

Original Research Article

The Role of Interferon-gamma (IFN- γ) and Interleukin-10 (IL-10) in Iraqi Patients with Visceral Leishmaniasis (VL)

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A total of 50 blood samples of active visceral leishmaniasis was identified clinically by fever of more than two-week duration, hepatosplenomegaly, and pancytopenia then confirmed by rK39 Dipstick. Fifty bone marrow smears showed moderate to severe megaloblastosis, an increased number of plasma cells and megakaryocytic hyperplasia with abnormal morphology. Amastigotes appeared as round or oval bodies found intracellular in monocytes and macrophages in the stained smears. Peripheral blood samples and lymph nodes were obtained before and 30 days after, completion of treatment with sodium stibogluconate (Pentostam;UK). Enzyme immunoassay was carried for quantitative determination of human IFN- γ and IL-10 in serum of patient and control groups. Serum IFN-gamma level showed the distribution of serum levels of IFN-gamma in patients with VL and control group. The levels in the pretreated VL group were (60.87 ± 3.6) pg/ml higher than control (5.12 ± 0.98) pg/ml with a significant difference ($p < 0.01$). There was a significant decrease in serum levels of IFN-gamma in post treated group (9.83 ± 1.1) pg/ml, which were significantly lower than that in the pretreated group. There was no significant difference between post treated and control group ($p > 0.05$). Serum levels of IL-10 in the control were (2.98 ± 0.52) pg/ml significantly lower than that in patients with VL (110.8 ± 4.2) pg/ml. In the post-treated VL group the levels were (4.75 ± 0.86) pg/ml which were significantly lower than pretreated VL group ($p < 0.01$). There was no significant difference between post treated and control group ($p > 0.05$).

Keywords: VL, Human, IL-10, IFN- γ

INTRODUCTION

Human visceral leishmaniasis, caused by *L. donovani* or *L. infantum* in Africa, Asia, and Europe, or by *L. chagasi* in Latin America, is most often recognized as an acute infection with severe morbidity and high mortality in untreated cases. Iraq is endemic to cutaneous leishmaniasis (CL) and visceral leishmaniasis (VL). These patients are characterized immunologically as having high levels of anti-leishmania antibody and low or absent *Leishmania* - elicited T cell proliferation and cytokine (IL-2 and IFN- γ) production, responses which become positive upon recovery from acute disease⁽¹⁻³⁾. Delayed hypersensitivity responses to *Leishmania* antigens parallel the in vitro lymphocyte response patterns^(4, 5).

Cytokine production patterns in human visceral leishmaniasis, particularly with regard to the production of down-regulating Th2 cytokines, have not been well documented. Of particular interest is the production of cytokine or cytokine mRNA in lymphoid tissue, as well as in leukocytes of the peripheral circulation. In the present study, we have determined that an important Th2 cytokine, IL-10, is produced during human infections with *L. donovani* and provide evidence for a role for IL-10 in regulating aspects of T cell responses in visceral leishmaniasis patients. INF-gamma is a potent activator of mononuclear phagocytes during respiratory burst, promotes

the differentiation of CD4+ T-cell to Th1 phenotype and inhibits the proliferation of Th2^(6,7).

In spite of long history of presence of the disease in the region and increasing numbers of reported cases, the information of VL incidence, endemic foci, clinical aspects and reservoir animals are not well known in Iraq⁽⁸⁾. The present study aimed to determine the role of Th1 and Th2 in VL patients by measuring IL-10 and IFN-gamma in Iraq.

MATERIALS AND METHODS

Sample Collection

5 ml of blood was collected from suspected individuals. They were categorised as Kala-azar cases and controls by physicians, according to clinical signs and symptoms. The confirmed cases of Kala-azar have fever, hepatosplenomegaly, anaemia and weight loss from hospitals and central laboratories in different parts of Baghdad. The controls included clinically healthy people.

Microscopical Examination

A fifty bone marrow smears showed moderate to severe megaloblastosis, an increased number of plasma cells and megakaryocytic hyperplasia with abnormal morphology. Amastigotes appeared as round or oval bodies found intracellularly in monocytes and macrophages in the Giemsa-stained smears⁽⁹⁾.

rK39 Dipstick test

The rK-39 immunochromatographic test (In Bios International, USA), was performed and interpreted according to the manufacturer's instructions. The test was considered positive when two bands (a control and a test band) appeared within 10 min and negative when only the control band appeared⁽¹⁰⁾.

ELISA

Cytokines were measured by a sandwich ELISA using commercial kits according to the manufacturer's instructions (Immunotech Beckman Coulter Company). Absorbance was read at 450 nm using a spectrophotometer and the results are expressed in pg/mL based on a standard curve. The sensitivity of the test was 4 pg/mL for IFN- γ and 2 pg/mL for IL-10. The statistical analysis was performed using Graph-Pad software, because the cytokine levels did not show a normal distribution. Differences were considered to be statistically significant when $p \leq 0.05$.

RESULTS

Figure (1) showed bone marrow smear with moderate to severe megaloblastosis, an increased number of plasma cells and megakaryocytic hyperplasia with abnormal morphology. Amastigotes appeared as round or oval bodies found intracellularly in monocytes & macrophages.

The serum IFN- γ and IL-10 levels in patient (pretreated & posttreated VL) and control groups are shown in figures (2,3).

DISCUSSION

In most parasitic diseases, a predominantly cellular Th1 or humoral Th2 immune response offers the best control over pathogens, the induction of an appropriate T-helper cell

response is essential in determining a successful immune reaction. Th1 cells are effective mediators for delayed type hypersensitivity reaction (DTH) and secrete IL-2 and IFN-g, the prime effectors of cell - mediated immunity⁽¹¹⁾.

In active VL, the immune system is highly activated and produce both the macrophage-activating cytokines IFN-g and the macrophage-deactivating cytokines IL-10⁽¹²⁾. Plasma levels of IFN are influenced by numerous endogenous factors beside *IFN*- gene activity⁽¹³⁾.

Many studies have indicated the suppressive role of IL-10 to immune response via blocking Th1 activation and subsequently down regulation of IL-12 and IFN- γ production^(13,14). It is because this immunosuppressive property, it has been postulated that IL-10 can contribute to escape *Leishmania* from immune surveillance and favor infection.

High levels of IFN-g have been detected in the serum of patients with VL in comparison with control. High levels of IFN-g are necessary in the maintenance of the balance between Th1 and Th2 responses. These results matched the results of previous researchers Gomes and Dos (1998) who found a mixed Th1/Th2 response of parasite-specific T - cells from both acute and chronic murine visceral leishmaniasis. This was supported by the later study of Kemp and Theander (1999) who found an elevation of both IL-10 and IFN-g mRNA in patients with VL^(15,16).

Also the result of our study of IL-10 confirmed the results showed by Margaret and David, (2001) who stated that VL patients have increased expression of IL-10 mRNA as an important strategy for down-regulating T-cells response⁽¹⁷⁾. *Leishmania donovani* infection is known to induce endogenous secretion of IL-10 as a mechanism of parasitism because IL-10 seems to be responsible for inhibition synthesis of IFN-g the main macrophage -stimulating cytokine involved in the defense against *Leishmania* which facilitated the intracellular survival of parasite by down-regulation of oxidative and inflammatory response⁽¹⁸⁾. In fact in human, severity of VL has been closely associated with increased levels of IL-10 and the use of anti-IL-10 antibody to block the IL-10 activity or IL-10 receptor blocked can be effective approach for the treatment of leishmaniasis⁽¹⁹⁾.

Figures (2,3) showed highly significantly increased in serum IFN- γ patients as compared to control group, which agreed with the⁽¹⁸⁻²⁰⁾, also demonstrated that IL-10 was significantly higher in patients than in healthy controls which agreed with results from studies^(21,22). These results confirmed that high IL-10 level in combination with high parasite associated with persistence and severity of VL, it is also one of the reasons why children are more susceptible to leishmaniasis⁽²²⁾. A different observation showed that in American Visceral Leishmaniasis, the IL-2 and IFN-gamma production were absent upon stimulation with *Leishmania* antigens. Also Bogden *et al.*, (2001) noted that IL-10 blocks Th1 activation and consequently a cytotoxic response by down-regulation IL-12 and IFN-production, IL-10 also inhibits macrophage activation and decreases the ability of these cells to kill *Leishmania*⁽²³⁾. In this study, serum levels of IL-10, IFN-gamma, returned to normal levels at the end of therapy. This result agreed with results of Karp *et al.*, (1993) who reported that high concentration of IL-10 and IFN-gamma estimated in the sera of patients with VL at the beginning of the infection, return to the normal range at the end of therapy⁽²⁴⁾.

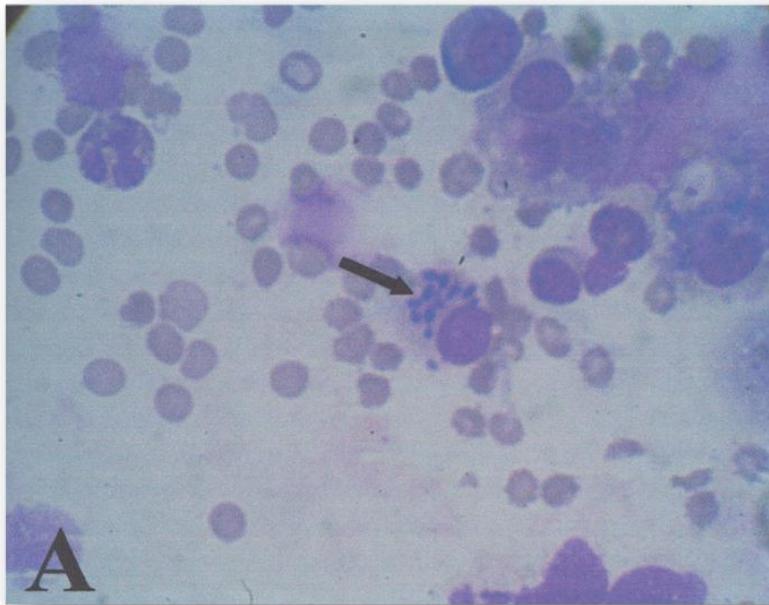


Figure (1) Bone marrow smear showed megaloblastosis and amastigotes appeared as round or oval intracellular bodies

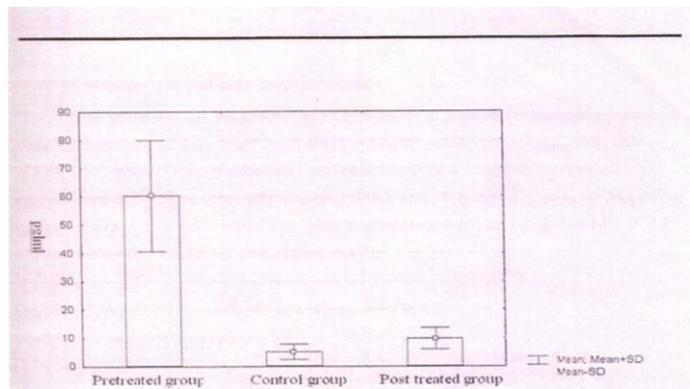


Figure (2): Serum levels of IFN- γ , measured by ELISA in healthy control and patients with VL before and after sodium stibogluconate therapy

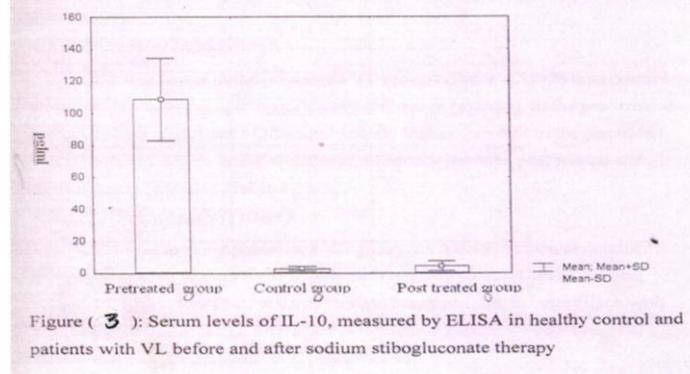


Figure (3): Serum levels of IL-10, measured by ELISA in healthy control and patients with VL before and after sodium stibogluconate therapy

CONCLUSIONS

Studies in human leishmaniasis confirm the relevant roles of IFN-g and IL-12 as the major cytokines involved in host protection, whereas IL-10 takes the place as the leading cytokine responsible for parasite survival and disease progression. Other, less explored cytokines may also prove important in immunoregulation in human leishmaniasis. Future strategies for vaccination or immunotherapy must take into account such findings, which do not always parallel mouse studies.

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