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Investigation Of Il-10 And Ifn-Γ In Visceral Leishmaniasis's Patients Iniraq

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Abstract:

Background: Visceral leishmaniasis is a reticuloendothelial system infection that can have an impact on the immune system

Aim of the study: The currentstudy aimed toStudy the immunity and pathogenesis of visceral leishmaniasis through determining host-related factors levelsthat likely lead to parasite destruction and patient recovery and may be useful both for designing and evaluating immunoprophylaxis

Material and Methods: A case-control study was conducted among 50 cases of patientswith VL and 50 healthy control.who were admitted to maternity and children's hospitalsin Iraqin the period from 1 October 2020 to 5 May 2021. Patients were diagnosed with visceral leishmaniasis according to history and clinical findings including persistent fever, hepatosplenomegaly, and pancytopenia might be seen in patients and by the IFAT test to confirm the disease's presence then Sandwich.Immunosorbent.The assay was employed forthe quantitativedetermination of human serum IL-10 and IFN-γ levels.Data were analyzed with (SPSS) version 23,Whitney, Mann test, and The Chi-square test.

Results: In the present study,The majority of the most common clinical signs observed in all hospitalized patients were fever 50(100%),pallor 50(100%),Hepatomegaly 45(90%) and Splenomegaly 48(96%),The cytokine levels of IL-10 and IFN-γ were higher significantly in patients than control groups($P < 0.01$)

Conclusion: the current study has shown that IL-10 &IFN-γ are most likely to be involved in determining the fate of disease and can use as a diagnostic tool to estimate the severity of the injury as well as it may also use As criteria for follow-up treatment.

Keywords: VL, Human, IL-10, IFN-γ

Introduction:

Leishmania parasite is developed by William Leishman, who found one of the forms of flagellate protozoa around 1900. Leishmania invaded the reticular endothelium systems tissues of the (vertebrate host), and it transmits through one host to another through sandfly insect bites.(Al-maeahi&

Marhoon,2018). Visceral leishmaniasis is a disseminated parasite illness spread by female Phlebotomine sandflies and caused by the Leishmaniainfantum. It is a major public health problem in Mediterranean countries (Barani et al,2020). is fatal in approximately 95% of cases if left untreated (Altayeb et al,2021). It primarily affects newborns and young children aged 1–4 years, with dogs serving as the primary reservoir host in these regions (Torres et al, 2017).Leishmaniasis can cause an enlarged abdomen, as well as general.symptoms such as prolonged fever, appetites, and weight loss, malaise, pallor of the mucous membranes, and high levels of gammaglobulinemia in the bloodstream.(Karimi et al., 2016).In the defense against the Leishmania parasite, both innate and adaptive immunity play critical roles (Dos-Santos et al,2016). Host immune system players must work together to successfully eradicate Leishmania.parasites from an affected host. During Leishmania's life cycle, the parasite's ability to change and/or escape the host's immune responses will play a significant part in the fight between parasite destruction and disease establishment.Elements of the innate and adaptive immune systems are among the host defensive components required for parasite elimination (Ikeogu et al,2020). Infection with Leishmania results in the presence of macrophages. Even thoughLeishmania parasites can infect both neutrophil and macrophage cells, macrophages are the main infected cells because they survive longer than neutrophils.(Tomiotto et al,2018).Classical macrophage activation is mediated by interferon-gamma (IFN-γ), effector cytokines that release by CD4+ T helper type 1 (Th1) cells, CD8+ T cells, and natural killer (NK) cells(Wu et al,2014). Macrophages produce inducible nitric oxide synthase (iNOS) in response toIFN-γ stimulation which converts L-arginine to nitric oxide (NO),a crucial effector molecule for killing intracellular amastigote.(Salim et al,2016). IFN-γ is the most cytokine in host defense, and it plays an important role in macrophage priming(Ivashkiv,2018).Transforming growth factor-beta.and Interleukin-10 concentrations in human VL correlate with parasite burden and the absolute number of FoxP3 Treg cells, indicating the T regulatory has a considerable role in cytokine release. (Bhattacharya et al,2016). Many studies have shown that IL-10 suppresses immunological response by blocking Th1 activation and, as a result, downregulating IL-12 and IFN-γ production. Because of this immunosuppressive property, it has been hypothesized that IL-10 can let Leishmania evade immune monitoring and favor infection. (Al-Autabbi et al,2015). Iraq is considered an endemic area for leishmaniasis, with both cutaneous (Baghdad boil) and visceral (Kala-azar) forms of the disease present. In Iraqi governorates, leishmaniasis is widespread; the first cases were reported in Mosul and Baghdad.(Al-Hayali,2021)

Materials and Methods:

Patients and sample collection:

A case-control study was conducted in AL-Diwaniyah province. Based on 50 patients with VL which include (20 males and 30 females), who were admitted to maternity and children's hospitals in(Al-Diwaniya, Hilla,Diyala,Baghdad,Kirkuk,Nasiriyahand Basrah) in the period from 1 October 2020 to 5 May 2021 under the supervision of a pediatric physician. Their ages were from 4 months to 7 years. Patients were diagnosed with visceral leishmaniasis according to historyand clinical findings including persistent fever, hepatosplenomegaly, and pancytopenia might be seen in patients. As soon as the samples are transferred to Baghdad's central public health laboratory, the IFAT test will be used to confirm the disease's presence.

The study also included a control group of around 50 people, 20 males, and 30 females,Their ageswere from 4 months to 7 years who appeared to be healthy and had never been exposed to any parasites. Fifty patients with visceral leishmaniasis and fifty healthy controls were venipuncture and 5 ml of venous blood were extracted with an aseptic disposable syringe. A gel tube was filled with three ml of blood and allowed to coagulate for five minutes before being centrifuged at 1500 rpm for five minutes separated the serum. Eppendorf tubes were used to collect the serum, which was subsequently kept at 20°C for use in the ELISA test. to determine the concentration of IL-10 andIFN-γ.

Principle of ELISA test:

Kits of The ELISA test wasfrom Elabscience companyUSA (No for IL-10 kit(18DTQWZN3D) and for IFN-γ kit (Z5AW57EG3M) whichis dependent the Sandwich-ELISA principle and following the manufacturer's instructions then The ELISA results were calculated depending on the average of the duplicatereadings for each standard and sample's optical density. Then created a standardcurve by plotting the mean OD value each standard on the y-axis against theconcentration on the x-axis and draw a best fit curve through the points on thegraph in excelofficeprogram.

Statistical analysis:

The SPSS(23) Program was used to collect, summarize, analyze, and present data,Mann Whitney U test was used to study differences in mean rank between any two groups provided that the variable was non-parametric,Chi-square test was used to study the association between any two categorical variables.The level of significance was considered at a P-value of 0.05 or less and a highly significant level at 0.01 or less (Daniel 2009).

Result :

Distribution of Visceral Leishmaniasis patients according to the some clinical feature

Visceral leishmaniasis is typically a chronic parasitic disease associated with prolonged fever, splenohepatomegaly, and anemia.Anemia was severe in all of the cases on admission, and fever was present in all of the patients with VL clinical features. In the current study, The clinical feature accompanying Visceral Leishmaniasisis shown in table (1).

Table (1): Frequency distribution of some Associated Clinical features in a patient with Visceral Leishmaniasis.

Characteristic	Patients	
	N	%
Fever		
Present, n (%)	50	100.0 %
Absent , n (%)	0	0 %
Pallor		

Present, n (%)	50	100.0 %
Absent , n (%)	0	0 %
Hepatomegaly		
Present, n (%)	45	90.0 %
Absent , n (%)	5	10.0 %
Splenomegaly		
Present, n (%)	48	96.0 %
Absent , n (%)	2	4.0 %

Interleukin-10 level in patients and control groups:

The comparison of serum IL-10 levels between patientswith VisceralLeishmaniasis and control groups has been carried out and the results were demonstrated in a table (2) and figure (1), Median levels of serum IL-10in patients with Visceral Leishmaniasis were higher than in comparison the median levels of control groups, 107.80 (39.58) pg/ml versus 12.45 (8.65) pg/ml, the difference was highly significant (P < 0.01).

Table (2): Frequency distribution of patients with Visceral Leishmaniasis and control subjects according to the level of Serum IL-10.

	Case-control comparison		
IL-10(pg/ml)	**Patients n = 50**	**Control n = 50**	**P**
Range	9.62 – 211.05	9.01 – 43.22	**< 0.01 † HS**
Median (IQR)	107.80 (39.58)	12.45 (8.65)	

n: number of cases; IQR: interquartile range; †: Mann Whitney U test; HS: Highly significant at P ≤ 0.01

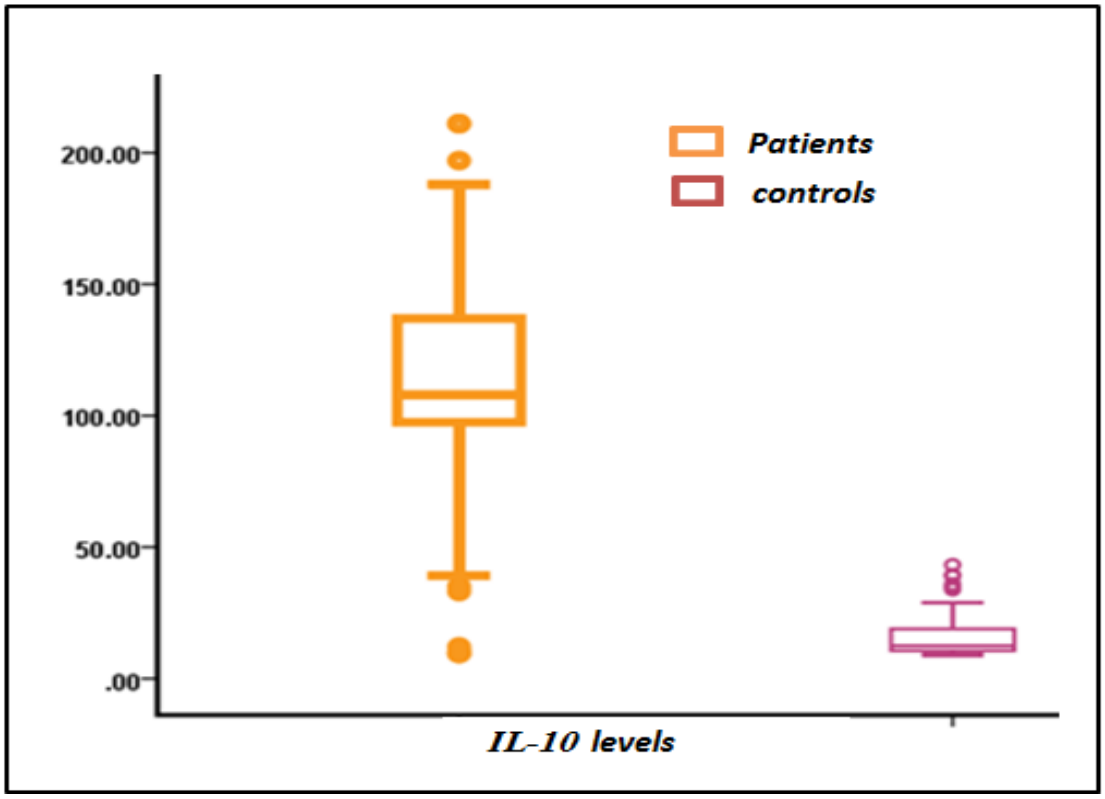

Figure (1): Box plot showing the comparison of median serum IL-10 level among patients with Visceral Leishmaniasis and control subjects

Serum IFN-y level in patients and control groups:

The comparison of serum IFN-y level between patientswith Visceral Leishmaniasis and control groups has been carried out and the results were demonstrated in the table (3) and figure (2),Median levels of serum IFN-yin patients with Visceral Leishmaniasis were higher than in comparison the median levels of control groups, 143.39 (32.91) pg/ml versus 29.128 (10.97) pg/ml, the difference was highly significant ($P < 0.01$).

Table (3): Frequency distribution of patients with Visceral Leishmaniasis and control subjects according to a level of Serum IFN-y.

	Case-control comparison		
IFN-y (pg/ml)	**Patients n = 50**	**Control n = 50**	**P**
Range	32.50 – 217.43	19.99 – 78.30	**< 0.01 † HS**
Median (IQR)	143.39 (32.91)	29.128 (10.97)	

n: number of cases; IQR: interquartile range; †: Mann Whitney U test; HS: Highly significant at P ≤ 0.01

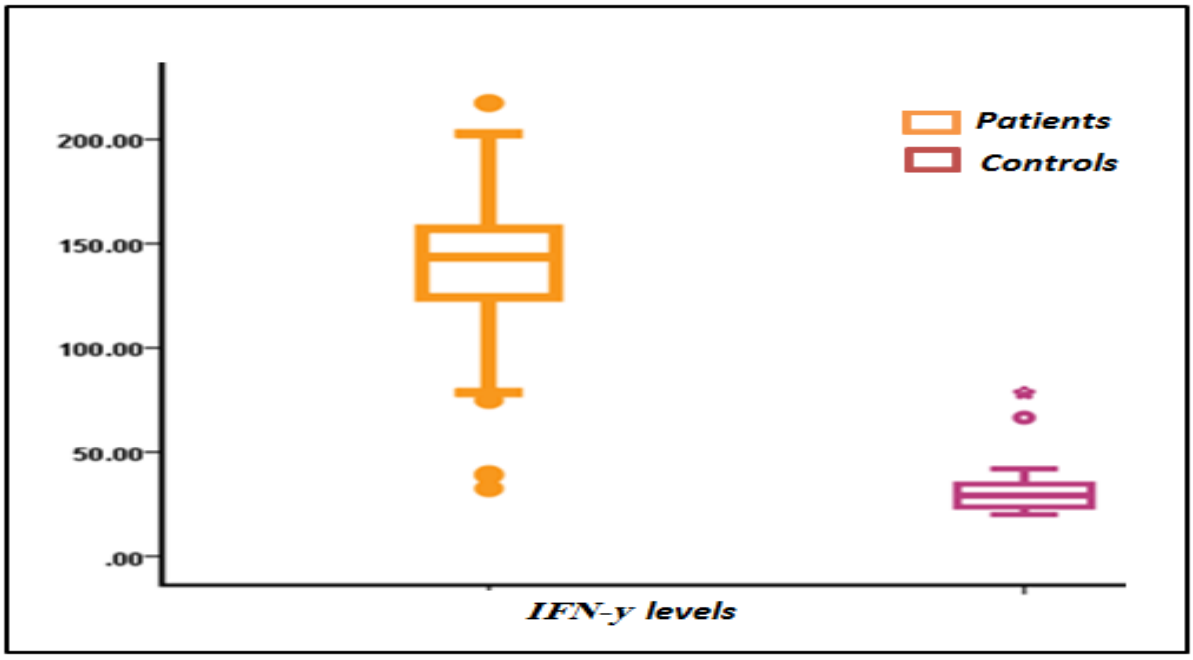

Figure (2): Box plot showing a comparison of median serum IFN-γ level among patients with Visceral Leishmaniasis and control subjects

Discussion:

Visceral leishmaniasis is widely disseminated in many regions of Iraq, which is considered an endemic area, particularly in the middle and southern parts, with the main reason being the adaptability of the vector sandfly in these places. (Al-Saqur et al,2008). In the present study,The majority of the most common clinical signs observed in all hospitalized patients with VL were fever and pallor, which were observed in all patients 50 (100%) The frequency distribution of patients according to Hepatomegaly was as follows, were shown a high frequency of patients have hepatomegaly 45 (90.0%). According to splenomegaly inpatient with Visceral Leishmaniasis, the present study show 48 (96.0 %) of the patients have splenomegaly, and these symptoms were similar to other the results (Abid,2019) found that most of those patients presented with fever(96.6%) ,Splenomegaly(86.6%) , pallor(83.3%) ,hepatomegaly (60%),Al-Ani&Al-Hamwandi(2012) record that The major presenting clinical manifestations were; fever (100%), hepatosplenomegaly (100%),Pallor (96.9%),(Al-Hamash,2012) show that All patients presented with fever(96.6%) ,Splenomegaly(92%) , pallor(70%) ,hepatomegaly (80%). According to the cytokine data in this study, an increase in serum levels of IL-10 and IFN-γ during active disease occurs, as shown by Kamil et al. (2013), who found a significantly higher serum level of IFN-γ and IL-10 in VL patients compared to healthy controls.also confirmed by Hummadi (2010), reported thatThere were significant increases in the levels of all studied cytokines (IFN-γ, IL-10, and TNF-a) in the sera of patients with VL during active disease as compared to the control group.also confirmedIn a study in Iran by Khoshdel et al,(2009), a recorder thatSerum cytokine levels, IL-12, IL-10, and IFN-γ were greater in patients than in family members and control individuals.Also, in a previous study by Karp et al, (1993), the height of both

IL-10 mRNA and IFN-γ levels in the lesion environment of the bone marrow in patients with kala-azar before treatment, results from that may be important in understanding how this parasite can resist immune-mediated destruction by host macrophages.as showed Al-Autabbi et al. (2015) that The immune system is highly stimulated in active VL, producing both macrophage-activating cytokines IFN-γ and macrophage-deactivating cytokines IL-10.In a typical Th2 and Th1 response, IL-13, as well as other cytokines like IL-12, IL-10, and IFN-γ have been discovered to be significant components in the regulating of immune responses. (Mahmoodi et al., 2005).IL-10 has been systematically correlated to VL pathogenesis; this cytokine has been considered as the key regulatory cytokine in the VL due to its pleiotropic effects related to the suppression of microbicidal activities in infected macrophages. (Santos et al., 2017).IL-10 can inhibit Th1 cell and macrophage activation; therefore, increased levels in VL sera may be expected. IL-10 also plays an important role in the regulation of inflammatory response and is important for the parasite's survival and persistence inside macrophages.(Costa et al., 2012).Other studies have found that IL-10 plays a role in adapting host cells so that they are less responsive to high levels of IFN-γ for intracellular killing. (Belkaid et al., 2001).

The current results indicated that active VL caused increasing amounts of IL-10, which is consistent with the findings of Gatto et al. (2015), who found that pre-treatment IL-10 levels were related to the expression of toll-like receptor2 (TLR2).Another study found that pretreatment with an anti-TLR2 antibody diminished IL-10 production in human macrophages infected with Leishmaniadonovani, suggesting that TLR2 may be related to immune response regulation. (Engwerda et al.,2004).The infection of Leishmaniadonovani is known to stimulate endogenous secretion of IL-10 as a parasitic mechanism because IL-10 appears to be responsible for inhibiting the synthesis of IFN-γ, the main macrophage stimulating cytokine involved in the defense against Leishmaniadonovani that facilitated intracellular parasite survival by downregulation of oxidative and inflammatory response. (Bhattacharya et al.,2007).

IFN-γ plays an important role in macrophage-mediated anti-leishmanial activity, parasite elimination, and subsequent infection resolution (Tripathi et al., 2007). Furthermore, the IFN-γ cytokine is the primary factor in stimulating iNOS transcription and NO generation (Shio and Olivier, 2010).IFN-γ causes tyrosine kinase phosphorylation, Janus kinase (JAK1 and JAK2) activation, and subsequently the phosphorylation and dimerization of signal transducer and activator of transcription (STAT). Activated STAT migrates to the nucleus and binds to iNOS promoter sequences, resulting in the production of NO (Shio et al., 2012).IFN-γ produced by NK cells has a more prominent role in host defense, promoting activated macrophages in killing intracellular parasites through the formation of reactive oxygen intermediates (ROI) or reactive nitrogen in the control of intracellular parasites by triggering IFN-γ production.(Nylen et al., 2003).However, reduction of NK cells during the first seven days of Leishmania infection in mice results in an increase in parasite burden due to insufficient IFN- production. (Mutiso et al., 2013).

Although IFN-γ has been shown to increase macrophage leishmanicidal activity, higher serum levels are detected in active cases of VL with large parasite loads. IFN-γ did not show anti-leishmanial action in sera from active VL patients, despite having been reported in sera from patients with tegumentaryleishmaniasis and recovered VL patients. Because IL-10 appears to be the predominant

macrophage-deactivating cytokine in human leishmaniasis, a lack of IFN-γ activity could be related to the presence of high levels of IL-10.

Conclusion:

the current study has shown that IL-10 &IFN-γ are most likely to be involved in determining the fate of disease and can use as a diagnostic tool to estimate the severity of the injury as well as it may also As criteria for follow-up treatment.

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