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Reduced Glutathione Peroxidase (GPx) Activity in Patients with Cutaneous Leishmaniasis

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Abstract

Background: Cutaneous leishmaniasis (CL) is a tropical parasitic infection that involves several factors in its pathogenesis, including oxidative stress. The oxidative stress in CL may deplete antioxidant nutrients.
Objectives: The aim of the study was to measure glutathione peroxidase (GPx) activity in patients with CL.
Methods: The investigation included 80 patients with CL and 80 healthy participants. A diagnostic kit was used to measure GPx activity.
Results: The results showed a significant decrease in GPx activity in the CL patient group compared to the control group ($P < 0.001$), which is related to increased oxidative stress in patients.
Conclusions: According to the results, GPx enzyme activity can serve as a biomarker for the diagnosis, prognosis, and monitoring of treatment in patients with CL.

Keywords: Cutaneous Leishmaniasis, Glutathione Peroxidase, Oxidative Stress, Case-Control

1. Background

Leishmaniasis is a tropical parasitic infection caused by the genus Leishmania spp. This intracellular parasite is transmitted via the bite of a sand fly vector. Cutaneous leishmaniasis (CL), the predominant form of infection, has a worldwide distribution and affects about 1 million people each year in endemic countries (1). Cutaneous leishmaniasis is a significant public health and social issue in Iran due to its high incidence (77 per 100,000 population), the absence of an effective vaccine, and the lack of effective antileishmanial drugs (2, 3). Although the pathogenesis of CL is not fully understood, evidence has shown that the host immune system response plays a vital role in the disease outcome (4).

Oxidative stress is important in the pathogenesis of leishmaniasis. During infection, skin macrophages phagocytize the promastigote form of the parasite, causing it to convert into the amastigote stage (5). Leishmania amastigotes induce an increase in macrophage oxygen consumption and the generation of reactive oxygen species (ROS) in the phagosome, leading to the eventual death of phagocytosed microorganisms. Reactive oxygen species are highly reactive molecules that can cause cell and tissue damage due to an oxidant-antioxidant imbalance (6). The host body possesses antioxidant defense systems, including antioxidant enzymes, to protect cells from ROS damage. Some antioxidant enzymes require a sufficient quantity of trace elements (TE) to function (7).

Copper, zinc, and selenium (Se) are key elements vital to the immune system. These trace elements support antioxidant enzymes that help the body eliminate harmful free radicals by acting as cofactors. Antioxidant levels vary in a wide range of illnesses, including leishmaniasis. These alterations are part of the organism's defense strategies and are induced by different cytokines (8, 9). Glutathione peroxidase (GPx), an enzyme that recycles glutathione and catalyzes the oxidation of reduced glutathione by H_2O_2 and other

hydroperoxides to generate oxidized glutathione and water, requires selenium, making it a vital nutrient for human health (10, 11). In the presence of reduced glutathione (GSH), this enzyme catalyzes detoxification (12). Previous research has found that CL patients have lower GPx enzyme activity and higher GSH concentrations (7).

2. Objectives

However, while the significance of Se and its redistribution during Leishmania infection is well-documented, information regarding GPx activity is limited. The aim of this study was to measure GPx activity in patients with CL.

3. Methods

3.1. Sampling and Patients

A total of 80 patients with CL and 80 healthy volunteers from the same areas who had not been exposed to the parasite were recruited for this investigation. All CL patients were recruited from health clinics affiliated with Abadan University of Medical Sciences. A physician verified the clinical, paraclinical, or combination of diagnoses made for the patients. Clinical confirmation of the diagnosis was obtained, in addition to laboratory confirmation of the parasite in the lesions using direct smears, cultures, or both. After cleaning with ethanol, sterile lancets were used to puncture the lesion at its margins. Exuding material was used to make smears, which were then air-dried and fixed in methanol. Giemsa's stain was then applied to them so that light microscopy could be used to examine them. When amastigotes were found in the smears, microscopic diagnoses were made. Patients with lesions for 6 months or longer were excluded from the trial due to spontaneous healing.

3.2. Ethical Statement

All participants voluntarily agreed to be tested for the cause of ulcers. This study was approved by the Behbahan Faculty of Medical Sciences Ethical Committee (IR.BHN.REC.1399.004).

3.3. Biochemical Assays

The chemicals used in this study were purchased from Merck Co. (Germany). Five milliliters of venous blood were collected from the subjects and transferred into tubes containing heparin. The activity of GPx was subsequently measured.

3.4. Determination of Glutathione Peroxidase Activity

Glutathione peroxidase activities were measured in whole blood as previously described (13, 14).

3.5. Statistical Analysis

Summary statistics (mean and standard deviation) were computed in this case-control study. One-way analysis of variance (ANOVA) was used to statistically compare values. A P-value of < 0.05 was considered statistically significant, and all results were reported as mean ± SD. SPSS 21 for Windows was used to conduct the statistical analysis.

4. Results

The matching process was strictly conducted by our team for age and two physical characteristics, including weight and body mass index, between the case and control groups to minimize bias in the outcomes. The data reveal that these parameters were comparable across the two groups ($P > 0.05$), indicating the absence of any confounding factors. In the study, a significant reduction in GPx activity was observed in CL patients compared to the control group (67.85 ± 12.67 vs. 100.80 ± 11.21 U/g Hb, $P < 0.001$).

5. Discussion

Because antileishmanial drugs have significant adverse effects and there is no vaccine to prevent the disease, finding biomarkers that interfere with disease pathogenesis can aid in the diagnosis and treatment of CL (15). The current study discovered that GPx activity was considerably reduced in CL patients.

The immune system protects the host from Leishmania infection through various mechanisms, including NADPH oxidase, inducible nitric oxide synthase (iNOS), and myeloperoxidase activation. In response to parasite invasion, activated macrophages produce ROS such as superoxide ($\bullet O_2$), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($\bullet OH$) (16). Recently, there has been a surge of interest in identifying oxidative stress indicators in CL patients to estimate disease severity (17). Several investigations have shown an increase in ROS production and a decrease in antioxidant defense levels in leishmaniasis patients, indicating that oxidative damage plays a significant role in disease pathogenesis (18-20). Prolonged exposure to

high levels of ROS leads to the development of leishmaniasis and tissue damage (17).

Minerals have been shown to affect the antileishmanial immune response through the production of antioxidant enzymes. Selenium has been linked to the pathophysiology of CL (21). This micronutrient element participates in free radical scavenging by functioning as a cofactor for many antioxidant enzymes such as GPx (22). Se deficiency can be caused by malnutrition or by various illnesses, including leishmaniasis (23). Prior studies have shown that patients with CL have reduced amounts of selenium in their serum (13, 24-26). Decreased serum Se during parasitic diseases has been related to the redistribution of Se from the plasma pool into the tissues, increased metabolism or consumption, stress, or hyperthermia (27). Furthermore, it has been proposed that Se deficiency, as a host defensive mechanism, kills parasites by lowering GPx activity and increasing the number of hydroperoxides (24). Several studies have found a link between low Se levels in the blood and the severity of leishmaniasis, suggesting that Se may have therapeutic potential in severe leishmaniasis (22, 26). We examined enzyme activity to test the hypothesis that lower Se levels would diminish GPx activity in patients.

Glutathione peroxidase, a selenoenzyme, plays a vital role in the antioxidant defense system by protecting membranes against peroxide damage (27). Numerous parasitic diseases, such as leishmaniasis, have been shown to modify antioxidant enzymes (5). In line with previous research, a decrease in GPx activity was observed in patients with CL, suggesting that this enzyme may be involved in the pathogenesis of CL (7, 18). Various studies have reported decreased GPx enzyme activity and elevated rGSH concentrations in CL patients, which were reversed after treatment (16-18). During the infection, increased production of ROS and reactive nitrogen species (RNS) leads to a decrease in GPx activity (28). The release of oxygen and nitrogen free radicals by neutrophils and macrophages reduces GPx activity and/or levels by consuming the enzyme during ROS scavenging (29, 30). The increased content of hydrogen peroxides in CL patients' erythrocytes was demonstrated in a previous study (16). In addition, the lack of selenium in CL patients may lead to diminished GPx activity or suppression of GPx expression, resulting in an accumulation of H_2O_2 and an increase in lipid peroxidation (24). This result is consistent with Kocyigit et al.'s study, which demonstrated a considerable decrease in serum Se and GPx activity in patients with CL (13). Furthermore, a decrease in the amount of GSH, the substrate of GPx, may result in decreased GPx activity in CL patients (31). Therefore, it seems that GPx enzyme activity can be considered a diagnostic and prognostic biomarker in CL patients, as well as a treatment monitoring tool.

The main findings and strength of the present study were the possible role of GPx enzyme activity in the development of CL. However, there were some limitations in this study. First, while the association between GPx enzyme activity and CL progression is established, a comparative analysis of GPx enzyme activity in other forms of leishmaniasis would have provided more context for understanding the current investigation's findings. Second, the study only examined a small sample size; as a result, larger studies examining a greater number of samples are required.

5.1. Conclusions

This study suggests that monitoring the activity of the GPx enzyme in the serum of CL patients can be used to assess the effectiveness of treatment. Thus, the activity of the GPx enzyme can serve as a biomarker for diagnosis, prognosis, and treatment monitoring in patients with CL.

Footnotes

Authors' Contribution: SS and MS designed the study protocol; SS, MS, and MSK collected the data and involved in statistical analysis; SS performed the experiments; MP drafted the manuscript; MS and MF critically revised the manuscript. All authors read and approved the final version of the manuscript.

Conflict of Interests Statement: The authors declared no potential conflicts of interest with respect to the research, authorship, and or publication of this article. The second author Dr. Masoud Foroutan serves as the Director of Research and Technology of Abadan University of Medical Sciences.

Data Availability: The data used to support the findings of this study are available from the corresponding author upon reasonable request.

Ethical Approval: This study received the approval from the Behbahan Faculty of Medical Sciences Ethical Committee (IR.BHN.REC.1399.004).

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Informed Consent: All subjects voluntarily agreed to be participated. A written informed consent was obtained from all subject.

References

1. Gurel MS, Tekin B, Uzun S. Cutaneous leishmaniasis: A great imitator. *Clin Dermatol*. 2020;**38**(2):140-51. [PubMed ID: 32513395]. https://doi.org/10.1016/j.clindermatol.2019.10.008.
2. Panahi S, Abbasi M, Sayehmiri K, Sadegh S, Abdi J. Prevalence of Cutaneous Leishmaniasis in Iran (2000-2016): A Systematic Review and Meta-analysis Study. *Infect Disord Drug Targets*. 2021;**21**(2):173-9. [PubMed ID: 32479248]. https://doi.org/10.2174/1871526520666200601164850.
3. Kadkhodamasoum S, Bineshian F, KarimiPour A, Tavakoli P, Foroutan M, Ghaffarifar F, et al. Comparison of the Effects of Sambucus ebulus Leaf and Fruit Extracts on Leishmania major In Vitro. *Infect Disord Drug Targets*. 2021;**21**(1):49-54. [PubMed ID: 31858914]. https://doi.org/10.2174/1871526520666191220114540.
4. van Griensven J, Gadisa E, Aseffa A, Hailu A, Beshah AM, Diro E. Treatment of Cutaneous Leishmaniasis Caused by Leishmania aethiopica: A Systematic Review. *PLoS Negl Trop Dis*. 2016;**10**(3). e0004495. [PubMed ID: 26938448]. [PubMed Central ID: PMC4777553]. https://doi.org/10.1371/journal.pntd.0004495.
5. Chakraborti S, Chakraborti T, Chattopadhyay D, Shaha C. *Oxidative stress in microbial diseases*. Springer; 2019.
6. Van Assche T, Deschacht M, da Luz RA, Maes L, Cos P. Leishmania-macrophage interactions: insights into the redox biology. *Free Radic Biol Med*. 2011;**51**(2):337-51. [PubMed ID: 21620959]. https://doi.org/10.1016/j.freeradbiomed.2011.05.011.
7. Vural H, Aksoy N, Ozbilge H. Alterations of oxidative-antioxidative status in human cutaneous leishmaniasis. *Cell Biochem Funct*. 2004;**22**(3):153-6. [PubMed ID: 15124179]. https://doi.org/10.1002/cbf.1066.
8. Weyh C, Kruger K, Peeling P, Castell L. The Role of Minerals in the Optimal Functioning of the Immune System. *Nutrients*. 2022;**14**(3). [PubMed ID: 35277003]. [PubMed Central ID: PMC8840645]. https://doi.org/10.3390/nu14030644.
9. Pouresmaeil V, Al Abudi AH, Mahimid AH, Sarafraz Yazdi M, Es-Haghi A. Evaluation of Serum Selenium and Copper Levels with Inflammatory Cytokines and Indices of Oxidative Stress in Type 2 Diabetes. *Biol Trace Elem Res*. 2023;**201**(2):617-26. [PubMed ID: 35279796]. https://doi.org/10.1007/s12011-022-03191-w.
10. Oliveira-Silva JA, Yamamoto JUP, Oliveira RB, Monteiro VCL, Frangipani BJ, Kyosen SO, et al. Oxidative stress assessment by glutathione peroxidase activity and glutathione levels in response to selenium supplementation in patients with Mucopolysaccharidosis I, II and VI. *Genet Mol Biol*. 2019;**42**(1):1-8. [PubMed ID: 30776046]. [PubMed Central ID: PMC6428122]. https://doi.org/10.1590/1678-4685-GMB-2017-0334.
11. Battin EE, Brumaghim JL. Antioxidant activity of sulfur and selenium: a review of reactive oxygen species scavenging, glutathione peroxidase, and metal-binding antioxidant mechanisms. *Cell Biochem Biophys*. 2009;**55**(1):1-23. [PubMed ID: 19548119]. https://doi.org/10.1007/s12013-009-9054-7.
12. Rush JW, Sandiford SD. Plasma glutathione peroxidase in healthy young adults: influence of gender and physical activity. *Clin Biochem*. 2003;**36**(5):345-51. [PubMed ID: 12849865]. https://doi.org/10.1016/s0009-9120(03)00039-0.
13. KOÇYİĞİT ABDURRAHİM, EREL ÖZCAN, GÜREL MS, SEYREK ADNAN, AKTEPE NECMEDDİN, GÜR SELAHATTİN, et al. Decreasing Selenium Levels andGlutathione Peroxidase Activity in PatientsWith Cutaneous Leishmaniasis. *J Turkish J Med Sci*. 1999;**29**(3):291-6.
14. Paglia DE, Valentine WN%. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *The J Laboratory Clinical Med*. 1967;**70**(1):158-69.
15. Farzin L, Moassesi ME, Sajadi F, Amiri M, Shams H. Serum levels of antioxidants (Zn, Cu, Se) in healthy volunteers living in Tehran. *Biol Trace Elem Res*. 2009;**129**(1-3):36-45. [PubMed ID: 19066735]. https://doi.org/10.1007/s12011-008-8284-7.
16. Erel O, Kocyigit A, Bulut V, Gurel MS. Reactive nitrogen and oxygen intermediates in patients with cutaneous leishmaniasis. *Mem Inst Oswaldo Cruz*. 1999;**94**(2):179-83. [PubMed ID: 10224524]. https://doi.org/10.1590/s0074-02761999000200009.
17. Giudice A, Camada I, Leopoldo PT, Pereira JM, Riley LW, Wilson ME, et al. Resistance of Leishmania (Leishmania) amazonensis and Leishmania (Viannia) braziliensis to nitric oxide correlates with disease severity in Tegumentary Leishmaniasis. *BMC Infect Dis*. 2007;**7**:7. [PubMed ID: 17316450]. [PubMed Central ID: PMC1810296]. https://doi.org/10.1186/1471-2334-7-7.
18. Kocyigit A, Gurel M, Ulukanligil M. Erythrocyte antioxidative enzyme activities and lipid peroxidation levels in patients with cutaneous leishmaniasis. *Parasite*. 2003;**10**(3):277-81. [PubMed ID: 14535169]. https://doi.org/10.1051/parasite/2003103277.
19. Kabel AM. Free radicals and antioxidants: role of enzymes and nutrition. *World J Nutrition Health*. 2014;**2**(3):35-8.
20. Quintela-Carvalho G, Luz NF, Celes FS, Zanette DL, Andrade D, Menezes D, et al. Heme Drives Oxidative Stress-Associated Cell Death in Human Neutrophils Infected with Leishmania infantum. *Front Immunol*. 2017;**8**:1620. [PubMed ID: 29218050]. [PubMed Central ID: PMC5703736]. https://doi.org/10.3389/fimmu.2017.01620.
21. Chandra RK, Dayton DH. Trace element regulation of immunity and infection. *J Nutrition Research*. 1982;**2**(6):721-33.
22. Farzin L, Moassesi ME. A comparison of serum selenium, zinc and copper level in visceral and cutaneous leishmaniasis. *J Isfahan Univ Med Sci*. 2014;**19**(4):355.
23. Kocyigit A, Gur S, Erel O, Gurel MS. Associations among plasma selenium, zinc, copper, and iron concentrations and immunoregulatory cytokine levels in patients with cutaneous leishmaniasis. *Biol Trace Elem Res*. 2002;**90**(1-3):47-55. [PubMed ID: 12666825]. https://doi.org/10.1385/BTER:90:1-3:47.
24. Kocyigit A, Erel O, Gurel MS, Avci S, Aktepe N. Alterations of serum selenium, zinc, copper, and iron concentrations and some related antioxidant enzyme activities in patients with cutaneous leishmaniasis. *Biol Trace Elem Res*. 1998;**65**(3):271-81. [PubMed ID: 9892499]. https://doi.org/10.1007/BF02789102.
25. Kahvaz MS, Soltani S, Soltani S, Carvalheiro MC, Foroutan M. Low Serum Levels of Selenium, Zinc, Iron, and Zinc/Copper Ratio in an Endemic Region of Cutaneous Leishmaniasis in Southwest Iran. *Biol Trace Elem Res*. 2021;**199**(4):1291-6. [PubMed ID: 32607766]. https://doi.org/10.1007/s12011-020-02271-z.
26. Taghipour A, Abdoli A, Ramezani A, Abolghazi A, Mofazzal Jahromi MA, Maani S, et al. Leishmaniasis and Trace Element Alterations: a Systematic Review. *Biol Trace Elem Res*. 2021;**199**(10):3918-38. [PubMed ID: 33405078]. https://doi.org/10.1007/s12011-020-02505-0.
27. Chaudhuri S, Varshney JP, Patra RC. Erythrocytic antioxidant defense, lipid peroxides level and blood iron, zinc and copper concentrations in dogs naturally infected with Babesia gibsoni. *Res Vet Sci*. 2008;**85**(1):120-4. [PubMed ID: 18378268]. https://doi.org/10.1016/j.rvsc.2007.09.001.

28. Serarslan G, Yilmaz HR, Sogut S. Serum antioxidant activities, malondialdehyde and nitric oxide levels in human cutaneous leishmaniasis. *Clin Exp Dermatol.* 2005;**30**(3):267-71. [PubMed ID: 15807687]. https://doi.org/10.1111/j.1365-2230.2005.01758.x.

29. Mukbel RM, Patten CJ, Gibson K, Ghosh M, Petersen C, Jones DE. Macrophage killing of Leishmania amazonensis amastigotes requires both nitric oxide and superoxide. *Am J Trop Med Hyg.* 2007;**76**(4):669-75. [PubMed ID: 17426168].

30. Novaes RD, Teixeira AL, de Miranda AS. Oxidative Stress in Microbial Diseases: Pathogen, Host, and Therapeutics. *Oxid Med Cell Longev.* 2019;**2019**:8159562. [PubMed ID: 30774746]. [PubMed Central ID: PMC6350582]. https://doi.org/10.1155/2019/8159562.

31. Saif MA, Al-Mohammad HI. Evaluation of immune cellular enzymes, oxidative and nitrosative Stress in Saudi Patients with Cutaneous Leishmaniasis in Al-AHssa, before and after treatment. *King Faisal University College of Medicine.* 2020.