral leishmaniasis (VL) and control group . Fifty blood samples were confirmed parasitologically by immunochromatography (IC) with rk - 39 antigen strips test and their applications . Ten samples for control were obtained from apparently healthy children . The results of enzymes showed that there was a significant increase (P < 0.05) in the levels of adenosine deaminase (ADA), glutathione reductase (GHR) and superoxide dismutase (SOD) in VL patients compared to the healthy control group . The mean levels of hepatic enzymes; alkaline phosphatase (ALP), lactate dehydrogenase (LD), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) exhibited a significant elevation (P < 0.05) in VL patients compared to the control .

Key words: visceral leishmaniasis and enzymes (ADA, GHR, SOD, ALP, LD, ALT, and AST) .

INTRODUCTION

Leishmania donovani occurs primarily in young children in North Africa and East Africa, Asia, the Mediterranean area, and South America . In India and Bangladesh, the disease is found primarily in adults [1] . Two million new cases occur each year and 350 million people at risk of infection [2] . In Iraq, there has been an increase in the number of reported cases during the last few years, that there was 3267 children infected with VL in 2013 [3] . This is possibly due to many factors, as population movement ; the destruction of health and vector control [4] . VL is usually detected in infants and children in the first seven years of life [5] . Splenomegaly, hepatomegaly, lymphnode enlargement , thrompocytopenia, anemia, leukopenia and hypergammaglobulinemia are the most finding in pediatric leishmaniasis [6] . VL could be considered as an important cause of liver enlargement with an increase in enzymes activity [7] . So many researcher have been done on different aspects of leishmaniasis . In Iraq very little information is available about biochemical evidence on the involvement of *L.donovani* in evasion strategies and to clarify some of the factors may eventually lead to establishment of VL . Therefore, this study aims at carrying a biochemical aspects to monitor the level and prognostic values of some enzymes in VL as follow :

1 . Hepatic enzymes (ALP, LD, ALT and AST) .
2 . Adenosine deaminase (ADA) which reflects changes in the immune response .
3 . Glutathione reductase (GHR) which is important in antioxidant defense .
4 . Superoxide dismutase (SOD), this enzyme is important in the respiratory burst that occurs in host macrophage during phagocytosis .

MATERIALS AND METHODS

Between March and December 2014, 5ml blood samples were collected, from children < 6 years as they attend the children's hospitals in Al-Furat Al-Awsat district of Iraq . All samples were collected with permission of the Iraqi

Ministry of Health's Center for the control of communicable disease and the informed consent of the parents / guardians of each children investigated .As controls samples were collected from 10 apparently health children who had never lived in an area known to be endemic for VL . Another 50 samples came, pretreatment, from children with splenomegaly and VL that had been serologically confirmed (in dipstick assay) . Each blood samples obtained in a plain tube centrifuge as soon as possible and serum separated, liquated into portions to avoid repeate freezing and thawing then stored at-20 ℃ until used . Some information was taken for each patients such as name, gender, address and other subjects .

Biochemical Tests

Superoxide dismutase (SOD) assay

The SOD was determined in the serum of all patients and controlled according to the method of [8]

Adenosine deaminase (ADA) activity

The activity was determined in the serum according to the method of [9] .

Glutathione reductase (GHR) activity

The activity of this enzyme was determined in the serum according to the method of [10] .

Alkaline phosphatase (ALP) activity

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

Lactate dehydrogenase (LD) activity

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

Alanine aminotransferase (ALT) activity

The activity was determined in the serum; the assay was carried out using kit [11] .

Aspartate aminotransferase (AST) activity

The activity was determined in the serum; the assay was carried out using randox kit [11] .

RESULTS

Superoxide dismutase (SOD)

The activity of SOD enzyme in VL group was higher (48.60 ± 0.82 µg/l) as compared with its control group (44.36 ± 0.64 µg/l) . The differences between the two group were significant ($P < 0.05$) (Table : 1) .

Adenosine deaminase (ADA)

The mean concentration of ADA of healthy control (36.84 + 2.4 U/l) was significant lower ($P < 0.05$) than that of patients with VL (87.24 ± 4.20 U/l) (Table:1) .

Glutathione reductase (GHR) .

The mean concentration of GHR of healthy control (8.20 ± 2.3 µmol/ml) was significant lower ($P < 0.05$) than that of patients with VL (16.18 ± 1.5 µmol/ml) (Table:1) .

Alkaline phosphatase (ALP)

A highly significant increase ($P < 0.05$) in the levels of VL patients was seen compared to that of the normal healthy control group (180.23 ± 4.86 versus 82.62 ± 2.5 U/l) (Table:2) .

Lactate dehydrogenase (LD)

A highly significant increase ($P < 0.05$) in serum LD mean level of VL patients compared to the healthy control group (631.40 ± 2.54 U/l versus 146.62 ± 3.76 U/l) .

Alanine aminotransferase (ALT) or GPT

A highly significant increase ($P < 0.05$) in the level of ALT of VL patients was seen, compared to that of the normal healthy control mean (25.40 ± 2.20 U/l versus 8.60 ± 4.5 U/l) (Table:2) .

Aspartate aminotransferase (AST) or GOT .

A highly significant increase ($P < 0.05$) in serum AST mean level of VL patients compared to healthy control group (60.00 ± 8.2 U/l versus 10.46 ± 6.20 U/l) (Table:2) .

Table (1) : Mean concentration ± SE of ADA, GHR & SOD enzymes sera of 50 patients infected with VL and healthy control

Enzymes	Control	Patients	P-value
SOD(µg/l)	0.64±44.36	0.82± 48.60	< 0.05
ADA (U/l)	2.4± 36.84	4.2±87.24	< 0.05
GHR (µmol/ml)	2.3 ±8.20	1.5 ± 16.18	< 0.05

Table 2 : Mean concentration ± SE of ALP, LD, ALT & AST enzymes in sera of 50 VL patients & health control

Enzymes (U/l)	Control	Patients	P – value
ALP	2.5± 82.62	4.86±180.23	< 0.05
LD	3.76 ±146.62	2.54± 361.44	< 0.05
ALT (GPT)	4.5 ± 8.60	2.20 ±25.40	< 0.05
AST (GOT)	6.20 ± 10.46	8.2 ± 60.0	< 0.05

DISCUSSION

Superoxide dismutase (SOD)

The concentration of SOD in VL group was significantly higher than in the control group . Failure of macrophages which were infected by *Leishmania* parasites to produce high levels of respiratory burst as a mechanism defense of parasite against host led to persistence of leishmaniasis [12] . The high level of the antioxidant SOD in the VL group protect host cell from the damaging effect of free radicals that are released in different infected host cell especially in liver and spleen . The source of free radicals may be from the soluble agents like antibody antigen complexes [13] . The increase of SOD as a host defence mechanism is associated with the immune response . WBC_s membranes are composed of lipid, some of them unsaturated fatty acid which are highly susceptible to free radicals attacks that may be released from phagosomes that affect the integrity of the cell membrane and decrease its fluidity, consequently impairing their function . Also free radicals caused protein oxidation, affect DNA synthesis and may be involved in cell apoptosis [14]. All this effects cell growth and impairs the hosts immune response . This might have occurred in our study with VL patients, in the reduction of cell cycle parameters than in the control and CL group . In this study, the control groups, which represent different ages did not differ in SOD concentration [15] found that there was no change in SOD activity with age .

Adenosine deaminase (ADA)

ADA enzyme is one of the most essential immune enzyme, its function gives a clear picture of the immune status of the body . It was found to play a critical role in the normal development of immune system, and for the proper development of T and B – lymphocytes in mammals . The ADA activity in leishmaniasis was significantly higher in the VL group than in control . This may result from increased B-lymphocyte count or RBC_S hemolysis by immune complexes and complement . ADA was localized within the cell membrane of RBC_S and surrounding phagocytic vacuoles . These results are similar with those of [16] who reported that patient with infections or non infections disease in which high fever occurs showed slight or moderate increase in serum ADA activity like typhoid fever and Q fever pneumonia . Our result differ from previous studies with other parasitic infection [17] studied the activity of ADA enzyme in sheep infected with *F.gigantica* and [18] in women chronically infected with *T.vaginalis* . All above found a relatively decreased activity in this enzyme which correlated with the immuno - suppression state . The decrease in ADA specific activity could cause a state of immuno – suppression, for ADA plays a critical role in the normal development of the immune system [19] . B – cell dysfunction was also found to be associated with ADA deficiency . This was found to be due to the alteration in antigen receptors [20] . In our study ADA and SOD enzymes were found to be higher in VL group . This may be result from leak of infected tissue to the blood lysis . This agrees with [21] in their study with cancerous laryngeal tissue, that the source of elevated ADA and SOD results from leak to the blood stream and removal of the serum enzyme from the blood took long period (more than month) and the cancerous tissue is it the only source the enzyme and also the significant increase of both of them could be explained as a healthy stimulated host immune response .

Glutathione reductase (GHR)

The antioxidant defense system is sophisticated and adaptive, GSH is central constituent of this system [22] . GSH depletion may be the ultimate factor determining vulnerability to oxidant attacks, so intracellular GSH status appears to be asensitive indicatior of the cells overall health [23] . In this study there was a significant increase in glutathione reductase (GHR) enzyme activity in patients with leishmaniasis . Our study differ from study of GHR activity in hydatidosis [24] reported that the mean of GHR of healthy controls was significantly higher than that of patients with hydatidosis . This enzyme is responsible for maintaining the reduced glutathione GSH in the cell by glutathione peroxidase – glutathione reductase 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 higheat content of poly unsaturated lipid and / or the most meager antioxidant defenses are generally the most

vulnerable [25]. The liver is the organ most involved with the detoxificant of xenobiotics (substances foreign to the body), and also is the main storage local for GSH (actually exporting GHS to other organ) [26]. The increase in the activity of GHR indicated its formation in the liver with its damage cells causing a high release of enzyme in to the circulation . GSH is considered the free radical scavenger and repairer of radical mediated biological damage [27], so its' deficiency led to accumulation of free radicals [22] showed that the cumulative damaging effects of oxygen radicals and other oxidants are main contributors to degenerative disease .

Alkaline phosphatase (ALP)

The activity of ALP was found to be significantly higher in patients with leishmaniasis than in health control . Alkaline phosphatase enzyme was found in many tissues, but highest concentration were found in biliary tract epithelium and kupffer's cells of the liver [28]. Some reportes showed that any factor effecting the liver metabolism was found to cause an increase in ALP activity [29]. The infancy and childhood showed high ALP values as much as 3 - 4 times that of adult value [30]. A damage or injury which was caused by VL liver tissue (principally parenchymal cells) led to releasing ALP into the blood circulation [31].

Lactate dehydrogenase (LD)

Similar results also were obtained with LD enzyme . There was an increase in its activity in VL patients in comparison with healthy controls . In general this enzyme is usually used for detection of hepatocellular damage, whenever liver cells are damaged or injured, the liver enzymes leak out into the circulation across the damaged cell membrane [32]. As we mentioned previously ADA play a role in the liver function [33], so its deficiency lead to liver disturbance, hence, elevated levels of LD in VL disease patients will be attributed to hepatic involvement by diseases [34], 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 VL patients .

Alanine aminotransferase (ALT) or GPT

Serum ALT activity was significantly increrased in all cases of kala – azar studied in comparison to the normal controls . ALT was found predominantly in the liver, lesser quantities were found in kidneys, heart and skeltal muscle [28] . The rise of serum ALT in children affects with VL was caused by *Leishmania donovani* which damaged the liver and spleen cells, these injured cells released ALT into the circulation[35] . The increase in serum ALT activity could reflect the pathological picture of nonspecific granuloma formation in the liver of these patients [36] .

Aspartate aminotransferase (AST) or GOT

Serum AST was significantly increased in all cases of VL patients studied in comparison to the normal control . Serum AST levels were compared with ALT levels . The AST /ALT ratio is greater than one . This value may help in determining liver damage [28] . The rise serum AST in children affected with VL which damage the liver, these injuried cells release AST into circulation [35] .

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