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THE CELLULAR EVALUATION OF SOME OXIDATIVE - ENDOGENOUS ENZYMATIC ANTIOXIDANTS STATUS AMONG PATIENTS WITH CUTANEOUS LEISHMANIASIS

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ABSTRACT

The current study was conducted in order to investigate the cellular-physiological responses of some enzymatic endogenous antioxidants of parasitic infection that were performed as a cellular metabolically defenses through estimation of oxidative stress biomarkers as indicators in patients with cutaneous leishmania and evaluation of these responses of endogenous antioxidants. The study included an individual of both genders: 60 patients with cutaneous leishmaniasis (CL) and 30 healthy individuals. Study volunteers were distributed into three age groups (1-20), (21-40), and (41-60) years. The demographic data were collected for both the patient and healthy groups, including age and ABO-Rh blood groups. Also, the laboratory measurements were performed for all venous blood samples taken from patients and healthy subjects including measure the concentrations of oxidative stress biomarkers total oxidants status (TOS) and malondialdehyde (MDA), and the concentrations of enzymatic antioxidants superoxide dismutase 1 (SOD1) and glutathione peroxidase 1 (GPX1). The demographical study revealed that the incidence of CL disease more occurs in males than females within early and mid-ages. On the other hand, a higher percentage of CL incidences were an early age (1–20 years) and mid-age (21–40 years). In addition, the predominant blood type among CL patients was A+ followed by O+ > B+ > AB+ and B- > A- > O- > AB-. The serum biochemical assays of cutaneous leishmaniasis patients indicated for a significant increase in serum concentrations of (TOS) and (MDA), and a significant decrease in concentrations of SOD1 and GPX1 in comparison to the healthy group for all sample age ranges. It is concluded that the pathogenesis of cutaneous leishmaniasis caused cellular oxidative stress represented by the high production of free radicals which functionally depleted the cellular metabolically levels of serum endogenous enzymatic antioxidants.

Keywords: Antioxidants, Oxidative stress, *Cutaneous leishmaniasis* (CL).

Running title: The cellular evaluation of some enzymatic status among patients

Introduction

Oxidative stress is a state of imbalance in which the levels of oxidants (free radicals) increase and the levels of antioxidants decrease (Rahman, 2007). Free radical molecules are produced from mitochondria, as by-products to producing adenosine triphosphate (ATP) when cells use the oxygen to generate energy (Navarro-Yepes *et al.*, 2014). Also, it can produce from the endoplasmic reticulum, peroxisomes, and phagocytic cells, etc. (Phaniendra *et al.*, 2015). Since they are very unstable highly reactive molecules containing a single (unpaired) electron, free radicals interact quickly with other compounds to take the necessary electron for their stability leading to the disruption of living cells (Sarma *et al.*, 2010). The immune system in the body uses the lethal effects of oxidants to kill the pathogens (Babior, 1999). But these oxidants can cause injuries to the host tissue when high levels of them are generated (Rice-Evans and Gopinathan, 1995; Young and Woodside, 2001). Cutaneous leishmaniasis is one of a parasitic disease caused by many types of *Leishmania* species that belong to the genus *Leishmania* and characterized by a variety of clinical manifestations ranging from small dermal nodules to gross destruction of mucosal tissue (Reithinger *et al.*, 2007). This disease is widespread in the Mediterranean and Middle East,

including Iraq (Markle and Makhoul, 2004). It is transmitted to human from rodents, canines, and other mammals by the bite of the infected female sandfly (Allen and Liu, 2004).

So, oxidative stress is common in cutaneous leishmaniasis due to the overproduction of free radicals and decrease in the activities of endogenous antioxidants that over time become insufficient to counter the toxicity of increased amounts of free radicals (Culha *et al.*, 2007). When the parasites enter neutrophils, monocytes and macrophage cells in the host play an important defensive role via generating large amounts of the poisonous molecules such as "reactive oxygen species ROS" include superoxide radicals (O₂^{•-}), hydroxyl radicals (•OH), and hydrogen peroxide (H₂O₂); and "reactive nitrogen species RNS" such as nitric oxide (NO). The parasites, bacteria, and tumor cells stimulate macrophages to synthesize large amounts of NO that have cytotoxic effects on these stimulants (Kocyigit *et al.*, 2005).

ROS and RNS are able to break down many biomolecules, such as DNA, carbohydrates, and proteins. Moreover, ROS and RNS can attack the polyunsaturated fatty acids in membranous lipids causing peroxidation of lipid and disorder in cell construction and function (Djaldetti *et al.*, 2002). Lipid peroxidation is a well-known mechanism of cellular injuries and is used as a sign of oxidative stress in

the body (Magni *et al.*, 1994). Malondialdehyde (MDA) is widely used as an indicator of lipid peroxidation (Neupane *et al.*, 2008). Therefore, a significant rise in the MDA level was observed in the serum of cutaneous leishmaniasis patients, due to excessive increase in the production of ROS and RNS that causing an acceleration of lipid peroxidation (Asmaa *et al.*, 2017). In contrast, the body of a human has many mechanisms to counteract the deleterious effects of oxidative stress via endogenous and exogenous antioxidants (Pham-Huy *et al.*, 2008). The current study aimed to follow-up the biological effects of cutaneous leishmaniasis infection as oxidative stress and the physiological roles of defensive response by some cellular enzymatic endogenous antioxidants during parasitic infection.

Materials and Methods

Sample and design of study: The participants of current study consist patients with cutaneous leishmaniasis (CL) coming to AL-Zahra and AL-Karama hospitalizes in Wasit province, Iraq and healthy individuals during the period from 19/12/2018 to 11/5/2019.

- Sixty **CL patients** as a patient's group (which were diagnosed by the specialist physician), 32 males (53%) with age range (3.5-44) years and 28 females (47%) with age range (2-55) years.
- Thirty healthy individuals as control group, 18 males (60%) with age range (8-52) years and 12 females (40%) with age range (11-44) years, who did not suffer from any parasites of cutaneous leishmaniasis and free from any signs or symptoms of diabetes mellitus, hypertension, liver disease, lipid disorders and others infections.

The clinical biography form was prepared for each patient and control subjects which were included (age, sex, blood type, place of residence, social status, a biography of disease, and other diseases if any). Blood samples for both groups were collected from the aforementioned hospitals and a swab was taken from the lesion of CL patients on a glass slide for laboratory diagnosis and confirmation presence the parasites of cutaneous leishmaniasis. Biomarkers according to the study design were measured at labs of Al-Zahra teaching hospital and Al-Kut hospital for Gynecology obstetric and pediatrics. The CL patients and healthy individuals were divided into three age groups (1-20), (21-40) and (41-60) years.

Blood samples collecting

Five milliliters of venous blood samples were collected from patients with cutaneous leishmaniasis and healthy subjects by disposable syringe and then the collected blood was divided into two parts. The separated serum from the first part was transferred immediately into Eppendorf tube and frozen at (-20 °C) for subsequent analysis including measurement of the oxidative status biomarkers total oxidant status (TOS) and malondialdehyde (MDA); some enzymatic endogenous cellular antioxidants (Superoxide dismutase1 SOD1) and (Glutathione peroxidase1 GPX1). While the second part of collected blood was put in EDTA K3 tubes for determination ABO blood group.

Statistical analysis

The data of the present study was made using SPSS 23 (statistical software package) and analyzed by ANOVA (one-way analysis). The results were expressed as mean \pm standard

error of the mean ($M \pm S.E.M.$). LSD was used for comparisons between the CL patients and the control group. $P < 0.05$ was considered to be statistically significant.

Results and Discussion

As shown in the figure (1), the age range of all CL participants' patients was (1-60) years, the percentage of CL infection according to sample gender was recorded with highly in males (53%) than females (47%) patients with CL. The highest age's percentage among CL patients sample according to the age range was recorded in the early age range (1-20) years with (27%) for both genders followed by 22% in males and 10% females at age range (21-40) years. While the lower percentages of age in the CL patients subject are observed in the late age range (41-60) years (5% males and 10% females).

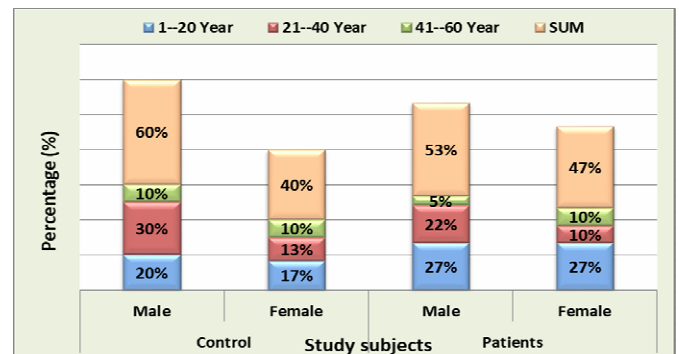


Fig. 1 : Distribution of percentages (%) of participants in study including the CL patients and control subjects for both genders according to the age range.

The results of the current study indicated that the incidence of CL disease more occurs in males than females within early and mid-ages. On the other hands, the higher percentage of CL incidence are in early age (1-20 year) and mid-age (21-40 year). This results agreed with the previous study by Rahi (2011) who refer that infection was detected in both sexes with a predominance in males and a similar recent study in Iraq, that refer most boys and men were found to be at higher risk for CL compared with girls and women in age groups 5-14 and 15-45 years old. So, the acceptable reasons to increase the percentage in early age groups are that most patients are from school student and workers in Iraq of both genders and they are more likely to participate in outdoor activities and be exposed to sand fly-associated environmental conditions than individuals from other age groups (Al-Warid *et al.*, 2017). Also, in supporting the study of Iranian total number of infected persons 51.7% were males and 48.3% females. The highest rate of leishmaniasis incidence was observed in the age group under 10 years old and the lowest in the age group 40 to 55 years (Talari *et al.*, 2012). In addition, the infection with cutaneous leishmaniasis in the early stages of life leads to the development of lifetime immunity (Mendonça, 2016), which contributes significantly to reducing the number of infections in the later stages of life (Karami *et al.*, 2013).

The distribution of the percentage of ABO blood groups among CL patients is shown in figure (2). The current data of ABO groups showed the highest percentage among CL patients with blood group A+ (33%), followed O+ (22%), B+ (20%), both AB+ and B- (8%), A- (4%), O- (3%), and the lowest percentage with AB- (2%). One of several questions related to these parasitic diseases that have been raised and

remained without an answer, is the ABO blood group consider as a risk factor? Especially in parasitic infections like cutaneous leishmaniasis (CL) that is a health problem in some tropical and subtropical regions. (Pereira *et al.*, 1979), (Barnes and Kay, 1997). The present study showed that the most prevalent blood group is A+ and the lowest is AB- among the total participants of CL patients. This finding comes conformable with a study done in Iraq by Qaddu *et al.*, (2006) on patients with cutaneous leishmaniasis arriving in Baghdad and its suburbs hospitals, it was found that patients carry blood type A+ > O+ > B+, while the negative group were percentage minor as A- > B- > O- and no blood group AB was recorded.

Besides, another local study was investigating the relationship between blood groups and visceral leishmaniasis (VL) among Thi-Qar in Iraq supported that high prevalence was found in blood group A (48.54%) and low prevalence in blood group O (8.73%) (Jarallah and Hantosh, 2016). Molaie *et al.*, (2013) also, showed the same finding in Iran in patients with kala-azar and *Leishmania infantum*. While, in contrary to our study on the same Wasit population By Al-Maamori (2008), who investigate the prevalence of ABO and Rh blood group system and their allele frequencies among a total of 1374 healthy subjects randomly selected which observe that overall picture of phenotypic frequencies of ABO blood groups is O > B > A > AB and the frequency of Rh+ve is more than Rh-ve convergent with our results. Also, a study by Boskabady *et al.*, (2005) in Iran showed that ABO blood group O was the most frequent among the total studied population in the city of Mashhad, Iran. We note from the above there is the relationship between the ABO blood group and cutaneous leishmaniasis. Nonetheless, some regional studies in Syria and Iran by Shanehsaz and Ishkhanian (2010) and Talari *et al.* (2012) respectively showed no association between ABO blood groups and cutaneous leishmaniasis (CL).

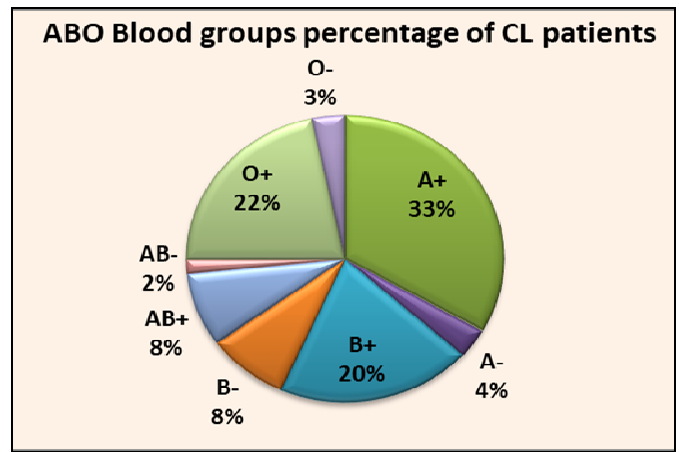


Fig. 2 : Distribution of ABO blood groups percentages (%) among CL patients.

Tables (1) and (2) illustrate the data of serum total oxidant status (U/ml) and serum malondialdehyde (ng/ml) concentrations respectively in both CL patients and control subjects. The results exhibit a significant increased ($p \leq 0.05$) in serum (TOS) and serum (MDA) concentrations for both genders in all age groups (1-20, 21-40, and 41-60 years) of CL patients compared with the control group. While the findings were showed non-significant ($p > 0.05$) differences within the age range among both CL patients and healthy study participants. Additionally, the serum TOS U/ml levels also showed no significant differences between males and females of CL patients and healthy participants. Although the serum MDA ng/ml levels were showed increased concentrations in female CL patients no significant differences observed between female and male of CL patients and also in the healthy participants.

Table 1 : Serum total oxidant status (U/ml) levels of CL patients and control subjects for both genders according to age range.

Age range (Year)	Total oxidant status (U/ml)			
	Control		Patients	
	Male	Female	Male	Female
1-20	5.47±0.10 ^a	5.19±0.21 ^a	7.07±0.18 ^b	7.34±0.11 ^b
21-40	5.43±0.27 ^a	5.34±0.02 ^a	6.96±0.14 ^b	7.17±0.22 ^b
41-60	5.45±0.01 ^a	5.35±0.18 ^a	7.23±0.06 ^b	7.29±0.29 ^b
LSD = 0.47				

Data= Mean ± S. E. M.

The different superscript letters mean significant differences ($P \leq 0.05$).

The similar superscript letters mean non-significant differences ($P > 0.05$).

LSD = Least significant difference

Table 2 : Serum malondialdehyde (ng/ml) levels of CL patients and control subjects for both genders according to age range.

Age range (Year)	Malondialdehyde (ng/ml)			
	Control		Patients	
	Male	Female	Male	Female
1-20	101.71±0.58 ^a	100.39 ± 0.73 ^a	242.68 ± 11.98 ^b	248.26 ± 9.30 ^b
21-40	100.93±0.56 ^a	102.25 ± 0.62 ^a	260.63 ± 23.06 ^b	267.92 ± 43.21 ^b
41-60	100.96 ± 0.89 ^a	101.29 ± 0.69 ^a	274.84 ± 0.64 ^b	276.88 ± 49.82 ^b
LSD = 48.68				

Data= Mean ± S. E. M.

The different superscript letters mean significant differences ($P \leq 0.05$).

The similar superscript letters mean non-significant differences ($P > 0.05$).

LSD = Least significant difference

The rising of serum total oxidative state in patients with cutaneous leishmaniasis for both genders may be due to the pathogenesis of the parasite itself, which considered as source of cellular stress in the CL patients causing an increase in ROS. Several exogenous factors stimulate the production of ROS free radicals *in vivo*, including such as the pathogen infections. The primary sources of endogenous ROS production are the mitochondria, plasma membrane, endoplasmic reticulum, and peroxisomes (Moldovan and Moldovan, 2004). At the onset of infection, *Leishmania* promastigotes are phagocytized by mammalian macrophages and the toxic oxidants such as hydrogen peroxide (H₂O₂) and superoxide (O₂⁻) generated during phagocytosis (Wilson *et al.*, 1994). The innate immunity is the first line of host defense that acts via general antimicrobial mechanisms like acidification, production of antimicrobial peptides or enzymes, and the generation of toxic molecules including ROS/RNS (Steck and Grassl, 2014). So leishmaniasis parasites (obligate intracellular protozoa) infect mammalian macrophages and stimulate them to generate large amounts of reactive oxygen species and reactive nitrogen species. This overproduction of reactive oxygen species and reactive nitrogen species leads to stimuli the oxidative stress in CL patients (Serarslan *et al.*, 2005).

On the other hand, the high serum malondialdehyde (MDA) among CL subjects (table 1-2) may be due to the overproduction of ROS that causes lipid peroxidation. ROS and RNS are capable to break down many biomolecules, including carbohydrates, proteins, and DNA, and can attack

the polyunsaturated fatty acids in membrane lipids causing the peroxidation of lipid and disorder of cell function and construction (Djalchetti *et al.*, 2002). They cause the killing of the cutaneous leishmaniasis parasite and also induce oxidative damage for non-infected cells (Kocyigit *et al.*, 2005). So, the high levels of free radicals can cause direct damage to lipids (Moldovan and Moldovan, 2004) causing lipid oxidation, a series of reactions that produce multiple breakdown molecules, like MDA (Ayala *et al.*, 2014). Thus the recent study by Asmaa *et al.*, (2017) supported the present results of increased MDA levels in cutaneous leishmaniasis patients by provides a clear sign on the role of malondialdehyde as an early biochemical indicator of peroxidation damage occurring during cutaneous leishmaniasis. Additionally, Serarslan *et al.*, (2005) also noticed a significant increase in serum MDA and NO levels in CL patients when compared to controls.

Tables (3) and (4) show a statistically significant decrease (p<0.05) in serum SOD1 and GPX1 (pg/ml) levels of CL patients in all age range (1-20, 21-40, and 41-60 years) for both genders as comparison of healthy subjects. While the data showed no significant (p >0.05) in serum SOD1 and GPX1 (pg/ml) levels between CL patients and healthy subjects within age range groups. Additionally, depended on the gender the serum SOD1 (pg/ml) levels were showed decreased concentrations in female CL patients but no significant differences when compared to the male of CL patients and also in the healthy participants.

Table 3 : Serum superoxide dismutase 1 (pg/ml) levels of CL patients and control subjects for both genders according to age range.

Age range (Year)	Superoxide dismutase 1 (pg/ml)			
	Control		Patients	
	Male	Female	Male	Female
1-20	101.141±2.19 ^a	102.253±0.83 ^a	84.038 ±1.57 ^b	82.714 ±1.71 ^b
21-40	103.311 ±0.92 ^a	99.165 ±1.68 ^a	83.781 ±2.23 ^b	81.670 ±3.70 ^b
41-60	100.958 ±1.95 ^a	100.926 ±2.08 ^a	83.823 ±0.64 ^b	80.589 ±2.97 ^b
LSD = 5.18				

Data= Mean ± S. E. M.

The different superscript letters mean significant differences (P≤0.05).

The similar superscript letters mean non-significant differences (P• 0.05).

LSD = Least significant difference

Table 4 : Serum glutathione peroxidase 1 (pg/ml) levels of CL patients and control subjects for both genders according to age range.

Age range (Years)	Glutathione peroxidase 1 (pg/ml)			
	Control		Patients	
	Male	Female	Male	Female
1-20	96.426±2.61 ^a	94.832±1.92 ^a	72.623±2.31 ^b	74.061±2.26 ^b
21-40	95.855±1.35 ^a	93.781±2.58 ^a	75.616±2.07 ^b	73.071±3.16 ^b
41-60	95.824±3.15 ^a	93.970±3.88 ^a	72.231±0.58 ^b	75.062±3.05 ^b
LSD= 6.23				

Data= Mean ± S. E. M.

The different superscript letters mean significant differences (P≤0.05).

The similar superscript letters mean non-significant differences (P• 0.05).

LSD = Least significant difference

The data indicated to a clear reduction in the level of serum endogenous antioxidant enzyme (SOD1) in patients with cutaneous leishmaniasis of both gender, that may be associated with ROS as cellular oxidative status that was previously documented by the highly serum levels of TOS and MDA in tables (1) and (2) respectively and also, for its

role as a cellular antioxidant. Antioxidant enzyme superoxide dismutase (SOD) plays a fundamental role in the antioxidant defensive ability of biological systems against deleterious free radical (Ighodaro and Akinloye, 2018). In this regard, the study by Khouri *et al.*, (2009) about the *L. amazonensis* and *L. braziliensis* revealed that enzyme SOD1 has an

antioxidant function: it converts superoxide anion ($\cdot\text{O}_2$) into molecular oxygen (O_2) and hydrogen peroxide (H_2O_2), the latter degraded by catalase and reveal a hitherto unknown IFN-beta/SOD1 axis in Leishmania infection and suggest that inhibition of SOD-associated pathways could serve as a strategy in the treatment of *L. amazonensis* as well as *L. braziliensis* infection, major human pathogens.

These results are consistent with Serarslan *et al.*, (2005) who refers to a significant decrease in SOD activity with significantly increased in serum MDA and $\text{NO}\cdot$ levels of CL patients compared to healthy, it may be suggested that the overproduction of ROS and RNS results in oxidative stress and the acceleration of lipid peroxidation in CL patients, resulting from altered enzymatic antioxidant activities. As well as, Giustarini *et al.* (2009) indicate to the inverse relationship between free radicals and antioxidants.

The data in the current study also showed a clear decrease in the levels of GPX1 in CL patients versus the control group, this may due to the protective role of cellular GPX1 from oxidative stress after infection with leishmaniasis. Glutathione peroxidase-1 (GPx-1) is an endogenous antioxidant enzyme that reduces the reactive oxygen species, such as superoxide ($\cdot\text{O}_2$) and hydrogen peroxide (H_2O_2) to limit its harmful effects. These reactive species when left unchecked can cause oxidative damage to deoxyribonucleic acid (DNA), membrane lipids, and proteins (Lubos *et al.*, 2011). These reactions occur mainly in the mitochondria and sometimes in the cytosol (Góth *et al.*, 2004). This outcome of reduced concentration of serum GPx-1 is consistent with Giustarini *et al.*, (2009) who indicated to the inverse relationship between free radicals and antioxidants, as well as with studies done by both Vural (2004) and Kocyigit *et al.*, (2003) which demonstrated low levels of glutathione peroxidase in the serum of cutaneous leishmaniasis patients versus the healthy group. The significant decrease in glutathione peroxidase (GSH-Px) activity in the CL patients may be due to the overproduction of ROS and RNS that result in oxidative stress and acceleration of lipid peroxidation in CL patients (Serarslan *et al.*, 2005).

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