The Measurement of Serum Tumor Necrosis Factor-alpha Levels in Patients with Lichen Planus

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Abstract

Background: Lichen planus is a common mucocutaneous inflammatory skin disease with a multifactorial etiology. Cytokines have a key role in its pathogenesis. In our study, we aimed to investigate the relationship between the disease severity and levels of the tumor necrosis factor alpha (TNF-α) cytokine which was considered as a primary cytokine that initiates the cytotoxicity. Materials and Methods: A total of 34 patients with lichen planus who were 18 year or older and gender-matched healthy controls were included in the study. Serum TNF-α levels were measured by human TNF-α enzyme-linked immunosorbent assay test kits and the values in the two groups were statistically compared. Results: The mean serum TNF-α levels were higher in the patient group than that in the control group. Serum TNF-α levels were not associated with oral mucosal involvement and gender. However, it was observed that the level of TNF-α was higher in older ages, both in patient and in control groups. Conclusion: It is thought that TNF-α, a proinflammatory cytokine, may have an important role in the pathogenesis of lichen planus.

Key Words: Cytokines, lichen planus, tumor necrosis factor alpha

Introduction

Lichen planus is an itchy papulosquamous dermatosis characterized by typical papules located on the body, oral and genital mucosa and the flexor regions of the extremities. Although the etiology of the disease is not completely known, various theories have been put forward, focusing on endogenous – genetic, immunological, and exogenous – environmental factors (drugs, infection, and psychogenic).[1,2] Recently, however, T-cell-mediated immune response has been shown to play a role in the process. Studies in which monoclonal antibodies are used against T lymphocyte subtypes have added a new dimension to the disease. In early lesions, there are often helper lymphocytes in the dermal infiltrate. Langerhans cells in the infiltrate process the antigen and provide them to Th lymphocytes. Activated T lymphocytes lead more T lymphocytes through tumor necrosis factor alpha (TNF-α), interleukin (IL)-2, and interferon gamma (IFN-γ) and cause cytokine release from keratinocytes.[7] TNF-α is a molecule with 17 kD weight, polypeptide structure, and antitumor immunity. Furthermore, it is a prototype molecule of a family related to central mediator of the acute inflammation.[1] TNF is released from primarily stimulated monocytes and macrophage cells. Langerhans cells and active keratinocytes are other cellular sources of TNF-α. In the trauma area, it leads to adhesion of both neutrophil and endothelial cells and migration of leukocytes.

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In this study, we aimed to compare the TNF-α levels, which were thought to be one of the proinflammatory cytokines that initiated autoimmune cytotoxicity in the etiopathogenesis, between the patients with lichen planus and the healthy control group.

Materials and Methods
A total of 34 clinically and histopathologically proved patients of lichen planus were included in the study. The control group included twenty healthy persons who were randomly selected. An ethical clearance from the institutional review board was obtained and all patients were informed about the study, and their consent was obtained. Detailed health histories of the patients were recorded. Physical examinations were performed. Patients with autoimmune disease, malignancy, and familial lichen planus and patients who used drugs that might cause lichenoid eruption were excluded from the study. The patients were advised not to use topical or systemic corticosteroid or other immunosuppressive treatment until at least 3 months prior to inclusion in the study. Attention was drawn to the fact that participants did not have upper respiratory tract or other infectious diseases until 3 weeks before the selection of the subjects. Whole blood count, sedimentation, and acute phase reactance measurements were done for both the groups.

Serum levels of TNF-α were assessed by the human TNF-α (hTNF-α) enzyme-linked immunosorbent assay (ELISA) test (Biosource International, hTNF-α, Belgium). Blood samples of patients were centrifuged at 3000 rpm for 5–10 min in 10 ml sterile tubes, and the serum was removed and stored at −20°C until the test day. Biosource International hTNF-α kit was a solid-phase sandwich ELISA method in which the antigen was conjugated with the specific antibody for hTNF-α in the first incubation and the biotin-coated antibody was added after washing. In the second incubation, this antibody was bound to the hTNF-α that was detected in the first incubation. After the removal of the excess secondary antibody, streptavidin–peroxidase was added. The four-layered sandwich was completed after the biotin-coated antibody was bound. Substrate solution was added after the third incubation and after removal of the unbound enzymes. The color was obtained after it was bound to the enzyme. The density of the colored product was directly proportional to the concentration of hTNF-α. Then, absorbance levels were read at 450 nm wavelength and the samples were evaluated. The significance of the difference between the averages of the two groups was tested using the Mann–Whitney U test.

Results
The results of the patient group and that of the control group are summarized in Table 1. Of 34 patients with lichen planus, 20 (58.8%) were female and 14 (41.2%) were male. The ages of the patients ranged from 18 to 82 years. Of 20 subjects in the control group, 12 (60%) were female and 8 (40%) were male. The age of the control group was between 21 and 52 years. The disease duration of the patient group was between 1 month and 8 years. Lesions were observed in the lower extremities of 23 patients, in the upper limb of 25 patients, in the upper body of 24 patients, in the oral mucosa of 16 patients, and in the genital mucosa of 3 patients. When TNF-α levels were compared between the patients with lichen planus and that of the healthy control group, a statistically significant difference was observed (P<0.001). TNF-α levels were not statistically different for those with oral mucosal involvement (P=0.254) [Tables 1 and 2]. There was no statistical difference between the genders. When the TNF-α levels of patients and control groups were compared in terms of age, the level of TNF-α was higher in the elderly group.

Discussion
A number of studies have been conducted on the etiopathogenesis of lichen planus, and the emphasis has been made on the predominance of cell-mediated autoimmune response and epithelial cytotoxic cell injury. Lichen planus is a dermatosis that involves the local release of cytokines, the retention of lymphocytes, and death of basal keratinocytes through lymphocyte-mediated immunological mechanisms. The late hypersensitive immune reaction in the etiology has been shown to result in a type 1 T helper immune response [Figure 1]. It has been reported that there is IL-2, IFN-γ, and TNF-α release around the dermal...
vessels, which allow adhesion molecules and T cells to accumulate. The cytokines released from activated T cells accumulate inflammatory cells in the region, and this leads to cell-mediated cytotoxicity and keratinocyte destruction. In this context, it has been shown that TNF-α and IFN-γ are the major cytokines in keratinocyte damage as well as in the action of lymphocytes and other inflammatory cells. These two cytokines, together with other cytokines that release locally and systemically, cause the liquefaction degeneration in keratinocytes. In epithelial cells, TNF-α is cytotoxic at high concentrations and antiproliferative at low concentrations. In prolonged release, it inhibits the proliferation of keratinocytes by the primer cytokine role. TNF-α has a key regulatory effect in the onset and progression of lichen planus.

Langerhans cell activation has increased, especially in those with oral involvement. In 1997, Simon and Gruschwitz conducted a study with 15 patients who had acute eruptive lichen planus and they examined the soluble form of the TNF-α receptor (TNFR) in the serum and the expression of TNFRI and TNFRII in the lesion immunohistochemically. According to their results, serum TNFRI was significantly different from that of the normal, and both receptor levels were found to be higher in infiltrating lymphocytes. They indicated that the expression in epidermal cells or in the T lymphocytes was correlated with apoptosis in skin lesions of lichen planus. Nickoloff et al. demonstrated an in vitro synergistic interaction of TNFRs with IFN-γ in the basal layers. They supported the idea that it led to apoptosis in lichen planus. They also reported that serum TNFRI levels might be markers of activity in patients with lichen planus. Studies had examined TNF-α, IL-2, IFN-γ, IL-6, IL-1β, and lymphotoxin levels in serum of patients with oral lichen planus, and they found that only TNF-α and IL-6 levels were higher in patients with oral lichen planus compared to that of the control group. Recent studies have shown that TNF-α production was high in mononuclear cells

Table 2: Tumor necrosis factor alpha distribution of patients with lichen planus according to the group with oral mucosal involvement

|                      | TNF-α (pg/ml) |
|----------------------|---------------|
|                      | n             | Mean | Standard deviation | Median | Minimum | Maximum |
| Patients with oral lesion | 16            | 52.29 | 17.78               | 61.5   | 11.7    | 94      |
| Patients without oral lesion | 18            | 68.25 | 20.99               | 63.25  | 44      | 144     |

TNF-α: Tumor necrosis factor alpha

Figure 1: Immunological theory in lichen planus

Retraction
and keratinocytes when the patients with nonlesional or normal oral mucosa and oral lichen planus were compared to each other. In a study conducted by Kaur and Jacobs in Korea in 2016, serum and saliva TNF-α concentrations in patients with oral lichen planus were higher than those of the control group. This suggested that TNF-α might be a diagnostic marker for oral involvement. However, our study failed to find any difference between the TNF-α levels between the patients with oral lesion and those without it.

In recent years, studies have focused on the importance of TNF-α as a major mediator of inflammation in conjunction with IL-1, thereby inducing T-cell activation, induction of apoptosis, and an increase in the expression of adhesion molecules (intercellular adhesion molecule-1, vascular cell adhesion molecule-1, endothelial leukocyte adhesion molecule-1). In our study, we found that the TNF-α levels of patients with lichen planus were significantly higher than that of the control group. We observed that the patient group with oral involvement was not statistically different when compared to the group without involvement. For this reason, we believed that in those with oral mucosal involvement, the release of TNF-α might be more likely to occur when the activity of the disease was increased and thus might be dependent on the phase of the disease. Measuring TNF-α levels in the serum of patients of lichen planus during active phase of the disease and in a larger group of patients may throw more light on these issues.

When patients with multiple oral lichen planus resistant to systemic steroids and immunosuppressant treatments, were treated with anti-TNF-α antagonists agents there was a favorable response. Successful treatment with infliximab and adalimumab has been reported in two patients with oral lichen planus. The efficacy of these drugs in the treatment of this disorder has been shown the importance of TNF-α in lichen planus.

**Conclusion**

We found that high serum TNF-α levels in patients with lichen planus in our results were consistent with the findings of the previous studies. Based on these findings, we believe that TNF-α is not only an intermediate mediator in the pathogenesis of the disease. It may be a marker of disease activity and follow up.

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Nil.

**Conflicts of interest**

There are no conflicts of interest.

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**What is new?**

TNF-α is a significant mediator in the pathogenesis of the lichen planus and serum TNF-α levels might act as diagnostic markers for activity of oral lichen planus.

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