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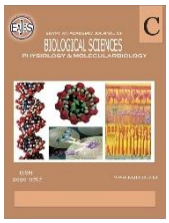

Study the Role of Some Immune Markers in Patients Infected with Cutaneous Leishmaniasis

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ABSTRACT

The total reported cases of Leishmaniasis infections in prior from 2008 to 2015 were 17001 which ranged from (2.9 - 10.5) / 100,000 individuals in Iraq. The highest cases were noted in the year 2015 (4000 cases). The study aimed to evaluate the serum levels of the IL17 cytokine, TGFβ1, MCP-1 and PGE2 in patients with Leishmaniasis and control groups. The results of the current study showed a higher significant $P<0.05$ in a concentration of IL17 cytokine in the patients' group 360.92 ± 82.766 pg/ml compared with the control group with a concentration of 38.974 ± 4.9219. The concentration of TGFβ1 level was higher significantly $P<0.05$ in the patients' group 309.57 ± 60.313 pg/ml than in the control group 124.17 ± 31.329 pg/ml. The results were significantly higher in the concentration of MCP-1 in the patients' group if compared with control group and noticed higher levels of MCP-1 as (218.87 ± 36.137) pg/ml and (78.541 ± 28715) pg/ml respectively as well as the concentration of PGE2 level were significantly highest in patients group with (1015.4 ± 107.41) pg/ml than the control group (308.76 ± 46.879) pg/ml.

INTRODUCTION

Leishmaniasis is a vector-borne disease transmitted by biting of the female Sand fly (Karimi and Nabipour, 2015) caused by different species of *Leishmania* It is manifested by three major clinical forms: cutaneous, mucocutaneous and visceral Leishmaniasis (Alemayehu and Alemayehu, 2017). Prevalent in tropical and subtropical regions; about (12) million people are affected in 98 countries these diseases with (350) million people at infection risk worldwide (Alvar *et al.*, 2012, Khazaei S *et al.*, 2015), mostly reported from Afghanistan, Algeria, Pakistan, Saudi Arabia, Iran, Iraq, Peru and Brazil (Organization, 2016).

In Iraq, the total number of reported cases of Leishmaniasis infections in prior from 2008 to 2015 was 17001 which ranged from (2.9 to 10.5) / 100,000 individuals. The highest number of cases was noted in the year 2015 (4000 cases) (Al-Obaidi *et al.*, 2016). Cutaneous Leishmaniasis is a very old disease in Iraq called Baghdad boil, less severe form of disease which manifests self-healing ulcers, *Leishmania major* and *Leishmania tropica* causative agents of cutaneous Leishmaniasis in Iraq (Alavinia *et al.*, 2009), according to species of parasite and immune response of the patients, the symptoms different in regions, that begin as erythematous papule, increase in size produce a nodule, ulcerate and crusts (Control and Prevention, 2015). A zoonotic type caused by *L. major* and an anthroponotic type caused by *L. tropica* (AlSamarai and AlObaidi, 2009).

The recognition and characterization of *Leishmania* spp. are essential for the accurate, rapid and sensitive diagnosis of Leishmaniasis, which has a major effect on effective treatment and control methods (Medley *et al.*, 2015). The type of cutaneous pathology is determined in part by the infecting *Leishmania* species and also by a combination of inflammatory and anti-inflammatory host immune response factors resulting in different clinical outcomes (Scorza *et al.*, 2017) these infections induced both cellular and humoral immune response and the balance of expression varies with the type of disease, previous studies in Iraq based on some immunological effect such as interleukins and interferon by showed immune value especially in patients serum infected with cutaneous Leishmaniasis and differentiation of other skin diseases (Al-Aubaidi, 2011; Al-Hadraawy & Hessen , 2017)

MATERIALS AND METHODS

Study Design and Patients:

The samples were collected from patients with cutaneous Leishmaniasis attending Al-Hakim and Al-Manathira Hospitals in Al-Najaf province during the period from September 2018 to January 2019 after clinical diagnosis.

Serum Collection:

Five ml of blood were collected from healthy and infected patients. Blood samples were drawn in sterile plain tubes and left at room temperature for 30 min. Centrifugation was done at 3000 rpm for 5 min (Memmert, Germany**).** The serum was collected and kept in sterile tubes at deep freeze at -20 until use.

Serum Biomarkers Detection:

Three human biomarkers were used in this study: the IL17 cytokine, TGFβ1, MCP-1 and PGE2. All these biomarkers kits were provided by Elabscience Company, Bulgaria and the level of biomarkers in serum was determined by using an ELISA device (Human reader, Germany) according to the Manufacturer Company.

Statistical Analysis:

T-test was used in this study for comparison between samples by using Graph-pad Prism version 10 computer software. *P* -valueless than 0.05 is considered statistically significant (Al-Hadraawy, 2016); Aljanaby AAJ &Alhasnawi HMRJ, 2017).

RESULTS

The mean concentration of the cytokine IL-17 has a significant increase (P< 0.05) between patients and control groups, (360.92 ±82. 766) pg /ml versus (38.974± 4.9219) pg /ml respectively as seen in Table (1)and Figure (1).

Table 1: Serum cytokines levels in the study group**.**

Cytokines type	Clinical group	M ± SD(pg/ml)	(P value)
Interluken-17 (IL-17)	Patients	360.92 ± 82.766	P<0.0074**
	Control	38.974 ± 4.9219	
Transform Growth Facctor beta1 (TGF-B1)	Patients	309.57 ± 60.313	P<0.0387*
	Control	124.17 ± 31.329	
Monocyte Chemoattractant-1 (MCP-1)	Patients	218.87 ± 36.137	P<0.0126*
	Control	78.541 ± 28715	
Prostaglandin E2 (PGE2)	Patients	1015.4 ± 107.41	P<0.0001***
	Control	308.76 ± 46.879	

* Represent significant difference at P ≤ 0.05 level, chi-square test

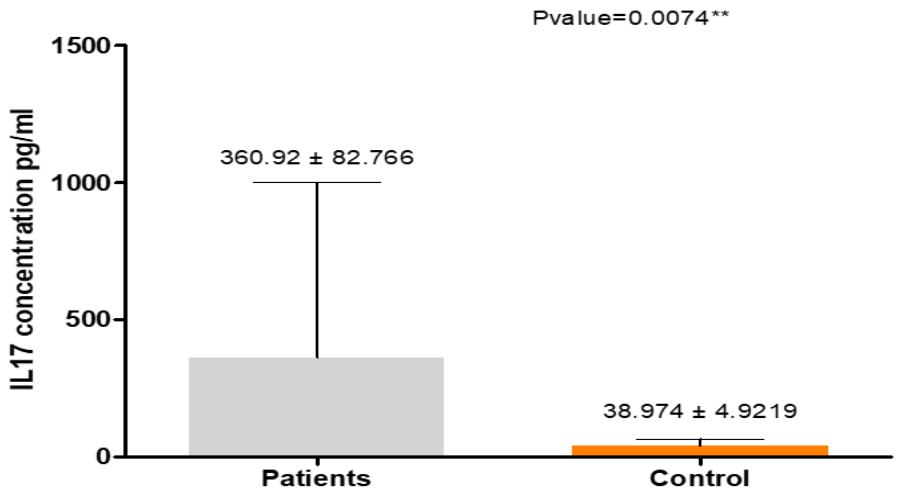

Fig. 1: Comparison of mean concentration serum IL-17 cytokine between patients and control Group.

According to gender in patients and control groups, it was found that the mean concentration of IL-17 significantly increased between males and females in patients and control groups. The data showed that IL-17 mean concentration in males was (45280 ±144.63) pg /ml and 43.151 ± 8. 4006 pg /ml) in patients and control group respectively, while the IL-17 mean concentration in females (269.03± 79.837) pg/ml and 34.797± 5.2250) pg /ml in patients and control group respectively. as seen in Figure (2).

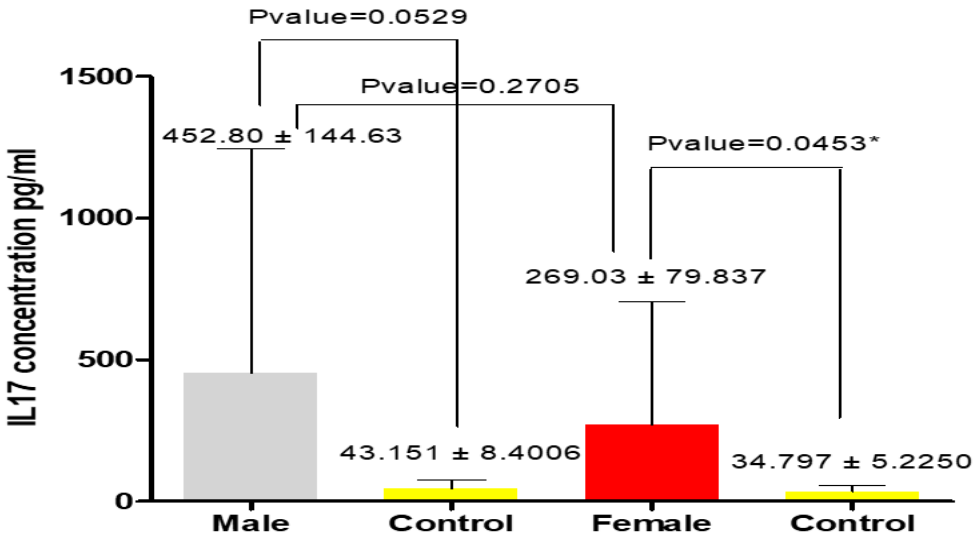

Fig. 2: Comparison of mean concentration serum IL-17 between males and females in patients and control.

TGF-β1 Concentration:

The present study revealed a significant increase (P≤0.05) (309.57 ±60.313 pg /ml), (124.17± 31.329 pg/ml) between patients and control groups respectively as seen in Table (1) and Figure (3).

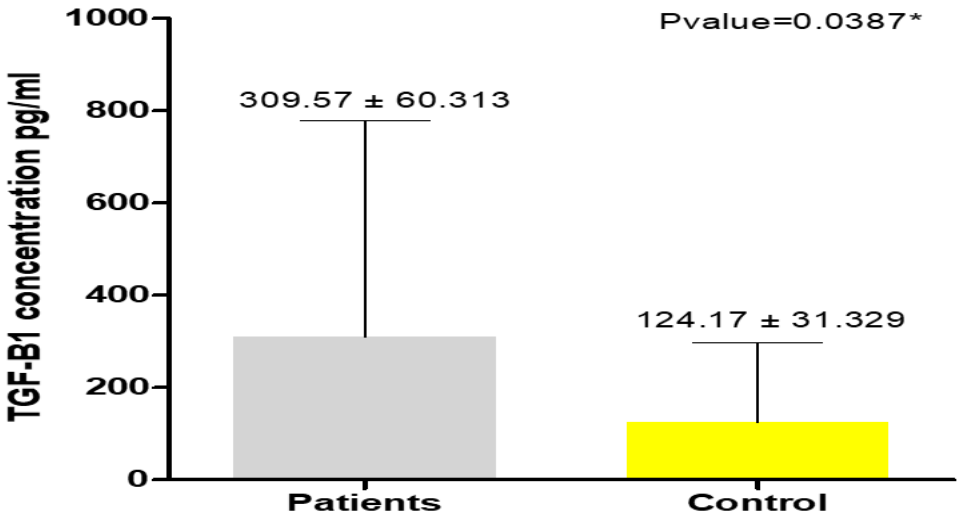

Fig. 3: Comparison of mean concentration serum TGF-β1 cytokine between patients and control Group**.**

The mean concentration of TGF-β1 level significantly increased between males and females in patients and control groups, the data showed that TGF-β1 mean concentration in males was (485.9 ±110.85 pg /ml) and 173.17 ± 59.746 pg /ml) in patients and control group respectively, while the TGF-β1 level mean concentration in female (134.5± 19.716 pg/ml) and (75.171± 12.400 pg/ml) in patients and control group respectively. as seen in Figure (4).

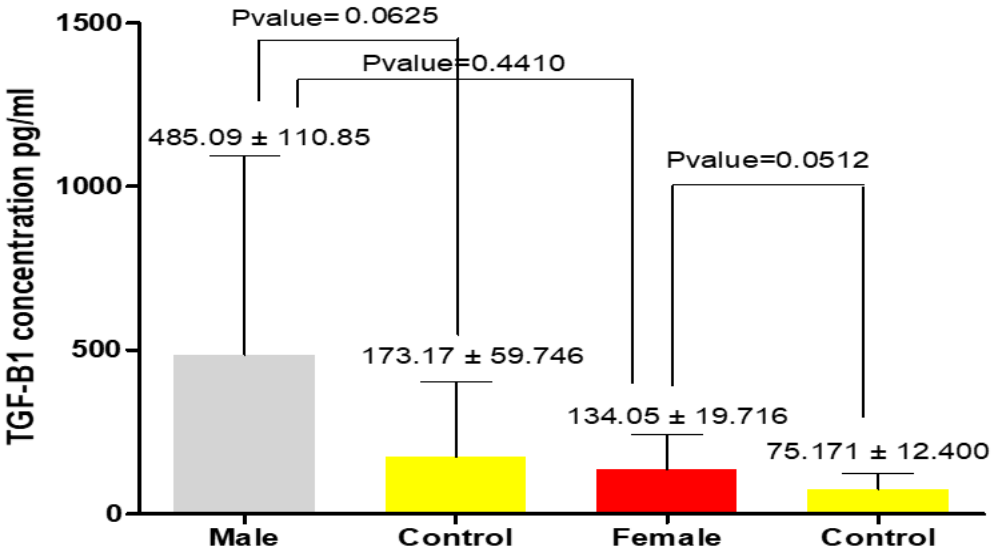

Fig.4: Comparison of mean concentration serum TGF-β1 between male and female in patients and control.

MCP-1Concentration:

The present study revealed a significant increase (P≤0.05) (218.87 ±36. 137 pg /ml), (78.541± 28.715 pg /ml) between patients and control groups respectively as seen in Table (1) and Figure (5).

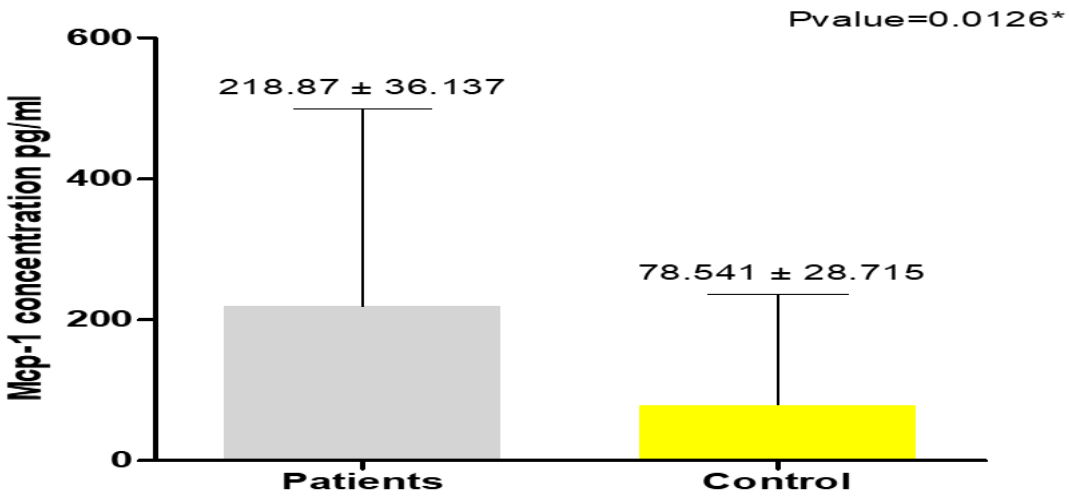

Fig. 5: Comparison of mean concentration serum MCP-1cytokine between patients and control Group.

The mean concentration of MCP-1 level significantly increased between males and females in patients and control groups, the data showed that MCP-1mean concentration in males was (209.61 ±57.959 pg /ml) and (103.09 ± 56.317 pg /ml) in patients and control group respectively, while the MCP-1 level mean concentration in female (228.14± 44.141pg/ml) and (53.987± 12.583 pg/ml) in patients and control group respectively. as seen in Figure (6).

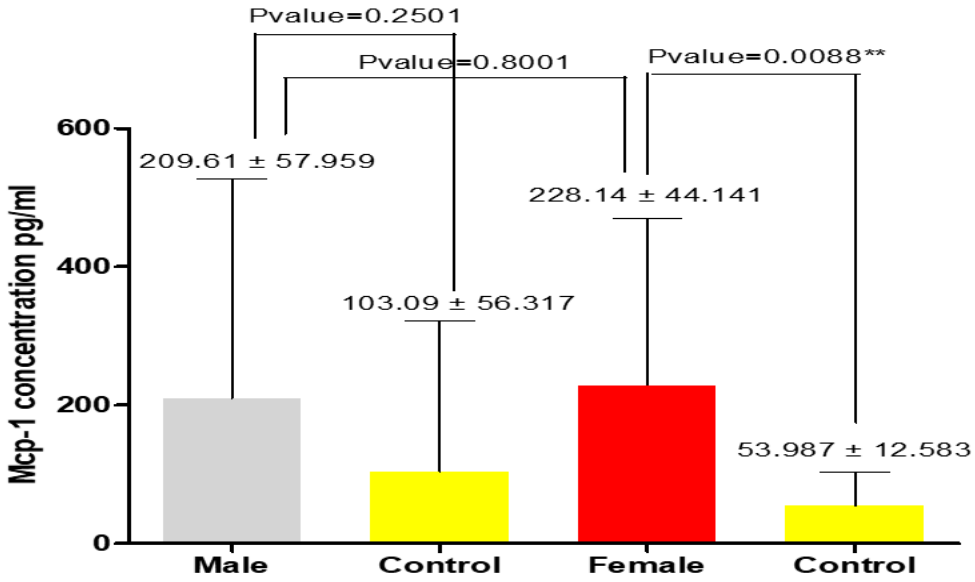

Fig. 6: Comparison of mean concentration serum MCP-1 between males and females in patients and control.

Prostaglandin PGE2 Concentration:

The present study revealed a significant increase (P≤0.05) (1015.4 ±107.41 pg /ml), (308.76± 46.879 pg/ml) between patients and control groups respectively as seen in Table (1) and Figure (7).

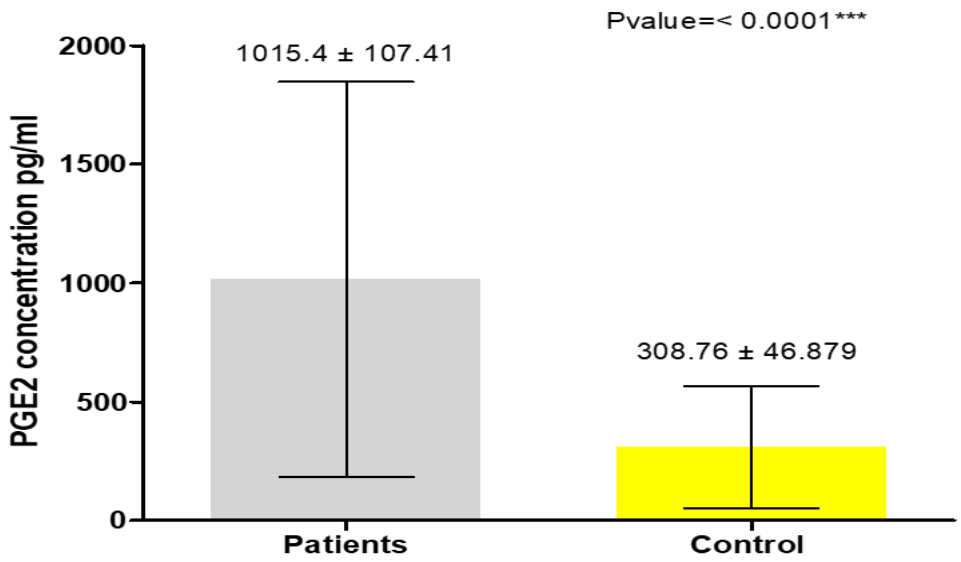

Fig. 7: Comparison of mean concentration serum PGE2 cytokine between patients and control Group.

The mean concentration of PGE2 level significantly increased between males and females in patients and control groups, the data showed that PGE2 mean concentration in males was (1163.0 ±150.40 pg /ml) and (211.38 ± 64.865 pg /ml) in patients and control group respectively, while the PGE2 level means concentration in female (867.71± 151.07pg/ml) and (406.15± 59.516 pg/ml) in patients and control group respectively. as seen in Figure (8).

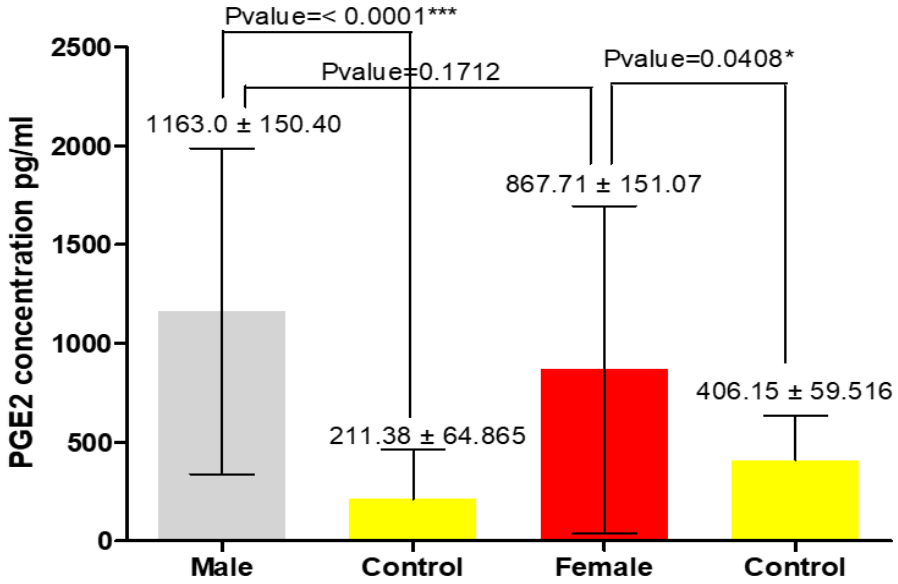

Fig. 8: Comparison of mean concentration serum PGE2 between male and female in patients and control.

DISCUSSION

The mean concentration of IL-17 was highest significant (P< 0.05) between patients and control groups, (360.92 ±82. 766 pg /ml)versus (38.974± 4.9219) respectively ; current study revealed that concentration of IL-17 there was significant increase($p<0.05$) between male and female in CL patients group , this increased expression may be due to an increased level of cellular activation or a relative increase in the number of cytokine-producing cells, the results agreement with

(AL-Saady, 2014) in Diyala province in Iraq ,where found increase of IL-17 concentration in patients with CL and control group (67.02. ± 38.3 pg/ ml) , (38.21±1.87 pg / ml) respectively as well as in Tunis (Kammoun-Rebai *et al.*, 2016) documented participants divided in three group of healed , asymptotic and naïve individuals, according to Leishman skin test LST response and presence of scar, significant *Leishmania* response in LST+SCAR+ , LST+SCAR- (but not in naïve subject) produced in higher significant with ($p<0.05$) in infected donors compare to naïve individuals While (Kammoun-Rebai *et al.*, 2016) in Brazil were found that IL-17 concentration in patients infected with lymphocytes from mucosal Leishmaniasis and cutaneous Leishmaniasis produce higher levels of IL-17 than uninfected controls, these support for the role of IL-17 in the pathogenesis of the inflammatory reaction in Leishmaniasis , in addition to (Katara *et al.*, 2013) showed that IL-17 levels were found to be significantly higher in CL samples compared to controls. this finding result was consistent with a study of (Gonzalez-Lombana *et al.*, 2013) found that increased IL-17 production is responsible for immunopathology in IL-10-deficient C57BL/6 mice infected with *L. major* by infiltration of neutrophils at the site of infection.

The mean concentration of total TGF-β1 in all CL patients was a significant increase in comparison to that observed in their control groups The present study revealed a significant increase ($P<0.05$) (309.57 ±60.313 pg /ml), (124.17± 31.329) between patients and control groups. Respectively. TGF-β1 expression plays a role in the immunopathogenesis of chronic cutaneous Leishmaniasis because of inhibitory effects on macrophage function (Melby *et al.*, 1994).

The present result is consistent with the study Barral *et al.* (1995) who showed that the involvement of TGF-β1 in human Leishmaniasis in vitro and in patients with cutaneous Leishmaniasis that human macrophages produce active TGF- β1 after infection by many species of *Leishmania* higher concentration of TGF- β1 level by *L. amazonensis* (480 +/- 44.7 pg/ml, *L. donovani* (295 +/- 7.6 pg/ml) and *L. braziliensis* (196 +/- 15.7 pg/ml) however TGF- β was added to cultures of human macrophages infected with Leishmaniasis led to an increase with 50% in parasite numbers as compared with untreated cultures, TGF-β recognized as an important immunoregulatory in murine Leishmaniasis which increases susceptibility to disease, several studies have reported TGF-β modulates lymphocyte proliferation and production of inflammatory cytokines as it limits increased inflammatory reactions that are responsible for tissue damage (Hejazi *et al.*, 2012). Other study have showed a significant increase in the expression of TGF-β in the patients with infected cutaneous Leishmaniasis with late lesions (more than four months) compared with patients infected with early lesions less than two months (Melby *et al.*, 1994).

The murine model explains TGF- β production by *Leishmania*-infected macrophages results from increased progression and susceptibility of murine Leishmaniasis which affects cells of the immune system by downregulation of macrophage functions (Al-Hadraawy, 2017). The mean concentration of total MCP-1 in all CL patients was a significant increase in comparison to that observed in their control groups. High MCP-1 expression in the skin is described in localized, self-healing cutaneous lesions (Valencia-Pacheco *et al.*, 2014).

Our result finding was in agreement with (Ritter *et al.*, 1996) who explain that MCP-1 leads to self-healing cutaneous Leishmaniasis in humans since other groups have already discovered the presence of MCP-1 in self-healing cutaneous lesions and the absence of MCP-1 in the non-healing diffuse cutaneous leishmaniasis, other study with (Ritter and Körner, 2002) were showed that self-healing localized cutaneous leishmaniasis is associated with higher levels of MCP-1, which may stimulate macrophage microbicidal mechanisms.

Another study found a significant

increase of MCP-1 levels in serum patients with another disease than CL such as VL disease and showed MCP-1 levels to be significantly increased after VL cure (Ibarra-Meneses *et al.*, 2017).

The mean concentration of total PGE2 in all CL patients was a significant increase in comparison to that observed in their control groups. PGE2 played a role in the determination of the Th1/Th2 balance at both the induction and effector phases of the immune response by modulating cytokine and chemokine production it is a critical inhibitory factor of infected macrophage to decrease their anti-leishmanial activity (Nahrevanian *et al.*, 2009). Our result finding was consistent with the study (França-Costa *et al.*, 2012) which found that PGE2 levels were higher in patients' serum with diffuse cutaneous leishmaniasis compared with patients with localized cutaneous leishmaniasis or with controls from an area of endemicity in other studies such as (Kuroda and Yamashita, 2003) they indicate in a murine model that the greater production of PGE2 by macrophages which is related to the suppression of Th1 cytokine production as well as (Penke *et al.*, 2013) who showed that PGE_2 play crucial roles in various infections and implicated as a susceptibility factor in *Leishmania* infection and cells from infected mice BALB/c produce more PGE2 and the expression of COX2.

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