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Determination of some Sera Chemokines in Iraqi Patients infected with Cutaneous Leishmaniasis

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ABSTRACT

Cutaneous leishmaniasis (CL) is caused by a protozoan parasite of the genus *Leishmania* that infects macrophages of many mammals including humans. Their infection induces both humoral and cellular immune responses, but the balance of their expression varies with the type of the disease. The aim of the present study is to determine levels of serum chemokines that include granulocytes-macrophages colonies stimulating factor (GM-CSF), intercellular adhesion molecule1 (ICAM-1), interferon-gamma inducible protein-10 (IP-10) and vascular endothelial growth factor (VEGF) in Iraqi patients with CL lesions which can be considered as immunological markers for CL infection. Ninety people were included in the present study; sixty of them were infected with CL lesions while 30 uninfected people were considered as control. Patients were diagnosed on the basis of clinical and parasitological criteria. Sera (GM-CSF, ICAM-1, IP-10 and VEGF) levels were determined by ELISA using a quantitative sandwich enzyme immunoassay technique. The results showed no significant differences between males and females ($p>0.05$) in the infection with CL, while highly significant difference ($p<0.01$) for distribution of CL lesion according to site of infection were recorded. The prevalence of multiple lesions was more than single lesions with a highly significant differences ($p<0.01$) .Sera levels of GM-CSF, ICAM-1, IP-10, and VEGF were significantly higher in patients group than healthy subjects ($P< 0.01$).

Keywords: Cutaneous leishmaniasis, Chemokines, GM-CSF, ICAM-1.

1. INTRODUCTION

Leishmaniasis is a parasitic disease, clinically divided into three forms: cutaneous, mucocutaneous and visceral. It is caused by parasitic protozoa of the genus *Leishmania*. Humans are infected via the bite of phlebotomine sandflies, which breed in forest areas, caves, or the burrows of small rodents. Cutaneous leishmaniasis (CL) is a complex disease with wide spectrum of clinical manifestations. More than 90 % of CL cases live in the following countries: Afghanistan, Saudi Arabia, Aljazeera, Brazil, Iran, Iraq and Syria. The number of new cases of CL reached to 1.5 million people in the world (1, 2).

In Iraq, *L. major* and *L. tropica* are the causes of cutaneous leishmaniasis infections. *Leishmania* species are intra-cellular parasites invading monocytes, macrophages, and Langerhans's cells (3). Their infection in humans induces both humoral and cellular immune responses, but the balance of their expression varies with the type of the disease (4). A variety of inflammatory mediators are produced by monocytes/macrophages during the course of infection (5). The importance of cytokines during leishmanial infection comes from the demonstration (on experimental murine leishmaniasis) of the existence of two distinct CD4+ Th1 and Th2 subsets (6). Chemokines are small (8-10-kDa) chemoattractant cytokines that play important roles during immune responses by triggering integrin activation and inducing the recruitment of antigen-specific lymphocytes to peripheral tissues in response to inflammation (7). At present, approximately 50 human chemokines and 20 chemokine receptors have been characterized (8). The objective of the present work was to determine levels of serum chemokines that include granulocytes-macrophages colonies stimulating

factor (GM-CSF), intercellular adhesion molecule 1(ICAM-1), interferon-gamma inducible protein-10 (IP-10) and vascular endothelial growth factor (VEGF) in Iraqi patients with CL lesions, which can be considered as immunological markers for CL infection.

2. MATERIALS AND METHODS

2.1 Patients

The study was conducted at Al-Karama Teaching Hospital, Baghdad/ Iraq, during the period from October 2016 to April 2017. Ninety people were included, 60 patients admitted with CL (25 were males, 35 were females) and 30 as control. Their ages ranged from 10-50 years. The clinical diagnosis by dermatologists was confirmed by laboratory demonstration of the parasite in the lesions by direct smears. Lesions were cleaned with 70% ethanol, and punctured at the margins with a sterile lancet. Exudate material was smeared, dried in air and fixed by methanol. The smears were stained with Giemsa's stain and examined by light microscope. Positive microscopic diagnosis was made when amastigotes forms were identified in the smears.

2.2 Blood Collection

Five milliliters of venous blood were collected from each studied individual by using 5ml disposable syringe. The blood samples were immediately transferred to a plain tube and left to clot at room temperature (20-25°C) for 15 minutes. Then, they were centrifuged at 1000 rpm for 10 minutes to separate sera, which were distributed into aliquots (0.25 ml) in tightly closed Eppendorf tubes, and then the tubes were stored at -20ºC until assayed by the chemokines GM-CSF, ICAM-1, IP-10 and VEGF was done.

2.3 Serum Level of Chemokine

Sera of CL patients and control assessed for levels of four chemokines GM-CSF, ICAM-1, IP-10 and VEGF by ELISA using human GM-CSF, ICAM-1, IP-10 and VEGF kit (PeproTech; USA).

2.4 Statistical Analysis

Descriptive and statistical data analyses were performed by using Statistical Package for Science SPSS version 2010, while Chi square ($\chi 2$) test, Monte Carlo test (MC) was applied to demonstrate any significant differences of gender, number and sites of lesions of CL patients and means of chemokines levels were compared between groups using Student t-test.

3. RESULTS AND DISCUSSION

Table (1) shows that there were no significant differences ($p>0.05$) in the percentage of infection with CL between males 25 (42%) and females 35 (58%).

Table 1: Number and percentage of CL infection according to Gender.

Gender	No.	%
Males	25	42
Females	35	58
Total	60	100
$\chi 2$= 1.667 p>0.05	NS	

NS: Not significant

Cutaneous leishmaniasis is one of the important health problems in many countries of the world's which are given top priority in the WHO program (9).

The present study recorded a high percentage of infection in females than males (58% and 42%, respectively) without any significant difference (Table 1). The major cause of this result may be due to numbers of infected cases that were reviewed from hospitals and health centers and increase the number of population, urbanization and agricultural and environmental changes that provide well-activity of the insect vectors. Also, the immunity status in females were less than in males due to pregnancy and childbirth (10, 11).

The results showed highly significant differences ($p<0.01$) for distribution of CL lesions according to site of infection (Table 2): the face recorded highest percentage 23(38%), followed by upper limbs 16(27%), lower limbs 15(25%) ear 3(5%), neck 2(3%) and shoulder 1(2%).

Table 2: Distribution of CL patients according to site of lesions.

Site of lesions	No.	%
Face	23	38
Upper limbs	16	27
Lower limbs	15	25
Ear	3	5
Neck	2	3
Shoulder	1	2
Total	60	100
MC p<0.01	HS	

MC: Monte Carlo test.
HS: Highly significant at P< 0.01

The results of the study indicated that most affected part of the body (Table 2) was the face (38%) with highly significant difference (p<0.01) The reason may be that the face is the most exposed part of the body, making it more susceptible areas for a bite of the sand fly (12). These results are with agreements with other studies in Iraq (13) and other countries (14, 15, 16).

The number of CL lesions were explained in table (3) and recorded prevalence of multiple lesions 42(70%) were more than single lesions with highly significant differences (p<0.01).

Table 3: Distribution of CL patients according to number of lesions.

Number of lesions	No.	%
Single	18	30
Multiple	42	70
Total	60	100
$\chi 2$= 11.267 p<0.01 HS		

HS: Highly significant at P< 0.01

Also, incidence of multiple lesions (Table 3) was higher (70%) than single lesions (30%) with highly significant differences (p<0.01) in this study. This result could be attributed to the long periods of exposure to Plebotomine sand flies and the bites times are very close which did not allow the composition of a strong immune response (17). These results are in agreement with those of Al-Samarai and Al-Obaidi (18). Serum level of GM-CSF (Table 4) was significantly (P< 0.01) increased in CL patients compared with control (27.428±4.204 vs. 19.700±1.487 pg/ml).

Table 4: The level of GM-CSF (pg/ml) and descriptive statistics and t- test of CL and control groups.

Groups	No.	Min.	Max.	Mean ± SD.	t-test	P-value
CL patients	60	20.733	37.418	27.428±4.204	9.306	0.000
Control	30	17.321	22.673	19.700±1.487		HS

HS: Highly significant at P< 0.01.

GM-CSF as a factor responsible for activation and increase in the number of large granule cells and macrophages, as well as direct and indirect effects in multiple types of cells, including cell proliferation and maturation (19). Levels of GM-CSF chemokine (Table 4) was assessed in the serum of CL patients (27.428±4.204 pg/ml) and control (19.700±1.487 pg/ml). GM-CSF may decrease the healing time of cutaneous leishmaniasis ulcers by three potential mechanisms: increasing parasite killing by directly activating macrophages (20, 21, 22), enhancing scar formation (23) and modulating immunologic balance (24). Previous studies have shown that GM-CSF activates macrophages to kill *Leishmania* in vitro. (20, 21). GM-CSF has been described to improve healing and scarring of cutaneous lesions caused by agents other than *Leishmania* (25).

Serum level of ICAM-1(Table 5) was significantly (P< 0.01) increased in CL patients compared to controls (8.596±1.190 vs.4.824±0.782 pg/ml).

Table 5: The level of ICAM-1 (pg/ml) and descriptive statistics and t- test of CL and control groups.

Groups	No.	Min.	Max.	Mean ±SD.	t-test	P-value
CL patients	60	6.311	10.821	8.596±1.190	14.470	0.000
Control	30	3.325	5.998	4.824±0.782		HS

HS: Highly significant at P< 0.01

ICAM-1 also known as CD54 (Cluster of Differentiation 54) is a protein that in humans is encoded by the ICAM-1 gene (26). This gene encodes a cell surface glycoprotein which is typically expressed on endothelial cells and cells of the immune system. It binds to integrins of type CD11a / CD18 or CD11b / CD18 and is also exploited by rhinovirus as a receptor (27). ICAM-1 can be induced by interleukin-1 (IL-1) and tumor necrosis factor (TNF) and is expressed by the vascular endothelium, macrophages, and lymphocytes, as well as ICAM-1 is a ligand for LFA-1 a receptor found on leukocytes (28) When activated leukocytes bind to endothelial cells via ICAM-1/LFA-1 and then transmigrate into tissues (29). Level of ICAM-1 chemokine was detected in this study (Table 5). Patient of CL group recorded higher level (8.596±1.190 pg/ml) than control group (4.824±0.782 pg/ml). In a study that performed by Elhassan et al. (30) about biopsies from

skin lesions and draining lymph nodes of patients suffering from CL caused by Leishmania major were venular endothelium in the skin lesions expressed ICAM-1 which is the ligand for LFA-1, and in other study (31) showed an increase expression of ICAM-1 molecules in localized CL.

Serum level of IP-10 (Table 6) was significantly (P< 0.01) increased in CL patients compared to controls (23.577±2.409 vs. 13.246±1.522 pg/ml).

Table 6: The level of IP-10 (pg/ml) and descriptive statistics and t- test of CL and control groups.

Groups	No.	Min.	Max.	Mean ±SD.	t-test	P-value
CL patients	60	17.734	28.882	23.577±2.409	15.067	0.000
Control	30	10.033	16.690	13.246±1.522		HS

HS: Highly significant at P< 0.01

IFN-inducible protein 10 (IP-10, CXCL10), a chemokine secreted from cells stimulated with type I and II IFNs and LPS, is a chemoattractant for activated T cells. Expression of IP-10 is seen in many Th1-type inflammatory diseases, where it is thought to play an important role in recruiting activated T cells into sites of tissue inflammation (32). As seen from Table (6) the results of the present study detected an increase in the levels of IP-10 in CL group (23.577±2.409 pg/ml) in comparison with control group (13.246±1.522 pg/ml). Vargas-Inchaustegui *et al*. (33) found an elevated amount in IP-10 concentration in CL group (180 pg/ml) in comparison with control group (150 pg/ml). The role of CXCL10 in human leishmaniasis is not clear, although CXCL10 is well-known for its involvement in recruiting monocytes, macrophages , T cells and NK cells (34) during human viral and bacterial infections (35, 36) and organ transplantation (37). By using a murine model of *L. amazonensis* infection, it found that exogenous CXCL10 promotes parasite killing in macrophages cultures *in vitro* (38) and enhances the antigen-presenting function in infected DCs (39). The local injection of CXCL10 significantly delays the onset of cutaneous lesions (38). Similarly, a study of pulmonary tuberculosis revealed a positive correlation between increased sera levels of chemokine CXCL10 and the active status of the disease, as well as a positive correlation between the high levels of CCR1, CCR2 and CXCR2 on the surfaces of T and NK cells and disease severity (36).

Serum level of VEGF (Table 7) was significantly (P< 0.01) increased in CL patients compared with controls (29.587±3.875 vs. 24.770±1.051 pg/ml).

Table 7: The level of VEGF (pg/ml) and descriptive statistics and t- test of CL and control groups.

Groups	No.	Min.	Max.	Mean ±SD.	t-test	P-value
CL patients	60	22.653	39.418	29.587±3.875	8.575	0.00
Control	30	22.768	26.874	24.770±1.051		HS

HS: Highly significant at P< 0.01

Vascular endothelial growth factor (VEGF) which was originally known as vascular permeability factor (VPF) (40), is a signal protein produced by cells that stimulates vasculogenesis and angiogenesis. It is part of the system that restores the oxygen supply to tissues when blood circulation is inadequate such as in hypoxic conditions (41). VEGF's normal function is to create new blood vessels during embryonic development, new blood vessels after injury, muscle following exercise and new vessels (collateral circulation) to bypass blocked vessels (42). The results of VEGF concentrations are highly significant at P< 0.01 (Table 7) of CL group (29.587±3.875 pg/ml) in comparison control group (24.770±1.051 pg/ml) in this study. Weinkopff *et al.* (43) found that infection with *L. major* increases the expression of VEGF-A and VEGFR-2. This is associated with significant changes in the blood and lymphatic vasculature at the site of infection. Antibodies blockade of VEGFR-2 during infection led to a reduction in lymphatic endothelial cell proliferation and simultaneously increased lesion size without altering the parasite burden. These data show that *L. major* infection initiates enhanced VEGF-A/VEGFR-2 signaling and suggest that VEGFR-2-dependent lymph angiogenesis is a mechanism that restricts tissue inflammation in leishmaniasis.

4. CONCLUSION

The present study suggested that Chemokines play an important role in the resolution of CL infection.

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