Impact of Some Immunological Parameters (antioxidant – cytokines) in Cutaneous leishmaniasis in a Sample of Patients in the Al-Ramadi City.

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Received: 23/10/2023
Accepted: 16/11/2023
Online: 13/05/2024

ABSTRACT

Background: Cutaneous leishmaniasis is one of the endemic diseases in Iraq. Both types of cutaneous leishmaniasis that cause different ulcers are present, namely *Leishmania major* and *Leishmania tropica*, The disease also represents a major public and global health problem all over the world, **objective:** Investigation of the relationship between parasitic infection with cutaneous leishmaniasis and some antioxidants Superoxide, Nitric oxide and immune cytokines tumor necrosis factor-alpha, Interferon-gamma. **Materials and Methods:** The demographic and epidemiological study included 55 cases suspected of being infected with cutaneous leishmaniasis. They visited Ramadi Teaching Hospital, with 25 volunteers. After obtaining their consent, they were used as control samples, where blood serum samples were obtained from both study groups. After that, work was done in laboratories. Department of Biology - College of Education for Pure Sciences - Anbar University. **Results**: The patients and healthy people were of different genders, and among the 55 samples from the infected, the number of males was 36 (65.5)% and females 19 (34.5)%, with statistically significant differences $p<0.05$, and the age groups of the patients were from one year to 60 years. With an average age of (31 years), they were distributed into 6 groups. Also, among the 25 samples from the control group, the number of males was 12 (48%) and females were 13 (52%), Their ages also ranged from one year to 60 years, with an average age of (13) years, the enzyme-linked immunoabsorbent assay (ELISA) technique was used to detect the level of immunological indicators TNF-α, IFN-γ, SOD, NO, where the results showed High levels in the serum of cutaneous leishmaniasis patients (120.00 ± 52.11, 123.00 ± 44.21, 3.10 ± 1.21 and 121.93 ± 33.22), respectively compared to healthy subjects (72.86 ± 14.94, 21.91 ± 45.40, 0.88 ± 0.12, and 81.46 ± 4.50) respectively With statistically significant differences at the level of $p < 0.05$, $p < 0.001$. **Conclusions**: The inflammatory cytokines TNF-α, IFN-γ play a vital role in increasing or decreasing the severity in the pathogenesis of the disease and eliminating it.

Keywords: Immunity of *Leishmian*, Superoxides, Nitric oxide, interferon-gamma

https://doi.org/10.24126/jobrc.2024.18.1.781

1-INTRODUCTION

Leishmaniasis is a disease transmitted by the bite of female sandflies and caused by various *Leishmania* species, manifesting itself in three main epidemics: cutaneous leishmaniasis, and systemic leishmaniasis. It is spread in natural and semi-natural areas (1), About 12 million people in 98 countries were affected by these diseases, and the total number of disease cases that peaked in the period from 2008 to 2015 in Iraq reached 17,001 cases, amounting to between (2.9 - 10.5) / 100,000 individuals, the highest cases recorded in the year 2015 (4000 cases) (2, 3), Due to the complexity of the life cycle due to the lack of human presence and the presence of many vectors and reservoir hosts, disease surveillance remains without solutions, so scientific eradication remains the main choice, In Iraq, there are two types of cutaneous Leishmaniasis, *L. tropica* or anthroponotic cutaneous

leishmaniasis (ACL), and *L. major* (zoonotic cutaneous leishmaniasis (ZCL), In Iraq, there are two types of sandflies namely *Phlebotomus papatasi*, *Phlebotomus sergenti*, (4, 5), The innate natural killer (NK) cells, macrophages, dendritic cell (DC), neutrophils, cytokines as well as complement proteins. are recruited to the infection site early after infection(6). Leishmaniasis is generally initiated by immune cells. Innate cells are involved in the call-in against *Leishmania* infection, They are well characterized in the detection of germline-encoded document detection cases, They are involved in host immune cooperation in Cutaneous leishmaniasis. Also in all immunopathology protection, which means it may speed up treatment(7) Proinflammatory cytokines are produced primarily to amplify the immune response to *Leishmania* infection. Major proinflammatory cytokines include Tumor necrosis factor alpha, Interferon gamma,Interleukin1,Interleukin2, Interleukin8, Interleukin12, Interleukin15, and Interleukin18,while anti-inflammatory cytokines are immune regulatory molecules, that counteract the effects of pro-inflammatory cytokines to reduce inflammation caused by excessive production of pro-inflammatory cytokines as described in these major anti-inflammatory cytokines include IL-5, IL-6 , IL-4, IL-10, IL-13, and Transformation growth factor beta .Experimentally, it was investigated that IFN-γ-deficient C57BL/6 mice infected with *L. amazonensis* exhibited larger lesions, increased parasite burden, and developed Thymaic helper cell type2(Th2-type) immune responses associated with IL-4 elevations compared to wild-type mice Administration of cytokines is a potential way to modulate biological effects associated with immune diseases and hence, cytokines may be addressed as potential therapeutics in the future(8, 9).

Many researchers have indicated through experiments that *Leishmania* parasites are associated with the production of cytokines and the activation of Cluster Differentiation 4 T Helper Cell On the other hand, people become infected when they produce T helper 2 cytokines that facilitate damage due to the disease. In addition, some studies have also indicated that CD8+ T cells are a common part in immune defense through the production of cytokines, their cytotoxic activity, and the excessive production of proinflammatory mediators promoting cell amplification. This can be associated with excessive inflammatory reaction and ultimately lead to tissue destruction and development of immune pathogenesis. Consequently, there are contradictions regarding the role of immune responses in the protection and immunopathogenesis of CL(10).

2- MATERIAL AND METHODS

Collection of Blood sample

Five ml of peripheral blood was drawn from all patients and healthy individuals by using a disposable sterile syringe. The blood was divided into parts: 2 ml placed ethylene diamine tetra acetic acid(EDTA) tube for a complete blood count test, and 3 ml were placed in gel tube, stand at room temperature then centrifuged 3000 rpm for 5 minutes to obtain serum, The serum was transferred into white sterile tubes (white tube), Then the serum samples were preserved until use.

Estimation of Serum Levels of Some Immunological Parameters

The serum levels of immunological parameters(TNF-α, IFN-γ, Nitric Oxidic, SOD), were estimated by sandwich ELISA kits manufactured by SunLong Biotech, China .

Complete Blood Picture(CBC)

Peripheral blood is used for CBC test that counts all blood components (red blood cells, white blood cells, and platelets). A small amount of blood sample was analyzed by the Sysmex XP-300 hematological analyzer.

Statistical analysis

Statistical analysis was carried out via using Statistical Package for Social Sciences, version 22(SPSS), described in the form of numbers and percentages, and the comparison was made using the Chi-square test. As for the variables with a numerical form, they were described using the mean and the standard deviation, and the comparison between the groups was done using the(t-test) between two groups(11)

3-RESULTS

Distribution of patients and healthy people according to immune and hematological parameters, according to age and gender.

Table (1): shows the distribution of age groups and genders for the study patient group

GROUPS(AGE AND SEX)	NO OF CASES CL AND PERCENT(%)	TOTAL	GROUP TYPE
1-10	22(40.0%)	55	PATIENTS
11-20	9(16.4%)		
21-30	15(27.3)		
31-40	4(7.3%)		
41-50	3(5.5)		
51-60	2(3.6)		
MALE	36(65.5%)		
FEMALE	19(34.5%)	55	

Table (2): shows the distribution of age groups and gender for the study control group

Groups (age and sex)	NO of cases control and percent(%)	Total	Group type
1-10	7(28%)	25	Healthy
11-20	6(24%)		
21-30	6(24%)		
31-40	2(8%)		
41-60	4(16%)		
Male	12(48%)	25	
Female	13(52%)		

Distribution of results of immune parameters between the two study groups

The results of the current study showed statistically significant differences ($p<0.05$) between patients and controls for the parameters INF-γ, TNF-α, NO and SOD, as the results showed high levels in patients (120.00 ± 52.11, 123.00 ± 44.21, 3.10 ± 1.21 and 121.93 ± 33.22) respectively, compared to healthy subjects (72.86 ± 14.94, 21.91 ± 45.40, 0.88 ± 0.12, and 81 ± 4.5 0), as in Table (3).

Table (3): Results of estimating immune parameters using ELISA technology between two groups.

Immunological parameters	Groups	Mean± SD	p-value
INF-γ (pg/ml)	patients	120.00±52.11	p<0.05*
	control	72.86±14.94	
NO (µmol/L)	patients	123.00±44.21	p<0.001***
	control	45.40±21.91	
SOD (ng/ml)	patients	3.10±1.21	p<0.001***
	control	0.88±0.12	
TNF-α (pg/ml)	patients	121.93±33.22	p<0.05*
	control	81.46±4.50	

Distribution of hematological parameters between the two study groups.

The results of the current study show that there are statistical differences ($p<0.05$) between the blood variables in the two study groups. The blood variables recorded monocytes, lymphocytes, and white blood cells (WBCs) high levels in patients (3.11 ± 1.23, 9.22 ± 2.62 0.21, ± 1.32), respectively. Compared to healthy subjects (0.65, 7.00 ± 1.39, 1.83 ± 0.19, ± 0.58) respectively, while basophils, neutrophils, and eosinophils did not record significant differences between the two study groups as shown in the table(4).

Table (4): Results of CBC test.

Groups		Mean± SD	p value
Basophil 10^3/µl	Patients	0.05±0.001	p>0.05
	Control	0.04±0.01	
Eosinophil 10^3/µl	Patients	0.29±0.11	p>0.05
	Control	0.23±0.11	
Monocytes 10^3/ µl	Patients	1.32±0.21	p<0.01**
	Control	0.58±0.19	
Lymphocytes 10^3 /µl	Patients	3.11±1.23	p<0.01**
	Control	1.83±0.64	
Neutrophil 10^3/µl	Patients	4.31±1.38	p>0.05
	Control	4.33±1.22	
WBCs 10^3/µl	Patients	9.22±2.62	p<0.05*
	Control	7.00±1.39	

Correlation Between Immunological Parameters

The results of the current study showed that there were significant differences with statistical significance between the physiological and demographic variables and the immune variables, as shown in Table (5).

Table (5): The relationship between immune variables and other variables for patients.

Parameters	TNF-α	IFN –γ
SOD	0.251*	0.118 NS
Nitric oxide	0.022 NS-	-0.064 NS
Age	0.500**	0.182*
Gender	0.389**	0.246NS
Number lesion	0.320*	0.121NS
Type lesion	0.492**	0.201 NS
Location lesion	0.319*	0.208NS

*positive correlation $p<0.05$

**positive correlation $p<0.01$

NS: Negative correlation $p>0.05$

4-DISSCUSION

The results showed significant levels and differences in the serum of patients and controls for the immune variable IFN-γ, These results are consistent with the study (12)in Iraq, where the concentration of IFN-γ in patients (67.30 ± 59.41 pg/ml) was higher than control (3.81±1.79 pg/ml) And the study (13) in Iraq, which indicated a higher concentration of interferon-gamma for both types of *L. major* and tropicalis in infected people compared to healthy people, as well as the study (14) in Iraq, where they recorded a higher percentage for infected people (40.94 ± 1.17). pg/m) compared to healthy controls (3.04 ± 0.53 pg/ml), Natural killer cells (NK) increase the expression of pro-inflammatory cytokines IFN-γ and TNF-α, which are one of the innate immune mechanisms to eliminate *Leishmania*. Control of *Leishmania* infection and resolution of the disease depend critically on sending interferon-gamma signals, (IFN-γ) secreted by activated T cells and natural killer cells in response to cytokinetic signals IL-12. Interferon-gamma also stimulates and expresses (iNOS), which produces nitric oxide (NO) by phagocytes that harbor the parasite and is, therefore, necessary to activate macrophages to kill and dissolve the parasite's infection, so a current study agreed with it, as it was observed that there were high levels of nitric oxide production among infected people(17-15).

A significant increase in the serum of people with cutaneous leishmaniasis for tumor necrosis factor (TNF-α) was observed in this study, as the results of this study agreed with (14)in Iraq, as the researchers recorded a higher concentration for those infected (24.4±125pg/ml) compared to healthy people (18.5±34pg/ml), Elevated TNF-α levels during active disease contribute to the emergence of separate clinical manifestations in patients. IFN-γ and TNF-α have synergistic killing effects against Leishmania major infection by stimulating macrophages to increase NO production(18), One of the parasite's mechanisms for evading immune and evasion mechanisms is to enhance tumor necrosis factor gene expression that induces tissue damage, as found(19).

As for secreted antioxidants, high levels of nitric oxide (NO) were observed for patients compared to healthy people (123.00 ± 44.21 µmol/L), Thus the results were consistent with the study(20), as they recorded high levels in patients **.**(12.11 ± 0.33 mmol/l) compared to healthy people (3.23 ± 0.10). A study was also recorded in the Kingdom of Saudi Arabia (21)to evaluate some indicators of oxidation and antioxidants, as a significant increase in nitric oxide was observed in those infected before and after receiving treatment, The study recorded high levels of superoxide dismutase (SOD) in patients (3.10±1.21) compared to healthy people (0.88±0.12), and thus these results are consistent with the study (21)as the researchers noted that levels of antioxidants were low in non-patients, Treatment recipients compared to control. These results also agreed with the study(22), as high levels were recorded for patients **.**(0.0314±0.026 pg/ml) compared to healthy controls (0.128±0.09).

IFN-γ synergizes with TNF to activate inducible nitric oxide (iNOS or NOS2) to produce nitric oxide (NO), leading to the elimination of intracellular parasites. Nitric oxide (NO) is the most important mediator that mediates a wide range of physiological and pathophysiological processes. Nitric oxide regulates the constriction of small and medium-sized blood vessels, promotes smooth muscle relaxation, has anticoagulant properties, inhibits platelet activation, and mediates immune response and neurotransmission. In contrast, when treating CL patients, significantly higher SOD activities were observed. Most forms of SOD fall Within cells, which could be the source of the different activity observed in previous studies, increased SOD activity leads to increased conversion of the superoxide anion to hydrogen peroxide (H2O2)(23, 24).

The results of the current study show that there is a positive relationship between the physiological variable SOD with the immune variable TNF-α ($r = 0.251$) at the probability level of $P < 0.01$. At the same time, there was a negative relationship between the physiological variable SOD with the immune variable IFN-γ ($r = 0.188$) at Probability level $p<0.05$, Tumor necrosis factor-alpha represents a central mediator of inflammatory responses, and its small amount is essential for host defense against infection. Local and systemic TNF-α levels increase in poor healing and chronic wounds in Leishmania infection (25), Conversely, SOD expression is associated with differentiation and replication processes. In Leishmania spp, higher SOD activity is observed during amastigote differentiation. In contrast, lower SOD activity and ROS accumulation are observed during the log phase. Local and systemic TNF-α levels increase in poor healing and chronic wounds(26), Th1 responses involving nitric oxide and cytokines including IL-12, γ-IFN, and TNF-α kill parasites. In contrast, Th2 responses are characterized by the production of IL-4, IL-13, and IL-10, leading to susceptibility to infection(27). The results showed an inverse negative relationship between the physiological variable NO with both immune variables TNF-α and IFN-γ (r=-0.064,r=-0.022). This is explained by the increase in anti-inflammatory cytokines such as IL-5, IL-6 and IL- 13 and

TGF-β, as it has a role in reducing the secretion of pro-inflammatory cytokines TNF-α and IFN-γ(8),Our study showed a negative relationship between gender with the immune variable IFN-γ (r = 0.246), while a positive relationship appeared with the immune variable TNF-α (0.389) at a statistical significance of $p<0.01$. A study using animal models showed that T cells in females have a The T cells from males showed mixed pictures with the inflammatory cytokine IL-17A, the eosinophil-recruiting cytokine IL-5 and the anti-inflammatory cytokine IL-10. About half of the genes were overexpressed. T cells from females contained estrogen-responsive elements, suggesting a regulatory role for sex steroids. However, the mechanisms underlying the immune responses of sex-biased T cells have not been clarified (28)(29).

There was a positive relationship between age and the immune variable IFN-γ (r = 0.182) at the probability level of $p < 0.05$. At the same time, there was a high positive relationship between age and the immune variable TNF-α (r = 0.500) at the probability level of $p < 0.01$, This is explained by the secretion of the immune cytokine IFN-γ in varying proportions between the age groups that we recorded in the study. This is consistent with the findings that elderly people have a decrease in the response to the IFN-γ antigen, and therefore they lack the main cytokine responsible for macrophages (activation and killing), while secretion of the immune cytokine tumor necrosis factor was high among different age groups in the study (30).

5-CONCLUSION

The inflammatory cytokines TNF-α, IFN-γ play a vital role in increasing or decreasing the severity in the pathogenesis of the disease and eliminating it.

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تأثير بعض المعايير المناعية (مضادات الأكسدة – السيتوكينات) في الإصابة بداء الليشمانيا الجلدي لدى عينة من المرضى في مدينة الرمادي.

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الخلاصة:

خلفية عن الموضوع: داء الليشمانيات الجلدي هو أحد الأمراض المستوطنة في العراق. يوجد كلا النوعين من الليشمانيا الجلدي الذي يسبب تقرحات مختلفة، وهما L.major وL.tropica، ويمثل المرض أيضًا مشكلة صحية عامة وعالمية كبرى في جميع أنحاء العالم، **الهدف من الدراسة**:دراسة العلاقة بين العدوى الطفيلية بداء الليشمانيات الجلدي ومرض الليشمانيات الجلدي. بعض مضادات الأكسدة سوبر أكسيد وأكسيد النيتريك والسيتوكينات المناعية وعامل نخر الورم ألفا وإنترفيرون جاما. **المواد وطرق العمل:** شملت الدراسة الديموغرافية والوبائية 55 حالة يشتبه بإصابتها بداء الليشمانيا الجلدي. قاموا بزيارة مستشفى الرمادي التعليمي مع 25 متطوعاً. وبعد الحصول على موافقتهم، تم استخدامهم كعينات مراقبة، حيث تم الحصول على عينات مصل الدم من مجموعتي الدراسة, وبعد ذلك تم العمل في المختبرات, قسم الأحياء - كلية التربية للعلوم الصرفة - جامعة الانبار **النتائج:** المرضى والأصحاء كانوا من جنسين مختلفين، ومن بين الـ 55 عينة من المصابين كان عدد الذكور 36 (65.5)% والإناث 19 (34.5). %، مع وجود فروق ذات دلالة إحصائية $p<0.05$، وكانت الفئات العمرية للمرضى من سنة إلى 60 سنة. وبمتوسط عمر (31 سنة) تم توزيعهم على 6 مجموعات. كما أنه من بين 25 عينة من المجموعة الضابطة كان عدد الذكور 12 (48%) والإناث 13 (52%)، كما تراوحت أعمارهم من سنة إلى 60 سنة، بمتوسط عمر (13) سنة, تم استخدام تقنية مقايسة الامتصاص المناعي المرتبط بالإنزيم (ELISA) للكشف عن مستوى المؤشرات المناعية TNF-α، IFN-γ، SOD، NO، أظهرت النتائج ارتفاع مستوياتها في مصل مرضى داء الليشمانيا الجلدي (120.00 ± 52.11، 123.00 ± 44.21، 3.10 ± 1.21 و 121.93 ± 33.22)، على التوالي. مقارنة بالأشخاص الأصحاء (72.86 ± 14.94، 21.91 ± 45.40، 0.88 ± 0.12، و 81.46 ± 4.50) على التوالي مع وجود فروق ذات دلالة إحصائية عند مستوى $p <0.05$، $p <0.001.1$ **الاستنتاجات:** السيتوكينات الالتهابية TNF-α وIFN-γ تلعب دورا حيويا في زيادة أو تقليل شدة المرض وفي التسبب في المرض والقضاء عليه.

الكلمات المفتاحية: المناعة ضد اللشمانيا، السوبر أوكسيد ،أوكسيد النتريك ،أنترفيرون-غاما، عامل نخر الورم-الفا.