Salivary $\beta_2$- microglobulin Levels in Patients with Oral Lichen Planus and Squamous Cell Carcinoma

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Abstract

Objective

β2 microglobulin one of the biomarkers which are used to oral malignancy and pre-malignance lesion diagnosis. The components of the microglobulin system can help in growing and development of tumors directly or indirectly. The current study aims to evaluate the salivary β2- microglobulin level in malignance and pre-malignance lesion. The statistical population of research consists of 75 people which 25 people were healthy, 25 were infected by oral Lichen Planus (OLP) and remaining were infected by squamous cell carcinoma (SCC). The salivary data was performed by none –stimulated spitting method. The sample was performing during 9-12 AM and samples were transmitted to the laboratory immediately and salivary beta2microglobin was recorded based on the factory instructions by ELISA optical density method with 45Nm wavelength for each sample. Data analysis was performed by SPSS 24 software and ANOVA and post-hoc least significant difference (LSD) test.

Results

The salivary β2 microglobulin level in squamous cell carcinoma (SCC) and oral lichen plan (OLP) patient is significantly higher than the healthy group. Therefore, we can use this index as a modern treatment protocol for malignancy and a pre-malignance oral lesion.

Introduction

Lichen planus is a common chronic inflammatory mucocutaneous disease and the involvement of oral mucosa is often seen in 50% of cases. So far, the etiopathogenesis of oral lichen planus (OLP) has still been poorly understood, but T cell-mediated immunity and inflammatory pathways play a role in its pathogenesis [1]. 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-toxicity cells cause them to be selected for research about inflammation and autoimmunity [2] From the first reported squamous cell carcinomas (SCC) developed from LP so far, several studies have focused on malignant transformation of OLP lesions to oral SCC (OSCC), as it has become a concerning topic in the world’s health. Nowadays, the term “oral lichen planus” is known as a precancerous condition, using the World Health Organization (WHO) classification. The molecular
mechanisms underlying the development of oral 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 is a serious worldwide health problem; so, understanding the SCC biomarkers is essential for early diagnosis as well as better prognosis and prevention of disease recurrence and is a good way to decrease the mortality of patients. Malignant transformation of oral mucosa due to gene mutation in cell growth and its regulation causes increased proliferation of cells, abnormal keratinization, epithelial dysplasia, increased cell motility, and angiogenesis. Cancer occurs by genetic changes that cause deregulation of protein, poor cell division, and tissue differentiation, invasion, and metastasis [4].

Tumor indicators have been recently recognized for the early diagnosis of malignancy. In oral cavity carcinomas, different serum indicators including oncofetal proteins (alpha photoprotein CEA), B proteins and enzymes (LDH) have been studied. One of the most significant indicators is β2 microglobulin which is a protein with low molecular weight (11800 kDa). It is found on every cell’s surface except for erythrocytes which are considered as a light unchangeable chain of compatible histologic antigens [5]. It is abundant in monocytes and lymphocytes [6]. In the normal physiologic state, some amounts of β2microglobulin can be secreted to the cell or serum due to intracellular release and it is often extracted from the blood by kidneys [7-11]. Thus, β2net concentration of microglobulin (β2M) is measured by its production and secretion to serum and the amount of extraction by kidneys [12]. β2M concentration increases as the kidney’s function reduce and cells' turnover increases [13]. Thus, in individuals with a healthy kidney, an increase in the β2M amount indicates the proliferation of the changed cells. Increasing β2M amount in serum was observed in some pathologic cases including kidney diseases, immunity deficiency, and autoimmune disease. Besides, there was a high level of β2M in some solid and hematologic cancers in the time of diagnosis [14, 15]. Saliva-based analysis has been proposed in recent years and the potentially abnormal markers of oral cavity appear in saliva directly or indirectly. Therefore, its application as a diagnostic fluid can be of special significance. Saliva is a diagnostic tool to assess markers due to its several
advantages: a low-cost tool for monitoring, safe collection, non-invasive, convenient, simple and reproducible without causing discomfort for the patient [16-18].

Baliah et al., (2017) determined the β2M level in serum in patients with oral leukoplakia, oral submucous fibrosis and oral squamous cell carcinoma and compared to the control group. In total, 100 individuals were classified in four groups: the first group contained patients with oral leucoplakia based on the clinical and histopathological reports; the second group consists of patients with oral submucous fibrosis; the third group was diagnosed to have oral squamous cell carcinoma (OSCC) and the last one was the control group. Results have indicated that the mean level of β2M in the serum of the leukoplakia, oral submucous fibrosis, OSCC patients and in the control group was 2597 ± 148.6, 2187.68 ± 678.6, 3166.04 ± 357.7, and 1542.60 ± 377.70 ng/mL, respectively. There was a significant increase in the mean level of β2M concentration in the first and the third groups compared to the control group. However, an increase in β2M concentration in patients with oral submucous fibrosis was not statistically significant. The present study has supported the hypothesis of using β2M concentration as an indicator in patients with oral leukoplakia and oral squamous cell carcinoma [19]. Diwan et al., (2016) studied the role of β2M as a tumor indicator in OSCC and leukoplakia. For this purpose, 30 OSCC patients, 23 leukoplakia patients, and 20 normal individuals in the control group were analyzed. Using the logistic regression model, the effect of age and gender was removed from samples due to their influence on β2M concentration. Results showed that β2M concentration was higher in OSCC and leukoplakia patients compared to the control group. Thus, β2microglobulin in serum can be used as an indicator in the diagnosis of these diseases. Increasing the concentration of β2microglobulin was positively correlated with grading the histology of OSCC [18].

Thus, considering the high prevalence of oral cancers and oral lichen planus in Zahedan, the lack of similar study, and proving that saliva is safe and useful as a diagnostic method of oral cancer and Lichen planus, we have analyzed the β2M concentration in these patients.

Materials And Methods
75 patients were referred to the Department of Oral Medicine at School of Dentistry in Zahedan University of Medical Sciences, including 25 patients with clinical lesions of OLP (bilateral lesions,
popular and reticular lesions, and Wickham lines) and, if necessary, they had histological confirmation (characterized by band-like inflammatory infiltrate 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); they had no other oral lesions (group A), 25 patients with new SCC and reports of pathologist proving SCC and except for SCC, they had no oral lesions (group B), and 25 individuals with no history of SCC or LP (group C). Unstimulated whole saliva was collected in a quiet room between 10 to 12 am under resting conditions and they were banned from eating, drinking and smoking at least 120 minutes before sampling; 0.5 mL unstimulated saliva samples were collected by spitting without chewing movements. One of the best ways is spitting method to collect whole saliva (2500 g, 10 minutes). After centrifuge, the superficial layers of saliva immediately separated and it was stored at -70 °C for further assessment. Laboratory measurements of salivary concentration for β2M concentration were performed by an immuno-sorbent enzyme-linked method and ELISA kits and BOSTER biological made in France with a sensitivity of 95%. The determination of the levels of β2M concentration was performed according to the manufacturer's instructions. OSCC and OLP patients received treatment and follow-up. The data entered into SPSS version 24 software were analyzed using descriptive, Kruskal-Wallis and Mann-Whitney and Pearson’s correlation coefficient.

Result
In the present study, 25 patients with oral lichen planus and 25 patients with Oral Squamous Cell Carcinoma were investigated. Considering the gender and mean age of cases, in a healthy group, 48% of patients were male and 52% were female with the mean age of 49 years. In patients with oral lichen planus and Oral Squamous Cell Carcinoma, 36% and 48% of patients were male, and 64% and 52% were female, respectively, also the mean age in both groups was 43 and 57 years. The examination of involved tongue’s surfaces suggested that in patients with Squamous Cell Carcinoma, cheeks (40%), gum (32%), dorsum (16%), both sides of tongue (8%), undersurface of the tongue (4%); and in patients with oral lichen planus, gum (68%), dorsum (12%) and sides of tongue (20%) were involved.

Table 1. The Mean and Standard Deviation (SD) of β2- microglobulin in Participants of Each Group
(Healthy Controls, SCC, OLP)

| Group | Mean | SD    | Min | Max |
|-------|------|-------|-----|-----|
| Control | 0.6918 | 0.21934 | 0.09 | 0.97 |
| OLP     | 1.4408 | 1.41455 | 0.25 | 5.45 |
| SCC     | 1.3729 | 1.31760 | 0.28 | 4.93 |

ANOVA Analysis suggested a significant difference among these three group

**Table 2.** LSD test to compare the groups in pairs in terms of the level of β2- microglobulin.

| I    | J    | Mean difference (I - J) | p value | 95% confidence level |
|------|------|--------------------------|---------|----------------------|
| Control | OLP  | -0.74898                 | 0.042   | -1.5501 0.0522       |
| Control | SCC  | -0.6810                  | 0.048   | -1.4900 0.1278       |
| OLP   | SCC  | 0.06788                  | 0.977   | -0.7153 0.8511       |

Post-hoc Tukey test-pairwise comparison- suggested that β2- microglobulin in healthy group was significantly less than that in Oral lichen planus and Oral Squamous Cell Carcinoma groups (P = 0.042); while it was the same in both groups of patients (P = 0.997).

**Discussion**

In many studies, saliva has been used by dentists and physicians as a diagnostic medium. A decrease and increase in cytokines and inflammatory mediators' level with different diagnostic factors in saliva makes sampling possible which can also be done by the patient himself. Some experts considered saliva and its derivate as a reflection of the physiologic and pathologic changes in the body [20, 21]. B-2 microglobulin is a kind of protein with 11800 kDa molecular weight synthesized by cells containing a nucleus. Its normal amount in the saliva is 0.038 ng/lit [22]. The present study was performed to analyze the level of β2M in the saliva of OSCC and oral lichen planus (OLP) patients. The results have indicated that β2M concentration in control group was significantly less (0.691±0.21 mg/mL) than patients with OSCC (1.1±44.41 mg/mL) and OLP (1.1±37.31 mg/mL). The amount of β2M in the saliva of these patients is the same.

The study of Rupkar Pratic et al., (2016) indicated that serum level of β2M is a valuable diagnosis factor in the OLP and OSCC patients [17]. The results of their study are completely consistent with those of the present study.

Baliah et al., (2017) reported that serum beta-2 microglobulin in patients with Oral Squamous Cell Carcinoma was significantly more than that in healthy people. Their study confirmed that beta-2
microglobulin can be applied as an indicator of the tumor for Oral Squamous Cell Carcinoma [19].
Various studies such as that of Diwan et al., (2016) [18], Agrawai et al., (2016) [18], and Singh et al. (2014) [16] suggested that beta-2 microglobulin is considered as a sensitive and specific indicator and Oral Squamous Cell Carcinoma
Kadam et al., (2011), has indicated that the serum level of these biomarkers has increased during the development of the disease from the first to the fourth step. These biomarkers are efficient for oral cancer [22].
Viashali and Tupkari (2005) have also shown a significant relationship between β2M serum level and histological grading of SCC and introduced this biomarker as a sensitive test for diagnosis, analysis and prognosis as well [23]. Based on the results of the present study, the involved areas in OSCC patients were cheeks (40%), gum (32%), dorsal and side surface of tongue (16% and 8%, respectively) and under the tongue area (4%); and in OLP patients were cheeks (68%) and side and dorsal surfaces of tongue (20% and 12%, respectively)
However, some studies indicated different results. In this regard, Rasool et al., (2014) have suggested that the β2M blood level is a better indicator comparing to its saliva level [24]. Though, in the present study, the β2M saliva level was significantly higher in patients rather than in normal people. This contradiction could be related to the low sample volume, the intensity of the disease, and the patient's age and sex. Thus, it is essential to determine the differentiated degree of the disease in measuring of β2M saliva in metastatic patients.
Because the saliva is a non-invasive tool, cheap, simple and duplicable, proving that it can be used as a diagnostic marker is highly important. It has a potential role in analyzing the premalignant and malignant complications. The present study showed that the β2M level in normal people is significantly lower compared to OLP and OSCC patients; β2M concentration in patients with those diseases was the same.

Limitations
It is suggested to perform the studies with higher sample volume and considering different age groups and genders. It is recommended to measure the β2M level in different grades of the disease
and different pre-malignant and malignant complications.

Abbreviations

β2M: β2- macroglobulin

OLP: oral Lichen Planus

SCC: squamous cell carcinoma

OSCC: OLP lesions to oral SCC

Declarations

Ethics approval and consent to participate

The study is approved by ethics committee of Zahedan university of Medical Sciences. All participants signed a written consent.

Availability of data and materials

Data are available upon request from corresponding author.

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There is no acknowledgment for the present study.

Consent for publication

Not applicable.

Competing interests

There is no conflict of interest to declare.

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Authors Contributions

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FN and TN contributed to the conception and design of the study and the study protocol. TN and ea managed the day-to-day running of the study. SSR conducted data analysis and all authors helped with data interpretation. This manuscript was written by FN and EA with input from all co-authors. All authors read and approved the final manuscript.

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