

Biochemical and Hematological Levels in Patients with Cutaneous Leishmaniasis in Yemen

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ABSTRACT:

Background & objectives: This study was conducted in Sharab, Yemen to evaluate the oxidative- anti oxidative status of patient with cutaneous leishmaniasis (CL) and to establish the data base line on biochemical and hematological parameters alteration among infected patient.

METHODS: In This study, a total of 150 individuals aged from 1 to 60 years; 99 of them (47 males and 52 females) had cutaneous leishmaniasis (CL) and 51 (27 males and 24 females) free from CL were used as a control group. Serum malondialdehyde (MDA) and uric acid levels, catalase activity, liver function tests, lipid profile and blood indices were assessed in this study.

RESULTS: The results showed a significant increase in serum MDA, uric acid and a significant decrease in albumin, total cholesterol, low-density lipoproteins and high-density lipoproteins in patients with CL as compared with controls.

CONCLUSION: The high MDA level strongly indicated the event of oxidative stress and lipid peroxidation as a mechanism of tissue damage during infection with CL and also the results suggest that decrease in lipid profile levels may be a valuable information in the diagnosis of the CL besides the clinical and laboratory features.

KEYWORDS: Blood indices, cutaneous leishmaniasis, lipid profiles, liver function tests, oxidative-ant oxidative.

I. INTRODUCTION

Leishmaniasis refers to a group of infectious disease endemic in tropical, Asian and southern European countries. It is caused by obligate intra macrophage protozoa and is transmitted by the bite of infected female sand flies. The disease phenotypes include visceral leishmaniasis, post-kala-azar dermal leishmaniasis, cutaneous leishmaniasis, and mucosal leishmaniasis [1] Cutaneous leishmaniasis (CL) often refers to a group of diseases because of the varied spectrum of clinical manifestations, which range from small cutaneous nodules to gross mucosal tissue destruction. CL is a chronic granulomatous infection that invades the skin. The clinical signs of the disease are attributed to the severity of the immune response of the host. The cellular immune response against the disease is fundamental and vitally important [3]. Macrophages, neutrophils and other phagocytes cells are key components of the antimicrobial and tumoricidal immune responses. These cells are capable of generating large amounts of highly toxic molecules, such as reactive oxygen species (ROS), including superoxide radicals (O₂.), hydrogen peroxide (H₂O₂) and hydroxyl radicals (OH.), and reactive nitrogen species (RNS) [3,12]. Possible roles of the highly reactive oxygen, free radicals in the pathogenesis of parasitic infections have been an active area of research [3, 4] ROS and RNS are capable of degrading numerous biomolecules including DNA, carbohydrates and proteins. In addition, ROS and RNS can attack the polyunsaturated fatty acids of membrane lipids causing lipid peroxidation and the disorganization of cell structure and function [3]. Highly reactive oxygen free radicals have been implicated in the pathogenesis of various parasitic infections including leishmaniasis [4]. Lipid peroxidation is, a well-established mechanism of cellular injury, is used as an indicator of oxidative stress in cells and tissues. It is derived from polyunsaturated fatty acid, which is unstable and can decompose to form a complex series of numerous degradation products [6]. Increasing levels of lipid peroxidation products have been associated with a variety of chronic diseases including parasitic infections [4]. The determination of MDA level and antioxidants enzyme activities are major criteria concerning the severity of possible peroxidation, which takes place in cell membranes. There are several intracellular defense mechanisms that prevent potential oxidative damage, and can be either enzymatic (e.g. catalase, superoxide dismutase and glutathione peroxidase) or non-enzymatic (such as vitamins, uric acid, albumin and bilirubin) [7]. The antioxidant in its highest concentration in human blood is uric acid, which provides half of the total antioxidant capacity of human serum [8, 16]. The role of lipids and their metabolical

mechanisms in protozoan and helminthes infections have shown that cholesterol is a major constituent of eukaryotic membranes and plays a crucial role in cellular membrane organization, dynamics, function and sorting. Cholesterol is required for efficient attachment and internalization of the parasite in macrophages, leading to infection [20]. Leishmaniasis is endemic in Yemen and has been recognized as a public health problem. Although its prevalence has not been fully documented, most of the cases have been registered in Lahj, Abun, Hajh, Taiz and Saadah Governorates in Yemen [2]. Therefore, this work has been carried out to investigate the status of CL and its impact on blood levels in patients suffering from CL.

II. MATERIAL AND METHOD

2.1 Case preparation

This study has been conducted in Sharab District (Taiz, Yemen) in the period from April 2010 to October 2011. Cases were selected from Banny-Ziad Health Center in Sharab. A total of 99 patients infected with CL (47 males and 52 females) aged between 1 to 60 years were subjected to investigation. The study population's ages and genders were matched. Out of the total sample, 51 healthy persons (27 males and 24 females) functioned as control group. Diagnosis was confirmed clinically, as well as by laboratory demonstration of the parasite in the lesions by direct smears.

2.2 Sample collection

Ten milliliters of venous blood were collected from patients as well as from controls after an overnight fast. The collected blood samples were immediately transferred to two different test tubes, the blood fraction in the first test tube containing ethylenediaminetetraacetic acid (EDTA) was used to determine blood indices, whereas the separated serum from the other fraction of blood were used for biochemical analysis. The study was performed in accordance with the Helsinki Declaration and approved by ethical Committee approved by Ethical Committee

2.3 Biochemical Analysis

Catalase (CAT) activity was determined in the serum according to the method of Goth [9]. It was a combination of optimized enzymatic conditions and the spectrophotometric assay of hydrogen peroxide based on formation of its stable complex with ammonium molybdate. Results were expressed as Katal unit per liter (KU/l). Malondialdehyde (MDA) level was measured by using thiobarbituric acid tests according to the Fong et al. [10] method. Results were expressed as nanomole per mill (nM/ml).

The biochemical tests includes assays of alanine: 2-oxoglutarate aminotransferase (ALT), aspartate: 2-oxoglutarate aminotransferase (AST), direct bilirubin (DB), total bilirubin (TB), triglycerides (TG), total cholesterol (Tcho), low density lipoprotein (LDL), high density lipoprotein (HDL), uric acid (UA), albumin (Alb) and total protein (TP) were estimated following the instructions of commercial kits provided by Spinreact, Spain.

2.4 Hematological Study

Hemoglobin level (Hb), white blood cells count (WBC's), platelets count (PLT) and erythrocyte sedimentation rate (ESR) was measured in EDTA samples according to the methods of Dacie and Lewis [11].

2.5 Statistical Analysis

The data obtained from this study was analyzed with SPSS program. The results were expressed as mean $\pm$ standard error of the mean (SEM). Statistical significance was assessed by student t-test. Correlations among different parameters were carried out using Pearson's linear correlation analysis. P- Value less than 0.05 was considered statistically significant.

III. RESULTS

Table (1) Means $\pm$ SEM of MDA, CAT, UA, Alb, TB and DB in the serum of patients and controls

	MDA nM/ml	CAT kU/l	UA mg/dl	Alb mg/dl	TB mg/dl	DB mg/dl
Patients	3.40 $\pm$ 0.06*	72.53 $\pm$ 4.5	8.5 $\pm$ 0.27*	3.05 $\pm$ 0.1*	0.47 $\pm$ 0.03	0.16 $\pm$ 0.12
Controls	1.38 $\pm$ 0.07	83.11 $\pm$ 4.91	5.6 $\pm$ 0.17	3.71 $\pm$ 0.3	0.43 $\pm$ 0.07	0.20 $\pm$ 0.07
* P < 0.001 compared to control group						

Results are presented as mean $\pm$ SEM. Data was analyzed by student -t-test. * P < 0.001 was considered significant.

Table (1) reveals that serum MDA and UA levels were high in patients, but the mean level of Alb decreased. The differences were significant as compared with controls, whereas the levels of CAT, TB and DB not change.

Table (2) Means $\pm$ SEM of serum TG, Tcho, HDL and LD in patients and controls

<i>Lipid profile (mg/dl)</i>	<i>Controls</i>	<i>Patients</i>
<i>Tcho</i>	163.70 $\pm$ 5.5	135.12 $\pm$ 4.1*
<i>TG</i>	153.15 $\pm$ 01	154.36 $\pm$ 1.3
<i>HDL</i>	41.69 $\pm$ 1.5	32.40 $\pm$ 1.6*
<i>LDL</i>	91.3 $\pm$ 4.6	73.44 $\pm$ 3.1*
* P < 0.001 compared to control group		

Results are presented as mean $\pm$ SEM .Data were analyzed by student -t-test. * P < 0.001 was considered significant.

Table (2) shows significant decrease in serum Tcho, HDL and LDL concentrations in patients whereas, serum TG level did not change significantly as compared to controls.

Table(3) Means $\pm$ SEM of serum TP, AST and ALT in Patients and controls

<i>Parameters</i>	<i>Patients</i>	<i>Controls</i>
<i>TP(mg/dl)</i>	8.30 $\pm$ 0.20*	6.52 $\pm$ 0.20
<i>ALT(U/l)</i>	19.35 $\pm$ 0.56	19.82 $\pm$ 0.61
<i>AST(U/l)</i>	23.71 $\pm$ 0.61	24.52 $\pm$ 0.63
* P < 0.001 compared to control group		

Results are presented as mean $\pm$ SEM .Data were analyzed by student -t-test. * P < 0.001 was considered significant.

Table (3) reveals that the mean levels of AST and ALT were not significantly changed whereas, the mean level of TP was significantly increased in patients compared to controls (P<0.001).

Table (4) significant correlation coefficients among various biochemical parameters in Patients

<i>Parameters</i>		<i>R</i>	<i>p-Value</i>
TP	<i>Alb</i>	0.293	0.004
	<i>TCho</i>	-0.252	0.001
	<i>LDL</i>	-0.413	0.001
MDA	<i>Alb</i>	-0.239	0.003

Correlations among different parameters were carried out using Pearson's linear correlation analysis.

Table (4) shows significant negative correlations between the levels of MDA and Alb (R=- 0.293, P <0.003), between TP and Tcho (R= - 0.252, P < 0.001), and between total protein and LDL (R=- 0.413, P < 0.001). It also shows a positive correlation between Alb and TP (R= 0.293, P <0.004).

Table (5) Means $\pm$ SEM of hematological Parameters in patients and controls

<i>Parameters</i>	<i>Patients</i>	<i>Controls</i>
Hb(g/dl)	12.41 $\pm$ 0.11	12.80 $\pm$ 0.14
WBC's ($\times 10^9$/dl)	6219.83 $\pm$ 22*	5011.76 $\pm$ 16
ESR (mm/hr)	31.61 $\pm$ 1.9*	24.68 $\pm$ 2.4
PLT ($\times 10^9$/dl)	348.51 $\pm$ 8.80	321.18 $\pm$ 13.3
* P < 0.001 compared to control group		

Results are presented as mean $\pm$ SEM .Data were analyzed by student -t-test. * P < 0.001 was considered significant.

Table (5) shows significant increase in WBC's count, ESR, but no significant changes in PLT count and Hb concentration in patients compared to controls.

IV. DISCUSSION

The significant increase of MDA level in the present study strongly reflects an increased lipid peroxidation initiated by reaction of free radicals to polyunsaturated fatty acids in biological membranes. Lipid peroxidation is produced by oxidative stress resulting from the over-production of ROS and RNS [3]. The high serum MDA value in CL reflects the host defense against the parasite infection. Moreover, the rapid production of oxygen free radicals depletes the protective antioxidant enzymes, which is attributed to cell injury caused by Leishmania [3,4]. A significant decrease in catalase activity and increased MDA levels in patient with cutaneous leishmaniasis have been reported [3].The non-significant decrease of catalase activity in our study may have happened as a result of the parasite itself being protected to some extent against toxic oxygen metabolites.Amastigotes appears to contain catalase and superoxide dismutase and is poorly endowed in GPX, where they serve to mop up H₂O₂ evolved during infection. However, due to the difference in Km, the concentration of GPX and CAT in detoxification of H₂O₂ is different. GPX acts at low H₂O₂ concentration whereas CAT plays a role when GPX pathway reaches saturation [13]. In this study, the lack of significant decrease in CAT activity may be attributed to low levels of GPX and increased generation of H₂O₂ above the capacity of GPX, which has led to an increase in CAT activity. High levels of uric acid were reported in patients with parasite infection [16, 17]. Uric acid is an important contributor to total antioxidant capacity; it provides a significant antioxidant defense against nitration by peroxynitrite [15]. It is an important oxidative stress marker and has a potential therapeutic role as an antioxidant [7]. Increased level of uric acid may contribute much more to scavenging of singlet oxygen and other free radicals. Significant decrease of albumin level in the present study may reflect its role as a non-enzymatic scavenger; it participates in Fenton reactions, by preventing H₂O₂ from escaping into free solution [18]. Moreover, during the acute-phase, response levels of negative acute phase proteins such as albumin and transferrin decreased whereas levels of positive acute-phase proteins as C-reactive protein and serum amyloid A increased [6].

Acute-phase protein plays important roles in the repair and maintenance of the body tissue including the immune system during infection and inflammation [21]. The significant negative correlation between MDA and albumin levels in our study may suggest that the significant decrease in albumin level supports its role as a non-enzymatic scavenger and not due to malnutrition or another clinical status. Similar suggestions have been reported in patients infected with CL [3] and in patients suffering from visceral leishmaniasis (VL) [5]. Levels of uric acid, albumin and bilirubin are often used as major non-enzymatic antioxidant biomarkers [7]. They prevent free radical reaction by sequestering transition metal ions by chelating in plasma, providing the primary extracellular defense against oxidative stress [14]. Significant alterations in lipid were reported in patients with parasites infection [20] and the role of cholesterol in parasitic infection has been reported [20]. Elevated lipoproteins like HDL and LDL and total cholesterol have been shown in patients suffering from malaria infection [25]. Decreased HDL, LDL and total cholesterol levels were observed in pediatric VL [26, 27]. The presence of good negative correlation between TP and both LDL and total cholesterol in our study may suggest that there are special links between protein and lipids. During infection and inflammation, which are acute phase reactions, it is well known that some alterations in lipoprotein metabolism occur and result in a variety of changes in the plasma concentrations and composition of lipids and lipoproteins. Infection and inflammation are associated with a decrease in HDL and cholesterol levels [6]. The cause of reduced HDL levels is especially associated with the decrease of HDL-associated ApoA-I and ApoA-II levels, decrease in lecithin cholesterol acyl transferase activity, and increased levels of serum amyloid [6,23].

The alterations in lipid have not been fully understood in patients suffering from CL. The lack of significant increase of AST, ALT and bilirubin in the present study reflect the normal liver function and absence of VL among patients. Kashani et al. [22] reported non-significant change in the levels of bilirubin, AST and ALT in CL patients. Whereas, significant increase in liver function tests has been observed in patients with VL [19, 24]. The increased WBCs count in the present study may be due to infection. WBCs increase in response to allergic reaction and parasitic infection [20]. By contrast, the increase in ESR rat may probably be due to the release of the acute phase reactants [28]. Severe types of hematological disorders including pancytopenia, hemolysis and hemophgocytic syndrome were found in patients with VL [28, 30] and in patients with VL who suffer from cutaneous lesions [24]. Parasite proliferates in the mononuclear phagocytic system especially in spleen, liver and marrow of patients with VL, and leads to hyperplasia of phagocytic system with resultant disturbances in phagocyte bearing organs, producing hematological manifestations. Hence, this condition is of interest to hematopathologists, because the reticuloendothelial system is the target of parasitization [28]. Peripheral blood picture evolution has often been discussed in patients with VL but it has not been widely investigated in CL. However, significant decrease of blood indices were reported by Ayatollahi[29] in patients infected with CL after treatment with Glucantime, whereas pre-treatment blood indices of such patients appeared within reference value and near the result obtained in our study.

V. CONCLUSION

Based on the present study, we conclude that CL patients are affected by oxidative stress, which may contribute to the progression of the disease. These results clearly show that the high level of MDA together with the decrease in Tcho, HDL and LDL could be a clue for diagnosis of CL besides the clinical and laboratory features. This study is the first of its type in Taiz, Yemen. Further, more studies are needed on leishmaniasis and its effect on biochemical and hematological values in humans in the targeted area (Taiz) and other related areas in the country.

Conflict of interest

No financial, personal or other conflict of interest.

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