American Journal of Medicine and Medical Sciences 2023, 13(12): 1953-1958
DOI: 10.5923/j.ajmms.20231312.29

Role of Cytokines in the Regulation of Immune Response in Patients with Cutaneous Leischmaniasis

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Abstract To study the role of some cytokines in the regulation of the immune response in patients with cutaneous leishmaniasis, taking into account the clinical form. Clinical; immunological (IL-4, IL-6, IL-8, TNF-α, IFN-α and IFN-γ), statistical research methods were used. Blood plasma from 96 patients with various clinical manifestations of cutaneous leishmaniasis were selected for the study. Of these, thirty patients were diagnosed acne form of cutaneous leishmaniasis, 42 – and ulcerated leishmaniomas, 24 – and ulcerated leishmaniomas with tubercles of inoculation and lymphangitis. The control group consisted of 20 practically healthy individuals aged 25 to 40 years. In patients with various clinical forms of cutaneous leishmaniasis, a significantly pronounced decrease in the synthesis of IL-4 and IFN-γ and high concentrations of proinflammatory cytokines, in particular TNF-α, are observed. In complicated forms of cutaneous leishmaniasis with lymphangitis and lymphadenitis, the levels of IL-6 and IL-8, as well as the synthesis of TNF-α, increased significantly relative to the control. A pronounced imbalance of cytokines was most typical in patients with a disease duration of more than 1 year. In cutaneous leishmaniasis, changes in the content of cytokines are detected, expressed by a deficiency in the concentration of the anti-inflammatory cytokine IL-4 and an increase in the content of proinflammatory cytokines IL-6, IL-8 and TNF-α, which are directly dependent on the clinical form and duration of the disease, which indicates active inflammatory process and the presence of immune deficiency, while anti-inflammatory and immune factors are insufficient for a favorable outcome of the disease. Invariation in the concentration of pro- and anti-inflammatory cytokines in various clinical forms of cutaneous leishmaniasis can be considered as a result of the immune response to the leishmania antigen, depending on the severity of the course, the development of complicated forms and the duration of the onset of the disease. The results obtained indicate the important regulatory role of cytokines in the manifestation of the immune response to leishmania and serve as a rationale for immunomodulatory, as well as rational and effective elimination antibiotic therapy.

Keywords Cutaneous leishmaniasis, Clinical forms, Cytokine profile

1. Introduction

Cutaneous leishmaniasis (CL) (Leishmaniosi cutis), or Borovsky's disease (synonyms: cutaneous leishmaniasis of the Old World - Borovsky's disease; cutaneous leishmaniasis of the New World - American cutaneous leishmaniasis; visceral leishmaniasis - kala-azar) is a vector-borne, protozoal disease with endemic distribution in countries with a hot, tropical and subtropical climate [2,3,11,16]. Depending on the region of distribution, leishmaniasis causes damage to the skin, mucous membranes and internal organs. Cutaneous leishmaniasis affects the skin of exposed areas of the body, causing mainly the formation of ulcers, which can leave permanent scars, cause severe disability and lead to stigmatization of survivors. About 95% of cases of CL are observed in the countries of America, the Mediterranean basin, the Middle East and Central Asia. The annual number of new cases of this form of the disease is estimated to range from 600,000 to 1 million, but only about 200,000 cases are reported to WHO. In Central Asia, the main endemic zones are located in Uzbekistan and Turkmenistan [1,4,6,9]. In the endemic regions of Uzbekistan (Bukhara, Surkhandarya, Kashkadarya, Jizzakh, Karakalpakstan), there is a fairly high prevalence of zoonotic cutaneous leishmaniasis, where dozens of new cases of this disease are recorded annually [13,14].

Currently, existing methods of combating CL, including extermination of pathogen reservoirs and vectors is labor-intensive and does not always give significant results. Due to the increase in incidence, registration of complications and atypical forms, insufficient preventive measures and pathogenetically based method streatment make the problem of CL extremely relevant [1,2,4,5,6,10,12, 15,17,18,19].

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Received: Nov. 29, 2023; Accepted: Dec. 16, 2023; Published: Dec. 18, 2023
Published online at http://journal.sapub.org/ajmms

An important link in the pathogenesis of CL is the state of the immune reactivity of the patient's body, the strength and degree of the immune response of which determines the prognosis and outcome of the disease.

It is known that the cytokine network is the most important regulatory mechanism of intercellular interactions. An imbalance in cytokine production can affect both immunopathogenesis and the chronicity and progression of the disease. Recently, interest in the synthesis and secretion of cytokine molecules, their receptors, and natural antagonists has grown significantly and is of high importance due to the possibility of their use to determine etiopathogenesis, diagnosis, and monitoring the effectiveness of treatment [7,8].

Pro- and anti-inflammatory cytokines, primarily mediators that influence the function of neutrophils and macrophages, play an important role in the regulation of antifungal, antiparasitic and anti-inflammatory resistance. An important role is played by IL-4, IL-6, IL-8 and TNF-α [8,16,18,20,21].

Studying the levels of cytokines allows us to obtain information about the functional activity of various types of immunocompetent cells; about the severity of the inflammatory process, its transition to the systemic level and prognosis; on the relationship between activation processes Th1- and Th2-, which is very important in the differential diagnosis of a number of infectious and immunopathological processes; about the stage of development of a number of allergic and autoimmune diseases. In this regard, the study of the state of cytokines in patients with CL at the present stage seems extremely relevant.

The purpose of the study: to study the role of some cytokines in the regulation of the immune response in patients with cutaneous leishmaniasis, taking into account the clinical form.

2. Materials and Methods of Research

The work used clinical data (clinic and course of CL in the endemic region of Bukhara region, Republic of Uzbekistan); immunological (IL-4, IL-6, IL-8, TNF-α, IFN – α and IFN – γ), statistical research methods. Blood plasma from 96 patients with various clinical manifestations of CL were selected for the study. Of these, thirty patients were diagnosed acne form of cutaneous leishmaniasis, 42 – and ulcerated leishmaniomas, 24 – and ulcerated leishmaniomas with tubercles of contamination with lymphangitis. The control group consisted of 20 practically healthy individuals aged 25 to 40 years.

3. Results and Discussion

We studied the content of anti- and pro-inflammatory cytokines in patients depending on the clinical forms of CL (IL-4, IL-6, IL-8, TNF-α, IFN-α and IFN-γ). These cytokines are important in the regulation cell-mediated immune response and play a decisive role in modulating the immune system, carrying out a complex of protective reactions of the body during the introduction of a pathogen. Thus, proinflammatory cytokines typically regulate the growth, activation and differentiation of immune cells, as well as the targeting of immune cells to sites of infection to control and destroy pathogens, initiating an inflammatory response. Anti-inflammatory cytokines regulate immune responses and limit the development of inflammation in many pathological conditions and infections [7,8].

As the results of the study showed (Table 1), the initial data on the level of pro-inflammatory cytokines were significantly increased. Thus, the level of IL-6 was 2 times higher than the values in the control group, averaging 305.83± 1.61pg/ml ($p<0.001$). The level of IL-8 was 1.9 times higher than the control values. Noteworthy are the high levels of TNF-α, which was 3.6 times higher than the control values. On the contrary, the content of the anti-inflammatory cytokine IL-4 in patients in this group was 2.7 times lower than in the control, amounting to0.69±0.06pg/ml ($p<0.001$). Similar changes were characteristic of IFN-α and IFN-γ. In this case, IFN-γ deficiency was more pronounced compared to the control (2.53 times), and amounted to8.43 ± 0.25pg/ml ($p<0.001$).

Table 1. Indicators of some cytokines in patients with CL (M±m)

Parameters	Control group n=20	Group with cutaneous leishmaniasis (general group) n=96
IL-4 (pg/ml)	1,86 ± 0,13	0,69±0,06**
IL-6 (pg/ml)	150,66 ± 1,66	305,83± 1,61**
IL-8 (pg/ml)	52,06 ± 0,88	98,91± 1,34**
TNF-α (pg/ml)	14,25 ± 0,55	51,34± 1,06**
IFN-α(pg/ml)	44,70 ± 1,77	29,79 ± 0,62**
IFN-γ (pg/ml)	21,35 ± 0,66	8,43 ± 0,25**

Note: p – reliability of data in relation to control at
** - $p<0.001$

In further studies, we studied cytokine levels in patients with CL depending on the clinical form of the disease.

Table 2. Some indicators of cytokines in patients with angular form of CL(M±m)

Parameters	Control group n=20	Patients with acne form of cutaneous leishmaniasis n=30
IL-4 (pg/ml)	1,86 ± 0,13	0,78 ± 0,08**
IL-6 (pg/ml)	150,66 ± 1,66	271,18 ± 2,39**
IL-8 (pg/ml)	52,06 ± 0,88	88,50 ± 1,74**
TNF-α (pg/ml)	14,25 ± 0,55	31,35 ± 1,48**
IFN-α(pg/ml)	44,70 ± 1,77	31,92 ± 1,19*
IFN-γ (pg/ml)	21,35 ± 0,66	11,24± 0,44**

Note: p – reliability of data in relation to control at
* - $p<0.01$; ** - $p<0.001$

As can be seen from the presented data (Table 2), that in the blood plasma of patients with angular form of CL here was a significant increase in the concentration of cytokines IL-6, IL-8 and TNF-α compared to the control group (p<0.001) and on average they were 271.18 ± 2.39 pg/ml, 88.50 ± 1.74 pg/ml ml and 31.35 ± 1.48 pg/ml, respectively, with 150.66 ± 1.66 pg/ml, 52.06 ± 0.88 pg/ml and 14.25 ± 0.55 pg/ml, respectively, in the control. At the same time, the level of these cytokines was 1.8; 1.7 and 2.2 times higher than in the control, respectively. The increase in the content of pro-inflammatory cytokines was accompanied by a decrease in the level of anti-inflammatory IL-4, the level of which was reduced by 2.4 times (p<0.001) relative to the control.

It was found that in patients in this group, the levels of IFN-α and IFN-γ were also reduced, amounting to 31.92 ± 1.19 pg/ml p < 0.01 and 11.24 ± 0.44 pg/ml (p <0.001), respectively, that is, 1.4 and 1.9 times lower than in healthy controls. Thus, in patients with angular form of CL in the blood plasma, a significantly expressed decrease in the synthesis of IL-4 and IFN-γ, high concentrations of pro-inflammatory cytokines, in particular TNF-α, were revealed, which indicates an active inflammatory process and the presence of immune deficiency.

Table 3. Some indicators of cytokines in patients with and ulcerated leishmaniomas (M±m)

Parameters studied	Control group n=20	Patients with and ulcerated leishmaniomas n=42
IL-4 (pg/ml)	1,86 ± 0,13	0,68 ± 0,07**
IL-6 (pg/ml)	150,66 ± 1,66	316,38 ± 2,25**
IL-8 (pg/ml)	52,06 ± 0,88	104,12 ± 1,88**
TNF-α (pg/ml)	14,25 ± 0,55	58,42 ± 1,32**
IFN-α(pg/ml)	44,70 ± 1,77	29,81 ± 1,01**
IFN-γ (pg/ml)	21,35 ± 0,66	7,86 ± 0,43**

Note: p – reliability of data in relation to control at
* - p<0.001

Next, we examined the state of cytokines in patients with and ulcerated leishmaniomas. The results obtained showed (Table 3) that in this group of patients even more significant changes in cytokine levels are observed. Thus, the level of IL-4 in patients with and ulcerated leishmaniomas upon admission in the blood plasma was 2.7 times lower than in the control and amounted to 0.68 ± 0.07 pg/ml versus 1.86 ± 0.13 pg/ml (p <0.001), while IL levels -6, IL-8 and TNF-α were 2.1; 2.0 and 4.1 times significantly higher than in the control group. At the same time, a low level of IFN-γ was observed (2.7 times), amounting to 7.86 ± 0.43 pg/ml (p <0.001) versus 21.35 ± 0.66 pg/ml in the control.

Our data show that in patients with and ulcerated leishmaniomas pronounced inflammatory process is observed, and anti-inflammatory and immune factors are insufficient for a favorable outcome of the disease.

Table 4. Some indicators of cytokines in patients with and ulcerated leishmaniomas, tubercles of infestation and lymphangitis(M±m)

Parameters	Control group n=20	Patients with and ulcerated leishmaniomas with tubercles of inoculation and lymphangitis n=24
IL-4 (pg/ml)	1,86 ± 0,13	0,58 ± 0,11**
IL-6 (pg/ml)	150,66 ± 1,66	331,45 ± 3,44**
IL-8 (pg/ml)	52,06 ± 0,88	109,33 ± 3,45**
TNF-α (pg/ml)	14,25 ± 0,55	65,55 ± 2,42**
IFN-α(pg/ml)	44,70 ± 1,77	27,93 ± 1,52**
IFN-γ (pg/ml)	21,35 ± 0,66	7,15 ±1,67**

Note: p – reliability of data in relation to control at
* - p<0.001

In subsequent studies, we examined the state of cytokine levels in patients with and ulcerated leishmaniomas, tubercles of infestation and lymphangitis. As can be seen, in patients with and ulcerated leishmaniomas, tubercles of infestation and lymphangitis cute changes in cytokine levels were revealed (Table 4). Thus, the level of IL-4 was reduced by more than 3.2 times, amounting to 0.58 ± 0.11 pg/ml versus 1.86 ± 0.13 pg/ml (p <0.001) in healthy controls. Also, initially low values were characteristic of IFN-γ and IFN-α cytokines within reliable limits (2.98 and 1.6 times, respectively, relative to control values). On the contrary, the levels of anti-inflammatory cytokines increased significantly (p<0.001), IL-6 by 2.2 times and IL-8 by 2.1 times relative to the control. Noteworthy is the pronounced increase in TNF-α synthesis (4.6 times), amounting to 65.55 ± 2.42 pg/ml (p <0.001) versus 14.25 ± 0.55 pg/ml in the control.

It should be noted that the identified changes in cytokine levels in patients in this group were more pronounced than in patients with angular form of CL and ulcerated leishmaniomas.

The next stage of our research was to study the state of cytokine indicators in patients with CL, depending on how long the pathological process has existed.

The results of the study showed (Table 5) that in patients, regardless of how long the pathological process had existed in all examined groups, there was a significant increase in the concentration of all pro-inflammatory cytokines in the blood plasma. Thus, the level of IL-6 in patients with the disease duration from 7 months to 1 year was increased by 2.2 times, and with a prescription of more than 1 year, by 2.3 times relative to the control. The same trend was observed for IL-8 levels. However, the level of TNF-α turned out to be significantly high when the disease duration was from 7 months to 1 year and more than 1 year, amounting to 48.45 ± 2.45 pg/ml and 51.3 ± 3.62 pg/ml (p< 0.001) respectively. The level of IL-4 cytokine 2.8 was lower in the group with a disease duration of more than 1 year than in the control group and amounted to 0.66 ± 0.18 pg/ml (p <0.001) versus 1.86 ± 0.13 pg/ml ml in control. The values of IFN-α and IFN-γ also remained low in the group of patients with dermatosis lasting more than 1 year, amounting to 26.29±2.34 pg/ml (p<0.001)

and 8.21±2.57 pg/ml (p <0.001) respectively. As can be seen, the identified changes in cytokine levels were more pronounced in patients with a disease duration of more than 1 year.

4. Discussion

Thus, analysis of the data obtained shows that in cutaneous leishmaniasis, changes in the content of cytokines are detected, expressed by a deficiency in the blood plasma concentration of the anti-inflammatory cytokine IL-4 and an increase in the content of proinflammatory cytokines IL-6, IL-8 and TNF-α, which are directly related on the clinical form and duration of the disease.

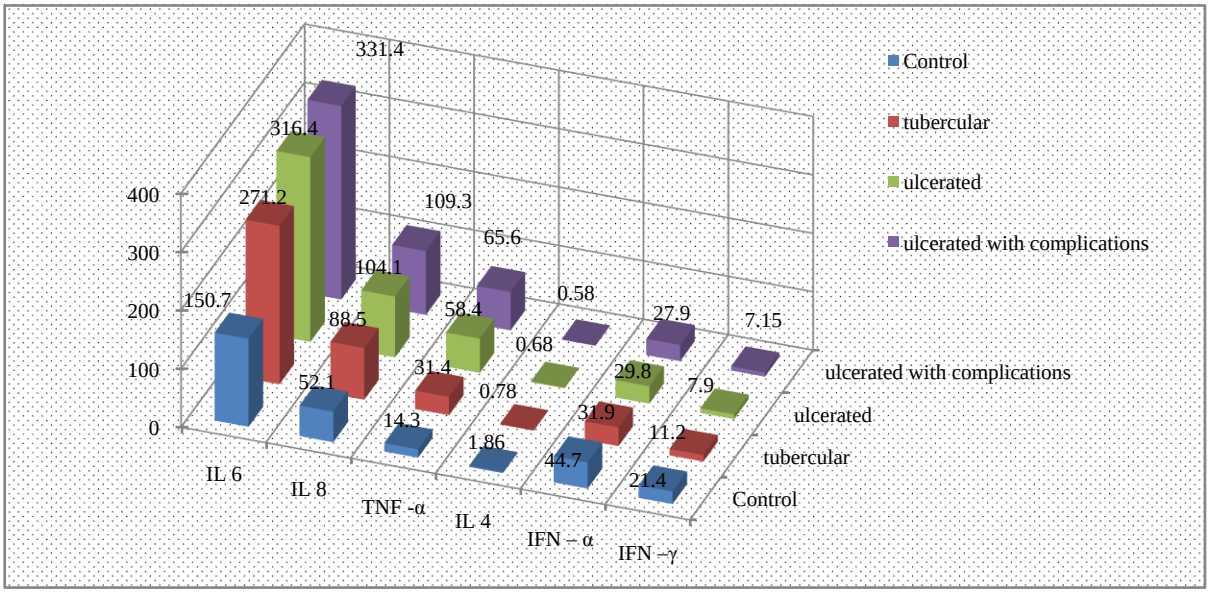
Analyzing the results obtained, the following should be noted. Cytokines, key modulators of inflammation, are produced in response to invading pathogens by stimulating, recruiting, and proliferating immune cells. Proinflammatory cytokines are secreted by CD4+ T cells, macrophages and dendritic cells and are characterized by the production of several interleukins, in particular the one we studied TNF-α, IL-6, IL-8, IFN-γ and IFN-α (last the cytokine is involved in both pro- and anti-inflammatory processes). These cytokines regulate the growth, activation and differentiation of immune cells, as well as the direction of immune cells to foci of infection in order to control and destroy the pathogen. In our studies, elevated levels of cytokines, in particular TNF-α indicates the development of a powerful inflammatory response in the body in response to the introduction of Leishmania. In our opinion a high level of these cytokines is a reflection of the activity and severity of the pathological process. Moreover, in our case, with complicated clinical forms of CL, the levels of these cytokines were significantly high (Pic 1).

Anti-inflammatory cytokines, in particular the one we are studyingIL-4, regulates specific immune responses and helps limit the development of inflammation. In our studies, this cytokine was secreted in quantities insufficient to relieve the inflammatory response. Moreover, the same trend was observed: the level of this cytokine was lower, the more complex and prolonged the course of cutaneous leishmaniasis, that is, it depended on the clinical form of CL.

Table 5. Some cytokine indicators in patients with CL depending on the duration of the disease (M±m)

Parameters	Control group n=20	Duration of CL disease		
		up to 6 months n=26	from 7 months up to 1 year n=17	more than 1 year n=8
IL-4 (pg/ml)	1,86 ± 0,13	1,03± 0,79**	0,81± 0,18**	0,66 ± 0,18**
IL-6 (pg/ml)	150,66 ± 1,66	286,25 ± 2,34**	331,45 ± 2,43**	346,51 ± 2,17**
IL-8 (pg/ml)	52,06 ± 0,88	93,71 ± 2,37**	104,12 ± 2,29**	114,53 ± 3,62**
TNF-α (pg/ml)	14,25 ± 0,55	35,62 ± 2,78**	48,45 ± 2,45**	51,3 ± 3,62**
IFN-α(pg/ml)	44,70 ± 1,77	31,92 ± 3,17*	29,80 ± 3,16**	26,29 ± 2,34**
IFN-γ (pg/ml)	21,35 ± 0,66	10,67 ±2,14**	9,28 ±2,45**	8,21 ±2,57**

Note: p – reliability of data in relation to control at
* - p<0.01; ** - p<0.001



Picture 1. Blood cytokine levels in patients with various clinical forms of cutaneous leishmaniasis

It is known that an acute inflammatory reaction is characterized by a rapid onset and short duration. If the agent that caused the inflammation is not removed, the inflammatory process continues and becomes chronic, leading to significant tissue destruction and the development of complications. Moreover, in case of failure of local protective reactions, that is, at the site of introduction of leishmania, the inflammatory reaction continues to develop, the synthesis of cytokines increases, they enter the circulation, and their action manifests itself at the systemic level, which leads to the development of a systemic inflammatory reaction. In cutaneous leishmaniasis, the pathological process is characterized by a long course, which also depends on the immunological reactivity of the patient, in particular, on the insufficient activity of cellular immunity, and, accordingly, the immune response in various clinical forms of CL.

5. Conclusions

1. The variation in the concentration of pro- and anti-inflammatory cytokines in different clinical forms of CL is quite understandable and can be considered as a result of the course of the inflammatory process caused by Leishmania, depending on the severity of the course, the development of complicated forms and the duration of the onset of the disease.
2. Our data indicate the important regulatory role of cytokines in the manifestation of the immune response to leishmania and serve as a rationale for immunomodulatory therapy, as well as rational and effective eliminating antibiotic therapy.

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