Gene expression of some cytokines in patients with Cutaneous leishmaniasis in Al-Diwanyah province

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Abstract. The current study was conducted in the Department of Biology - Faculty of Education for Girls - University of Kufa for the period from March 2018 to August 2018, which aims to detect the genetic expression of the mRNA of some of the cytokines in the peripheral blood of patients with cutaneous leishmaniasis using RT-qPCR technique. The results showed that the relative quantification of gene expression of mRNA for IFN-γ, IL-4, IL-10 and TNF-α in the blood of patients with cutaneous leishmaniasis compared to control. The highest amount was TNF-α, followed by IFN-γ, IL-10 and IL-4, which was 15.9±4.9, 10.5±4.6, 7.6±3.7 and 3.7±1.1 respectively, compared with control of 2.3±1.2, 2.6±1.2, 2.6±1.2 and 1.9±1.3 respectively, with a statistically significant difference at P <0.05. The correlation results showed that all cytokines (IFN-γ, IL-4, IL-10, TNF-α) were significantly correlated with each other in terms of gene expression and the association was strong between IFN-γ and IL-10 (r = 0.88), followed by IFN-γ and TNF-α (r = 0.75) and IL-10 and TNF-α, (r = 0.63) and then IL-4 and IL-10 (r = 0.54). The conclusion from this study that a cellular immune response is achieved by increasing the genetic expression of cytokines, IFN-γ, IL-4, IL-10 and TNF-α in the peripheral blood of patients with cutaneous leishmaniasis and all these cytokines are positively correlated with each other.

Keywords: gene expression ; IFN-γ ; IL-4 ; IL-10 ; TNF-α ; Cutaneous leishmaniasis

Introduction

Cutaneous Leishmaniasis is an epidemic disease caused by two types of Leishmania parasites Leishmania tropica and Leishmania major, which differ from each other in the clinical and immunological aspects. The disease is described in the Middle East and Iraq [1]. The incidence of the disease has increased in the last few decades as a result of human migration, deforestation and adaptation of leishmania parasites to new vertebrate hosts and vectors [2]. Leishmaniasis has a global prevalence of 12 million cases with nearly 2 million new cases each year (1.5 million cases of cutaneous leishmaniasis and 500 000 cases of visceral leishmaniasis). The disease occurs in 88 countries of Europe, Asia and America. There are 350 million people at risk of leishmaniasis [3,4,1]. The parasites that cause the disease are transmitted by the phlebotomus sand fly, where the parasite is injected into the skin of the healthy person. The patient is exposed to ulcers in different parts of the skin (face, head, arms, lower extremities, etc.) whose size and distribution depend on the immune conditions and skin sensitivity of the infected person [5,6]. The infection of Leishmania parasites stimulates the genetic expression of a number of cytokines and chemokines [7,8] which have a great benefit for the parasite by mobilizing cells such as macrophages that grow and multiply parasites.
within them [9]. Macrophages play an important role in the presentation of parasite antigens into T-helper cells, which in turn is subdivided into Th1, which is important in resistance to infection through the release of IFN-γ and IL-12, and Th2 that respond to sensitive hosts by producing IL-4 and IL-10 [10]. Thus, cytokines and chemokines play a key role in shaping the nature of the immune response of a host infected with Leishmania parasites [11,12]. Due to the lack of studies on the gene expression of some Leishmania-related cytokines, this study aimed at detecting the genetic expression of the mRNA of some cytokines in the peripheral blood in patients with cutaneous leishmaniasis using RT-qPCR technique.

Materials and methods

The current study was carried out on 45 patients with cutaneous leishmaniasis and 25 healthy individuals. The amastigote stages were diagnosed by microscopic examination of the Giemsa-stained specimens under 100X [13]. The Trizol kit equipped by Korean Bioneer Company was used to extract total RNA from 1.5 milliliters from each sample of blood which placed in EDTA-containing tubes. The DNA extract was treated with DNAase I to dispose of the DNA residue in the extraction process and according to instructions attached to the enzyme kit. The concentration and purity of the RNA was measured by the Nanodrop spectrophotometer and stored at -70 °C. The RNA was converted to cDNA by the Accupower Rockscript RT Premix kit, which is supplied by Korean Bioneer Company. This method was done in accordance with the instructions attached to the kit. The primers used in this study were designed according to NCBI GenBank Data and using Primer3 plus. This primers are equipped by Korean Bioneer Company as in Table (1).

Table (1) The primers used in the study

| Primers | Sequence | Amplicon |
|---------|----------|----------|
| TNF-α   | F AAGTGCTGGCAACCACCTAAG | 132bp    |
|         | R TCAAGTCCTGCAGCATATTCTG |          |
| IFN-γ   | F TCCTTTGGACCTGATCAGCTTG | 124bp    |
|         | R AACCCAAAACGATGCAGAGC    |          |
| IL-10   | F ACATCAAGGCAGCATGGAAC   | 106bp    |
|         | R ACGGCCTTGTCTTGTCTTTC |          |
| IL-4    | F TTTGCTGCCTCCAAGACAC    | 149bp    |
|         | R AATCGGATCAGCCTGTGTC    |          |
| GAPDH   | F AATTCATGGCACCCTGCAAG   | 104bp    |
|         | R ATCGCCCCACTHTTGTG     |          |

The RT-qPCR was tested for patients and healthy samples using the Accupower 2x Green Star qPCR kit supplied by Korean Bioneer Company according to the instructions attached with the kit. The relative quantification of gene expression of IFN-γ, IL-4, IL-10, TNF-α was calculated using the CT method using a reference gene, as in the following equation:

\[
\text{Ratio (reference/target)} = 2^{\Delta \text{CT(reference)} - \text{CT(target)}}
\]

Where, CT = Threshold Cycle
Reference = Standard gene
Target = Target gene (cytokine gene)

statistical analysis
The results were statistically analyzed using SPSS version 22, where t-test was used to determine
the significant differences between patients and healthy at P <0.05 [14].

Results and Discussion
The results of the gene expression were shown using RT-qPCR, Increase the relative quantification of
gene expression of cytokines, IFN-γ , IL-4, IL-10, TNF-α in patients with cutaneous leishmaniasis ,
compared to healthy individuals and the highest amount of TNF-α, followed by IFN-γ, IL-10 and then IL-4, which was 15.9±4.9, 10.5±4.6, 7.6 ± 3.7 and 3.7±1.1, respectively, compared to healthy individuals of 2.3±1.2, 2.6±1.2 , 2.6±1.2 and 1.9±1.3 respectively, with a statistically significant
difference at P <0.05, as shown in Table (2).

The results of the current study in Table (3) showed that all cytokines (IFN-γ, IL-4, IL-10, TNF-α) were significantly correlated with each other in terms of relative quantification of gene expression. The correlation was strong between IFN-γ and IL-10, (r = 0.88) followed by IFN-γ and TNF-α, (r = 0.75) and IL-10 and TNF-α, (r = 0.63) and then IL-4 and IL-10, (r = 0.54).

Table (2) Relative quantification of gene expression of cytokines, IFN-γ, IL-4, IL-10, TNF-α in Patients with cutaneous Leishmaniasis and control

| Cytokines | Healthy M±SD | Patients M±SD | Calculated t | Table t | Result of statistical analysis |
|-----------|--------------|---------------|--------------|---------|-------------------------------|
| IFN-γ     | 2.6±1.2      | 10.5±4.6      | 8.1          | 1.98    | Significant differences at P <0.05 |
| IL-4      | 1.9±1.3      | 3.7±1.1       | 6.2          | 1.98    |                                |
| IL-10     | 2.6±1.2      | 7.6 ± 3.7     | 3.16         | 1.98    |                                |
| TNF-α     | 2.3±1.2      | 15.9±4.9      | 13.5         | 1.98    |                                |

Table (3) The correlation coefficient (r) between the relative quantification of genetic expression of IFN-γ, IL-4, IL-10 and TNF-α in patients with cutaneous leishmaniasis

| Cytokines | IFN-γ | IL-4 | IL-10 | TNF-α |
|-----------|-------|------|-------|-------|
| IFN-γ     | -     | -    | -     | -     |
| IL-4      | 0.24  | -    | -     | -     |
| IL-10     | 0.88  | 0.54 | -     | -     |
| TNF-α     | 0.75  | 0.31 | 0.63  | -     |

T-cell-mediated immunity in patients with cutaneous leishmaniasis is an important study as a result of the urgent need to find new ways to prevent and treat this disease quickly. The analysis of gene expression by RT-qPCR technique is a modern method for determining the immune mediators (cytokines) of a disease because of its sensitivity and high accuracy [15]. [16] found the genetic expression of cytokines, IFN-γ, IL-10, TNF-α in all skin lesion samples in patients with cutaneous leishmaniasis and increased gene expression of IL-10, TNF-α in late skin lesions compared to early skin lesions. Because of its inhibitory effects of macrophages, IL-10 has an important role in the immunopathology of chronic leishmaniasis. [17] studied the localized immune response in skin lesions in patients with L. tropica by determining a relationship between the number of parasites and the genetic expression of IL-4 in both early and late skin lesions. The increase in gene expression of IL-4 was associated with the large number of parasites in early skin lesions and concluded that this cytokine has an important role in the pathogenesis of cutaneous leishmaniasis by inhibiting the protective
immune response [18] used RT-qPCR technique to measure the relative quantification of IFN-γ, IL-4, IL-10, and TNF-α in biopsy samples from patients with cutaneous leishmaniasis in Sri Lanka, found increased gene expression of IFN-γ, produced from TH1 cells while the relative amount of gene expression of IL-4 produced from TH2 cells, decreased in patients compared to healthy control. Also there was an increase in gene expression of IFN-γ and TNF-α in skin lesions in the late period of the infection, while IL-4 and IL-10 remained prevalent in skin lesions that did not respond to treatment, [15] studied the genetic expression of IFN-γ and IL-10 in biopsy specimens for patients with American leishmaniasis, there was an increase in the relative amount of these cytokines compared with non-infected individuals.

The results of the present study agreed with the results of a number of studies that found a positive and strong relationship between the amount of gene expression of IFN-γ and IL-10 and that these cytokines had contrasting effects on host response against intracellular pathogens [19,20]. This explains the balance between pro-inflammatory cytokines and immune regulation response in patients with cutaneous leishmaniasis [18]. The findings of the present study, together with the results of a number of studies, confirmed that there is a positive and significant correlation between IFN-γ and TNF-α, which work together to activate the macrophages to kill the parasites present within them by stimulating them to produce nitric oxide (NO) [20,18]. The results of the present study were also concurred with the results of the [20], which found significant correlation between gene expression of IL-10 and TNF-α. A positive correlation was observed between gene expression of IL-4 and the time taken by the lesions to healed, which indicates the role of IL-4 in interference in the healing of skin lesions. The IL-10 also possesses anti-inflammatory properties and is expressed with IL-4, demonstrating an important positive relationship between these two cytokines [21,12]. In the same vein, previous studies have described the high genetic expression of IL-10 in skin lesions that are slowly cured in patients with cutaneous leishmaniasis caused by L. major [22], and promotes the continuation of the disease in people infected with L. Mexicana [23]. The conclusion from this study that a cellular immune response is achieved by increasing the genetic expression of cytokines, IFN-γ, IL-4, IL-10 and TNF-α in the peripheral blood of patients with cutaneous leishmaniasis and all these cytokines are positively correlated with each other.

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