

Measurement of Cytokines Concentration (IFN- γ and IL-10) in the Serum of Cutaneous leishmaniasis patients.

Hussein S. Aswed Al-Obaidi¹ and Azad Kamal²

¹Department of Microbiology, College of Medicine, University of Tikrit, Tikrit, Iraq

²Al-Hawija general hospital, Ministry of Health, Iraq

Abstract:

A total of 107 patients with cutaneous leishmaniasis (CL) were included in this study. They were 57% males, while 43% females. Their ages ranged from 1-60 years old.

Estimation of IL-10 and IFN- γ concentration in patients and control groups revealed the mean level of IL-10 and IFN- γ was significant increased in patients groups ($P < 0.05$) in comparison with control groups. Even that the mean level of IFN- γ in patients groups was less than the mean level of IL-10, since the mean of IL-10 in patients groups were 28.33 ± 0.36 Pg/ml, 36.76 ± 0.27 Pg/ml and 2.92 ± 0.28 Pg/ml respectively, while it was 0.04 ± 0.002 Pg/ml, 0.08 ± 0.008 Pg/ml, 0.027 ± 0.001 Pg/ml in control groups respectively, while the mean of IFN- γ level in patients groups were 6.8 ± 0.06 Pg/ml, 6.0 ± 0.008 Pg/ml and 0.52 ± 0.008 Pg/ml in patients groups respectively, while it was 0.06 ± 0.002 Pg/ml, 0.06 ± 0.008 Pg/ml and 0.005 ± 0.002 Pg/ml in control groups respectively.

Introduction:

Cutaneous leishmaniasis caused by parasites considered being the simplest organisms in the animal kingdom. They are all single cell ⁽¹⁾. This parasite is taxonomically classified as belonging to the order *Kinetoplastidae* and the family *Trypanomastidae*, genus *Leishmania* owing to the presence of kinetoplast mitochondrion and other trypanosomal feature. It exists in two forms, the promastigote and the amastigote ⁽²⁾. Temperature triggers the change of one from to the other ⁽³⁾.

Within the vector, the *Leishmania* parasite exists into two forms, the non-infectious procyclic promastigote found in the insect mid-gut develops into an infectious metacyclic promastigote found in the foregut and pharynx of vector. The sand fly transmits the infectious form to the mammalian host during a blood meal. Once transmitted, the metacyclic promastigote infects local tissue macrophages and rapidly transforms into an amastigote which multiplies in a parasitophorous vacuole. These organisms replicate through binary fission and ultimately escape the macrophage and re-infect other tissue macrophage, which can be then in turn picked up by feeding sand flies to continue the cycle by changing form once again into the procyclic promastigote ⁽²⁾. The genus of *Leishmania* which like *Trypanosoma*, are peculiar in having extracellular DNA in an organelle the kinetoplast. *Leishmania* of mammals are obligatory intracellular protozoan which infect cells of the reticuloendothelial system. They are transmitted from one vertebrate host to other by small biting flies belonging to genus *phlebotomus*. The life cycle of *Leishmania* includes a regular alteration of hosts between the *phlebotomus* vector and vertebrate host. Profound biological change must accompany this oscillating cycle in its course the organism is repeatedly transformed, back

and forth from a non-motile amastigote in the vertebrate macrophage to a motile extra cellular promastigote in the lumen of the insect gut ⁽⁴⁾. When Sand flies of the genus *phlebotomus* obtain a blood meal from infected individual or animal, they ingest the amastigotes. In the midgut of the insect, these organisms, change to spindle shaped promastigotes which multiply by binary fission and within 4-5 days extend to the foregut of the insect ⁽¹⁾. The promastigotes are about blocking the proventriculus at the time of the next meal, which does not hinder the forward migration of the flagellates that accumulate in the pharynx and mouth parts of the insect about the ninth or tenth day after the original feed ⁽⁵⁾. The sand fly feed by thrusting their styles into the capillary plexus of the dermal rets. This sharp painless incision causing a small hemorrhage about the lacerated capillary. The proboscis is extended into the wound and the esophageal bolus of promastigotes regurgitated into the dermal puncture before the insect can ingest blood ⁽⁶⁾.

Materials & Methods:

A total of 107 patients infected with CL were included in this study. Although CL infect all age groups, the patients were divided in to two groups according to development of immune system ⁽⁷⁻⁸⁾ and due to different requirement of Zn diet for maturation and growth for all age groups ⁽⁹⁾.

Sixty healthy individuals were used as control group in this study. Control group divided to two groups, according to age, first group include 20 individuals their ages ranging from 1-15 years and second group include individuals above 15 years their ages ranging from 16 to 60 years and sub-divided into 20 males and 20 females.

After taken the history and careful examination of patients, skin smears were done for CL patients. The skin lesion area was sterilized with 70% alcohol and then 0.2 ml of Lock's solution by using sterile hypodermic needle syringe was injected sub-cutaneously beneath the pus area. Their injected solution was re-aspirated. This aspirate was used to prepare a direct smear and culture to identification of leishmania parasite ⁽¹⁰⁾.

For screening of IFN- γ , the area of aspiration of blood from cubital fossa vein were sterilized by alcohol 70%, a blood sample (3ml) was collected from each patients and control groups. Then the blood were transferred to plain tube (serum tube) for serum isolation for screening of cytokines IFN- γ and IL -10 levels and immediately stored under -20°C .

ELISA technique was used for measurement of cytokines levels ⁽¹¹⁾. The kit that used for screening IFN- γ manufactured was provided from Mabtech company.

Measurement of IFN- γ concentrations were done in (Medical Research Center) College of Medicine, (AL-Naharin University).

Results:

A total of 107 patients with CL were included in this study .

Figure (1) shows the mean concentration of IL-10 in male patients above 15 years old group and control groups. It was found that the mean of IL-10 level was significant increased in patients group in comparison to that observed in control group since the mean of IL-10 level in patients group was 28.33 ± 0.36 Pg/ml while it was 0.04 ± 0.002 Pg/ml in control group. There was a significant difference between both groups since ($P < 0.05$).

The same was observed in female patients groups. Figure (2) shows the mean concentration of IL-10 in female patients above 15 years old and control groups. It was found that the mean of IL-10 level was $(36.76 \pm 0.27$ Pg/ml in patients group while it was 0.08 ± 0.008 Pg/ml in control group. There was a significant difference between both groups since ($P < 0.05$).

Figure (3) shows the mean concentration of IL-10 in patients ≤ 15 years old and control groups. It was found that the mean of IL-10 level were 2.92 ± 0.28 Pg/ml, 0.02 ± 0.002 Pg/ml in patients and control groups respectively. There was a significant difference between both groups since ($P < 0.05$).

The results that sorted out by ELISA reader (optical density) were applied on standard curve in order to sort out the IL-10 concentration. Figure (4) shows the standard curve that used for sorted out IL-10 concentration.

Figure (5) shows the mean concentration of IFN- γ level in male patients above 15 years old and control groups. The mean of IFN- γ significant increased ($P < 0.05$) since the means of IFN- γ were 6.8 ± 0.06 Pg/ml, 0.06 ± 0.002 in patients and control groups respectively.

Figure (5) shows the mean concentration of IFN- γ in female patients above 15 years old and control groups. The mean of IFN- γ level was in significant increased ($P < 0.05$) in patient group in comparison to that observed in control group since the means of IFN- γ level in patients group was 6.0 ± 0.09 Pg/ml, while it was 0.06 ± 0.008 Pg/ml in control group.

The same was observed in patients ≤ 15 years old. Figure (6) shows the mean concentration of IFN- γ in patients ≤ 15 years old and control groups. It was found that the mean of IFN- γ level were 0.52 ± 0.008 Pg/ml, 0.005 ± 0.002 Pg/ml in patients and control groups respectively since ($P < 0.05$).

The results that sorted out by ELISA reader (optical density) were applied on standard curve in order to sort out the IFN- γ concentration figure(7).

Figure (1): IL-10 Concentration in male Patients and Control Group their Ages above 15 Years Old

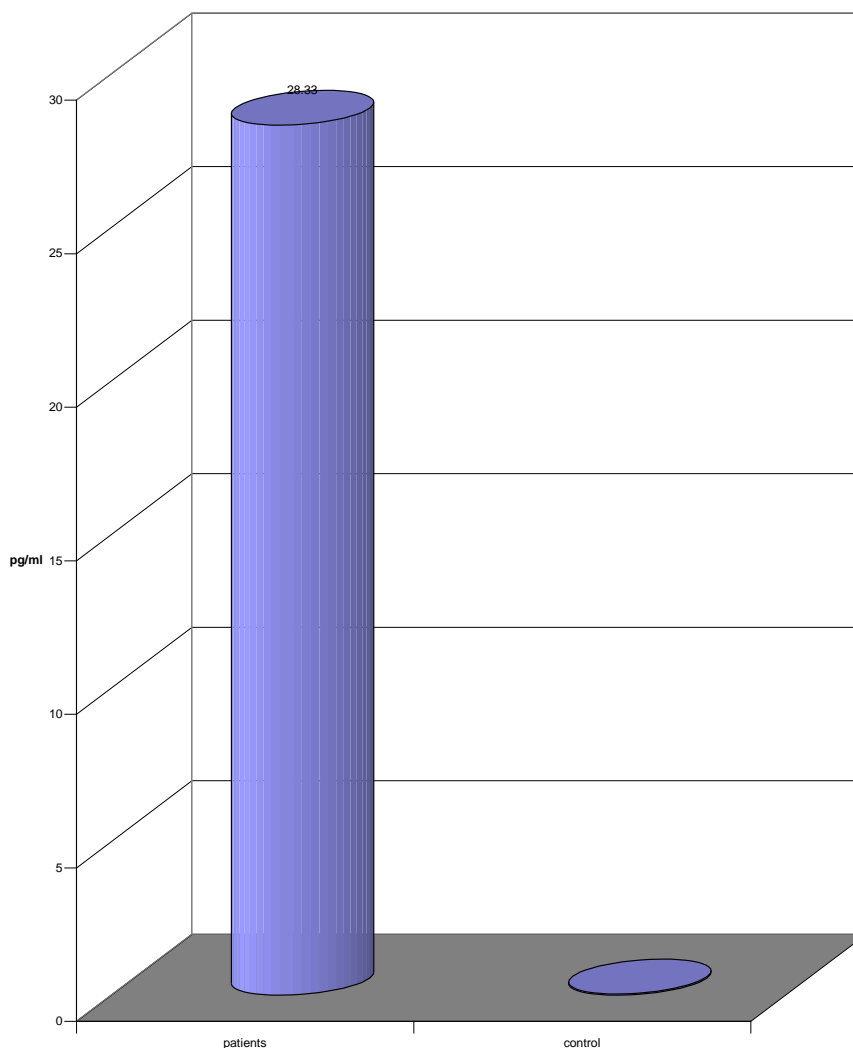


Figure (2): IL-10 Concentration in female Patients and Control Group their Ages above 15 Years Old

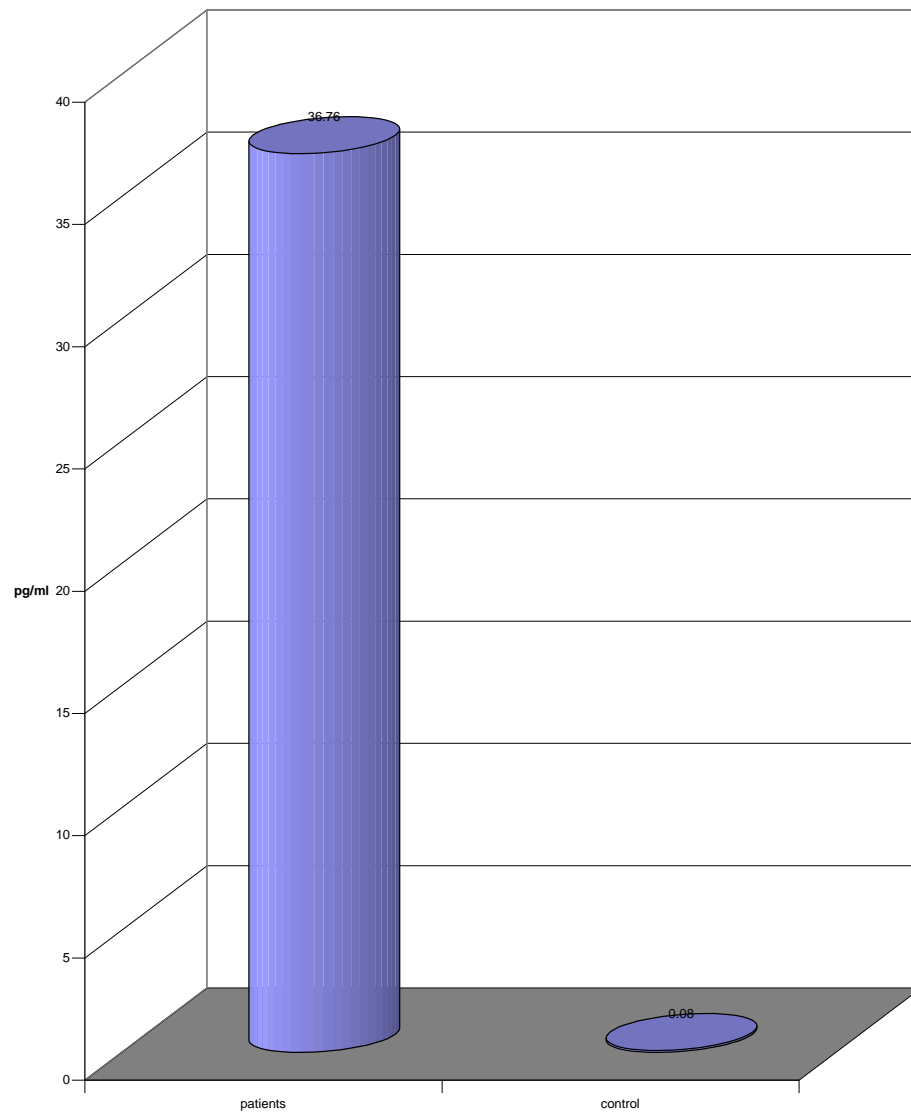


Figure (3): IL-10 Concentration in Patients and Control Groups their Ages ≤ 15 Years Old

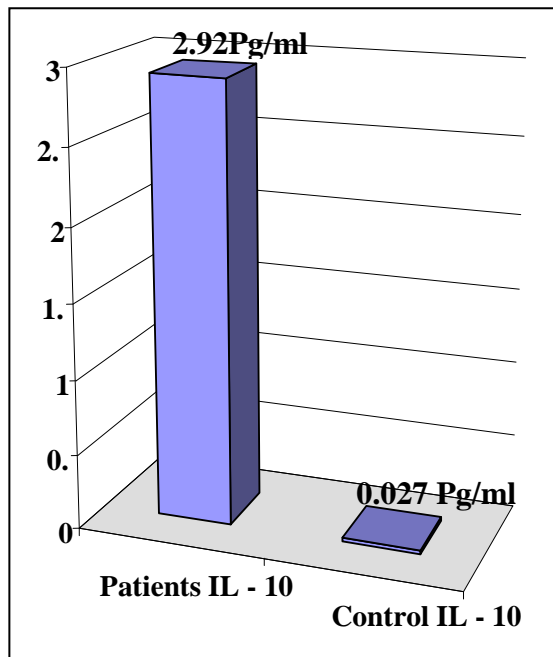


Figure (4): Standard Curve of IL-10 Concentration

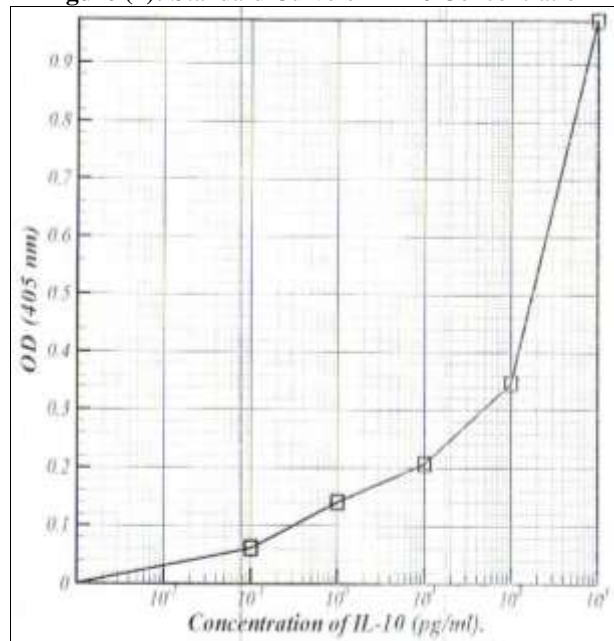


Figure (5): IFN- γ Concentration in male and Female Patients and Control Groups their Ages above 15 Years Old

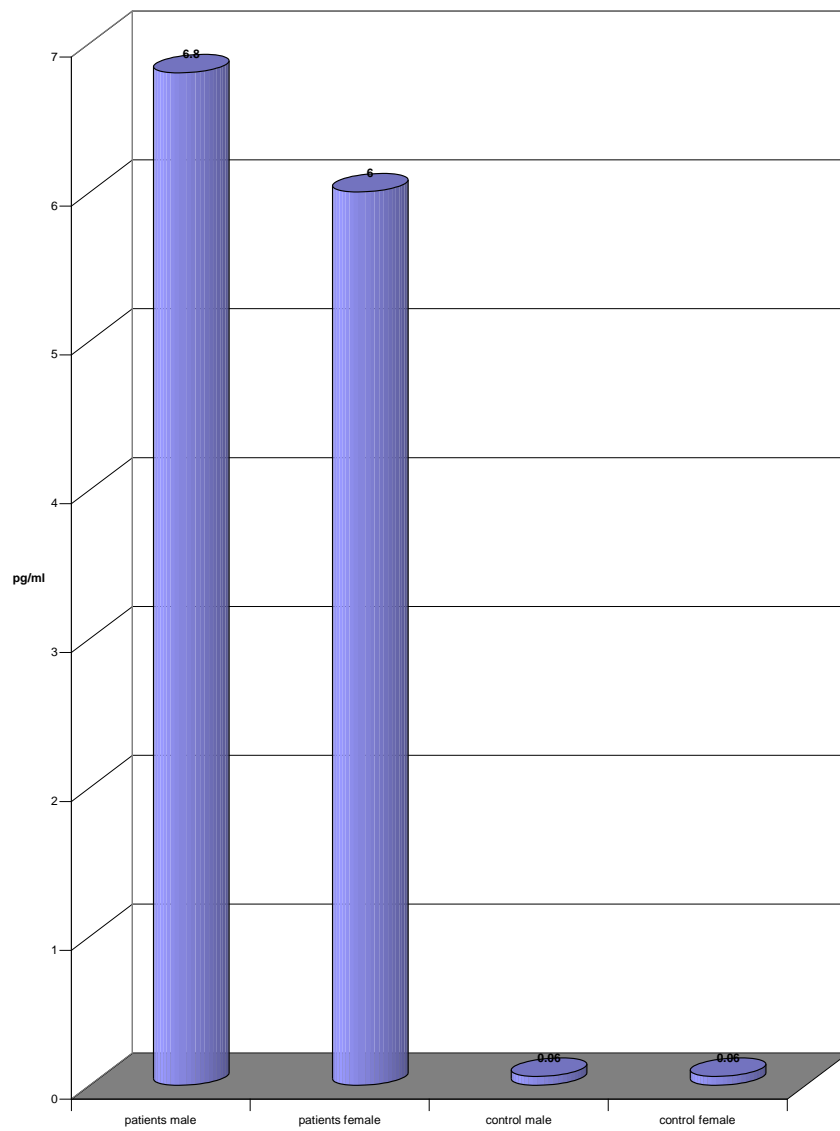


Figure (6): IFN- γ Concentration in Patients and Control Groups their Ages ≤ 15 Years Old

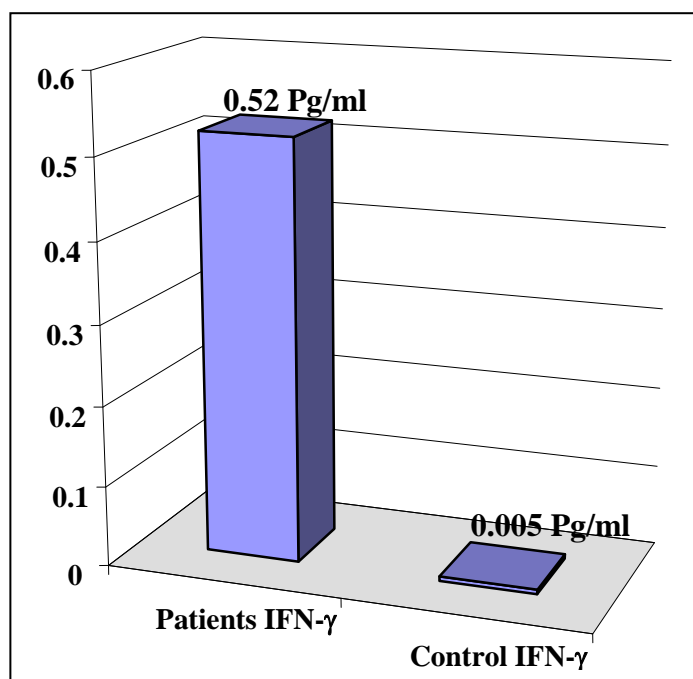
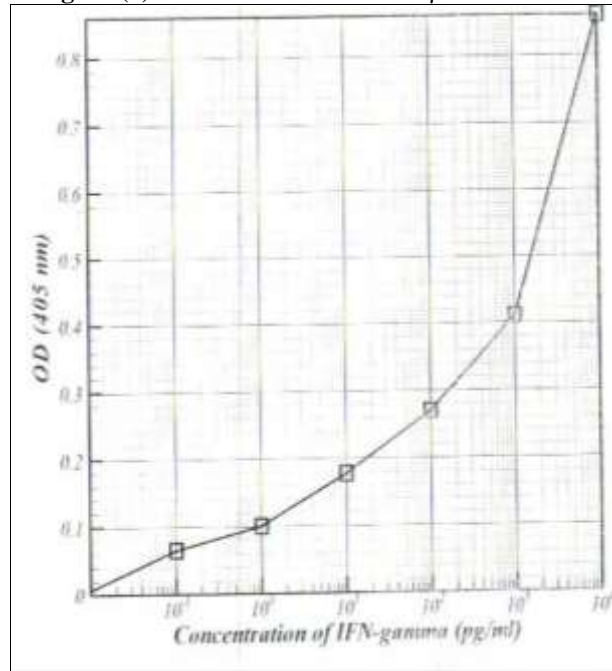


Figure (7): Standard Curve of IFN- γ Concentration



Discussion:

Cutaneous leishmaniasis is vector-borne parasitic disease endemic in many parts of the tropics, subtropics and Southern Europe. The World Health Organization estimated that 1.5 million cases of CL in the World. Both *L. major* and *L. tropical* are common etiologic agents of CL in Iraq. CL is endemic in Iraq especially in rural and periurban area ⁽¹²⁾. The disease is a self limiting but it is usually takes several months and even years for spontaneous recovery leaving an ugly scar.

Our data revealed that IL-10 concentration in serum of CL patients in male CL were significant increased in all ages groups in comparison to that observe in their control groups since ($P < 0.05$).

Regarding to our data we found that the mean level of IFN- γ in serum of all age groups were significantly increased in comparison to that observed in their control groups since ($P < 0.05$).

Regarding to the reference CD4⁺ T-lymphocyte is subpopulation in to Th-1 cells and Th-2 cells since Th-1 cell secretes IL-2, IFN- γ and TNF- α while Th-2 secretes IL-4, IL-6 and IL-10. IFN- γ is responsible in activation of macrophage to produce nitric oxide to elimination of intracellular microorganisms like amastigotes form of *Leishmania*, while IL-10 is responsible in deactivation of IFN- γ ⁽¹³⁾. Through our results indicate that the mean concentration of IFN- γ is less than the mean of IL-10 this might be related to Zn deficiency in all CL patients and an imbalance occurred between Th-1 and Th-2. This is in agreement with that found by Prasad ⁽¹⁴⁾, who found that cytotoxic T-cells, T-helper (Th) cells (CD4⁺) are affected by Zn and Zn deficiency, causes an imbalance between Th-1 and Th-2 function and altered secretion of the typical Th-1 and Th-2 cytokines during Zn depletion, through Th-1 cytokines levels is decreased, while cytokines levels of Th-2 are remain unchanged during Zn deficiency and is in agreement with that found by Weyenbergh *et al.* ⁽¹⁵⁾ they found that an imbalance occurred between Th-1 and Th-2 in Zn deficiency CL

patients, Furthermore is in agreement with that found by Solomons NW. He was found that mild Zn deficiency produce an imbalance between cell-mediated and humoral immunity ⁽¹⁶⁾. Even that is in agreement with that found by Sprietsma JE he was found that Zn-controlled Th1/Th2 switch significantly determines development of diseases ⁽¹⁷⁾.

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قياس تركيز السايوتوكاين في مصل المرضى المصابين باللشمانيا الجلدية

حسين ساهر اسود العبيدي^١ و آزاد كمال^٢

^١ فرع الاحياء المجهرية، كلية الطب، جامعة تكريت، تكريت، جمهورية العراق

^٢ مستشفى الحويجة العام، وزارة الصحة، جمهورية العراق

الملخص:

تم دراسة ١٠٧ مريضاً باللشمانيا الجلدية في قسم الجلدية للعيادة الخارجية لمستشفى الحويجة العام في قضاء الحويجة. كانت نسبة الذكور ٥٧% بينما نسبة الإناث ٤٣%. تم قياس تركيز السايوتوكاينات (Cytokines) (IL-10, IFN-γ) في مصل مجاميع المرضى ولوحظ ارتفاع معنوي ($P < 0.05$) في معدل تركيز السايوتوكاينات في مجاميع المرضى مقارنة بمعدل تركيز السايوتوكاينات في مجاميع السيطرة. ولوحظ في مجاميع المرضى أيضاً أن معدل تركيز السايوتوكاين (IFN-γ) هو اقل من تركيز السايوتوكاين (IL-10)، وحيث كان معدل تركيز (IL-10) في مجاميع المرضى $28.33 \pm$

٣٦، بيكوغرام / مل، 36.76 ± 0.27 بيكوغرام / مل، 2.92 ± 0.28 بيكوغرام / مل، بينما كانت 0.02 ± 0.04 بيكوغرام / مل، 0.08 ± 0.08 بيكوغرام / مل، 0.27 ± 0.02 بيكوغرام / مل في مجاميع السيطرة على التوالي وكان معدل تركيز IFN-γ في مجاميع المرضى 6.8 ± 0.6 بيكوغرام / مل، 6.0 ± 0.08 بيكوغرام / مل، 0.52 ± 0.08 بيكوغرام / مل بينما كان 0.6 ± 0.02 بيكوغرام / مل، 0.6 ± 0.08 بيكوغرام / مل، 0.05 ± 0.02 بيكوغرام / مل في مجاميع السيطرة على التوالي.

