Original Article

Evaluation of Oxidative Stress in Cutaneous Leishmaniasis in Iraqi Patients

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Abstract

Background: This study evaluated the oxidative—antioxidative status of patients with cutaneous leishmaniasis (CL) and to establish the data alteration among the patients and the control. **Objectives:** To determine serum levels of malondialdehyde (MDA), serum uric acid (UA), and catalase enzyme as a markers of oxidative stress in the patients suffering from CL. **Materials and Methods:** A total of 46 patients with CL (22 males and 24 females) aged between 5 and 60 years were subjected to investigations. Out of the total sample, 42 healthy persons (28 males and 14 females) functioned as control group, patients who received anti-leishmaniasis treatment locally or systemically for their cutaneous lesions and also patients with chronic history of medical disease or drug history for systemic or dermatological diseases were not included in this study. **Results:** For comparative evaluation of oxidative stress markers between patients and control; there were a significant increase in lipid peroxidation marker; serum MDA and non-enzymatic antioxidant marker—serum UA in the patient group in comparison to the control group, with non-significant decrease in enzymatic antioxidant—serum catalase enzyme in patients as compared with control groups. There is negative significant relationship for decrease of catalase enzyme for patients with CL above age of 15 years. The study of the patients with CL that is, associated with secondary bacterial infection shows a significant decrease in serum catalase enzyme when compared with control group. **Conclusions:** 1. The results of this study clearly demonstrate the importance of serum MDA as an early biochemical indicator of peroxidative damage resulting from CL. 2. Increased serum UA, and decrease in serum catalase enzyme provided a free radical scavenger's action.

Keywords: Cutaneous leishmaniasis, Iraqi patients, oxidative stress

Introduction

Numerous Leishmania species can infect both humans and other mammals, leading to the vector-borne disease leishmaniasis. It is a complex disease, with heterogeneous clinical manifestations ranging from asymptomatic infections to lesions at cutaneous sites (cutaneous leishmaniasis [CL]), mucosal sites (mucocutaneous leishmaniasis) or in visceral organs (visceral leishmaniasis), depending on the species and host characteristics.[1]

The severity of the host's immune response to the disease is linked to its clinical manifestations,[2] and the cellular immune response to it is crucial and fundamental. Major players in the antimicrobial immune responses include macrophages, neutrophils, and other phagocyte cells. Reactive oxygen species (ROS), including superoxide radicals (O_2), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH), and reactive nitrogen species (RNS), are among the highly toxic molecules that these cells are capable of producing.[2-4]

It has been actively researched whether these highly reactive oxygen free radicals play a role in the pathogenesis of parasitic infections. Numerous biomolecules, such as DNA, carbohydrates, and proteins, can be broken down by ROS and RNS. In addition, ROS and RNS can attack the polyunsaturated fatty acids in membrane lipids, which

Access this article online

Quick Response Code:

Website: https://journals.lww.com/mjby

DOI: 10.4103/MJBL.MJBL_553_23

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Submission: 14-May-2023 **Accepted:** 02-Jan-2024 **Published:** 24-Sep-2024

How to cite this article: Al-Hattab MK, Nahi NH, Al-Kalidi JH. Evaluation of oxidative stress in cutaneous leishmaniasis in Iraqi patients. Med J Babylon 2024;21:538-43.

results in lipid peroxidation and the breakdown of cell structure and function.[2]

Lipid peroxidation is a well-established mechanism of cellular injury and is used as an indicator of oxidative stress in cells and tissues, with increasing levels of lipid peroxidation products have been associated with a variety of chronic diseases including parasitic infections.[4]

Malondialdehyde (MDA) is the main product of lipid peroxidation and is used commonly as an oxidative stress marker.[5]

There are several intracellular defense mechanisms that prevent potential oxidative damage (known as antioxidants), that can be either enzymatic (e.g., catalase, superoxide dismutase, and glutathione peroxidase) or non-enzymatic (such as vitamins, uric acid [UA], albumin and bilirubin),[6] the highest antioxidant concentration in human blood is UA, which provides half of the total antioxidant capacity of human serum.[7,8]

Proteins are important targets for oxidation reactions described as a mismatch in how free radicals are formed and the antioxidant reaction within cells, because they are abundant in tissues, extracellular cells and physiological fluids as well as their quick reaction rates with oxidants, furthermore oxidative stress can cause.[9]

Natural antioxidants can be useful in preventing or slowing the progressing of oxidative stress-related degenerative disease.[10] The determination of MDA level and antioxidant enzyme activities are major criteria regarding the severity of potential peroxidation which occurs in cell membranes.

The serum concentration of MDA was suggested in studies in humans with CL to establish its connection to the pathological mechanism of the disease.

Aim of the study

To determine the levels of serum MDA, serum UA, and catalase enzyme as a markers of oxidative stress in the patients suffering from CL.

Materials and Methods

Case preparation lesions

This study was performed in Al-Sader Teaching Hospital in Al- Najaf city in Iraq during the period from October 2017 to January 2018. A total of 46 patients infected with CL (22 males and 24 females) aged between 5 and 60 years were subjected to investigations. Out of the total sample, 42 healthy persons (28 males and 14 females) functioned as a control group. These patients were complained from skin lesion in exposed parts of the body mostly in the face, leg, arm, and diagnosed clinically by dermatologist as CL.

Patient's profiles including; name, date of sampling, age, sex, address (urban or rural), number of lesions, duration of lesion, type of CL infection (dry or wet), location of the lesions, associated bacterial infection, medical history, and drug history.

Patients who received anti-leishmaniasis treatment locally or systemically for their cutaneous lesions and patients with chronic history of systemic or dermatological diseases are not included in this study.

Clinical and bacteriological examination

The history and clinical examination involving appearance of skin lesion are the most important point in the diagnosis of CL[11] with bacteriological identification of clinically suspected secondary bacterial infection by swab and culture of CL.

Blood samples

Venous blood samples (5 mL) were drawn into plastic test tube without any anticoagulant in the early morning after at least 8 h overnight fasting. Samples were centrifuged at 3000×*g* for 10 min at 4°C to obtain serum and stored at −80°C until being analyzed all together at the same time later on.

Biochemical analysis

The serum levels of MDA, UA, and catalase enzyme were determined with a spectrophotometer according to the manufacturer's recommendations of commercial colorimetric assay kits with serum concentrations of MDA, catalase activity, and UA were expressed as nmol/mL, KU/L and mg/L respectively.

Statistical analysis

Analytical Statistics The results of the analysis of the study's data were presented as mean standard error of mean (SEM) in the SPSS program version 23. Statistical significance was assessed by independent Student *t* test and one way ANOVA test and regression analysis, the *P* value less than 0.05 was considered statistically significant.

Ethical approval

Ethical approval has been provided from the scientific council of Dermatology and Venerology-Arb Board of Medical Specialization. All participants in this study have been enlightened with signed consents. The date for this approval (October 2017 to January 2018).

Results

A total of 46 patients presented with CL (24 females and 22 males) with a mean age of 36.4 ± 6.2 years (range 5–60 years) and 42 healthy *controls* (18 females and 24 males) with a mean age of 34.1 ± 5.8 years (range 5–57 years) were included in our study.

Table 1: Patients are sorted into variable subgroups with their numbers and percentages

Variable	Number	Percent
Sex: male/female	22/24	48%/52%
Age: ≤15/>15	16/16/30	35%/65%
Urban/rural	19/27	41%/59%
Lesion number: (single, multiple)	17/29	37%/63%
Type of lesion: dry/wet	13/33	28%/72%
Secondary bacterial infection of lesion/not infected	9/37	20%/80%

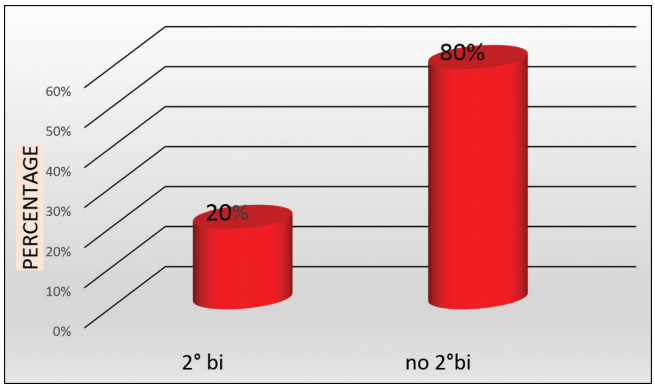

Figure 1: Percentage for patients with cutaneous leishmaniasis associated with secondary bacterial infection or not (2˚bi or no 2˚bi)

Demography of patient groups

The classification of patients with CL in this study were demonstrated as in Table 1.

The control group was also classified into subgroups according to their age (age ≤ 15 years = 14, age >15 years = 28) and address to (urban 20 and rural 22) for comparative study later on with the patients subgroups.

Associated secondary bacterial infections

Form swab and culture of the clinically suspected secondary bacterial infection of CL lesions and the result was nine patients (20%) associated with secondary bacterial infection (mostly *Staphylococcus aureus* and *Staphylococcus epidermidis*) from the total number of the patients [Figure 1].

Evaluation of oxidative stress markers in CL

For comparative study between patients and control group for oxidative stress makers; serum MDA (lipid peroxidation marker), serum UA (non-enzymatic antioxidant), and serum catalase enzyme (enzymatic antioxidant).

The results were serum level of MDA and UA were significantly higher in the patient with CL than that of the control group ($P < 0.05$), while catalase enzyme level was decreased in patients when compared with control group but to a non-significant value ($P > 0.05$) as in Table 2.

Significant effect of different variables on oxidative stress markers

The study also assessed the effects of variables like (age, gender, address) on oxidative stress markers (MDA, UA, and catalase enzyme) for the patients and the control group and also study was included comparison between each subgroups within the patients (associated secondary bacterial infection or not, type of lesion wet and dry, number of lesion—single/multiple) with the control group.

All of the above suggested study was non-significant except for following;

1. The results show significant negative relation of decrease catalase enzyme with increasing of age in subgroup of the patients (>15 years) as in Table 3.
2. The result also show significant decrease of serum catalase enzyme in secondary bacterial infection subgroup when compared with the control group as Table 4.

Discussion

The present study showed changes in lipid peroxidation during CL infection with some free radicals' scavengers as part of balanced oxidant and antioxidant mechanism during oxidative process of infection.

Serum lipid peroxidation marker; MDA levels

Lipid peroxidation is a physiologically necessary process that occurs on a constant basis, but evidence from several different directions suggests that peroxidation plays a significant role in the pathogenesis of a number of parasitic diseases.[12]

The significant increase in MDA level (P 0. 0.05) in the CL patient in our study strongly reflects an increased lipid peroxidation caused by oxidative stress brought on by the excessive production of ROS and RNS.

Lipid peroxidation is produced by the reaction of free radicals with polyunsaturated fatty acids in biological membranes.[12] As a result, its balanced mechanism between oxidant and antioxidant with a high serum MDA value in CL may reflect the host defense against parasite infection.

Table 2: Comparison of malondialdehyde (MDA), uric acid (UA) and catalase enzyme (cat.) for the patients and healthy control

Test	Number	Mean ± SEM	*P* value
MDA nM/mL	Patient (46)	Patients 3.40 ± 0.06	<0.05
	Control (42)	Control 1.38 ± 0.07	
UA mg/L	Patient (46)	Patient 8.5 ± 0.27	<0.05
	Control (42)	Control 5.6 ± 0.17	
Cat. kU/L	Patient (46)	Patients 70.5 ± 4.5	>0.05
	Control (42)	Control 88.1 ± 4.9	

Table 3: Significant negative relation of catalase enzyme when the age >15 years from the patients group

Test	Age		Number SEM	*P* value
Cat. KU/L	Patients > 15 years	16	68.39 ± 0.014	<0.05
	Control > 15 years	28	80.1 ± 0.32	>0.05
	Patients ≤ 15 years	30	73.100 ± 0.021	>0.05
	Control ≤ 15 years	14	86.55 ± 2.1	>0.05

Table 4: Serum level of catalase enzyme within the patients' subgroups (secondary bacterial infection or not) as compared with the control group

Variable/Cat. KU/L		Patients		Control	*P* value
		No.	(46)	(42)	
			Mean ± SEM	Mean ± SEM	
Associated bacterial infection	Yes	9	67.14 ± 0.013	88.1 ± 4.9	<0.05
	No	37	74.20 ± 0.022	88.1 ± 4.9	>0.05

In addition, the rapid production of oxygen free radicals depletes the protective antioxidant enzymes, which then contribute to cell injury caused by Leishmania.[13,14] Kocyigit *et al*.[13] and Ozbilge *et al*.[14] noted comparable outcomes, they demonstrated a significant increase in LPO, superoxide dismutase peroxidation (SOP), glutathione, and a decrease in catalase enzyme levels in patients with active CL than those of healthy control. Serarslan *et al*.[15] also found a significant increase of serum MDA and nitrous oxide (NO) in CL patients, as compared to their control.

The results of this study revealed that no effect of age and sex and other variable on MDA levels in the patients and the control group, similar findings were reported by Quassim[16] and Al-Shamiri[17] as they found no significant changing in MDA levels by effect of age on patients and control group but this disagree with the results of Hassan[18] who observed a significant increase in plasma MDA in the age of control group of (27–44 years) as compared to older age groups (45–58 years) and he suggested that this increase in MDA level due to decrease SOD scavenger.

Free radical scavengers

Enzymatic scavenger's catalase

In our study, there is decrease in serum catalase enzyme in the patient infected with CL but was not significant as compared to control and these results were in agreement with result of Asmaa *et al*.[19] Salwa *et al*.[20] and also these results were in disagreement with results of Erel *et al*.[21] and Kocyigit *et al*.[22] that they found that there was a significant decrease of serum catalase enzyme and increased MDA levels in patients with CL as compared to control.

The mechanism of decrease serum catalase enzyme was due to catalase is responsible for detoxification of H_2O_2 into water and molecular oxygen[23] and in an oxidative stress of infection can this mechanism causes consumption of catalase in serum as an enzymatic antioxidant, the non-significant decrease of catalase enzyme may have been happened as a result of altered susceptibility—resistance of parasite with human immunity as explain by Murray HW.[24]

The bases on which classification of the age into those ≤15 years and those >15 years this depend on many researches that show significant differences of this age subgroups for prevalence and severity of CL infection.[19,25]

Niwa *et al*.[26] reported different findings regarding the level of serum catalase enzyme. they discovered that the basic levels of the H_2O_2 scavenging enzymes catalase, glutathione peroxidase, and dglucose-6-phosphate dehydrogenase were significantly higher in younger adults than in elderly people. Similarly, these scavenging enzymes were found to be decreased in leukocytes of older adults

in comparing with younger adults and this may explain our study results that catalase enzyme level affected by increasing age group.

For significant decrease of serum catalase enzyme with secondary bacterial infection this may suggest more tissue destruction and invasion deplete catalase enzyme and this result agreed with result of Kocyigit *et al*.[22]

Non-enzymatic scavengers' uric acid

As a significant antioxidant defense against nitration by proxy nitrite, UA contributes significantly to total antioxidant capacity. It also plays a crucial role as a marker of oxidative stress and has the potential to be used therapeutically.[27]

Our study found that there was a significantly higher level of UA in the patient groups than in the control groups ($P < 0.05$), and this higher level of UA may contribute significantly more to the scavenging effect of free radicals. Glantzounis *et al*.[28] also reported a similar outcome.

This increase in UA level may reflect physiological activity and the influence of catabolism or destruction,[29,30] and evidently may support the potent antioxidant function of UA in scavenging singlet oxygen and other free radicals.[31,32] UA may protect against oxidative stress, or it may act as a pro-oxidant and contribute to the damage caused by these diseases.[31,32]

There was no significant effect on serum UA regarding the age, sex and other variables in our study and this may be due to its linear scavenger activities.[32]

Conclusions

1. This study gives strong support for MDA's function as an early biochemical marker of peroxidation damage occurring during CL.
2. Increased levels of serum UA, and catalase activity provided free radical scavengers' action.

Recommendations

Assessment of oxidative and antioxidant activity during oxidative stress of CL put promising base for diagnostic and therapeutic approach for CL infection.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Galluzzi L, Marcello C, Aurora D. Real-time PCR applications for diagnosis of leishmaniasis. Parasites Vectors 2018;11: 273.
2. Moschella SL, Hurley HL. Disease of the mononuclear phagocytic system. In: Dermatology. 2nd ed. Philadelphia: W.B. Saunders Co.; 1985. p. 890100.
3. Sharquie KE, Najm RA, Farjou IB, Al-Timimi DJ. Oral zinc sulfate in the treatment of acute cutaneous leishmaniasis. Clin Exp Dermatol 2001;26:21-6.
4. Sukkar F. Epidemiology of leishmaniasis in Iraq. Bull Endem Dis (Baghdad) 1983;228:33-52.
5. Ayala A, Muñoz MF, Argüelles S. Lipid peroxidation: Production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxid Med Cell Longev 2014;2014:31.
6. Sharquie KE, Hassen AS, Hassen SA, Al-Hamami IA. Evaluation of diagnosis of cutaneous leishmaniasis by direct smear, culture and histopathology. Saudi Med J 2002;23:925-8.
7. El-Sohemy A, Baylin A, Kabagambe E, Ascherio A, Spiegelman D, Campos H. Individual carotenoid concentrations in adipose tissue and plasma as biomarkers of dietary intake. Am J Clin Nutr 2002;76 :172-9.
8. Guirges SY. Natural and experimental reinfection of oriental sore. Ann Trop Med 1971;65:197-205.
9. Zainal IG. Study the profile of some antioxidant markers in Diabetes Mellitus and non-Diabetes Mellitus patients with cardiovascular disease. Med J Babylon 2022;19:653.
10. Oubaid EN, Ab-Raghif AR, Al-Sudany IM. Phytochemical screening and antioxidant activity of Uncaria tomentosa extract: In vitro and in vivo studies. Med J Babylon 2023;20:136.
11. Aronson N, Herwaldt BL, Libman M, Pearson R, Lopez-Velez R, Weina P, *et al*. Diagnosis and treatment of leishmaniasis. Am J Trop Med Hyg 2017;96:24-45.
12. Bagchi M, Mukherjee S, Basu MK. Lipid peroxidation in hepatic microsomal membranes isolated from mice in health and in experimental leishmaniasis. Indian J Biochem Biophys 1993;30:277-81.
13. Kocyigit A, Gurel M, Ulukanligil M. Erythrocyte antioxidative enzyme activities and lipid peroxidation levels in patients with cutaneous leishmaniasis. Parasite 2003;10 :277-81.
14. Ozbilge H, Aksoy N, Kilic E, Saraymen R, Yazar S, Vural H. Evaluation of oxidative stress in cutaneous leishmaniasis. J Dermatol 2005;32:7-11.
15. Serarslan G, Yılmaz HR, Söğüt S. Serum antioxidant activities, malondialdehyde and nitric oxide levels in human cutaneous leishmaniasis. Clin Exp Dermatol 2005;30:267-71.
16. Quassim MM. Oxidative stress in hypertensive patients on different types of treatment. M. Sc Thesis. AL-Mustansirriyah University. Iraq. 2001. (Arabic reference).
17. Al-Shamiri SAA. Evaluation of some biochemical markers of the oxidative damage and myocardial injury. Ph. D. Thesis Medical College, University of Baghdad, Iraq. 2003. p. 95-102. (Arabic reference).
18. Hassan NAR, Antioxidant activities of free radical scavengers in ischemic heart disease, doctors of philosophy in biochemistry. A Thesis, University of Baghdad, Iraq. 1996. p. 34-56. (Arabic reference).
19. Asmaa Q, Al-Shamerii S, Al-Tag M, Al-Shamerii A, Li Y, Osman BH. Parasitological and biochemical studies on cutaneous leishmaniasis in Shara'b District, Taiz, Yemen. Ann Clin Microbiol Antimicrob 2017;16:47.
20. Salwa AG, Adam H. Biochemical and hematological levels in patients with cutaneous leishmaniasis in Yemen. Int J Pharm Sci Invent 2014;3:2319-6718.
21. Erel O, Kocyigit A, Bulut V, Gurel MS. Reactive nitrogen and oxygen intermediates in patients with cutaneous leishmaniasis. Mem Inst Oswaldo Cruz 1999;94:179-83.
22. Kocyigit A, Keles H, Selek S, Guzel S, Celik H, Erel O. Increased DNA damage and oxidative stress in patients with cutaneous leishmaniasis. Mutat Res 2005;585:71-8.
23. Kono Y. Superoxide radical and superoxide dismutase. Arch Biochem Biophys 1978;186:189.
24. Murray HW. Susceptibility of leishmania to oxygen intermediates and killing by normal macrophages. J Exp Med 1981;153:1302-15.

25. Feleke T, Workalemahu A, Getasew M. Magnitude and associated factors of cutaneous leishmaniasis; in Mekelle City, Ayder Referral Hospital, Tigray, Northern Ethiopia. Clin Med Res 2014;3:189-99.
26. Niwa Y, Iizawa O, Ishimoto K, Akamatsu H, Kanoh T. Age-dependent basal level and induction capacity of copper-zinc and manganese superoxide dismutase and other scavenging enzyme activities in leukocytes from young and elderly adults. Am J Pathol 1993;143:312-20.
27. Teng RJ, Ye YZ, Parks DA, Beckman JS. Urate produced during hypoxia protects heart proteins from peroxynitrite-mediated protein nitration. Free Radic Biol Med 2002;33:1243-9.
28. Glantzounis GK, Tsimoyiannis EC, Kappas AM, Galaris DA. Uric acid and oxidative stress. Curr Pharm Des 2005;11:4145-51.
29. Mahajan M, Kaur S, Mahajan S, Kant R. Uric acid a better scavenger of free radicals than vitamin C in rheumatoid arthritis. Indian J Clin Biochem 2009;24:205-7.
30. Verde FA, Verde FA, Veronese FJ, Neto AS, Fuc G, Verde EM. Hyponatremia in visceral leishmaniasis. Rev Inst Med Trop Sao Paulo 2010;52:253-8.
31. Chuang CC, Shiesh SC, Chi CH, Tu YF, Hor LI, Shieh CC, *et al*. Serum total antioxidant capacity reflects severity of illness in patients with severe sepsis. Crit Care 2006;10:R36.
32. Bayiroğlu F, Cemek M, Ilaksen H, Cemek F, Dede S. Altered antioxidant status and increased lipid peroxidation in children with acute gastroenteritis admitted to a pediatric emergency service. J Emerg Med 2009;36:227-31.