Effect of cutaneous leishmaniasis on antioxidants and some biochemical parameters of patients with single and multiple ulcers

Fahad Ibraheem Salih[1], Ohood Mozahim Shakir[2]
[1,2]College of Applied Sciences - Department of Pathological Analysis - University of Samarra/Iraq
Email: albadryfahad5@gmail.com

Abstract
This study examined the comparison between single ulcers and multiple ulcers of the cutaneous leishmaniasis parasite in their effect on some antioxidants. This study included (90) people infected with cutaneous leishmaniasis from Samarra General Hospital in Samarra city in Salah El-Din Governorate for the period from August 2021 to April 2022. (54) a person with multiple ulcers. The results showed a significant decrease (P≤ 0.05) in the activity of catalase in the single ulcer group (U/L 1.1±0.4) and the multi-ulcer group (U/L 0.8±0.2) in comparison with the control group (U/L 1.5±0.8), as well as the Significant differences between single ulcer group (U/L 1.1±0.4) and multiple ulcer group (U/L 0.8±0.2). The results showed a significant decrease (P≤ 0.05) in the activity of glutathione peroxidase in the single ulcer group (pg/ml 15.5±4.4) and the multi-ulcer group (9.5±2.9 pg/ml) in comparison with the control group (pg/ml 21.7±5.3). As well as the significant differences between the group of single ulcers (4.4 ± 15.5 pg/ml) and multiple ulcers (pg/ml 9.5 ± 2.9).
Keywords Cutaneous leishmaniasis, single ulcer, multi-ulcer, catalase, glutathione peroxidase, antioxidants

1. Introduction

Leishmaniasis is a widespread parasitic disease that is endemic in more than 92 countries, including Iraq, Iran, Brazil, Afghanistan, Syria, India, Bangladesh and Sudan, and more than one million cases of leishmaniasis are recorded annually (WHO, 2020.(
Cutaneous leishmaniasis is a parasitic infection caused by a protozoa belonging to the haemoflagellate of the genus Leishmania, which compulsorily parasitizes within the cells of the reticuloendothelial system of the skin. Macrophages are the host cells of the leishmaniasis parasite (Ghosh, 2018).
The parasite of the genus Leishmania goes through an indirect life cycle, as its life cycle is completed by two phases, the first phase is called the amastigote phase, which parasitizes inside the cells of the macrophages of the vertebral host represented by humans and mammals (rodents, canine family, cats), and the second phase is represented by the promastigote phase, which It is found in the invertebrate host represented by the vector insect (the female sand fly) of the genus Phlebotomus (Jamal et al., 2020).
Cutaneous leishmaniasis is a global health problem that causes permanent skin deformities and scars in most affected cases. The first symptoms of infection appear as a small red papule that may disappear within weeks or develop into an ulcer ulcer, and two ulcers may join to form a large ulcer. Cutaneous leishmaniasis leads to the occurrence of one or more skin ulcers caused by the Leishmania parasites belonging to the type L. tropica, which self-heal within a year or more without the appearance of side effects, or the infection is represented by wet ulcers of animal origin caused by the parasite Leishmaniasis of the type L. major. It lasts for months and then heals by itself, leaving a permanent scar (Roberts et al., 2009)
It has been shown that the phagosomes containing Leishmania parasites fuse with the lysosome and this process does not seem to affect the parasite, which continues to multiply inside the phagolysosomes resulting from this fusion (Pinkovich et al., 2019).
The basic elements are among the important components in the human body, as they participate in many vital activities in the human body, such as: growth and development, as well as their importance in the immune system and their entry into the synthesis of hundreds of important enzymes in the human body (Al-Fartusie and Mohssan, 2017). Several antioxidant enzymes, such as catalase and glutathione peroxidase, have the ability to break down the yield by in vivo and catalyze the oxidation of glutathione. Al-Hassani, 2015) for water and requires selenium, Se for serum activity and Zn, Cu in activity of cutaneous leishmaniasis (Lal et al., 2013).

2. Working Methods

Samples of the study

This study included (90) people infected with cutaneous leishmaniasis from Samarra General Hospital in Samarra city in Salah El-Din Governorate for the period from August 2021 to April 2022. (54) a person with multiple ulcers.

blood samples collection

Blood samples were drawn using a sterile (5) ml syringe. Divide the blood into two parts, put (3) ml into sterile test tubes free of any anticoagulant to obtain the serum, as it is incubated at a temperature of (37) C for half an hour until coagulation occurs, then the serum is separated using a centrifuge quickly (3000).) cycle/minute for a period of (15) minutes, and the serum was kept at a temperature of (-20) C until it is used in the necessary tests. As for the other section of blood, (1) ml was placed in tubes containing an anticoagulant substance Ethylene diamine tetra acetic acid (EDTA.(

Estimation of catalase activity in serum

Working principle

The activity of catalase is estimated by measuring the

doi.org/10.31838/hiv22.02.675 Received: 11. 06.22, Revised:24.07.22, Accepted: 15.08.22

decrease in absorbance due to the consumption of the substrate.

The solutions used

1 -Phosphate buffer solution 50 mmol concentration at [7 = pH]: This solution was prepared by mixing solution A and B. A solution: A- was prepared by dissolving 6.81 g of KH2PO4 in 20 cm3 of distilled water and then completing the volume to 1000 cm3.
*Solution B :- Prepared by dissolving 6.90 of K2HPO4 in 20 cm3 of distilled water and then completing the volume to 1000 cm3. Adjust the pH by taking 400 cm 3 of solution A with 600 cm 3 of solution B.

Measurements:

The activity of the enzyme catalase was calculated using the following equation:
The activity of Catalase (U/L) = Log A1/A2 × 13.8
A1 = absorbance at 15sec
=Absorbance at 30 sec. A2

2 -Hydrogen peroxide 30 mM solution: The solution was prepared by dissolving 0.34 cm3 of 30% hydrogen peroxide in 20 cm3 of buffer solution and then completing the volume to 100 cm3 of buffer solution.

Methods of work

0.050 -1cm3 of serum is taken and diluted with 5 cm3 of the buffer solution.
2 2 -cm3 of the diluted serum is taken and 1 cm3 of the prepared peroxide solution is added to it.
3 _The absorbance intensity is measured after 15 seconds of adding peroxide, and the second absorbance intensity is measured after 30 seconds and at a wavelength of 240 nm. Using the spectrophotometer after zeroing the device on the equivalent.

competent	Samples	Solutions
1 cm3		buffer solution
2 cm3	2 cm3	diluted serum
1 cm3	1 cm3	hydrogen peroxide

Estimation of glutathione activity in serum

The ELISA technique was used to measure the concentration of glutathione in the serum. According to the test principle, the Sandwich quantitatve enzyme immune assay technique was used. The plate was pre-coated with the human GSH antibody, then the standard solutions and samples to be examined were added to the platelet bound to the antibody. plated on the pits, and then the biotreated human GSH antibody that binds to GSH is added to the sample.
Streptavidin-HRP is added and binds to a Biotinylated GSH antibody. After incubation the streptavidin-HRP is washed off, the substrate solution is added and the color develops in proportion to the amount of human GSH. The reaction is terminated by adding an acidic stopping solution and the absorbance is measured at 450nm

Measurement kit contents

- Standard Solution (48ng/ml) 0.5ml x1
- Pre-coated ELISA Plate 12 * 8 well strips x1
- Standard Diluent 3ml x1
- Streptavidin-HRP 6ml x1
- Stop Solution 6ml x1
- Substrate Solution A 6ml x1
- Substrate Solution B 6ml x1
- Wash Buffer Concentrate (25x) 20ml x1
- Biotinylated human GSH Antibody 1ml x1

Work method

Assay procedure

1 .Before starting work, leave the measuring kit at room temperature 18-25 ℃ for 30 seconds as mentioned in the method of work, and prepare all solutions and samples to the examination room for the purpose of conducting the examination at room temperature.
2 .We add 50 µl of Standard solution to the titration pits.
3 .We add 40 µL of samples and then 10 µL of anti-GSH antibody to the sample pits, then add 50 µL of red horseradish peroxidase solution HRP to the pits containing the samples and the pits containing the calibration solution. Then mix it well, cover and incubate for 60 minutes at 37°C.
4 .We remove the cover and then do the washing process, repeating the process.
5 .We add 50 µm of titrant solution A to each pit and then 50 µm of titrant solution B to each pit and incubate it after covering it for 10 minutes at 37°C in the dark.
6 .We add 50 microliters of the stopping solution, and the color begins to change from blue to yellow immediately.
7 .We determine the optical density of all the pits immediately at a wavelength of 450 nm within 10 minutes after adding the termination solution.

3. Results and Discussion

Estimation of catalase activity in serum

The results indicated a significant and clear decrease in the concentration of catalase at the probability level (Po ≤ 0.05) in the serum of patients with leishmaniasis compared with the control group, where the concentration of catalase in patients with single ulcer cutaneous leishmaniasis was (U/L 1.1±0.4). And in many ulcers (U/L 0.8 ± 0.2) compared with the control group (1.5 ± 0.8 U/L) as shown in Table (4-4) and Figure (11-4.(

Table (1): The concentration of catalase (U/L) in the serum of patients with leishmaniasis and the control group			
P value	mean±SD	Count	Study samples
0.05 a	1.5±0.8	40	Control group
0.05 b	1.1±0.4	36	single ulcer group
0.05 c	0.8±0.2	54	Multi ulcer group

The different English letters indicate the presence of significant differences at the level of Pp (≤0.5)

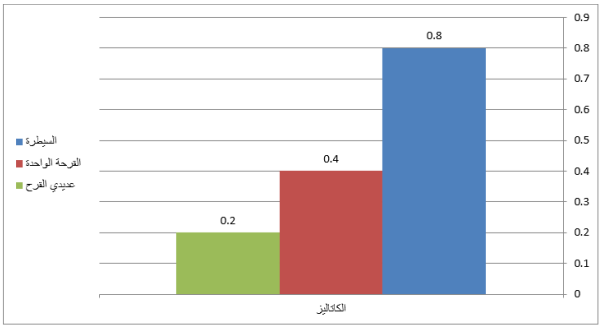

Figure 1: The concentration of catalase (U/L) in the serum of leishmaniasis patients and the control group

The current study showed a significant decrease in the levels of catalase in the serum of patients with cutaneous leishmaniasis compared to healthy people. The results of the current study agreed with a study conducted by

(Heidarpour et al., 2012; Abdul Ghani et al., 2014; Jafari et al., 2015; Rubio). et al., 2019)

This decrease is attributed to the fact that L. cutaneous infection leads to activation of Th2 cells that secrete cytokines, and cytokine-activated macrophages release a large number of reactive oxygen species (ROS), which are responsible for killing parasites in macrophages, and ROS can cause extensive damage to cellular proteins. lipids, and DNA. Antioxidant enzymes such as SOD and CAT constitute the first line of defense against ROS in the organism (Alzate et al., 2007). The decreased activity of SOD and CAT in the liver is due to the compensatory regulation of these antioxidants after the initial generation of ROS (Limon-Pacheco and Gonsehatt, 2009). Hydrogen H2O2 in this tissue, which may be a cause of oxidative stress (Sen et al., 2008), oxidative stress, is a signaling event, leading to programmed cell death (Jafari et al., 2015).

These enzymatic activities are to avoid the weakening of the oxidative defense formed in the host and to maintain the levels of ROS at non-toxic concentrations. It is in very high concentrations by destroying the cell structure, lipids, DNA and protein, and thus the occurrence of cell death, and that the catalase uses H2O2 and GSH-Px as a substrate, and therefore the decrease in the activity of catalase enables H2O2 to stay in the medium for a long time and at higher concentrations, and the researcher believes that the decrease is not significant in Catalase activity is either due to the mechanism of protection of the parasite itself from toxic oxygen metabolites or differences in methods or sample type. Or the significant decrease in CAT activity, decreased levels of glutathione peroxidase GPX, and increased generation of hydrogen peroxidase H2O2 may be greater than the clearance capacity of glutathione peroxidase GPX (Almohammed et al., 2021)

Estimation of glutathione activity in serum

The results indicated a significant and clear decrease in the concentration of glutathione at the probability level ($p<0.05$) in the serum of patients with leishmaniasis compared with the control group, where the concentration of glutathione in patients with single ulcer cutaneous leishmaniasis was (pg/ml 15.5±4.4). And in many ulcers (9.5±2.9 pg/ml) compared with the control group (21.7±5.3 pg/ml) as shown in Table (4-5) and Figure (12-4

P value	Mean ± SD	Count	Study samples
0.05 a	21.7±5.3	40	Control group
0.05 b	15.5±4.4	36	Single ulcer group
0.05 c	9.5±2.9	54	Multi ulcer group

Figure 2: The concentration of glutathione (pg/ml) in the serum of leishmaniasis patients and the control group

The results of the current study were in agreement with the study (Amzar and Iqbal, 2017; Rubio et al., 2019; Ma et al., 2020; Al-Hassani and Al-Mayali, 2020), where the results showed a decrease in the level of Gpx in the group of patients compared to the group of patients. the control.

Glutathione peroxidase plays a key role in the protection of cells exposed to oxidative stress, as the target of the glutathione peroxidase cycle is a major protective system for detoxification of ROS in red cells, where glutathione peroxidase stimulates the reduction of hydrogen peroxide H2O2 in the presence of rGSH, and a decrease in GSH-Px activities In cutaneous leishmaniasis patients compared with the control group in serum and erythrocytes, the reason for this is due to the poor utilization of glutathione rGSH in erythrocytes and thus sporadic activity and participation of the glutathione reductase cycle in detoxifying the damage by median radicals, where GSH-Px can be consumed during consumption However, it was not known whether the causes of this depletion were dependent on Se content or other factors, as it was observed that serum selenium concentrations and GSH-Px activities were significantly lower in patients. Several studies have shown that oxidants such as peroxide and hydrogen, which are products of respiratory macrophage explosion, were the main killing mechanism (the Hassani, 2020).

And the relationship of the reactive oxygen groups resulting from the activity of the phage phages of the parasite, and that hydrogen peroxide H202 is the active molecule against these parasites. The organism is expected to generate increased amounts of hydroperoxides to kill the protozoa as defense strategies for the host, and the absence of selenium and iron leads to a decrease in the activities of the enzyme GSH. -Px and CAT, and consequently a reduced ability to degrade H202 (Chen et al., 2018)

In a study conducted on cutaneous leishmaniasis patients who attended the Mustafa Kemal University Hospital in Turkey, the researcher Seraslan and his group (2005) indicated that there was a significant decrease in the levels of antioxidants in the infected compared to healthy people, and this was attributed to the generation of reactive oxygen species (H2O2, OH, 2O) works on the occurrence of oxidative stress and inactivation of GSH-Px as 2O can inhibit the function of peroxide and that GSH-Px is consumed during the elimination of ROS and thus decreases the enzyme activities during cutaneous leishmaniasis.

Zinc concentration in serum

The results indicated a significant and clear decrease in the zinc concentration at the probability level ($p<0.05$) in the serum of patients with leishmaniasis compared with the control group, where the zinc concentration in patients with cutaneous leishmaniasis with single ulcers was (µg/dL 90.5±14.2) And in multiple ulcers (88.5 ± 13.8 µg/dL) compared with the control group (.5 ± 14.8 118 µg/dL) as shown in Table (4-6) and Figure (13-4)

Table (3): Zn concentration (µg/dL) in serum of leishmaniasis infected and control group			
P value	mean±SD	Count	Study samples
0.05 a	.5± 14.8 118	40	Control group
0.05 b	90.5±14.2	36	single ulcer group
C 0.05	88.5±13.8	54	Multi ulcer group

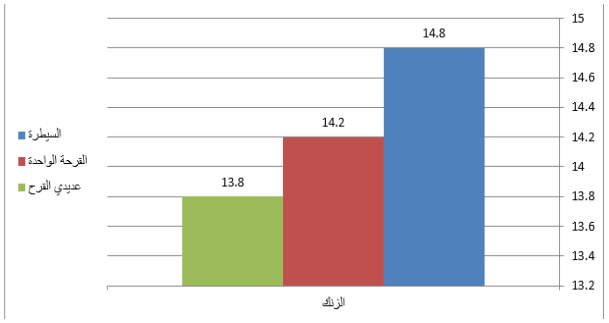

Figure 3: Zinc concentration (µg/dL) in serum of leishmaniasis infected and control group

The results of the current study showed agreement with (Al-Nasiri, 2009; Al-Jubouri, 2020) where zinc deficiency was observed in patients with cutaneous leishmaniasis. A significant decrease in the mean serum zinc concentration compared to the control group.

The results also showed that there is a significant difference between the single ulcer group and multiple ulcers, where its concentration was lower in the multi-ulcer group compared to the single ulcer group, and that the increase in the number of ulcers will lead to an increase in zinc consumption because of its role in protecting the skin and healing wounds, and the immune changes that occur In people with leishmaniasis, which is represented by immune deficiency, the deficiency in the concentration of this element, which is found in normal conditions in high concentrations in the lymph nodes and white blood cells, confirms the consumption of it by the parasite's metabolism, as it is one of the important elements in many of the auxiliary elements of many From enzymatic reactions, including protein synthesis, carbohydrate metabolism and the reactions of the Krebs cycle, as well as its entry into the synthesis of other enzymes (Al-Nasiri, 2009).

Where zinc plays an important and effective role in the process of the immune response and its regulation, where the activity of cells of acquired and intrinsic immunity is regulated by zinc, and an increase in the proliferation rate of these cells has been observed when an imbalance or change in zinc level occurs (Bonaventura et al., 2015).

The role of zinc in the immune response is tightly regulated by the group of ZnT-ZIP proteins and methylothionine stored in the body. -1) Which raises its level in a person infected with the cutaneous leishmaniasis parasite as a result of the immune response, and this leads to any defect in those immune mechanisms that will be associated with a decrease in the concentration of zinc in the body (Bonaventura et al., 2015).

Zinc is an essential trace element, which is required in minute quantities for the effective functioning of the immune system. Zinc protects host cells from oxidative stress and inflammation, and several enzymes play a key role in the cellular response to external infection based on its low concentration of catalytic action. Zinc is required for B cells and T cell growth and proliferation. Its deficiency affects the functioning of neutrophils, phagocytes, and causes a mismatch between Th1 and Th2 cells. Zinc deficiency causes stress and activates monocyte macrophages, as well as leads to an increase in the development of inflammatory cytokines such as IL-1b, IL-6, IL-8, and tumor necrosis factor. Zinc deficiency, even at low levels, can affect clinical, biochemical and immune functions (KT et al., 2022.(

The main concerns in endemic areas to reduce leishmaniasis are high cost, injection, toxicity, drug resistance, malnutrition due to poverty, comorbidities, and absence of an effective vaccine. Micronutrients such as vitamins and trace elements are necessary to stimulate the immune defense against pathogens that cause infectious diseases. A lower intake of micronutrients leading to malnutrition further influences disease progression, treatment efficacy, and relapse of leishmaniasis, since these micronutrients play a key role in the body's innate and adaptive immunity, thus reducing the risk of developing leishmaniasis. There is a lack of knowledge, awareness, and practice towards the use of micronutrient supplementation in leishmaniasis endemic areas.

Copper concentration in serum

The results indicated a significant and clear increase in the concentration of copper at the probability level ($p<0.05$) in the serum of patients with leishmaniasis compared with the control group, where the concentration of copper in patients with cutaneous leishmaniasis with single ulcers (µg/dL 85.5±11.2) And in multiple ulcers (115.5 ± 14.1) µg/dL compared with the control group (76.5 ± 9.8 µg/dL) as shown in Table (4-7) and Figure (14-4)

Table (4): Copper concentration (µg/dL) in serum of patients with leishmaniasis and control group

P value	mean±SD	Count	Study samples
0.05 a	76.5±9.8	40	Control group
0.05 b	85.5±11.2	36	Single ulcer group
0.05 c	.5± 14.1 115	54	Multi ulcer group
The different English letters indicate the presence of significant differences at the level of Pp ≤0.5))			

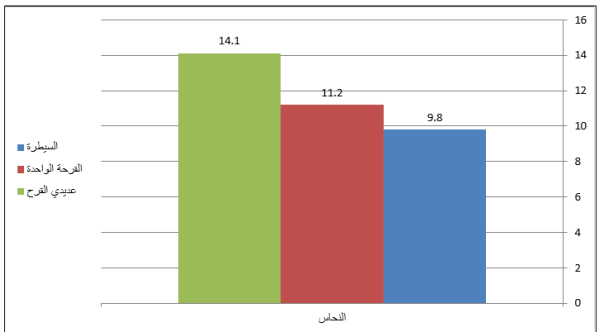

Figure 4: Copper concentration (µg/dL) in serum of leishmaniasis infected and control group

The results of the current study showed a higher copper concentration for the group infected with Leishmania parasite compared to the control group, and this study agreed with the results of (Van Weyenbergh et al., 2004 Araujo et al., 2008 Lal et al., 2013(;

Elevated blood copper levels may be a result of inflammation associated with infection and may increase resistance to leishmaniasis. It may also interfere with the immune response to leishmaniasis resistance, by causing a non-protective Th2/humoral immune response, which is known to be exacerbated in leishmaniasis, nor An increase in plasma copper can be considered merely as a marker of inflammation, as it was not observed in mucosal leishmaniasis, a chronic inflammatory condition characterized by elevated production of pro-inflammatory cytokines, such as TNF-α and -IFN, which may in fact be a primary event of increased humoral response to anti-leishmaniasis. A cross-correlation between copper/zinc levels and the humoral and cellular immune response has also been observed, in addition, copper imbalance may serve as a marker of reduced Th1 response and immunodeficiency in leishmaniasis, and serum copper associated with ceruloplasmin contributes to the

inflammation associated with 1-induced disease. IL increases with cutaneous leishmaniasis. An increase in the concentration of copper in the blood was recorded as it was associated with higher levels of ceruloplasmin, synthesized by the liver and containing seven copper atoms per molecule, induced by IL-1. The result of the organism's defense strategies, which are regulated through immune regulation

An increased level of copper may lead to an increase in resistance to leishmaniasis, meaning that under stress or attack (i.e., infection) serum copper increases more than usual to facilitate vital processes such as Cu-ATPases, which is crucial for the development of the central nervous system, liver function, connective tissues and many other Other physiological processes These characteristic changes in trace element metabolism are integral to the acute phase response. These changes are usually reflected in decreased serum zinc and iron and increased serum copper concentrations and consequently decreased IFN production.

It was also observed that there was a significant increase between the many ulcers and the single ulcer group, as this increase occurred as a result of the increase in the number of parasite and the severity of the virulence of these numbers led to an increase in the concentration of copper, as the increase of copper element is linked to the protein Ceruplasmin, which is stimulated by IL-1, which is secreted from immune cells to resist infections. Pathological, and the inflammatory processes resulting from parasite infection are linked to the immune response, where changes occur in the level of mineral metabolism as a result of the immune response associated with infection with the parasite. And that (90)% of the copper element in the blood is stored in the form of protein, the rise in the copper level can be observed in many injuries that have an effect on the protein, as in the acute phase reaction as a result of injury, and the increase in copper concentration is the result of an increase Cyroplasmin protein industry, as copper binds with this protein in the blood plasma when the parasite is infected, which increases its level in the blood (Al-Jubouri, 2020.(

selenium concentration in serum

The results indicated a significant and clear decrease in the selenium concentration at the probability level ($p<0.05$) in the serum of patients with leishmaniasis compared with the control group, where the concentration of selenium in patients with single ulcer cutaneous leishmaniasis was (µg/dL 108±15.4) And in multiple ulcers (100.15 ± 12.8 µg/dL) compared with the control group (120.5 ± 22.1 µg/dL) as shown in Table (4-8) Figure (15-4.(

Table (5): Selenium concentration (µg/dL) in serum of leishmaniasis infected and control group

P value	mean±SD	Count	Study samples
0.05 a	120.5±22.1	40	Control group
0.05 b	108±15.4	36	Single ulcer group
0.05 c	100.15±12.8	54	Multi ulcer group

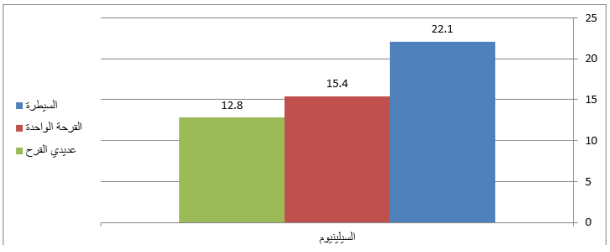

Figure 5: Selenium concentration (µg/dL) in serum of leishmaniasis infected and control group.

The results of the current study showed a significant decrease of selenium in the blood serum of the group infected with the cutaneous leishmaniasis parasite compared to the control groups. Our results agreed with what was confirmed by (Farzin et al., 2014; Farzin and Moassesi, 2014; Ghorbel et al., 2016; Kahvaz et al., 2020; Al-Hassani and Al-Mayali, 2020.(

The present results also showed a significant decrease between the single ulcer group and the multi-ulcer group, where the multi-ulcer group was lower than the single-ulcer group. The pathophysiological processes of cutaneous leishmaniasis and that decreasing levels of selenium in the blood may be the defense strategies of the infecting host organism, and that this decrease is necessary as a cofactor for some enzymes such as GSH-Px, a glutathione recycling enzyme that catalyzes the oxidation of reduced glutathione and other hydroperoxides by peroxide hydrogen (H2O2) to form oxidized glutathione and water, as any decrease in GSH-Px function leads to incomplete removal of H2O2 from cells. Defense against the threat of oxidation (Minich, 2022; Ha et al., 2019).

The baseline selenium levels of cutaneous leishmaniasis patients were much lower than those in the control groups, as many diseases cause selenium deficiency. The exact mechanism that causes this deficiency is still unknown, however, it is not known whether the causes of this depletion depend On selenium content or on other factors, selenium levels in human samples and characterization of selenium intake show high global variability due to variation in factors such as dietary habits, dietary selenium content, soil, race, gender, age, individual metabolism, daily exposure, exposure to coal and other sources of combustion and smoking. That there is a relationship between the level of selenium and the relationship between selenium and the activity of -GSH-Px in patients with cutaneous leishmaniasis, and that selenium deficiency may be malnutrition in protein calories and poor absorption from the intestine, and that this may be part of the defense strategies of the organism. More data is needed to properly define the role of selenium as a pathophysiological factor in patients with cutaneous leishmaniasis.

References

Al-Hassani, Mohamed Kamel Karim (2020). Study of the levels of some trace elements in blood serum and their relationship with some antioxidant enzymes and immune factors in patients with Cutaneous leishmaniasis and white rats. PhD thesis - College of Education - University of Al-Qadisiyah.

Al-Nasiri, Amal Kamel Daham Muhammad (2009). Cutaneous leishmaniasis, an epidemiological study, and some blood parameters in Salah al-Din Governorate, Master's thesis, College of Education for Girls, Tikrit University.

Al-Jubouri, Akram Abed Farhan (2020). Diagnosis of some intestinal parasites and cutaneous leishmaniasis and measurement of the level of a number of trace elements in the blood serum of infected people in the city of Shirqat, Master's thesis - College of Life Sciences - University of Tikrit.

WHO (World Health Organization) (2020). "Leishmaniasis and *Leishmania*/ HIV Co- infection." Fact Sheet No. 116.

Ghosh, S. (ed.), (2018). Paniker's textbook of medical parasitology. Jaypee, The Health Sciences Publisher, New Delhi: 256pp.

Jamal, Q.; Shah, A.; Rasheed, S. B. and Adnan, M. (2020).

In vitro assessment and characterization of the growth and life cycle of *Leishmania tropica*. Pakistan Journal of Zoology, 52(2): 447-455.
Pinkovich, A. G.; Kannan, S.; Nitzan-Koren, R.; Puri, M.; Bar-Avraham, Y.; McDonald, J. and Myler, P. J. (2019). The hunger games: sensing host arginine is essential for Leishmania parasite virulence. bioRxiv, 751610.
Al-Fartusie, F. S. and Mohssan, S. N. (2017). Essential trace elements and their vital roles in human body. Indian J Adv Chem Sci, 5(3), 127- 136.
Moustafa, S. R. (2015). Association of superoxide dismutase, glutathione peroxidase, catalse, and xanthine oxidase with incidence of bladder cancer. Cancer Research Journal, 3(2), 17-27.
Roberts, L. S.; Janovy, J.; and Schmidt, G. D. (2009). Gerald D. Schmidt & Larry S. Roberts' foundations of parasitology. Boston: McGraw-Hill: 701pp.
Heidarpour, M.; Soltani, S.; Mohri, M. and Khoshnegah, J. (2012). Canine visceral leishmaniasis: relationships between oxidative stress, liver and kidney variables, trace elements, and clinical status. Parasitol Res.; 111:1491-6. doi: 10.1007/s00436-012-2985-8. PubMed PMID: 22669694.
Abdul Ghani, S.; Hezam, A. and Asma, M. (2014). Biochemical and hematological levels in patients with cutaneous leishmaniasis in Yemen. Int J Pharmac Sci Inv, 3(5), 30-35.
Jafari, M.; Shirbazou, S.; Sadraie, S. H.; Kaka, G. and Norozi, M. (2015). The role of apoptosis in the cellular response of liver and spleen of BALB/c mice in cutaneous leishmaniasis. Iranian journal of medical sciences, 40(2), 133.
Rubio, C.; Escribano, D.; Hernández, J.; Cerón, J.; Tvarijonaviciute, A. and Martínez-Subiela, S. (2019). Blood Biomarkers of Oxidative Stress in Human and Canine Leishmaniosis Oxidative Stress in Microbial Diseases. Springer,21-31.
Alzate, J.F.; Arias, AA.; Moreno-Mateos, D.; Alvarez-Barrientos, A. and Jiménez-Ruiz, A. (2007). Mitochondrial superoxide mediates heatinduced apoptotic-like death in Leishmania infantum. Mol Biochem Parasitol. 2007; 152:192-202. doi: 10.1016/j. molbiopara.2007 .01.006. PubMed PMID: 17300844.
Limón-Pacheco, J. and Gonsebatt, M. E. (2009). The role of antioxidants and antioxidant-related enzymes in protective responses to environmentally induced oxidative stress. Mutation Research/Genetic Toxicology and Environmental Mutagenesis, 674(1), 137-147. doi: https://doi.org/10.1016/j.mrgentox. 2008.09.015.
Sen, G.; Mukhopadhyay, S.; Ray, M. and Biswas, T. (2008). Quercetin interferes with iron metabolism in Leishmania donovani and targets ribonucleotide reductase to exert leishmanicidal activity. J. Antimicrob. Chemother. 61, 1066–1075.
Almohammed, H. I.; Saif, M. A.; Albalawi, A. E. and Alanazi, A. D. (2021). Efficacy of pentavalent antimonial injection in improving the oxidative, nitrosative status and immune cellular enzyme activity in treating Saudi patients with cutaneous leishmaniasis. *Journal of the Egyptian Society of Parasitology*, *51*(1), 99-106.
Amzar, N. and Iqbal, M. (2017). The Hepatoprotective Effect of Clidemia hirta against Carbon Tetrachloride (CCl 4) – Induced Oxidative Stress and Hepatic Damage in Mice. Journal of Environmental Pathology, Toxicology and Oncology, 36(4).
Ma, C. I.; Tirtorahardjo, J. A.; Jan, S.; Schweizer, S. S.; Rosario, S. A. C.; Du, Y. and Andrade, R. M. (2020). Auranofin resistance in *Toxoplasma gondii* decreases the accumulation of reactive oxygen species but does not target parasite thioredoxin reductase. bioRxiv, 2020.2004.2023.058842. doi: 10.1101/2020 .04.23.058842.
Al-Hassani, M. K. K. and Al-Mayali, H. M. H. (2020). Evaluation of some biochemical levels in patients with Cutaneous leishmaniasis serum and their relationship with antioxidant enzymes. EurAsian J Biosci, 14(1), 1999-2006.
Chen, J.; Yu, B.; Chen, D.; Huang, Z.; Mao, X.; Zheng, P. and He, J. (2018). Chlorogenic acid improves intestinal barrier functions by suppressing mucosa inflammation and improving antioxidant capacity in weaned pigs. The Journal of nutritional biochemistry, 59, 84-92.
Serarslan, G.; Yılmaz, H. and Söğüt, S. (2005). Serum antioxidant activities, malondialdehyde and nitric oxide levels in human cutaneous leishmaniasis. Clinical and Experimental Dermatology: Experimental dermatology, 30(3), 267-271.
Farzin, L.; Moassesi, M. E. and Sajadi, F. (2014). Alterations of serum antioxidant trace elements (Se, Zn and Cu) status in patients with cutaneous leishmaniasis. *Asian Pacific Journal of Tropical Disease*, *4*, S445-S448.
Kahvaz, M. S.; Soltani, S.; Soltani, S.; Carvalheiro, M. C. and Foroutan, M. (2021). Low serum levels of selenium, zinc, iron, and zinc/copper ratio in an endemic region of cutaneous leishmaniasis in southwest Iran. *Biological Trace Element Research*, *199*(4), 1291-1296.
Bonaventura, P.; Benedetti, G.; Albarède, F. and Miossec, P. (2015). Zinc and its role in immunity and inflammation. *Autoimmunity reviews*, *14*(4), 277-285.
KT, M. F.; Sharma, A.; Bisht, P.; Dhingra, S.; Ravichandiran, V.; Ramesh, M. and Murti, K. (2022). The Possible Role of Selected Vitamins and Minerals in the Therapeutic Outcomes of Leishmaniasis. Biological Trace Element Research, 1-17.
Van Weyenbergh, J.; Santana, G.; D'Oliveira, A.; Santos, A. F.;Costa, C. H.; Carvalho, E. M., . . . Barral-Netto, M. (2004). Zinc/copper imbalance reflects immune dysfunction in human leishmaniasis: an ex vivo and in vitro study. BMC infectious diseases, 4(1), 50.
Araujo, C.F; Lacerda, M.V; Abdalla, D.S; and Lima ES. (2008). The role of platelet and plasma markers of antioxidant status and oxidative stress in thrombocytopenia among patients with Vivax malaria. Mem Inst Oswaldo Cruz; 103: 517-521.
Lal, C. S.; Kumar, S.; Ranjan, A.; Rabidas, V. N.; Verma, N.; Pandey, K. and Das, P. (2013). Comparative analysis of serum zinc, copper, magnesium, calcium and iron level in acute and chronic patients of visceral leishmaniasis. Journal of Trace Elements in Medicine and Biology, 27(2), 98-102.
Ghorbel, I.; Chaabane, M.; Elwej, A.; Boudawara, O.; Abdelhedi, S.; Jamoussi, K. and Zeghal, N. (2016). Expression of metallothioneins I and II related to oxidative stress in the liver of aluminium-treated rats. Archives of physiology and biochemistry, 122(4), 214-222.
Farzin, L.; Moassesi, M. E. and Sajadi, F. (2014). Alterations of serum antioxidant trace elements (Se, Zn and Cu) status in patients with cutaneous leishmaniasis. *Asian Pacific Journal of Tropical Disease*, *4*, S445-S448.
Minich, W. B. (2022). Selenium metabolism and biosynthesis of selenoproteins in the human body. Biochemistry (Moscow), 87(1), S168-S177.