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Determination of IFN -γ and TNF -α levels in serum of patients with Cutaneous leishmaniasis

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ABSTRACT

Cutaneous leishmaniasis, is common parasitic disease in Iraq, it is caused by unicellular protozoa that belong to the genus *Leishmania* which infecting human macrophages, and it is induce human humoral and cellular immune responses. Many cytokines are excreted during this response, however IFN -γ and TNF -α are the most important factors in the inception of immunity against *leishmania spp.* The present study aimed to estimate the serum level of TNF-α and IFN-Y in patients with cutaneous leishmaniasis (CL) by enzyme-linked immunosorbent assay (ELISA). Out of 62 patients with CL lesion the infection rate was higher in males 36 (58%) than females 26 (42%), also the highest infection rate 29 (46.8%) was in age group (less than 1 -10years), and the lowest infection rate 18 (2.4%) was in the age group (51-60). Blood samples collected from 62 patients with CL and 27 healthy individual. The results showed that the level of serum IFN-Y (38.23 pg/mL) in patients with CL was significantly higher than control group (21.98 pg/mL). As well as the results indicated a significantly increase in the levels of TNF-α in patients serum with CL (46.53 pg / mL) than control group (23 pg / ml).

Introduction

Cutaneous leishmaniasis (CL), is a health issue around world, and about 88 countries including Iraq, Iran, Brazil, Afghanistan, Syria, India, Bangladesh and Sudan are endemic area with cutaneous leishmaniasis [1]. *Leishmania* spp. which including *Leishmania major*, *Leishmania tropica*, and *Leishmania aethiopica* in old world and *Leishmania amazonensis* and *Leishmania mexicana* responsible for infection with CL[2]. *Leishmania* spp. are intracellular protozoa, and the life cycle includes distinct stages, the promastigote stage in the sand fly gut and the amastigote stage in human phagocytes and reticuloendothelial systems [3]. Interferon-γ (IFN-γ), and tumor necrosis factor-α (TNF-α) are most significant human proinflammatory cytokines secreted by Th cells CD4+ and CD8+, natural killer cells, and natural killer T cells. These cytokines invade intracellular pathogens and tumor cells [4]. In addition, IFN-γ stimulates production of nitric oxide in activated macrophages, represses growth of intracellular parasite [5], advanced differentiation of Th cells CD4+ T to the Th1 subset, and inhibits the advancement of Th cells 2 (Th2) and Th cells 17 (Th17) [6]. Generally macrophages produced TNF-α, which have a significant role in clearance of *Leishmania* spp. by increasing in macrophage action and nitric oxide (NO) synthesis. The cytokine TNF-α can enhances Th1/IFN-γ reactions against Leishmania *major* infection [7], also, it was seen that IFN-γ and TNF-α have synergistic killing effects against *Leishmania major* infection by activation of macrophages to increasing in production of NO [8]. The immunological range observed in patients with leishmaniasis, characterized by significant levels of IFN-γ. *Leishmania* spp. killed by IFN-γ activated macrophages and not neutralized by antibodies, additionally the resistance to *leishmania* infection relevant to T helper 1 development and production of proinflammatory cytokines such as interleukin 12, interleukin 1, interleukin 2, IFN-γ and TNF α, which lead to stimulation of macrophages against parasites [9]. The aim of the study is determining the serum levels of cytokines IFN γ, TNF α in patients have cutaneous leishmaniasis.

Material and methods

Patients

This study conducted in Kirkuk governorate from October 2018 to December 2019. Eighty-nine individuals were included in this study(62 patients with CL lesion and 27 healthy individuals as control group). The age patients ranged from less than year to 70 years. Out of the 62 patients with cutaneous leishmaniasis, 36 were male and 26 were female. Dermal lesion tissues and blood samples collected from 62 patients.

Microscopic examination: It used for diagnosis the amastigotes of cutaneous leishmania in the dermal lesion tissues smears from 62 patient. Cutaneous lesions cleaned with ethanol then punctured with sterile lancet, and the exudate smeared onto a clean slide, left air dried, and fixed with methyl alcohol for 1-2 minutes. The slide then washed with tap water, after that colored with Geimsa stain solution for 30 minutes, washed with tap water and left dry. The slides then examined microscopically for detection of amastigotes [10].

Determination of serum IFN-γ and TNF-α

Five ml venous blood withdrawn cautiously from patient whom Dermatologist suspected to have CL. Sera was separated by centrifuge for 10 minutes at 3,000 rpm, than kept at (-20˚C) until utilized [11].

Serum levels of IFN-γ and TNF-α, were determined by ELISA technique (ELISA kits for IFNγ and TNFα). All tests were done according to the manufacturer's instructions and levels of cytokines in sera were determined by reading the standard absorption and compared with levels of cytokines for control group samples.

Statistical analysis: χ^2-test utilized to assess relationships between categorical variables and T test in style of homogeneous used manually. Significance was characterized as P <0.05.

Results and Discussion

CL is a social problem in many countries، it is caused by different *Leishmania* species.This study wasstarting from the beginning of October 2018 till the end of December 2019. Sixty-two patients with CL who included in this study and 27 apparently healthy volunteers as control group، the infection confirmed with detection of amastigotes under 100X, as shown in figure-1 and figure-2.

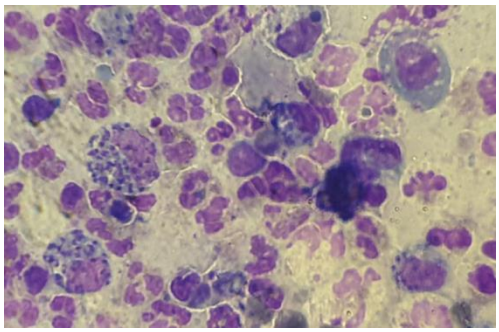

Fig. 1: Smear of skin lesion from patient with CL, stained with Giemsa stain shows amastigotes under 100X.

Fig. 2: Patient with multiple lesions of CL on hand.

Out of the 62 patients, 36 (58%) were males and 26 (42%) were females, as shown in Table- 1.

Table 1: Incidence of patients with CL regarding to sex.

Sex	No. of patients	%
Males	36	58
Females	26	42
Total	62	100
Chi-square value= 39.560	(P<0.05)	

Results of this study in agreement with this recently announced by [12], study in AL-Haweja city demonstrated that males of CL patients more than females (57%, 43% respectively). This result could be related to the incidence of working of males in regions (surfaces of houses), and more exposure to infected vectors than the females [13].

Regarding to age groups, the results of present study showed high significant differences (P<0.05) in distribution of infection within age groups. The age group 0-10 had the highest infection rate 29 (46.8%) followed by the age group 11–20 represented 14 (22.9%) while the lowest infection found in 51-60 age group 18 (2.4%), as showed in Table (2).

Table 2: Distribution of patients with CL in regarding to age groups.

Age (year)	No. of patients	%
10-0	29	46.8
20-11	14	22.9
30-21	7	11.2
40-31	5	8.0
50-41	4	6.4
60-51	1	1.5
70-61	2	3.2
Total	62	100
Chi-square value=92.638	(P<0.05)	

The results of this investigation in agreement with [14] who indicated that the high rate of infections was in age group under 12 years. Because of the adaptive immunity against the disease in enormous ages, also may be more chance of continuous exposer to the parasite during their life time [14].

The serum levels of the IFN-γ and TNF-α in patients and control groups determined by ELISA. The results indicated that the mean of serum interferon-gamma

(IFN γ) in patients (38.23 ± 13.95) was significantly ($p< 0.05$) higher than control group (21.98 ± 5.69), as shown in table-3.

Table 3: Mean of the serum levels of IFN-γ in patients with CL and control groups.

Groups	No.	Mean (pg/mL)	Std. Deviation
Patients	62	38.23	13.95
Control	27	21.98	5.69
Probability	-----	0.05	-----

Also, this study showed remarkable increase in the concentration of TNF-α in the serum of patient with CL (46.53±13.95) when compared with the control group (23.0±5.69) (Table-4).

Table 4: Mean of the serum concentration of TNF-α in patients with CL and control groups.

Groups	No.	Mean (pg/mL)	Std. Deviation
Patients	62	46.53	12.06
Control	27	23.0	4.86
Probability	-----	0.05	-----

Human proinflammatory cytokines such as IFN-γ and TNF-α are engaged in the immune-protection against cutaneous leishmaniases, and secreted by Th cells CD4+ and CD8+, natural killer Tcells, and natural killer cells. These cytokines invade intracellular pathogens and tumor cells [4]. High level of serum IFN γ detected in patients with CL when compared with control group. The high levels of IFN γ are important to keep up a balance between the responses of both Th1 and Th2. These results were indistinguishable from previous researchers, who found that the mixing of the response Th1 and Th2 of parasites is qualitative to T cell for both infection with *Leishmania*, whether acute or chronic condition [15]. TNF-α can promote T helper 1 cells and interferon gamma responses against infection to *L. major* [16]. In addition they have fundamental roles in control of intracellular pathogens and tumor cells [17].

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تحديد مستويات الانترفيرون كاما IFN-γ و عامل نخر الاورام TNF-α في مصل مرضى الليشمانيا الجلدية

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الملخص

داء الليشمانيا الجلدي هو مرض طفيلي شائع في العراق تسببه الاوالي احادي الخلية من جنس الليشمانيا الذي يصيب الخلايا البلعمية محدثا" استجابات مناعية خلوية وخلطية. تفرز العديد من السيتوكينات اثناء الاستجابة لاسيما (الانترفيرون كاما IFN-γ وعامل نخر الاورام TNF-α)، والتي تعد عوامل هامة في بدء المناعة الوقائية ضد داء الليشمانيات. الهدف من هذه الدراسة هو تقدير مستوى IFN-γ و TNF-α في مصل المرضى المصابين بداء الليشمانيا الجلدي عن طريق الفحص المناعي المرتبط بالانزيم (الاليزا). من بين 62 مريضاً مصابين بآفة CL كان معدل العدوى أعلى لدى الذكور 36 (58%) من الإناث 26 (42٪)، وأيضا أعلى معدل الإصابة 29 (46.8٪) كان في الفئة العمرية (أقل من سنة واحدة –10)، وأقل معدل إصابة 18 (2.4%) كان في الفئة العمرية (51–60). تم جمع 62 عينة دم من المرضى المصابين بداء اللشمانيا الجلدية بالاضافة الى 27 عينة دم من شخص سليم كمحموعة سيطرة. وأظهرت النتائج أن مستوى IFN-γ كانت(38.23بيكوغرام/مل) في مصل المرضى المصابين CL وكانت أعلى بكثير من مجموعة السيطرة (21.98 بيكوغرام/مل). ايضا اظهرت النتائج زيادة كبيرة في مستوى TNF-α في مصل المرضى المصابين بداء اللشمانيا الجلدي (46.53 بيكوغرام / مل) مقارنة مع مجموعة السيطرة اذ كانت (23 بيكوغرام / مل).