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Detection of IL-27 in Patients with Visceral leishmaniasis

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***Abstract*— Visceral leishmaniasis (VL), caused by the protozoan parasites of the *Leishmania donovani* complex, remains one of the most significant neglected tropical diseases. Immune response mechanisms play a critical role in both resistance and pathogenesis of VL.. This study included 32 VL patients (19 females, 13 males, 2 months to 5 years old) and 30 healthy controls (1 month to 11 years old). Blood samples were obtained from the Central Public Health Laboratories (CPHL). The serum was isolated by centrifuging blood samples at 1000g for 5 minutes. Human IL-27 levels were quantified using an ELISA kit (Elabscience Biotechnology Inc., USA; catalog No. E-EL-H2338) following the manufacturer's protocol. This study found significantly higher IL-27 levels in visceral leishmaniasis patients (54.120 pg/ml) compared to the control group (28.745 pg/ml). There was a significant difference between the median levels of IL-27 in patients and the control group, where the p-value was 0.03.**

IL-27 plays a significant role in the pathogenesis of visceral leishmaniasis. Its potential as a biomarker for active disease and for monitoring treatment response warrants further investigation, irrespective of the causative agent or geographic location.

***Keywords*—Biomarker, ELISA, Iraq, IL-27, Kala-azar disease, Visceral leishmaniasis.**

I. INTRODUCTION

Visceral leishmaniasis is a severe parasitic disease caused by species within the *Leishmania donovani* complex. It is characterized by extensive parasite replication in the spleen, liver, and bone marrow, resulting in significant morbidity and mortality. The balance between the parasite and the host immune response is crucial in visceral leishmaniasis development, while both effective and injurious immunity appear to be adopted by the host. The disease is prevalent in many parts of the world, including regions of Iraq and Ethiopia [1], and results in significant morbidity and mortality, as well as social and economic impacts. Leishmania–infected Phlebotomus sandflies are the sole reservoir, and climatic factors may be instrumental in variations in sandfly breeding cycles and transmission rates [2]. Interleukin-27 is a newly described cytokine, which is formed by p28 and Epstein-Barr virus-induced gene 3 , IL-27 is considered to play a pivotal role in the modulation of immune responses in different infections, including leishmaniasis. Since its discovery in humans, IL-27 has been produced mainly by dendritic cells and phagocytes in response to Toll- like receptor stimuli, interferons, and CD40 ligands and/or acts both as an autocrine and paracrine factor influencing both limbs of the immune system. However, IL-27 is not always protective, as it is produced in response to certain pathogens, has context-specific effects on immune responses, and can promote both protection and disease [3-5].

IL-27 is a cytokine composed of two subunits: p28 and Epstein-Barr virus-induced gene 3, which has been implicated in a number of roles in cell development/maintenance, differentiation, neuroimmune function, metabolism, and apoptosis , as well as chromatin remodeling and transcription regulation. These effects occur through stat-1, stat-3 , and stat-5 signaling pathways. Based on this context, the present study demonstrates that IL-27 stimulates the differentiation of CD4+ T cells. In addition, IL-27 is implicated in regulating the interactions between the host and the pathogen [6]. The multiple roles of IL-27 in *Leishmania* infection have been gradually revealed. IL-27 has been shown to augment the natural killer cell cytotoxicity through up-regulation of both perforin and granzyme B and stimulating IFN-γ production through T-bet, a mandatory inducer of T-bet and IL-18 sensitivity [7-9]. The increased plasma level of IL-27 provides recent and corroborative evidence in active visceral leishmaniasis from different geographical areas: India, Europe, and Brazil. These values increase are normalized after effective treatment, implying that IL-27 could be used as a biomarker of active visceral leishmaniasis and may be helpful in showing the efficacy of the treatment. Hence, while studying murine models of visceral leishmaniasis, it has been found that balancing immunopathology is important, and IL-27 plays a crucial role in the host modulation against the Leishmania parasite ; therefore, IL-27 contributes to both protective and pathological immune responses in visceral leishmaniasis [10-12].

Also, visceral leishmaniasis is diagnosed in the laboratory using microscopy, culture, serological tests, and molecular methods like PCR. Compared to microscopy and recombinant antigen dipstick tests, PCR demonstrates

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superior sensitivity (100%) [13,14,15]. Furthermore, Leishman bodies can be detected in lymph node, liver biopsy, or bone marrow aspirate samples, as well as in the buffy coat of peripheral blood. These appear as intracellular, round or oval bodies within monocytes and macrophages [16].

The aim of the present study is to explore the involvement of IL-27 in VL, summarizing its potential contributions to both host protection and immune-mediated.

II. Materials and Methods

The samples of the present study consisted of 62 patients (28 females and 34 males) infected with visceral Leishmaniasis (VL) their age range was between 2 months to 5 years ,and 30 healthy individuals group. The blood samples were collected from the contr at Central Public Health Laboratories (CPHL), their ages ranging between 1month to 11years. .An ELISA kit from Elab Science Biotechnology Inc./USA was utilized to detect the levels of human IL-27, catalog No. :E-EL-H2338. The blood sample was collected from each patient infected with VL , and the blood was centrifuged for 5 minutes at 1000 rpm to collect the serum to carry out the assay. The ELISA test was used in the present study, the *in vitro* quantitative determination of human IL-27 concentration in serum, according to the manual procedure provided with the kit.

Inclusion criteria: Every person infected with visceral leishmaniasis and confirmed by a doctor through clinical manifestation-was included in the present study.

Exclusion criteria: Every person without clear clinical symptoms of VL was excluded.

Ethical consent was taken from each patient and control group.

The current study was approved by the Ethics Committee.

III. Statistical Analysis

Data were analyzed using the SPSS statistical package for Social Sciences, version 20.0 for Windows, SPSS, Chicago, IL, USA. The normality of the statistical distribution was rechecked using the Kolmogorov-Smirnov test, and the IL-27 concentration was skewed. Quantitative data were expressed as Mean ± SD for normally distributed data and median IQR for data with skewed distribution. An Independent sample t-test was also used to investigate the difference in age between the two groups. Since the data of IL-27 were not normally distributed, the Mann-Whitney U test was applied to compare the two groups. The sensitivity and specificity of the tested IL-27 in patients and controls were determined by applying the ROC (receiver operating characteristic curve) test, the AUC , and cut-off values. An indication of significance was used with a p-value of <0.05.The statistical analyses was done by Prof. Dr. Zaid Al-Mudffaay.

IV. Results

The results of the present study recorded that the mean±SD of the age of patients infected with visceral leishmaniasis was 4.8±3.9 years, with range between 4 months to 14 years, while the mean±SD of the control group was 2.9±1.8 years, with a range between 1 to 5 years .There were no significant differences between the two groups, and p -value=0.503.

Table 1. The range of age in patients with VL and the control groups

Group							
Patient (32)				Control (30)			
Mean	SD	Min	Max	Mean	SD	Min	Max
4.8 y	3.9 y	4 m	14 y	2.9 y	1.8 y	1 y	5 y

Table(2) demonstrates that the median level of IL-27 in the patients group was 54.12pg/ml., while the median level of IL-27 in the control group was 28.75pg/ml. There was a significant difference between the median levels of IL-27 in patients and the control group, and p-value 0.03.

Table(2):. The levels of IL-27 in patients infected with VL and the control groups

	Group							
	Patient (62)				Control (20)			
	Median	IQR	Min	Max	Median	IQR	Min	Max
IL27	54.12	100.66	0	738.300	28.75	19.64	5.830	44.300

P = 0.002 (significant difference), Mann Whitney U test

Table 3. ROC measurement to study IL-27 in patients and controls

Area Under the Curve

Test Result Variable(s)	Area	Std. Error	Asymptotic Sig.	Asymptotic 95% Confidence Interval	
				Lower Bound	Upper Bound
IL 27	0.726	0.053	0.002	0.621	0.830

Table (3) show the region under the curve which was 72.6%─for IL-27 , and, the p- value was significant (P=0.002).

Figure (1) shows the value of the sensitivity, which was 61.3% ,and the specificity ,which was 90%, and the cut- off value was 42.25. The number of patients that had higher levels of IL-27 was 19. This value was higher than the cut-off 42.25, and there was one person in the control group who had higher level of IL-27 , which was 44.37pg/ml..

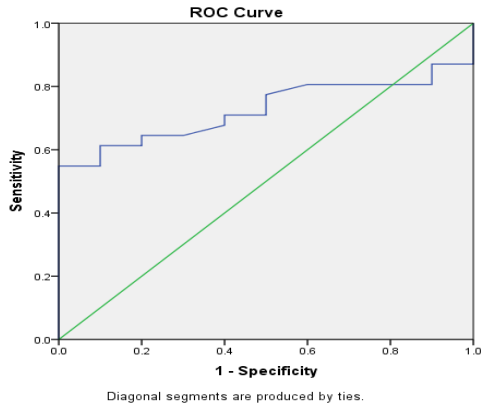

Fig. 1. The sensitivity and specificity of the ELISA test for IL-27.

Table(4): The median levels of IL-27(pg/ml) in males and females patients and the control groups.

	Gender							
	Male (N=34)				Female (N=28)			
	Median	IQR	Min	Max	Median	IQR	Min	Max
IL-27	42.660	52.99	5.010	738.300	56.160	116.21	0.000	241.530

Table(4) shows that the median levels of IL-27 in males were 42.66pg/ml(IQR)by Mann Whitney U test in males, males, while the median value of IL-27 in females were 56.16pg/ml(IQR116.2). There was no significant differences between males and females with IL-27 , and P=0.428

Table(5):The correlation between fever and IL-27.

	Fever							
	+ve (N=48)				-ve (N=14)			
	Median	IQR	Min	Max	Median	IQR	Min	Max
IL-27	59.850	111.40	0.000	738.300	35.290	48.46	0.000	56.570

.**P = 0.020** (significant difference), Mann Whitney U test.

Table (5) shows that there were 48 patients had fever and with IL-27 which was 59.85 pg/ml(IQR 111.4) ,while there were 14 patients had no fever with median value of IL-27 which was 35.29 pq/ml (IQR 48.46),there was a significant difference between fever and IL-27 ,P=0.02.

Table(6): The correlation between hepatosplenomegaly and IL-27.

	Hepatospleenomegaly							
	+ve (N=42)				-ve (N=20)			
	Median	IQR	Min	Max	Median	IQR	Min	Max
IL-27	54.120	90.64	0.000	241.530	52.070	113.89	0.000	738.300

P = 0.952 (no significant difference), Mann Whitney U test

Table(6) shows that there was no significant difference between hepatspleenomegaly and the median levels of IL-27 P=0.952, since there were 42 patients with VL had hepatospleenomegaly and had 54.12 pg/ml of IL-27 ,while there were 20 patients with VL did not have hepatosplenomegaly and had median value 52.07 pg/ml of IL-27 .

Table(7)shows that there was no significant difference between anemia and median value of IL-27, P=0.787,since there were 14 patients with VL had anemia and had 47.57 of the median value of IL-27, while there were 48 patients with VL did not have anemia and had 54.93 of the median value of IL-27.

Table(7): The correlation between anemia and IL-27.

	Anemia							
	+ve (N=14)				-ve (N=48)			
	Median	IQR	Min	Max	Median	IQR	Min	Max
IL-27	47.570	113.89	0.000	143.320	54.935	95.75	0.000	738.300

P = 0.787 (no significant difference), Mann Whitney U test

V. Discussion

The results of the present study showed that the levels of IL-27 were higher in patients with VL than that in control group, since patients with VL exhibited significantly higher median IL-27 levels which were(54.12 pg/ml) compared to controls which were (28.75 pg/ml) (P = 0.03) Table(2). This is in agreement with some studies reporting that increased IL-27 expression occurs in *L. infantum* infection in human and animal models. Specifically, some studies also show increased IL-27 in *L. infantum* infection in human and animal models. Specifically, also some studies show increased IL-27 in *L. infantum* infection during early stages in BALB/c mice [17-18]. others report increased IL-27 expression in *L. donovani*-infected BALB/c mice in spleenic CD8α+ and CD4+ cells at various time points post-infection [19]. Furthermore, increased IL-27 and receptor expression on infected macrophages and DCs has been observed in early *L. infantum infection*. Elevated IL-27 levels in active human VL cases, returning to baseline after treatment, suggest its potential as a marker of disease severity or prognosis [20-21]. The complex role of IL-27 is further highlighted by its involvement in both protective Th1 and Th2 responses, with the potential to influence susceptibility to infection [22- 23].

Symptomatic *L. donovani* infection is often prevented by developming of effective T-cell-mediated immunity, resulting in a relatively low prevalence of visceral leishmaniasis (VL) and protection against reinfection. However, susceptibility to VL is influenced by various factors, including genetic predisposition, helminth co-infections, and malnutrition . Following infection, white blood cells (WBCs), particularly neutrophils, monocytes, and T cells, infiltrate the liver, forming granulomas that play

a crucial role in controlling hepatic pathology [24]. IL-27 production, however, may be a consequence of parasite manipulation of the host immune response, potentially contributing to inflammation control and facilitating parasite establishment [25]. Table 1 shows that the mean age of patients was 4.8 ± 3.9 years (range: 4 months to 14 years). This is consistent with previous research, which shows a higher prevalence of visceral leishmaniasis (VL) among infants and children in rural areas of Iraq, especially in the south and center of the country[26]. ROC curve analysis Table(3) of IL-27 levels yielded an (area under the curve) AUC of 72.6% (P= 0.002), with a cut-o ff value of 42.25 pg/ml. The ELISA test demonstrated 61.3% sensitivity and 90% specificity. The lower sensitivity may be attributed to many factors which are in agreement with that reported by Aydin S.,2015 that factors that can interfere with appropriate ELISA testing can occur at any phase of the testing process, beginning with specimen collection. The quality and integrity of the assay plate, coating buffer, capture antibody, blocking buffer, target antigen, detection antibody, enzyme conjugate, washes, substrate, and signal detection can all interfere with proper ELISA testing [26-28]. In murine models, IL-27 appears crucial in preventing severe immunopathology following both cutaneous and visceral *Leishmania* infection [29].

The cause of low numbers of samples in the present study 62 patients was due to that reported by Hiro M. Obaid and Hager A. Shareef,2018 that the incidence of visceral leishmaniasis was very low (2.7%) comparing to that in cutaneous leishmaniasis (64.6%) in Kirkuk city [30]. The results in Table(4)of the present study revealed that the levels of IL-27 were high in female patients (56.16)pg/ml than in male patients (42.99)pg/ml . These results in agreement with that reported by Alidadi *et al..,* 2016)that IL-27 levels in female patients more than in male patients, which can be caused by interference of with sexual hormones called sex hormones [31].

Table(5)revealed that there was a significant correlation between fever and IL-27, P=0.02, since there were 48 patients with VL had fever and their levels of IL - 27 were 59.85 ,and these results in compatible with that reported by Priscila L, *et al.,*2016 that not only confirm the presence of high serum levels of inflammatory and anti-inflammatory cytokines in active VL, but describe for the first time high concentrations of IL-27 and its correlation with clinical profile [32].

VI. Conclusions

IL-27 plays a significant role in the pathogenesis of patients infected with visceral leishmaniasis (VL). It has the potential to serve as a valuable biomarker for identifying active human VL cases and for monitoring the efficacy of treatment. This holds true regardless of the etiological agent responsible or the geographical region affected, making IL-27 a crucial factor in understanding disease progression and therapeutic outcomes.

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Conflict of interest

Authors declare that they have no conflict of interest.

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