Journal of
Babol University of Medical Sciences
JBUMS
e-ISSN: 2251-7170 p-ISSN: 1561-4107

Comparison of Serum Interleukin-28 Levels in Patients with Squamous Cell Carcinoma and Lichen Planus versus Healthy People

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Article Type
Research Paper

Received:
Feb 28th 2025
Revised:
Apr 8th 2025
Accepted:
May 5th 2025

ABSTRACT

Background and Objective: Head and neck cancers are among the most common forms of cancer in the world and account for 2.4% of all cancer-related mortality. Since early detection of cancer is a key factor in improving prognosis and increasing patient survival, and given the diversity of results regarding the role of interleukins in the pathogenesis of lichen planus and oral squamous cell carcinoma, this study was conducted to compare serum levels of interleukin-28 in patients with squamous cell carcinoma and lichen planus versus healthy people.

Methods: This cross-sectional study was conducted over 5 years on 50 patients with oral squamous cell carcinoma (OSCC) and oral lichen planus (OLP) who referred to Khalili Hospital, Mother and Child Hospital, and the School of Dentistry, Shiraz University of Medical Sciences compared to 30 controls. 5 mL of blood was collected from the patients and controls in test tubes containing EDTA anticoagulant and was centrifuged. The collected sera were stored in sterile micro-tubes at -20ºC. Then, the concentration of IL-28 in serum was compared between the patients and the control group using the ELISA method.

Findings: 50 patients with oral lichen planus and oral squamous cell carcinoma with a mean age of 51.20±10.65 years participated in this study, including 19 men (38%) and 31 women (62%). The control group consisted of 12 men (40%) and 18 women (60%) with a mean age of 49.93±14.95 years. The mean age of male patients was 45.11±11.29 years and the mean age of female patients was 54.94±8.41 years (p=0.003). The mean serum level of IL-28 in the OLP group was 66.43±30.94 pg/ml, in the OSCC group 121.3±18.67 pg/ml, and in the control group 86.19±23.53 pg/ml (p<0.0001).

Conclusion: The results of the study showed that the concentration of IL-28 in patients with oral lichen planus was significantly lower compared to controls, while in patients with oral squamous cell carcinoma, it was higher than controls. Since the level of IL-28 is associated with OLP and OSCC, it can therefore be used as a biological marker for the diagnosis of this type of malignancy.

Keywords: *Oral Lichen Planus, Oral Squamous Cell Carcinoma, Interleukin-28, Cancer Biomarkers, Prognosis.*

Cite this article: Sadeghzade A, Fattahi MJ, Ghaderi A, Khademi B, Rezaee MJ, Karimi M, et al. Comparison of Serum Interleukin-28 Levels in Patients with Squamous Cell Carcinoma and Lichen Planus versus Healthy People. *Journal of Babol University of Medical Sciences*. 2026; 28: e8.

Introduction

Head and neck cancers are among the most common types of cancer worldwide, accounting for 2.4% of all cancer-related deaths. More than 90% of head and neck cancer cases are oral squamous cell carcinoma (OSCC), which is the most common cancer in male patients (1, 2). Various etiological factors, including age, gender, race, tobacco, alcohol, diet, and nutrition, contribute to the development of OSCC, with tobacco being the most common (1, 3, 4). Early detection of cancer is a key factor in improving prognosis and increasing patient survival rates. Although the oral cavity can be easily examined and evaluated through direct visualization, the conventional diagnosis of OSCC involves clinical examination of the mouth followed by biopsy of the suspicious tissue; unfortunately, most OSCC cases are not diagnosed early. This is probably because patients do not seek dental care regularly, and oral cancers are often asymptomatic in their early stages. Additionally, due to late diagnosis, metastasis is very common in OSCC, resulting in a five-year survival rate of less than 50% (5). In order to contribute to early diagnosis and improve cancer prognosis, patient awareness regarding regular visits to the dentist, as well as training for dental office staff and careful examination of patients, should be emphasized (6).

Oral lichen planus (OLP) is also a relatively common chronic inflammatory disease that affects 6-10% of the general population, particularly women over 40 years old. The diagnosis of reticular oral lichen planus is based on clinical findings. White reticular lines (Wickham striae) on the posterior buccal mucosa are its main characteristic (7). Most patients with lichen planus are middle-aged adults and, on average, it occurs in the fifth decade of life, and children are rarely affected. The female-to-male ratio is 3:2, and in another study, it is reported as 6:4 (8-10). Lichen planus is a branch of a wide spectrum of immune-mediated diseases known as lichenoid lesions. Therefore, there are numerous clinical and histological similarities between lichen planus and lichenoid dermatosis, drug stomatitis, some autoimmune disorders, and graft-versus-host disease (GVHD). Factors such as stress, diabetes, hepatitis C, trauma, and hypersensitivity to metals and drugs can be the cofactor triggers for lichen planus (11). Currently, biopsy is used for histopathological examination and definitive diagnosis of potentially malignant oral disorders. However, it has several drawbacks, including being time-consuming, costly, and destructive to human tissues. In addition, some adjunct clinical examinations, such as autofluorescence imaging and in vivo vital staining with toluidine blue are also diagnostic methods, but they have poor diagnostic accuracy and high rates of false positives. Therefore, finding a reliable diagnostic method with high sensitivity and specificity for detecting potentially malignant oral disorders is very important.

Cytokines are molecular messengers that have profound effects on cancer growth. Some cytokines are associated with both tumorigenic and anti-tumor activities, indicating that cytokines may play multiple complex roles related to cancer pathogenesis (12). New biological therapies and immunotherapies can cause cancer cell death and exacerbate inflammation. An inflammation that leads to stronger immune responses can be beneficial for anti-tumor immunity (13). However, some immune-stimulating cytokines may not be beneficial for cancer treatment and may unintentionally cause tumor regrowth or lead to immunosuppression (14). Dying tumor cells may stimulate cytokine production, which in turn increases resistance to cancer drugs (15). Cytokines, by intervening in the immune response as well as cancer development, have great potential in detecting the presence of cancer and monitoring its subsequent severity, such as during pharmacological interventions (16).

It has been shown that IL-28A plays an important role in the response to viral infections and may also play a role in tumor development (17-21). The IL-28 genes are located near IL-29 on human chromosome 19. The two isoforms of IL-28 (IL-28A and IL-28B) are 96% homologous. The functional differences between these two forms are unclear. The IL-28 receptor consists of a unique IL-28 alpha chain that pairs

with the IL-10 receptor beta chain, leading many researchers to classify IL-28 as a member of the IL-10 family. It has also been shown that IL-28 plays a role in the adaptive immune response (22). The role of IL-28A in tumor development appears unclear, as it has been shown to activate the JAK/STAT signaling pathway (23, 24) and increase STAT3 phosphorylation in cancer cells, subsequently elevating the secretion of angiogenic factors such as VEGF, MMPs, and IL-18 (25). Research has shown that exposure of mammary cancer cells in dogs and human bladder cancer cells to IL-28A increases cell migration in laboratory conditions, and tumors in patients with advanced bladder cancer exhibited higher expression of IL-28 (25, 26). Given that the etiology and pathogenesis of OLP and OSCC are not fully understood, and the role of interleukins in the pathogenesis of these diseases remains completely unclear with varying results, the present study was conducted to investigate serum levels of interleukin-28 in patients with oral lichen planus and oral squamous cell carcinoma in Shiraz, southwestern Iran, so that it can be used to predict susceptibility to these diseases.

Methods

After approval by the Ethics Committee of Shiraz University of Medical Sciences with codes IR.SUMS.DENTAL.REC.1400.131 and IR.SUMS.DENTAL.REC.1401.042, this cross-sectional study was conducted in 2022 on 50 patients with OSCC and OLP who referred to Khalili Hospital, Mother and Child Hospital, and the School of Dentistry, Shiraz University of Medical Sciences, compared to 30 controls, over a period of 5 years. The sample size was determined using similar studies (27) and after consultation with a statistics expert.

After obtaining written consent, patients were enrolled in the study based on initial diagnosis and according to clinical symptoms and confirmation by pathology reports (biopsy). Subjects with a history of radiation therapy or chemotherapy, unwillingness to participate, presence of other malignancies besides OSCC and OLP, any oral lesions, and immunological, inflammatory, or genetic diseases were excluded from the study. The inclusion criteria for entry into the control group included physical health and matching with patients in terms of age and gender. In case of any history of cancer other than OSCC or OLP, use of medications such as corticosteroids, transplants, and immunological or genetic diseases in themselves or first-degree relatives, participants were excluded from the study.

5 mL of blood was collected from the patients and controls in test tubes containing EDTA anticoagulant and was centrifuged. The collected sera were stored in sterile microtubes at -20°C. The concentration of IL-28 in the serum was then measured using the ELISA method with a commercial kit from Zellbio (Germany) and based on the kit's instructions. The concentrations of IL-28 were compared between patients and controls using the Kolmogorov-Smirnov statistical test.

Parametric tests (Independent T-Test and One-Way ANOVA) were used for normally distributed data, and non-parametric tests (Mann-Whitney and Kruskal-Wallis) were used for data that lack a normal distribution. To compare the frequency of gender between the two groups, the Chi-Square test (χ^2) was used, and to examine the correlation between data, the Pearson correlation test was used. ROC (Receiver Operating Characteristic) curve analysis was also performed, and $p<0.05$ was considered significant.

Results

Fifty patients with oral lichen planus and oral squamous cell carcinoma participated in this study, with a mean age of 51.20±10.65 years, including 19 men (38%) and 31 women (62%). The control group consisted of 12 men (40%) and 18 women (60%), with a mean age of 49.93±14.95 years. None of the patients reported

drug abuse, and only one patient consumed alcohol regularly. Among the patients, 29 out of 50 had a family history of cancer, particularly breast cancer in first-degree relatives (mother and sister), followed by gastric cancer. The mean age of male patients was 45.11±11.29 years and mean age of female patients was 54.94±8.41 years (p=0.003). The mean serum level of IL-28 in the OLP group was 66.43±30.94 pg/ml, in the OSCC group 121.03±18.67 pg/ml, and in the control group 86.19±23.53 pg/ml (p<0.0001). No significant difference was observed in serum IL-28A levels between male patients (118.3±18.10 pg/ml) and female patients (123.9±19.11 pg/ml). A linear and direct correlation was observed between serum IL-28A levels and age in patients with OLP (r=0.043), but this correlation was not statistically significant (Figure 1).

Serum IL-28A levels in OSCC patients over 60 years (127.3±13.74 pg/ml) were significantly higher than in patients under 60 years (114.1±21.88 pg/ml) (p<0.05). In OSCC patients, the optimal cutoff point achieved with the highest sensitivity (81.63%) and specificity (86.67%) was 1.106 ng/ml, and the AUC in this curve was 0.887 (Figure 2).

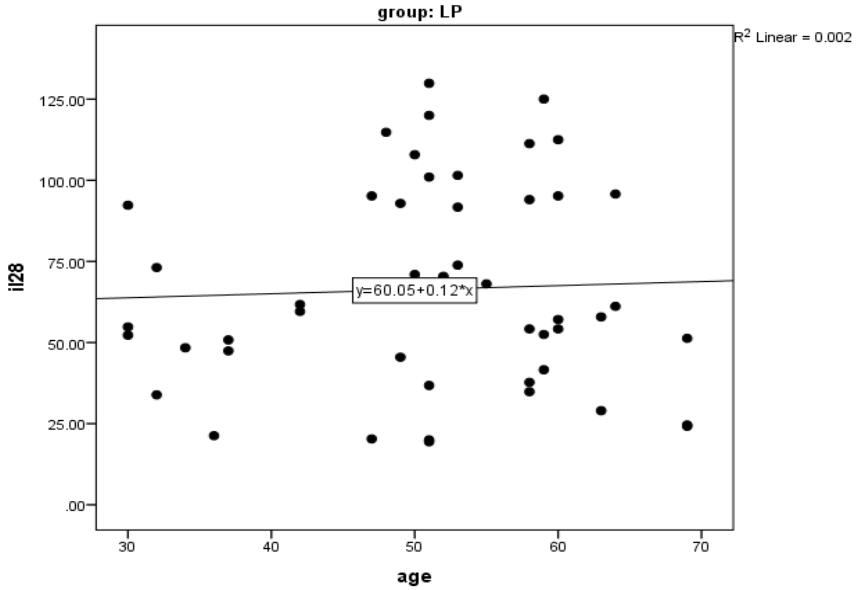

Figure 1. Scatter plot of serum IL-28 levels in OLP patients and its correlation with age

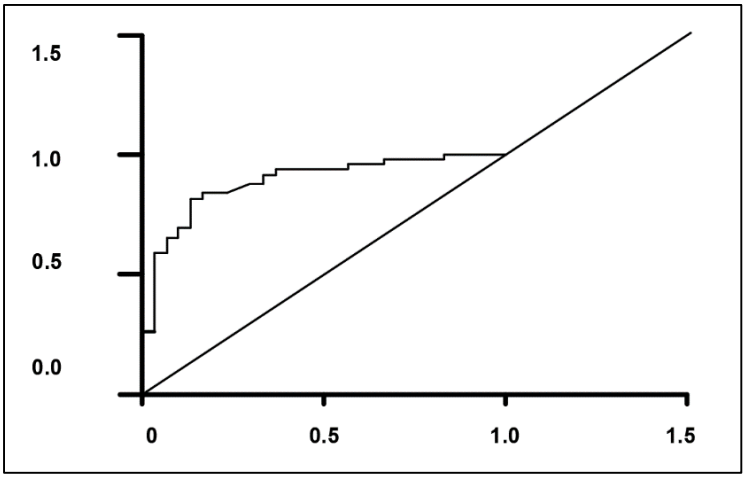

Figure 2. ROC for evaluating IL-28 in predicting OSCC

Discussion

The results showed that IL-28 levels were significantly higher in OSCC patients compared to controls, but they were significantly lower in OLP patients compared to controls. Additionally, serum IL-28A levels in patients over 60 years were higher compared to those under 60 years. On the other hand, there was no significant correlation between serum IL-28A levels and patient gender. This decrease in IL-28 may be due to the pathogenesis of OLP. The increase in IL-28 levels in OSCC patients compared to controls indicates that IL-28 concentration could be considered a potential biomarker for diagnosing OSCC. According to the results of the present study, there was no significant difference in mean age and frequency distribution of gender between the control group and patients with OLP. Based on this, it can be concluded that the two study groups had similar age and gender distributions. Most of the OLP patients in the study were women (62%), which does not align with reference textbooks (28, 29). In line with the results of the present study, most patients were women in a study by Mardani et al. (30). Results of studies have shown that the disease is more common in women and middle-aged individuals (31, 32). Contrary to the results of the present study, in the study by Mashayekhi et al., the prevalence based on gender was found to be 57.3% in men and 42.7% in women (33). In the studies by Romero et al. (34), Esmaeli et al. (35), Farshchian et al. (36), Shamsoddin et al. (37), and Esfandiarpour et al. (38), the prevalence was higher in men compared to women. Since OLP is an immune-mediated disease, the reason for the increased incidence of OLP with increasing age can be attributed to hormonal changes and the decrease in immune system-regulating hormones such as estrogen and progesterone. Concurrent with menopause in women, we also observe an increase in the incidence of immune diseases, which could be the cause of this occurrence due to the reduced inhibitory effect of estrogen on the immune system following a decrease in the circulating level of this hormone. This pattern is similar to other immune-mediated diseases, which either improve or worsen with the onset of menopause.

Studies by Lee et al. showed that various cancer tissues and cell lines express IL-28A (26). Novak et al., who investigated the role of IL-29 as a member of the IL-10 family and type III interferons (IFN-λ) on the cellular biology of myeloma tumor, found that myeloma cells bind to soluble IL-29, and IL-29 enhances the growth of myeloma cells, and protects against cell death induced by dexamethasone (39). The results of a study by Pingwara et al. showed that IL-28A makes mouse breast cancer cells prone to migration in laboratory conditions. It also increased angiogenesis induced by mouse breast cancer cells in laboratory conditions and in eggs (40). Mucha et al. identified increased activation of IL-28/IL-28RA signaling in dogs carrying mammary tumors. The highest expression of IL-28 was observed in dogs carrying stage III/IV mammary tumors. The secreted IL-28 led to increased expression of angiogenic factors and subsequently induced angiogenesis by endothelial cells, epithelial-mesenchymal transition (EMT), and increased tumor cell migration in laboratory conditions. Omission of the IL-28RA receptor reduced angiogenesis, tumor cell invasion, and migration (25). In this study, it was found that the concentration of IL-28 in OLP patients was significantly lower compared to controls. It was also shown that the IL-28 concentration in OSCC patients was higher than in controls. Additionally, there was no significant difference in IL-28 levels between male and female patients with OSCC. As a result, IL-28 levels may be associated with OLP and OSCC, and IL-28 could potentially be used as a biological marker for diagnosing this type of malignancy. Although IL-28 levels in OSCC patients were higher than controls, it is still unclear whether IL-28 is associated with prognosis. IL-28 levels were higher in patients over 60 years compared to younger patients. Overall, the prognosis of OSCC in younger patients is poorer, with a higher risk of metastasis. Therefore, serum IL-28 levels in older patients may be related to a defensive mechanism in these subjects.

Since salivary biomarkers are recognized as a non-invasive method for diagnosing OSCC and OLP, it is suggested that IL-28 levels in saliva be considered as a potential diagnostic factor.

Acknowledgment

We hereby express our gratitude to the Vice Chancellor for Research and Technology of the School of Dentistry, Shiraz University of Medical Sciences, and also to the colleagues in the Department of Oral and Maxillofacial Medicine and Oral and Dental Disease Research Center.

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