



Evaluation of IFN-Gamma and HSP70 Level in the Saliva of Behcet's Disease Patients With Active Oral Lesions

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Abstract

Introduction: Behcet's disease (BD) is a multi-systemic inflammatory disorder. Evaluating the production of cytokines such as interferon gamma and biomarkers such as heat shock protein-70 (HSP70) is an important way to study the pathogenesis and development of BD. This study aimed to compare the salivary level of interferon gamma and HSP70 between patients infected with BD and healthy individual.

Methods: This case-control study was performed on 35 patients with Behcet's syndrome and 70 healthy individuals as the control group, who were selected from those referring to the Department of Oral Medicine of Tabriz University of Medical Sciences. The levels of interferon gamma and HSP70 were measured in the whole unstimulated saliva through enzyme-linked immunosorbent assay (ELISA). In order to compare the quantitative variables between two groups, independent samples *t*-test or its nonparametric equivalent, Mann-Whitney U test, was used in SPSS software version 16.0. In this study, a *P* value less than 0.05 was considered statistically significant.

Results: There was no significant difference between the study groups in terms of age and gender, as well as salivary interferon gamma and HSP70 levels. Interferon gamma level was 15.16 ± 3.38 pg/mg in the case group and 5.27 ± 1.21 pg/mg in the control group, and salivary HSP70 level was found to be 45.50 ± 17 ng/mL and 19.5 ± 5.2 ng/mL in the case and control groups, respectively.

Conclusions: The results of this study showed that interferon gamma and HSP70 levels in patients with Behcet's syndrome are high and can be evaluated as an important tool for the treatment and evaluation of disease development in future studies.

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Keywords: Behcet's disease, Interferon gamma, HSP70, Saliva

Received Dec. 5 2019
Accepted Dec. 15 2019
Published Dec. 30 2019



Citation: Eslami H, Alizadeh Ghavidel L, Khabbazi A, Dolatkah H, Bonyadi M, Nasiri K, Bonyadi M, Khademnezhad S. Evaluation of IFN-Gamma and HSP70 Level in the Saliva of Behcet's Disease Patients With Active Oral Lesions Avicenna J Dent Res. 2019;11(4):120-124. doi: 10.34172/ajdr.2019.24.

Introduction

Behcet's disease (BD) is a multi-systemic inflammatory disorder with skin, mucosal, articular, vascular, and central nervous system involvement. BD is diagnosed based on the clinical symptoms, using the international criteria for BD (1). BD is a systemic vasculitis, and pro-inflammatory cytokines of TNF- α , IL-17, and IL-8 play important roles in the pathogenesis of the disease (2).

T helper cells (Th cells) are divided into two different subsets (Th1 and Th2) based on cytokine production (3,4). These two subsets of Th cells regulate their function through the antagonistic activity of their related cytokines that determine characteristics of the immune system (5,6).

It has been shown that the imbalance of Th1 and Th2 cells play an important role in the pathogenesis of many kinds of autoimmune diseases such as BD (7,8).

Highlights

- ▶ Evaluating the production of cytokines such as interferon gamma is an important tool in examining immune responses to stimuli such as pathogens and immune challenges, evaluating the process of pathogenesis, and following the disease progress. To date, no similar study has been done in Iran and on the population of the northwestern region of Iran, which is different from the studied populations in terms of genetic and geographic variations.

Interferon gamma is a dimerized soluble cytokine that is also called a macrophage activator. This cytokine is crucial for inherent and acquired immunity against intracellular bacteria and for tumor control (9).

Biologically, IFN- γ increases the activity of natural killer cells (NK), lysosomal macrophage function, leukocyte cell adhesion during intracellular migration,

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and intracellular defense factors (10).

Although many efforts have been made to clarify the role of cytokines in pathogenesis and inflammatory conditions of BD, the etiopathogenesis of the disease remains unknown (11,12).

Patients with BD show an increase in the serum levels of interferon gamma, and blood lymphocytes of these patients produce large amounts of interferon gamma; however, the cellular mechanism responsible for producing high levels of cytokines is still unclear (13,14).

Nowadays, cytokines have found their way to the treatment stage and have attracted the attention of many scientists (15)

Heat shock proteins (HSPs) are proteins that are expressed in the cell in stressful conditions. They prevent the structure of proteins from changing dealing with stressors and play a role in the immune system function. These proteins are divided into five main categories, one of which is HSP-70. According to the studies, HSP70 compared to the other HSPs plays an important role in the pathogenesis of BD. However, the mechanism of its activity is still unclear. The mechanism of pathogenesis based on the theory of molecular-mimicry in Behcet's patients was first introduced by Lehner et al. HSPs (including HSP60) have been suggested as an etiologic agent due to their similarity to the pathogenesis-related proteins in this field (16)

Saliva testing is accepted as a useful component in the diagnosis of systemic diseases, especially oral diseases (17) It has certain advantages over other methods such as serum analysis, which include easy access and non-invasiveness (18)

Evaluating the production of cytokines such as interferon gamma is an important tool in examining immune responses to stimuli such as pathogens and immune challenges, evaluating the process of pathogenesis, and following the disease progress. To date, no similar studies have been done in Iran and on the population living in the northwestern region of Iran, which is different from the studied populations in the previous studies in terms of genetic and geographic variations. Based on the articles obtained from other countries, in which levels of interferon gamma and HSP70 have been measured in the serum of patients, in this study, it was decided to examine the salivary levels of IFN- γ and HSP70 in patients with Behcet's syndrome and healthy individuals.

Materials and Methods

In this case-control study, 35 patients with BD and 70 healthy individuals were studied. The inclusion criteria included: being 20-70 years of age, being newly diagnosed with BD, and not receiving any related treatment. The diagnosis was done based on Iranian rheumatologist's criteria¹⁹ which include aphthous ulcers that at least appear three times within 12 months, recurrent genital ulcers, eye inflammation with blurry vision, skin lesions,

and positive pathergy test. The exclusion criteria included systemic disease and smoking, alcohol abuse, and medicine consumption. All patients with BD who first referred to the rheumatologist and received no medicine for the treatment were included in the study (19).

After obtaining informed consent and preparing a checklist, saliva sampling was carried out following the protocol of Navazesh (20). The participants were asked to avoid brushing, eating, and drinking 2 hours before saliva sampling. Volunteers were asked to wash their mouths 15 minutes before sampling and then their oral cavities were examined. The sample collection was performed in a sitting position from 8 AM to 10 AM and was carried out by spitting method and without chewing. Then, 2 mL of saliva was collected and transferred to the laboratory and stored immediately at -80°C. In order to measure IFN- γ and HSP70 levels, the ELISA kit was used.

In order to compare the quantitative variables between two groups, independent samples *t* test or its nonparametric equivalent, Mann-Whitney U test, was used in SPSS version 16.0. In this study, a *P* value less than 0.05 was considered statistically significant.

Results

Among the participants of the study, 23 men and 12 women with the mean age of 37.7 ± 8.2 were included as a case group and 43 men and 27 women with the mean age of $35 (\pm 9.3)$ were included as a control group. The results of chi-square test showed that there was no statistically significant difference between the case and control groups in terms of gender ($P=0.233$). The independent samples *t* test was used to determine the statistically significant difference in the mean age of participants between the two groups. The significance level of the test was 0.05. The results of this test showed that there was no significant difference between the two groups in terms of the mean age of the participants ($P=0.056$) (Table 1).

In the current study, the results of salivary testing of IFN- γ in the patients and control group showed that the concentration of IFN- γ in the patients and control groups was found to be 15.16 ± 3.38 pg/mg and 5.27 ± 1.21 pg/mg, respectively (Table 2). In order to evaluate the normal

Table 1. Demographic Information of the Participants in the Two Study Groups

| Variables | Case | Control |
|-----------|--------------------|------------------|
| Gender | Male | 12 (34.3%) |
| | Female | 27 (38.6%) |
| | Total | 23 (65.7%) |
| | 35 (100%) | 70 (100%) |
| Age | 37.7 (± 8.2) | 35 (± 9.3) |

Table 2. The Concentration of IFN- γ in the Studied Groups

| Study groups | IFN- γ Concentration (Mean \pm SD) |
|--------------|---|
| Case | 15.16 \pm 3.38 |
| Control | 5.27 \pm 1.21 |

distribution of IFN- γ level, Kolmogorov-Smirnov test was used. The significance level of the test was 0.05. The results of this study showed that the distribution of the mentioned variable was not normal, therefore, in order to compare the mean concentration of IFN- γ in the two groups, a non-parametric test had to be used ($P=0.042$). Mann-Whitney U test was used to examine the significant difference between the two groups in the mean concentration of IFN- γ . The results of the test showed that there is a significant difference between the two groups in the mean concentration of IFN- γ . Moreover, the mean concentration of IFN- γ in the case group was higher than that of the control group ($P<0.001$) (Figure 1).

The results of salivary testing of HSP70 in the patients and control group showed that the concentration of HSP70 in the patients and the control group was found to be 45.50 ± 17 (ng/mL) and 19.5 ± 5.2 (ng/mL), respectively (Table 3).

The results of Mann-Whitney U test showed that the difference between the two groups of patient and control was significant ($P<0.001$). In other words, the salivary HSP70 level in Behcet's patients was higher compared to the control group (Figure 2).

Discussion

BD is a multi-systemic inflammatory disorder with skin,

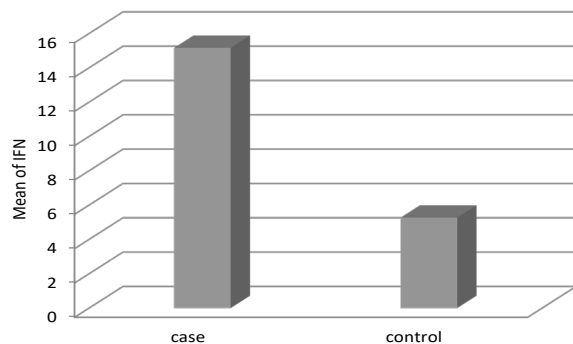


Figure 1. The Comparison of IFN- γ Level in the Studied Groups.

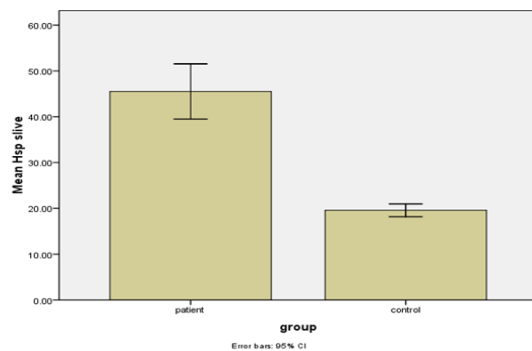


Figure 2. The Comparison of the Salivary HSP70 Levels in Patients and Control Group.

Table 3. The Concentration of HSP in the Studied Groups

| Groups | HSP concentration (ng/mL) (M \pm Sd) |
|----------|--|
| Patients | 45.50 ± 17 |
| Control | 19.5 ± 5.2 |

mucosal, articular, vascular, and central nervous system involvement. Considering the role of cytokines, such as interferon gamma, in immune response and pathogenicity, salivary laboratory test has certain advantages over other methods such as serum analysis, which include easy access and non-invasiveness (17). HSP70 is a type of HSP that may be a pro-inflammatory cytokine and causes tissue damage in this disease. In fact, HSP70 plays a role in the development of oral lesions, especially in BD (21,22).

In the present study, the level of interferon gamma and HSP70 in the saliva of patients with BD was measured and compared with that of healthy individuals.

The results showed that the interferon gamma and HSP70 levels in patients with Behcet's syndrome were high and there was no significant difference between the case and control groups in term of age and gender with high levels of salivary interferon gamma and HSP70.

Sugi-Ikai et al in a study on peripheral blood mononuclear cell (PBMC) in Behcet's patients concluded that the levels of interferon gamma CD4⁺ and CD8⁺ were higher in the case group than in the control group (23).

Belguendouz et al in their study on 42 patients with BD showed that interferon gamma level in the serum of Behcet's patients was higher than that in the control group, indicating a strong correlation between levels of IFN- γ and NO during the active phase of the disease (24).

Djaballah-Ider et al in a study on 34 patients with BD showed that IFN- γ is involved in the inflammatory and pathogenic processes of BD by stimulating the production of NO (25).

Malekzadeh et al investigated the level of salivary interferon gamma in 63 patients with lichen planus, and reported that the level of salivary interferon gamma in the case group was higher compared to the control group and that Th1 could play a significant role in the pathogenesis of oral lichen planus (26).

Touzot et al in a study on peripheral blood mononuclear cells in Behcet's patients using intracellular cytokine staining reported an increase in IFN- γ and IFN- α levels and stated that IFN- α promotes a regulatory Th1 response through IL-10 secretion. This effect may explain the efficacy of this cytokine in inflammatory disease (27).

Liu et al showed a significant positive correlation of cytokine levels between salivary and serum IFN- γ in patients with oral lichen planus (28).

Sahebari et al studied the serum level of HSP70 and its antibody using ELISA method in Behcet's patients with and without uveitis. The results showed that the serum level of HSP70 is higher in Behcet's patients with uveitis than in Behcet's patients without uveitis and idiopathic

uveitis, and it can be concluded that HSP70 is a sensitive indicator for the diagnosis of BD (19).

Birtas-Atesoglu et al examined the serum levels of HSP70 and anti-HSP-70 in patients with BD. The results showed that serum level of HSP-70 was significantly higher in Behçet's patients than in the control group (29).

Finally, Deniz et al in a review on the change in HSP-60 expression in individuals with Behçet's disease with recurrent aphthous ulcer showed that HSP-60 has an association with the etiology or chronicity of these inflammatory lesions in Behçet's patients.

In a study conducted by Mirfeyzi et al in Mashhad, a total of 68 patients with BD (31 with ocular involvement) were studied. It was reported that the serum level of HSP27 (7.7 ± 4.28 ng/mL) was significantly high in Behçet's patients (16).

Chen et al reported the reactivity of serum IgG against recombinant human HSP27 in 52 of 91 patients with BD (57%) (22).

The results of the mentioned studies are in line with the present study and there was no research against the results of the present study. Therefore, based on this study which was performed on the patients' saliva for the first time, which was a non-invasive method compared with other methods, the levels of interferon gamma and HSP70 were higher in patients with BD than in the control group.

According to the studies, interferon gamma and HSP70 play important roles in the pathogenesis of BD. However, their mechanism of action is still unclear. Since interferon gamma cytokines indicate the effectiveness of Th1 and HSP70 in the immune system performance, their high levels in the saliva of patients with BD indicate that there is a logical correlation between the expression of cytokines and salivary and serum HSPs, and the identification of salivary cytokines reflects the systemic expression profile of cytokine. Saliva collection is a cost-effective and non-invasive method for frequent monitoring of the disease as well as screening the large population in the studies of systemic diseases, especially those with oral symptoms.

Conclusions

The results of the present study showed that the level of interferon gamma and HSP70 is high in patients with Behçet's syndrome and can be utilized as an important tool for the treatment and development of disease in future studies.

Conflict of Interest Disclosures

There is no conflict of interests.

Ethical Statement

All the subjects signed informed consent forms. The study protocol was approved by the Ethics Committee of Tabriz University of Medical Sciences (IR.TBZMED.REC.1396,649).

Authors' Contribution

HE: Drafting the work or revising it critically for important intellectual

content; LAG: Preparing the kits and materials for IFN- γ and HSP70 levels; ARK: Evaluation of inclusion and exclusion criteria, Drafting the work or revising it critically for important intellectual content; HD: Perform laboratory steps, Drafting the work or revising it critically for important intellectual content; MB: Perform laboratory steps, Drafting the work or revising it critically for important intellectual content; KN: Evaluation of inclusion and exclusion criteria, Drafting the work or revising it critically for important intellectual content; NB: Salivary sample collection, Drafting the work or revising it critically for important intellectual content; SK: Statistical analysis, Drafting the work or revising it critically for important intellectual content; Final approval of the version to be published had been done by all authors.

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