Evaluation of Salivary Interleukin-12, Interleukin-23 Levels in Patients with Oral Lichen Planus and Squamous Cell Carcinoma

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Abstract

Objectives The present study aimed to evaluate interleukin-12 (IL-12) and IL-23 in the saliva of patients with oral lichen planus (OLP) and oral squamous cell carcinoma (SCC).

Materials and Methods Thirty cases with clinical and histopathological OLP (bilateral lesions, papular and reticular lesions, and Wickham lines) (Group A), 30 with oral SCCs (OSCCs) (Group B), and 30 with no history of oral cancer, other lesions, or lichen planus (Group C) were enrolled at the Department of Oral Medicine School of Dentistry, Zahedan, Iran. The whole unstimulated saliva was collected and the salivary concentration of IL-12 and IL-23 was measured using an enzyme-linked immunosorbent assay in the laboratory.

Statistical Analysis Data were analyzed using Kruskal–Wallis, Mann–Whitney tests, and Pearson's correlation coefficients.

Results In the present study, salivary IL-12 and IL-23 levels were higher in OSCC patients than in OLP and healthy individuals.

Conclusions The results show that although IL-23 and IL-12 cytokines have an important role in the pathogenesis of chronic immunity and inflammatory diseases, further studies are required to assess matrix metalloproteinase links with tumor invasion.

Keywords
- interleukin-12
- interleukin-23
- oral lichen planus
- oral squamous cell carcinoma

Introduction

Lichen planus is a common chronic inflammatory mucocutaneous disease and oral mucosa and is involved in 50% of cases. So far, the etiopathogenesis of oral lichen planus (OLP) has been poorly understood, but T cell-mediated immunity and inflammatory pathways play a role in its pathogenesis.[1,2] Many studies proposed autoimmune properties of OLP, such as interaction with other autoimmune diseases, reduced immune-suppressing activity in patients with OLP, and the presence of auto-toxic cells, and used these properties in conducting studies on inflammation and autoimmunity.[2] Since the first reported squamous cell carcinomas (SCC) developed from LP, several studies have focused on the malignant transformation of OLP lesions to oral SCC (OSCC), as it has become a health concern worldwide.[2] Nowadays, considering the classification by the World Health Organization (WHO), the term "OLP" is known as a precancerous condition. The molecular mechanisms underlying the development of oral

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cancer are not clearly known in patients with OLP, but OLP lesions can evolve from normal epithelium or precancerous lesions, and the basement membrane disruption may trigger the apoptotic keratinocytes.[3] OSCC is the most common neoplasia of the oral cavity and a serious worldwide health problem; thus, identifying the SCC biomarkers is essential for early diagnosis, better prognosis, and prevention of disease recurrence and is an effective method to decrease the mortality of patients. Malignant transformation of oral mucosa resulting from a gene mutation in cell growth and its regulation causes increased proliferation of cells, abnormal keratinization, epithelial dysplasia, increased cell motility, and angiogenesis. Cancer is caused by genetic changes resulting from the deregulation of proteins, poor cell division, and tissue differentiation, invasion, and metastasis.[4]

The suppressed immune system is an established phenomenon in patients with cancer, especially in those with squamous cell cancer, and can include changes in cytokines and the balance of immune cells. T-helper cells play a key role in the regulation of immune response. T-helper 1 cells are involved in cell-mediated immunity and are generally considered as the host of anticancer mechanisms, while T-helper 2 cells provide cell-mediated immunity and humoral reactions against extracellular pathogens. They are responsible for a broad range of activities, including angiogenesis, the proteolytic activity of growth factors, soft-tissue reconstruction, wound healing, and tumor invasion.[5,6]

Interleukin-23 (IL-23) has been identified in recent years and has caught attention in autoimmune diseases (5 and 4). The studies have shown that in autoimmune diseases, the IL-23 affects the donor lymphocytes (Th17) that are the actors of key autoimmunity processes and stimulates these cells to produce IL-17, subsequently inducing the production of other pro-inflammatory cytokines by IL-7 such as IL-6 and IL-1; thus, the tumor necrosis factor-α (TNF-α) would progress the inflammatory reactions.[7]

IL-23 is a heterodimer and pre-inflammatory cytokine produced by the activated macrophages and dendritic cells and formed by two p19 and p40 subunits. The p40 subunit is a common subunit placed between IL-12 and IL-23, though IL-12 and IL-23 have different activities. The IL-23 and IL-12 receivers have a common chain called β1 but the second chain in the IL-12 receiver is β2, and in the IL-23, is a subunit called IL-23R.[8]

Wide genomic studies have shown that the gene that codes for IL-23R has a significant relation with several autoimmune diseases, whereas the coding genes of IL-23P19 IL-12Rβ1 and IL-12P40 have shown no relation with these diseases. The IL-23 signaling is the same as that of IL-12 in terms of transferring signal molecules and the transcription factor (STAT) including STAT1, STAT3, STAT4, STAT5, and Janus kinase 2. Nevertheless, the activation of STAT4 in IL-23 signaling is weaker than that of IL-12 signaling.[9-11]

IL-12 is produced generally by the dendritic cells, macrophages, neutrophils, and B lymphoblastic human cells in response to antigenic stimulation and is related to autoimmunity. The prescription of IL-12 for patients with autoimmunity would exacerbate the circumstances. The IL-23 is known as the heterodimeric cytokine and is formed by a subunit called IL12P4 that is accompanied by IL-23P1 and IL-12.[12]

Kalogerakou et al have evaluated T cells in the secretion of Type 1 and Type 2 cytokines in the blood of patients with OLP (n = 80). The ELISA showed a significant decrease in the levels of IL-12 and interferon-γ (IFN-γ) in the OLP group compared with the control group. They have concluded that a decrease in the IL-12-secreting cells and IFN-γ and TNF-α in the OLP group indicates the suppressed and decreased function of Type 1 immunity. Whether these circumstances are involved in the immune process of OLP patients or not is unclear. They have acknowledged that the number of Type 1 and 2 secreting cells, as an indicator of interruption in the same immune system with different clinical appearances, differs.[13]

Chen et al have primarily evaluated the increase in IL-12 and IL-23 in OLP and SLP groups. Both LP (n = 42) and OLP (n = 38) groups included reported a higher percentage of increased IL-12 and IL-23 cells in the OLP than in SLP. This could be caused by Th2, which is an important part of immunity against oral mucosa microbes and tissue antigens, and could be the reason for the difference between the clinical behaviors of the two types of the disease.[14]

Lu et al have addressed that IL-23 is a pro-inflammatory cytokine that plays an important role in the pathogenesis of chronic immunity and inflammatory diseases including OLP. Their study indicated that IL-23 levels were higher in the OLP group than in the normal group.[15]

Ohyama et al stated that IL-23 is a necessary cytokine derived by the development of the Th17 and is accompanied by many tissue-destructing diseases related to the body's immunity. In the present study, IL-23 derived from Th17 plays the main role in periodontal diseases; therefore, the function of cytokine and related molecules in periodontal wastes has been measured. IL-23 and IL-12 in periodontal wastes were dramatically high; however, IL-23 receivers were significantly higher than IL-12 receivers in periodontal wastes.

Further, IL-17 in periodontal wastes, especially in tissues next to the bone, was significantly higher. Thus, in inflammatory periodontal wastes, the stimulation of TH-17 and the mediation of IL-23 were reported.[16]

Huang et al conducted a study on the importance of IL-12 and IL-27 in the pathogenesis of OLP and negative correlations of IL-12 and IL-27 levels with CD16+56 (NK) cells were observed.[17]

Concerning different results and the importance of cytokines in inflammatory chronic diseases, such as OLP, OSCC, and the high prevalence of OSCC in Sistan and Baluchestan areas, it is necessary to evaluate these markers for early diagnosis and proper medication. Thus, the present study aimed to evaluate IL-12 and IL-23 levels in the saliva of patients with OLP and OSCC.

Considering the high prevalence of oral cancers and OLP in Zahedan, the lack of such a study, and the safety and usefulness of saliva as a diagnostic method for oral cancer and Lichen planus, we have analyzed the β2M concentration in these patients.

Materials and Methods

A total of 90 patients were referred to the Department of Oral Medicine, the School of Dentistry in Zahedan University of Medical Sciences. They were classified into three groups including Group A (n = 30, patients with clinical lesions OLP [bilateral lesions, papular and reticular lesions, and Wickham lines], histological confirmation [characterized by a band-like inflammatory infiltrating cells, limited to the surface area of the connective tissue; they are predominantly mature lymphocytes, accompanied by vascular degeneration of the basal layer of the epithelium]); and with no other oral lesions Group B (n = 30, patients with new SCC and pathologist-proving SCC, with no oral lesions); and Group C (n = 30, cases with no history of SCC or LP. All participants signed informed consent after being clarified about the project.

The study was approved by the Ethics Committee of Research Deputy of Zahedan Medical Sciences University (IR. ZAUMS. REC.1395.60).

To collect the saliva samples in case and control groups, unstimulated spitting was used. Samples were collected between 9 and 12 am using a prepublished protocol.

They were asked to wash their mouth before spitting in certain tubes and sit upright 5 minutes after washing and spit in a 50 mL falcon. Samples were sent to the laboratory shortly after being collected and centrifuged at 2,600 gr for 15 minutes at 4°C. Proteinase inhibitors including 10 NL aprotinin (10 mg/mL), 10 NL phenylmethanesulfonyl fluoride (10 mg/mL), and 3 NL sodium orthovanadate (400,000 M, Na_2) were added to each milliliter of a floating solution to prevent deregulation of proteins. All samples were maintained at 80°C. Salivary concentrations for IL-12 and IL-23 were analyzed by enzyme-linked immunosorbent assay kits with a sensitivity of 95% (Boster, China). The levels of IL-12 and IL-23 were determined according to the manufacturer's instructions. OSCC and OLP patients received treatment and follow-ups.

Data were analyzed using descriptive, Kruskal–Wallis, Mann–Whitney, and Pearson's correlation coefficient (SPSS 21).

Table 1 The mean and standard deviation of interleukin-12 in participants of each group (healthy controls, squamous cell carcinoma, oral lichen planus)

Group	Mean ± deviation IL-12 (ng/mL)	Kruskal–Wallis test (p-Value)
Normal	2.52 ± 4.97	0.049
SCC	28.68 ± 5.27	
OLP	28.07 ± 6.56	

Abbreviations: IL, interleukin; OLP, oral lichen planus; SCC, squamous cell carcinoma.

Table 2 The mean and standard deviation of interleukin-23 in participants of each group (healthy controls, squamous cell carcinoma, oral lichen planus)

Group	Mean ± deviation IL-23 (ng/mL)	Kruskal–Wallis test (p-Value)
Normal	458.56 ± 421.89	0.919
SCC	340.05 ± 320.01	
OLP	358.16 ± 329.11	

Abbreviations: IL, interleukin; OLP, oral lichen planus; SCC, squamous cell carcinoma.

Results

In the present study, the data collected from 90 participants in three groups of 30 patients (healthy control, patients with OSCC, and patients with OLP) were analyzed.

The results from Kruskal–Wallis analysis (►**Table 1**) show a statistically significant difference in the levels of IL-12 in the saliva amongst the three groups (p = 0.049). The Mann–Whitney analysis showed that the difference between the healthy group and SCC group was significant (p = 0.024), but was not significant between the healthy group and OLP group (p = 0.052) and SCC group and the OLP group (p = 0.736).

The results from the Kruskal–Wallis statistical analysis (►**Table 2**) showed that the mean difference in the amount of IL-23 in the saliva amongst the three groups was not statistically significant (p = 0.919).

Discussion

Finding biological indicators for providing proper prognostic information on the metastasis of oral premalignant and malignant lesions is of great importance. These lesions represent a premalignant circumstance and are called OLP and SCC. Although these clinical diseases have normal symptoms and are diagnosable, a biopsy is needed for histopathologic evaluation, cellular atypia rejection examination, and malignancy signs.[18] However, as clinical or microscopic features do not determine the development and conversion of a premalignant–malignant lesion, recognizing the molecular markers characterized by these features seems to be essential.[19]

In this study, 90 patients were divided into healthy (n = 30, female = 76%, male = 48%), OLP (n = 30, female = 72%, male = 28%), and SCC (n = 30, female = 52%, male = 48%) groups. The mean ages in the control, OLP group, and SCC group were 40.68, 39, and 47.7 years, respectively. Pakfetrat et al reported that the mean age of OLP patients was 41 years and the frequency of female patients was 64%, and the results from age and gender variables were consistent with those obtained in the present study. Aghbali et al stated that the mean age of SCC patients was 63 years and the frequency of males to females was 1.7 to 1.18. The mean age in this study was less than that in Aghbali's study and the frequency of females was similar to that of males.

The results of the present study showed that the levels of IL-12 in the SCC group were significantly higher than those in healthy people ($p < 0.05$). The levels of IL-12 increased in the OLP group, and the difference with the healthy group was not significant. Further, the levels of IL-12 in SCC and OLP patients were similar.

Sensitive and specific biomarkers for SCC potentially decrease the morbidity and mortality of the disease. Elevated levels of cytokines have been found in the saliva of patients with OSCC. Furthermore, in a collaboration study, we identified enhanced levels of IL-8 protein and mRNA in the saliva of patients with OSCC as well as increased IL-6 in the serum samples of the same group.

Cytokines potentially serve as important angiogenic factors in the development of SCC. Assessments have revealed higher salivary IL-1α, IL-6, TNF-a, and vascular endothelial growth factor (VEGF)-a levels in both endophytic and exophytic groups of patients with SCC than control groups, while a higher level of IL-8 was observed in the endophytic group. Besides, smoker and smoker-drinker control groups revealed higher levels of IL-8 and VEGF-a than the healthy controls. These cytokines are potential candidates for salivary biomarkers because of their established role in angiogenesis, inflammation, and progression of diseases.

Chen et al have shown increased levels of IL-12 in OLP patients. In the recent study, although the difference in the levels of IL-12 between the healthy group and OLP group was not significant, the levels of IL-12 in the OLP group increased.

The reason for such a difference could be the types of patients included in the study. Based on the fact that OLP is a chronic disease and approximately related to gender (inclination to females), the time of disease and the abundance of the patients' gender could be effective in determining the levels of IL-12 in the saliva. But the important point is an increase in this marker in OLP patients.[14]

Huang et al have revealed a negative correlation between the levels of IL-12 and IL-27 with 56+ CD16 NK cells. In other words, with a decrease in killing cells caused by the occurrence and expansion of many cancers and autoimmunity diseases, the levels of IL-12 and IL-27 would increase, and these markers can be used in identifying autoimmunity diseases.[17]

In the present study, the levels of IL-12 in SCC and OLP patients were similar, indicating a similar malfunction in the immunity system of both diseases, but their clinical preferences were different.

The results of the present study showed that the levels of IL-23 in both groups of patients of (OLP and SCC) were similar to those of the healthy group.

Lu et al showed that the levels of IL-23 in the OLP group were higher than those in the normal group.[15] Chen et al have reported increased levels of IL-23 in OLP than in SLP.[14] Ohyama et al reported that IL-23 is an essential cytokine derived by the development of Th17 and is related to many devastating diseases.[16]

A previous study showed OLP occurrence by antigen-specific and nonspecific mechanisms. In antigen-specific mechanisms, basal keratinocytes with antigen-specific keratinocytes are killed by $CD8^+$ cytotoxic T lymphocytes. In nonspecific mechanisms, mast cells degranulate and matrix metalloproteinase (MMP) activates in OLP lesions. OLP is a T cell-mediated inflammatory disease. Through mast cell/T cell interactions in OLP lesions, mast cells release cytokines and chemokines, and MMPs can promote T cell activation, migration, proliferation, and differentiation.

The results of the present study are inconsistent with those of the previous study. The shortcomings of the present study included the limitation of the samples under study, restricted time of saliva IL measurements, and disease metastasis with different degrees.

Conclusions

The levels of salivary endothelin 1 in OLP and OSCC patients were significantly higher than those in healthy people. Hence, it can be used as a new biomarker for oral premalignant and malignant lesions.

Suggestions

To prove this hypothesis, further studies with a larger sample size of premalignant and malignant lesions are needed and the role of IL-23 and IL-12 markers should be measured in stages and different degrees of premalignant and malignant lesions.

Ethics Approval and Consent to Participate

All participants perceived the nature of the research project and provided written, informed consent to participate in this study. The study was approved by the Ethical Committee of Zahedan University of Medical Sciences (IR. ZAUMS. REC. 1395.60).

Conflicts of Interest

None declared.

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