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Effect of *NRAMP1* gene polymorphism on levels of (TNF-α1 and IL-1β) cytokines in cutaneous Leishmaniasis patients in Iraq

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Abstract

Background: Cutaneous leishmaniasis (CL) is vector-borne disease. and endemics in most regions of Iraq especially with poor populations. "*Natural resistance-associated macrophage protein 1* (*NRAMP1*)" gene play an essential role in susceptibility to CL and disease pathology, NRAMP1 influences a production and activation of pro-inflammatory cytokines like IL-1β and TNF-α. Pro- and anti-inflammatory cytokines play an essential role in susceptibility/resistance and the immunopathogenesis of Leishmania infection. These cytokines are crucial factors in the initiation and enhances of protective immunity against Leishmania infection. This study aimed to determine effect of polymorphism in *NRAMP1* genes on cytokines secretion and their effect in susceptibility to CL infection.

Materials and Methods: Samples of blood were collected from patients (n: 60) with CL and apparently healthy controls (n: 32). Polymorphism of *Nramp1* (*D543N*) detected by PCR-RFLP technique in patients and control groups while (TNF-α and IL-1β) cytokine concentrations detected by ELISA technique using a quantitative sandwich enzyme immunoassay technique.

Results: Results indicate to effect of *Nramp1* gene polymorphism on levels of (IL-1β and TNF-α) cytokines and this a clearly recorded in present study were A allele is associated with lower levels of (TNF-α and IL-1β) in patients and control groups compression to that absorbed in allele G with statically significant ($p \leq 0.05$).

Conclusions: Cytokines (IL-1β and TNF-α) plays an essential role in the resolution of CL infection, were its concentration in patients serum of all age groups were significant increase in comparison to that observed in their control groups. In polymorphisms of *Nramp1* (*D543N*) gene, were A allele is associated with lower levels of (IL-1β and TNF-α) compression to that absorbed in allele G, and this decreased production may be associated with susceptibility and proliferation of parasites in the macrophage.

Key words: *Cutaneous leishmaniasis, NRAMP1polymorphisms, TNF-α and IL-1β, Cytokine*

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Introduction

Cutaneous leishmaniasis (CL) is a parasitic disease transmitted by sand flies, and caused by obligate intra-macrophage protozoa, characteristic by a spectrum of cutaneous, mucocutaneous and visceral diseases that depend largely on the species of the parasite involved and host immune response (1, 2). CL is the most common form of leishmaniasis, with about 1.5 million cases every year, and about 50 to 70% of all cases in the world (2, 3). "*Natural resistance-associated macrophage protein 1* (*NRAMP1*)" gene is a member of the solute carrier family 11 (proton-coupled divalent metal ion transporter), member (A1) SLC11A1 (4, 5). "Homologs of natural resistance-associated macrophage protein (NRAMP) or solute carrier 11 (SLC11), conserved in eukaryotes and bacteria, form a family of proton-coupled transporters that maintain divalent metal (Me^{2+}, including Mn^{2+}, Fe^{2+}, Co^{2+}, and Cd^{2+}) homeostasis (6, 7). There are two *Nramp* genes that are associated with diseases in vertebrates, an *Nramp1* (also called SLC11A1), in humans, *Nramp1* gene is located in the chromosome region 2q35, containing 16 exons (8). NRAMP1 is protein transport divalent metal ions through the phagosomal membrane and might be an essential factor for resistance to some microbial infections. *Nramp1* gene plays an essential role in activation of the macrophage pathway. It has many effects on macrophages function as regulation of the CXC chemokine KC, synthesis and activation of pro-inflammatory cytokines as human "Tumor Necrosis Factor-alpha" and human Interleukin-1 beta (7, 9). During an intracellular infection, NRAMP1 protein transports essential elements (Mn^{2+}, Fe^{2+}, Co^{2+}) vital for the survival of the parasite, from the phagolysosome into the cytosol and hence starving and restricting their growth (10). Pro-inflammatory cytokine (TNF-α) primary produce by mononuclear phagocyte, fibroblast, B and T cell, macrophages participate in production of TNF- α, T cell induce macrophages to produce nitric oxide (NO), which cause control or killing parasites, TNF- α that secretion by macrophages also mediate in secretion of nitric oxide as well as activation of macrophages and parasite killing (11). IL-1β is primarily produced by several cells include the monocytes, mononuclear endothelial, keratinocytes, astrocytes, synovial cells, glial cells, osteoblasts, neutrophils, and numerous other cells, there are variant agent like endotoxins, microorganisms, antigens as well as other cytokines which mediate stimulation of IL-1β production, which contributes to the immunopathology effects observed in cutaneous leishmaniasis patients (12, 13).

Material and methods

Subjects and study design

A total 32 apparently healthy people and 60 patients with CL were included in this study during the period between February / 2017 to April/ 2017 in the out-patients clinic of the dermatology department in Al-Hussein Teaching hospital and specialized center of sensitivity in Al-Muthanna Province in Iraq. Cases diagnosed clinically by a special dermatologist as cutaneous leishmaniasis and confirmed as CL patients based on clinical symptoms and parasitological parameters (14).

Nramp1 (*D543N*) Typing

Genomic DNA from blood samples was extracted by using Geneaid DNA extraction kit (Whole Blood), according to the manufacturers' instructions. Polymerase chain reaction was used to amplify a 244 bp fragment. The forward primer was 5-ACT-AAGAAA-GAC-CCG-AGG-C-3 and the reverse primer was 5'-GGG-GCA-CGT-TGG-TGTTTA-C-3. The annealing temperature used was 58°C. Then REFLP-PCR master mix did according to instructions of the company (Biolabs/U.K). The PCR products were digested with Ava ll restriction endonuclease. After that, REFLP-PCR product was analyzed by electrophoresis (2.5%) agarose gel, there is three genotypes observed; GG, GA, and AA with band size 126/79/39 pb, 205/ 126/79/39 pb, and 205/39 pb respectively.

Determination of cytokines

Three milliliters of blood in the plain tube (serum tube), then the blood samples were centrifuged at (4700 rpm for 5 min) to obtain blood serum then frozen at -20 ºC until the time of test. Serum TNF-alpha and IL1-beta cytokines levels were identified by ELISA technique using a quantitative sandwich enzyme immunoassay technique (EASIA kits for TNF-α and IL-1β by PeproTech Company/Germany). All tests were done according to company's instruction. The results calculated by ELISA reader (optical density at 405nm immediately) and applied on a standard curve in order to sort out the cytokines concentration.

Statistical analysis

Statistical analysis was conducted by using SPSS version 23. Determine the statistical differences among different groups and associations between allelic and genotypes of *Nramp1* gene was performed by using the Pearson Chi-square ($\chi 2$) test and mean cytokine concentration were compared between groups using t-test (15). The probability of ($p \leq 0.05$) was considered to be statistically significant.

Results

Distribution of *Nramp1* (D543N) polymorphism was detected by PCR-RFLP technique, at this locus there is three genotypes; GG, GA and AA with band sizes 126/79/39 pb, 205/ 126/79/39 pb and 205/39 pb respectively. Allele GG was 44 (73.30%) in patients and 18 (56.25%) in control with (p=0.096), Allele GA was 14 (23.3%) in patients and 8 (25%) in control with (p=0.858), and Allele AA was 2 (3.3%) in patients and 6 (18.75%) in control with (p=0.012), in other hand Allele G was (85%) in in patients and (68.75%) in control with (p=0.01), and Allele A was (15%) in patients and (31.25%) in control with (p=0.01).

Figure 1 shows the mean TNF- α interleukin was significant increase in patients group in comparison to control subjects (2.698+0.122ng/ml) versus (0.414+0.015 ng/ml) respectively (p=0.000).

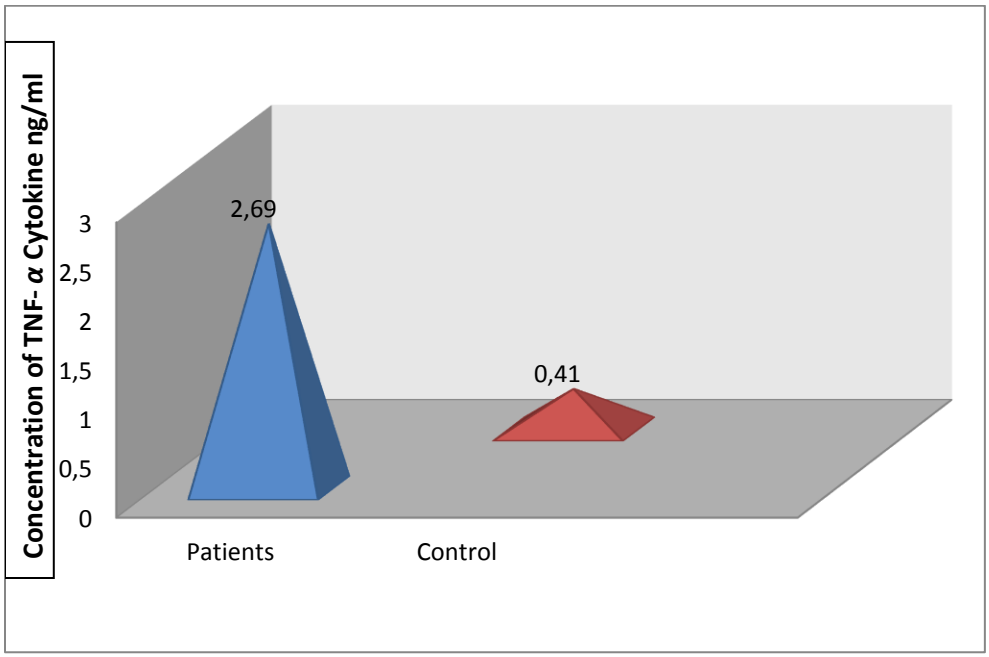

Figure 1. Comparison of mean serum TNF- α cytokine between patient and control groups.

Figure 2 shows the mean IL-1β interleukin was significant increase in patients group in comparison to control subjects (0.814+ 0.054 ng/ml) versus (0.482+0.020 ng/ml) respectively (p=0.000).

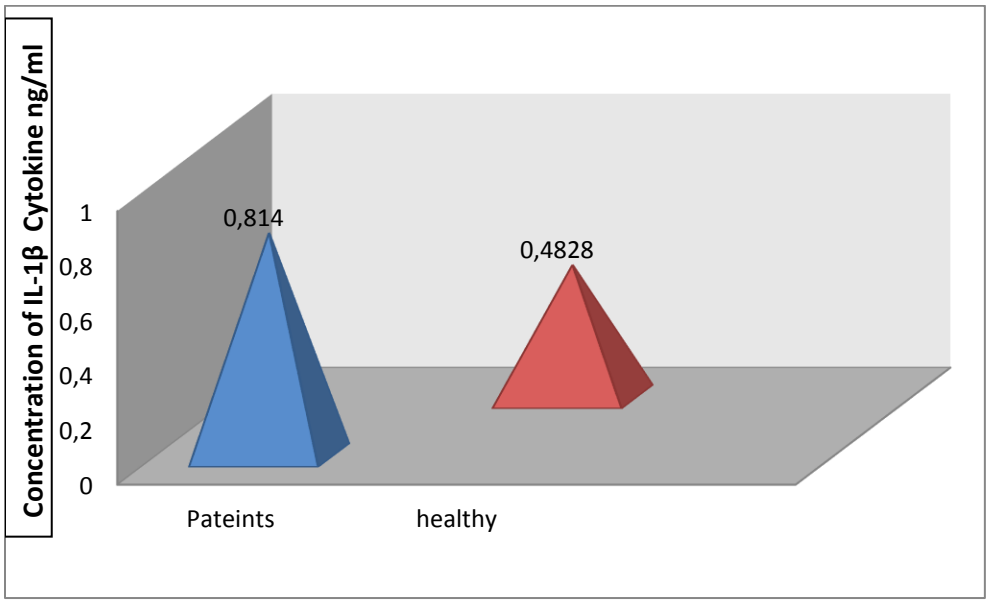

Figure 2. Comparison of concentration mean of IL-1β cytokine between patient and control groups.

Figure 3 shows the mean TNF-α cytokine concentration according to genotype in *Nramp1* gene. It was found that the mean of TNF-α cytokine level decreased in (*D543N*) A allele in patients groups (2.527±0.104 ng/ml) compression with G allele (2.761±0.162 ng/ml) in patient group (p≤0.05), also it decreased in (*D543N*) A allele

in control groups (0.402±0.0262 ng/ml) compression with observed in G allele (0.430±0.019 4ng/ml) in control group (p≤0.05).

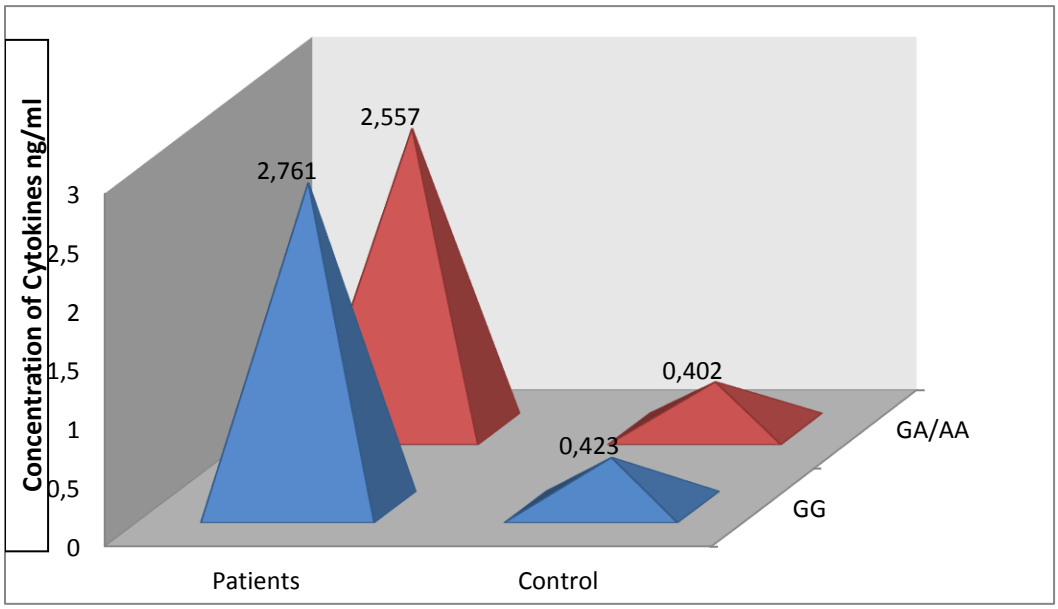

Figure 3. Correlation between *Nramp1* (*D543N*) genotype and serum TNF-α in patient and control groups.

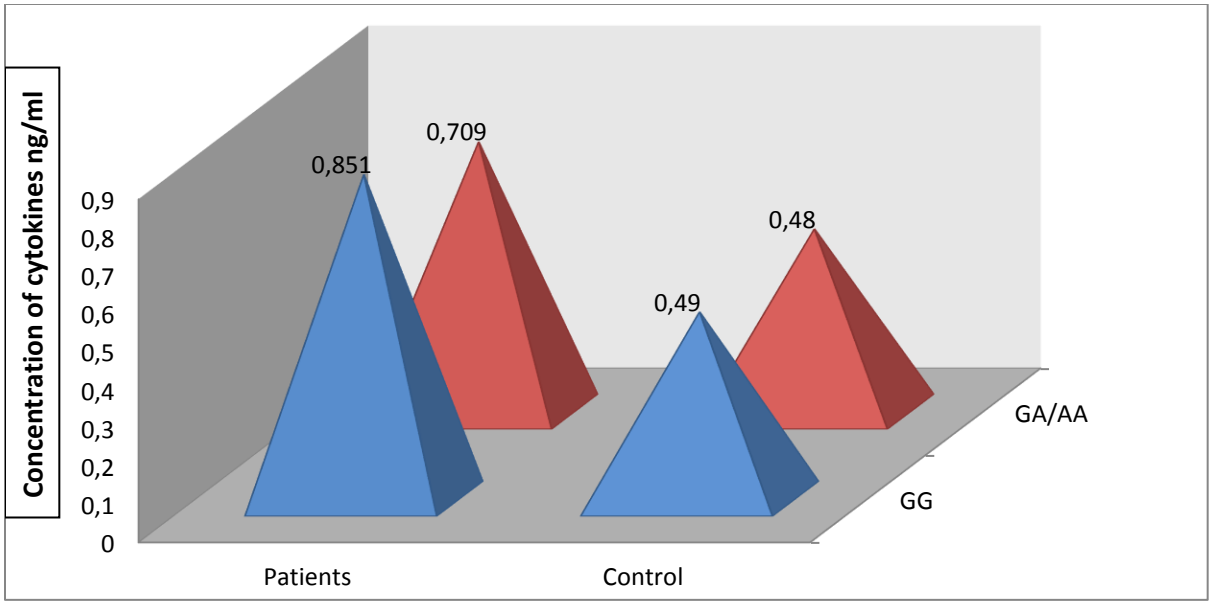

Figure 4. Correlation between *Nramp1* (*D543N*) genotype and serum IL-1β in patients and control groups.

Figure 4 shows the mean IL-1β cytokine concentration according to genotype in *Nramp1* gene. It was found that the mean of IL-1β cytokine level decreased in (D543N) A allele in patients groups (0.709±0.64 ng/ml) compression with G allele (0.852±0.069 ng/ml) in patient group (p≤0.05), also it decreased in (*D543N*) A allele

in control groups (0.468±0.028 ng/ml) compression with observed in G allele (0.490±0.030 ng/ml) in control group ($p \leq 0.05$).

Discussion

Present data revealed that TNF- α concentration was a significant increase in patients group in comparison to control group, Figure 1, "this increased expression in cytokine levels might be due to an increase of cellular activation or a relative increase in the number of cytokine-producing cells". The current study finding was agreement with other studies in in Iraq (16) and Turkey (17), the reason in higher concentration of TNF-α in serum of CL patients may be duo to responsive to treatment with sodium stibogluconate (pentostam), suggestion generally by that pentostam induce cytokines to activate macrophages (18).

Through present study were found that, the mean concentration of total IL-1β in all CL patients were significant increase in comparison to that observed in their control groups, figure 2, the current result finding was agreement with other studies in Iraq (16) and Turkey (17) were found serum levels of (IL-1β) was significant increase in patients group in comparison to control group, as well as (19) which found that IL-1β concentration in patients infected with Leishmania donovan more than control groups, this result may be due to stimulation essential immunological component in response to pentavalent antimonial (20,21). While, some studies refer to that cytokine secretion important in process of healing following treatment with pentostam (22,23).

In a resting macrophage, *Nramp1* gene encoded to protein which assembled into the membrane of late endosome, were phagocytosis it is relocated to the membrane of phagosome (24,25). NRAMP1 protein transport divalent metal ions through the phagosomal membrane and might be an essential factor for resistance to some microbial infections, NRAMP1 induce a variant types of antimicrobial responses of a macrophage, including induction of nitric oxide intermediates and radical oxygen, synthesis and activation of various pro-inflammatory cytokines such as (TNF-α and IL-1β) (7,26).

However, when mutations occur in the *Nramp1*gene result in a non-functional or unstable protein and then leading to an increased proliferation of parasites in the macrophage might be the reason by deficient antimicrobial responses that confer by NRAMP1 protein (27-29). In current study were result showed that allele A was able to induce less TNF-α secretion in patients and control groups compression with allele G, Figure 3, in another hand when mean serum IL-1β was studied in relation to allele A, there was decrease in mean serum IL-1β compression to that absorbed in allele G in patients and control group, figure 4, were unstable NRAMP1protein because mutation results in less expression in (TNF-α and IL-1β) and resulting in an increased susceptibility and proliferation of parasites in the macrophage, this result agreement with other studies on CL (27, 28).

Conclusion

Cytokines (IL-1β and TNF-α) plays an essential role in the resolution of CL infection, were its concentration in patients serum of all age groups were significant

increase in comparison to that observed in their control groups. In polymorphisms of *Nramp1* (*D543N*) gene, were A allele is associated with lower levels of (IL-1β and TNF-α) compression to that absorbed in allele G, and this decreased production may be associated with susceptibility and proliferation of parasites in the macrophage.

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