

EVALUATION OF SOME CHEMOKINES LEVELS IN IRAQI PATIENTS INFECTED WITH VISCERAL LEISHMANIASIS

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ABSTRACT

Visceral leishmaniasis (VL), or kala-azar, is one of the deadliest and most disregarded of all tropical maladies. The presence of inflammatory mediators in serum could hypothetically control the disease. However, there is likewise liberation of anti-inflammatory mediators that could intervene with the control of parasite multiplication. The objective of the current work is to determine levels of some serum chemokines that incorporate granulocytes-macrophages colonies stimulating factor (GM-CSF), interferon-gamma inducible protein-10 (IP-10) and vascular endothelial growth factor (VEGF) of Iraqi patients with VL which can be considered as a biomarkers for VL infection. Sixty people were included in the present study; thirty- five of them were infected with VL while 25 uninfected people were considered as control. Patients were recognized on the premise of clinical and parasitological criteria. Sera (GM-CSF, IP-10 and VEGF) levels were determined by ELISA using a quantitative sandwich enzyme immunoassay technique. The results showed that there were no significant differences (0.05) between males and females infected with VL, while sera levels of GM-CSF, IP-10, and VEGF were significantly higher in patients group than healthy subjects ($P < 0.01$).

Keywords: Visceral leishmaniasis, Chemokines, ELISA, GM-CSF, IP-10, VEGF

INTRODUCTION

Visceral leishmaniasis (VL), likewise called kala-azar, is a parasitic illness caused by infection with the intracellular protozoa *Leishmania donovani* and *L. infantum* among tropical ailments, VL is positioned as fourth in morbidity and second in mortality, with 20,000 to 40,000 deaths per year (WHO, 2010; Alvar, *et al.*, 2012). VL is the most serious type of leishmaniasis, it's deadly if not treated; 500,000 cases occurs each year, over 90% from cases detailed from 5 countries; India, Bangladesh, Brazil, Nepal and Sudan (WHO, 2003) and soon Iraq with 4,000-5,000 annual cases will be added as 6th country to the above list (Choi and Lerner, 2001). In Iraq, VL represents one of the serious public health problems in children especially those less than five years old. *L. donovani infantum* and *L. donovani donovani* are responsible for VL in Iraq (Al-Aubaidi, 2007). Leishmanial infection in man actuates both humoral and cellular immune responses. The organizing of cells movement from lymphoid to peripheral tissues plays a vital part in the immune response and chemokines and chemokine receptors are basic to the molecular techniques driving in this procedure (Laudanna, *et al.*, 2002). The mobilization of gullible and memory T cells to peripheral tissue is interceded by a mix of adhesion molecules and chemokine receptors it is likewise known that cytokines are immediately engaged with chemokine production and may forerun the

expression of chemokines (Díaz, *et al.*, 2013). Chemokines and chemokine receptors play a main part in immunity to *Leishmania sp.* by coordinating the recruitment and activation of anti-leishmanial immune cells (Oghumu, *et al.*, 2010). *Leishmania sp.* endeavor to change the chemokine expression profile in the infected tissue microenvironment will therefore participate to their capability to avoid the host's immune system (Teixeira, *et al.*, 2006). The present study aimed to determine some serum chemokines levels (GM-CSF, IP-10 and VEGF) of Iraqi patients with VL which can be considered as biomarkers for VL infection.

MATERIALS AND METHODS

Subjects: A total of 60 Iraqi children (age range; 10 months to 12 years) were enrolled in the study. They were distributed as 35 VL patients and 25 apparently healthy controls. The patients were hospitalized cases and they were admitted to central teaching hospital of pediatric in Baghdad during the period from November 2016 - January 2017.

Collection of Blood Samples: From each participating subject (patient and control), about 3 ml of venous blood were collected. The blood was dispensed in a plain tube and left to coagulate at room temperature (20-25°C) for 15 minutes. At that point, they were centrifuged at 1000 rpm for 10 minutes to isolate sera, which were distributed into

aliquots (0.25ml) in firmly closed Eppendorf tubes, and after that the tubes were put away at -20°C until was used for sero-diagnosis of VL and assessment of GM-CSF, IP-10 and VEGF levels.

Diagnosis of VL infection: After a clinical examination of the patient by the medical staff at the hospital, the serum was screened for anti-VL antibodies by rapid immune-chromatographic strip test (Kalazar Detect™ Test kit: InBios International, USA).

Serum Level of GM-CSF, IP-10 and VEGF: Serum level of GM-CSF, IP-10 and VEGF was determined by ELISA method using Pepro Tech kit (USA) which was designed for the quantitative measurement of GM-CSF, IP-10 and VEGF in human sera, and instructions of manufacturer were followed.

Statistical Analysis: Descriptive and statistic data analyses were performed by utilizing the Statistical Package for Social Science SPSS version 2010, while Chi square (χ^2) test, was applied to demonstrate any significant differences of gender, and mean chemokine levels were compared between groups using Student t-test.

RESULTS AND DISCUSSION:

Table 1 shows that the numbers of infected females 20 (57.2%) were higher than males 15 (42.8%) but without significant differences ($p>0.05$) in the percentage of infection. The major cause of the differences between the numbers of patients according to gender may be due to numbers of infected cases that were reviewed from hospital came from, urban and agricultural area and may be due to environmental changes that provide well-activity of the insect vectors, which can bring about differential illness results amongst males and females (Bail and Diana, 2007; Snider, *et al.*, 2009).

Table 1: Align Number and percentage of VL infection according to the gender

Gender	No.	%
Males	15	42.8
Females	20	57.2
Total	35	100
$\chi^2 = 0.714, p>0.05$		NS

NS: No Significant.

The concentration of GM-CSF (Table 2) was significantly ($P < 0.01$) increased in VL patients compared with controls (36.459 ± 1.253 vs. 25.377 ± 1.117 pg/ml). The hemopoietic development factor GM-CSF has numerous stimulatory effects on monocytes/macrophages that are useful during intracellular infections, for example, upgrading phagocytic and metabolic capacities and the liberation of other pro-inflammatory cytokines (Jones, 1996). Addition of GM-CSF to human monocytes *in vitro* expands their leishmanicidal impacts (Al-Zamel, *et al.*, 1996). Murray, *et al.* (1995) mentioned that when mice infected with *L. donovani* were treated with murine GM-CSF, they indicated expanded leishmanicidal action, and he also pointed that GM-CSF stimulates three impacts potentially helpful in VL, blood monocyte recruitment, macrophage activation, and improvement of granulocytopenia. GM-CSF induced by *Leishmania* antigens (Singal and Singh, 2005) might be either useful by actuating macrophages to become leishmanicidal (Murray *et al.*, 1995, Al-Zamel, *et al.*, 1996) or detrimental by animating inflammation through a T cell-driven delayed-type hypersensitivity response, in this way encouraging *Leishmania sp.* related kidney contribution (Naito, *et al.*, 1996; Kitching, *et al.*, 2002). GM-CSF was increased in the sera of Brazilian VL patients versus the insignificant levels in the sera of Brazilian controls (Duthie, *et al.* 2014); this result was agreement with present study.

Table 2: The levels of GM-CSF (pg/ml) in studied groups and descriptive statistics.

Groups	No.	Min.	Max.	Mean \pm Sd.	t-test	P-Value
VL patients	35	34.447	39.779	36.459 ± 1.253	28.284	0.000
Control	25	23.436	27.436	25.377 ± 1.117		(HS)

HS: Highly Significant at $P < 0.01$.

The level of IP-10 (Table 3) was significantly increased in VL patients compared to controls (29.878 ± 3.319 vs. 18.004 ± 1.075 pg/ml) with significant differences ($P < 0.01$). Interest has newly grown in the utilization of chemokines as substitutional biomarkers, when activated by IFN- γ , numerous cell types produce IFN- γ inducible IP 10 (Gasperini, *et al.*, 1999), which further increment the output of IFN- γ . IP-10 provide precise biomarker of a scope of infections, including those of mycobacterium tuberculosis, hepatitis C virus and

malaria parasites (Azzurri, *et al.*, 2005; Armah, *et al.*, 2007; Falconer, *et al.*, 2010). Recently, chemokines have been recognized in the host reaction against *Leishmania sp.* (Oghumu, *et al.*, 2010). Without a doubt, IP-10 has been shown basic in the mobilization of cellular immunity following vaccination against *L. donovani* and to assume a defensive part in decreasing the number of intracellular parasites in cutaneous sores (Vasquez and Soong, 2006; Fallahi, *et al.*, 2016). Further, IP-10 has been appeared to increment in patients treated

for *L. donovani* instigated VL (Hailu, *et al.*, 2004). The high sensitivity and specificity of IP-10 indicate it can be utilized as a biomarker for distinguishing asymptomatic people living in both *L. donovani* and *L. infantum* zones (Ibarra-Meneses, *et al.*, 2017), this is agreed with the results of the

current study. At a 50 ng/ml concentration, IP-10 was able to restrict intracellular parasite survival significantly (Gupta, *et al.*, 2009). In BALB/c infections of *L. donovani*, IP-10 mRNA was expressed at high levels during the period of the infection and involved CD⁴⁺ and CD⁸⁺ T cells (Cotterell, *et al.*, 1999).

Table 3: The levels of IP-10 (pg/ml) in studied groups and descriptive statistics.

Groups	No.	Min.	Max.	Mean ±Sd.	t-test	P-Value
VL patients	35	23.437	35.510	29.878±3.319	17.211	0.000
Control	25	16.123	19.598	18.004±1.075		(HS)

HS: Highly Significant at P< 0.01.

VEGF level (Table 4) was significantly higher (P< 0.01) in VL patients compared with control (23.268 ± 0.866 vs. 11.600 ± 0.783 pg/ml). Most *Leishmania sp.* promastigotes react with several controllers of pro - angiogenic factors including VEGF which is regulated by hypoxia. Since hypoxia regulates the infection of macrophages by the

parasites, these interactions might impact the infection of host cells by *Leishmania sp.* (Fatoux-Ardore, *et al.*, 2013). Duthie, *et al.*, (2014) indicates that level of VEGF is elevated inside the serum of VL patients from Bangladesh and Brazil, this result was agreement with current study.

Table 4: The levels of VEGF (pg/ml) in studied groups and descriptive statistics.

Groups	No.	Min.	Max.	Mean ±Sd.	t-test	P-Value
VL patients	35	21.351	24.789	23.268±0.866	52.442	0.000
Control	25	10.369	12.889	11.600±0.783		(HS)

HS: Highly Significant at P< 0.01.

Finally, our findings support most recent studies mentioned above that indicate GM-CSF, IP-10 and VEGF are important biomarkers in VL infection.

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