Full Upregulation of $\alpha_1$ and $\beta_1$ adrenergic receptors as an effective factor on hyposalivation of patients with OLP

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Research article

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Abstract

Objectives: Oral lichen planus (OLP) is one of the common chronic, autoimmune disorders in oral cavity. Regarding the role of adrenergic receptors in mediating stress and that's effect on salivary composition, the purpose of this study was to investigate salivary levels of α1- and β1-adrenergic receptors in OLP patients to response to this research question: Do agonist or antagonist of adrenergic receptors affect hyposalivation of OLP patients? Method: In this case-control study, stimulated and unstimulated saliva samples were collected from 33 patients and 33 healthy individuals. The salivary flow rate and levels of α1- and β1-adrenergic receptors were measured by ELISA assay. Data were analyzed using SPSS 25 and T-test. Results: The stimulated and unstimulated salivary flow rate was significantly lower in OLP patients than healthy subjects. The α1-adrenergic receptors in the unstimulated saliva of patients was significantly higher than in the healthy subjects (p<0.001). α1-receptor in unstimulated saliva in both groups of patients (p<0.001) and healthy subjects (p=0.006) was significantly higher than stimulated saliva in the same groups. The level of β1-adrenergic receptors in the patients was significantly higher in the unstimulated saliva (p=0.001) and lower in the stimulated saliva than in the healthy subjects (p=0.003). β1-receptor was significantly higher in the unstimulated saliva than stimulated saliva in the patients (p<0.001). Conclusion: high levels of α1- and β1-adrenergic receptors in saliva of OLP patients reduce salivary flow rate by increasing the salivary proteins, mucin and saliva viscosity. Selective antagonist of α1- and β1-adrenergic receptors Can improve hyposalivation of OLP patients. Keywords: Lichen Planus, Oral, Adrenergic Receptors, Adrenoceptors, saliva

Introduction

Oral lichen planus (OLP) is one of the commonly diagnosed chronic autoimmune oral mucosal disorders, which occurs through attacking autoreactive cytotoxic T lymphocytes to basal layer of oral epithelium[1]. It is commonly seen in middle-aged women, which affects not only the oral mucosa but also the skin and other mucosa such as vagina, esophagus and larynx[2]. In addition to its common symptoms like pain and irritation, OLP is associated with a number of other symptoms including xerostomia[3]. Various studies have documented the association of lichen planus with hyposalivation and xerostomia [4-7]. The prevalence of xerostomia in OLP patients is higher than that of healthy people. According to research, hyposalivation is the most common cause of xerostomia[8, 9]. In addition, changes in salivary composition, especially salivary proteins, are very important in the sense of xerostomia[10]. The etiology of OLP has not been fully understood so far. However, stress, anxiety and depression are considered as effective factors in the incidence and exacerbation of OLP courses[11]. Following stress, sympathetic nervous system and hypothalamic-pituitary-adrenal (HPA) axis are activated and then apply their effects to the body through their own specific receptors. Adrenergic receptors (ARs) are subtypes of G protein-coupled receptors (GPCRs) that react to releasing catecholamines in response to stress. These receptors are divided into two groups of α and β. Changes in the level or type of adrenergic receptors on the surface of immune and non-immune cells have been reported in some autoimmune diseases, although no reports were found on OLP patients[12]. The sympathetic nervous system (SNS) in the salivary glands exerts its
functions through the $\alpha_1$- and $\beta_1$-adrenergic receptors, and the parasympathetic nervous system (PNS) operates through vasoactive intestinal peptide (VIP) and acetylcholine (ACh)\cite{13}. The $\beta$-receptor has the central role in secretion of salivary proteins, and the $\alpha$-receptor has less active in the secretion of salivary fluid, electrolytes and proteins\cite{13}. Regarding the role of stress, SNS and adrenergic receptors in the etiopathogenesis of autoimmune diseases, including OLP \cite{11}, as well as considering the role of adrenergic receptors on the quality and quantity of saliva\cite{14}, the current study aimed to evaluate the salivary level of $\alpha_1$- and $\beta_1$-adrenergic receptors in OLP patients. According to best our knowledge so far this topic has not evaluated.

Materials And Methods

The Ethics Committee of Tehran University of Medical Sciences with the code of IR.TUMS.DENTISTRY.REC.1396.4182 approved the present study protocol. 33 patients with OLP from those referred to Department of Oral and Maxillofacial department, School of dentistry of Tehran University of Medical Sciences, and 33 healthy subjects with matched age and gender were selected for this study. All participants signed informed written consent form. Inclusion criteria were diagnosis of OLP based on modified WHO criteria, any systemic and local treatment in the past three months, and any sensory-motor or cognitive impairment such as MS, Myasthenia Gravis, Alzheimer's and Parkinson's diseases. Patients with systemic and autoimmune diseases, pregnancy or lactation, taking xerostomia-inducing drugs and agonist or antagonist of adrenergic and cholinergic receptors, gingivitis, toothache and Smokers excluded from study.

Saliva sample collection:

The unstimulated and stimulated saliva samples were collected from 9 am to 12 noon, from all healthy people and patients. They were asked to avoid eating 2 hours before sampling. When unstimulated saliva sampling, the participants were asked to sit on a chair and then spit into a plastic vial as often as possible within five minutes. To get the stimulated saliva sample, the participants chewed the same size and shape of chewing gum for a minute. After throwing the chewing gum and swallowing the saliva in their mouths, they spit their saliva in the vial within five minutes. The saliva flow rate was calculated by dividing the saliva content (in milliliters) by time (in minutes). Hyposalivation and xerostomia were detected when the stimulated saliva flow rate was less than 0.5ml/min, and when the unstimulated saliva flow rate was less than 0.1ml/min\cite{15}.

Laboratory methods:

The samples were centrifuged at 2000 rpm for 10 minutes to remove sputum and other additional salivary substances such as plaque and debris. The purified saliva (supernatant) was poured into 1-ml microtubes, kept at -20°C (as frozen), and sent to the laboratory to measure $\alpha_1$- and $\beta_1$-adrenergic receptors. Measurements were performed using ELISA kit (ELISA kit for adrenergic beta1, ELISA kit for adrenergic alpha1, manufacturer: USCN, the USA) to determine the exact level of receptors in each person.
Data were analyzed by SPSS version 25 software using t-test at the significance level of p-value<0.05.

**Results**

In this case-control study, were enrolled 33 OLP patients with mean age of 36.2 years (12 males and 21 females) and 33 healthy subjects with mean age of 35.6 years (12 males and 21 females) as case and control groups respectively.

The $\alpha_1$-adrenergic receptor level was significantly higher in unstimulated saliva of OLP patients to control group (p-value<0.001) although this result was not found in stimulated saliva (p-value=0.545). In Both case and control groups, levels of $\alpha_1$-receptor in unstimulated saliva were significantly higher than stimulated saliva in same group (p-value<0.001) (p-value=0.001) (Table 1).

The $\beta_1$-adrenergic receptor levels were significantly higher in unstimulated saliva (p-value=0.001) and significantly lower in stimulated saliva (p-value=0.003) of OLP patients.

In case group, $\beta_1$-receptor was significantly higher in the unstimulated saliva than stimulated saliva (p-value<0.001), although this result was not found in the control group (p-value=0.918) (Table 2).

The unstimulated and stimulated salivary flow rate was significantly lower in OLP patients than control group (p-value=0.005) (p-value=0.001). (Table 3).

**Discussion**

Autoimmune diseases, including OLP, have multifactorial pathogenesis. Genetic, hormonal and environmental factors seem to play a role in the development of diseases. Physical and emotional stresses are the main environmental factors in etiology of autoimmune diseases[16]. The two main hormonal pathways that are activated in response to stress alone or together are the Hypothalamic–pituitary–adrenal (HPA) axis and the sympathetic nervous system (SNS). The SNS activation directly and indirectly releases epinephrine and norepinephrine through adrenal medulla[17]. Several studies have shown a positive and significant relationship between psychogenic stress and OLP[18-21], while others did not find a meaningful relationship [22, 23]. The adrenergic receptors bind to the catecholamines secreted in response to stress, and lead to their effects in various cells of the body. Change in the level and/or the type of expressed receptor on the cell surface plays an important role in the etiopathogenesis of the disease[17]. Based on the results of this study, the levels of $\alpha_1$- and $\beta_1$-adrenergic receptors were significantly higher in unstimulated saliva of OLP patients compared to control group. In addition, the stimulated and unstimulated salivary flow rates were significantly lower in OLP patients to controls.

Various types of autoimmune diseases such as rheumatoid arteritis, systemic lupus erythematosus can be associated with dysfunction of salivary glands (regardless of the presence or absence of Sjogren's syndrome)[24]. The occurrence of hyposalivation as a common finding in OLP patients have been confirmed in various studies [4-7, 24, 25]. The autonomic nervous system has a critical role in the secretion of saliva. The $\beta$-receptor, mediated by cAMP, has high and very high performance in protein and
mucin concentrations of saliva respectively. The stimulation of these receptors leads to low, foamy and viscous saliva. Stimulation of $\alpha$-receptors leads to increased protein concentrations due to the induction of intracellular $\text{Ca}^{2+}$. The $\alpha$-receptors have low effect on volume, mucin and viscosity of the saliva[14]. In pathogenesis of autoimmune diseases, including OLP, chronic stress and increased SNS tone seems to be involved in reducing the salivary flow rate by increasing salivary mucin production ($\beta_1$-adrenergic receptor), increasing protein secretion ($\alpha_1$- and $\beta_1$-adrenergic receptors), vasoconstriction and decreasing blood supply of salivary gland parenchyma. Although the increase in salivary protein components can increase partially the salivary volume, but elevated salivary concentrations and viscosity highly decline the salivary flow rate (volume/min). As we know, the salivary flow rate is the main criterion to evaluation of hyposalivation, not the volume of saliva. The changes in the quality of saliva (increased mucin and protein) are one of the most effective factors in the development of xerostomia even in cases where the patient has no hyposalivation[13]. The high production of protein in the secretory cell requires a lot of energy and leads to stress in the endoplasmic reticulum (ER). The secretory cell response to ER stress by activating hypoxic signals, producing reactive oxygen species (ROS), autophagy, and cell death through internal apoptotic processes. This phenomenon, if chronic, leads to less secretion units in the salivary glands, resulting in reduced saliva production. During apoptosis, the production of inflammatory cytokines, such as IL-6 and type-1 IFNs, and the release and secretion of endogenous antigens and chaperon proteins via apoptotic buds of salivary cells can even be involved in the pathogenesis of OLP[16].

Despite the lack of previous studies on the correlation between adrenergic receptors and OLP pathogenesis, down regulation of muscarinic receptors in OLP patients have been confirmed [25]. It seems that the main role in reducing the salivary flow rate in the OLP patients is due to upregulation of SNS and downregulation of PNS. These two systems are not antagonists to each other in salivary secretion, but function together. The SNS has synergistic effects on secretory cells receiving parasympathetic fibers[9, 14]. It should be noted that the variable concentration of norepinephrine during SNS stimulation can differently stimulate the $\alpha_1$- and $\beta_1$-adrenergic receptors[26]. In the present study $\beta_1$-adrenergic receptor in the unstimulated saliva significantly increased and decreased in the stimulated saliva among the OLP patients. Several studies have also shown the contradictory effects of adrenergic agonist and antagonist drugs on the salivary flow rate [27-29]. Also lowering entrance of sympathetic nerve fibers to the parotid gland in comparison with other major salivary glands that cause to less expression of adrenergic receptors on the surface of the secretory cells of parotid gland [13], stimulation of saliva secretion through chewing instead of taste triggers that cause to preferably stimulation of PNS to SNS and more time opportunity for the exchange of acinar cells and ducts in unstimulated saliva to stimulated saliva [10, 16] justify lower levels of adrenergic receptors in the stimulated saliva compared to the unstimulated saliva in both control and OLP groups. Upregulation of salivary adrenergic receptor in OLP patients, probably source both receptors in the secretory cells of salivary gland and elevated level of these receptors in blood cells. However, this phenomenon has not yet been investigated in the OLP patients, such Studies have shown increased expression of the $\alpha_1$-adrenergic receptor in peripheral blood mononuclear cells (PBMCs) during chronic inflammation in patients with juvenile RA, which led to an
increase in IL-6 levels in these patients. These receptors are not expressed normally on the surface of the PBMCs [29-31]. Another study revealed that the adrenergic receptors on the surface of immune cells shifted from β2 to α1 in autoimmune diseases [29]. In healthy condition SNS, balance the pro-inflammatory and anti-inflammatory responses by stimulating different receptors. Chronic stress in genetically susceptible individuals led to an imbalance in the levels of adrenergic receptors on the surface of the immune and salivary cells that can be effective in reduced salivary flow rate and pathogenesis of autoimmune diseases such as OLP.

**Declarations**

**Ethics approval and consent to participate:** "Not applicable"

**Consent to publish:** In this study there is no details, images, or videos relating to an individual person that which needs to be agreed to publish.

**Availability of data and materials:** The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Competing interests:** There is not any competing interests in this study.

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Tables

Table 1. Level of α1 receptors

| α1 receptor (ng/ml) in saliva | N  | Minimum | Maximum | Mean    | Std. Deviation |
|------------------------------|----|---------|---------|---------|----------------|
| Case                         |    |         |         |         |                |
| Unstimulated                 | 33 | 15.55   | 29.20   | 24.3359 | 3.12060        |
| Stimulated                   | 33 | 11.57   | 26.87   | 17.4448 | 3.80048        |
| Control                      |    |         |         |         |                |
| Unstimulated                 | 33 | 11.14   | 23.76   | 18.8056 | 2.64409        |
| Stimulated                   | 33 | 12.78   | 21.75   | 16.9663 | 2.44620        |
### Table 2. Level of $\beta_1$ receptors

| $\beta_1$ receptor (ng/ml) in saliva | N  | Minimum | Maximum | Mean    | Std. Deviation |
|-------------------------------------|----|---------|---------|---------|----------------|
| Case                                |    |         |         |         |                |
| Unstimulated                        | 33 | 28.30   | 49.23   | 40.1883 | 5.01807        |
| Stimulated                          | 33 | 20.20   | 43.67   | 31.0703 | 6.89601        |
| Control                             |    |         |         |         |                |
| Unstimulated                        | 33 | 23.31   | 43.13   | 35.6515 | 5.56397        |
| Stimulated                          | 33 | 26.11   | 45.56   | 35.7397 | 5.40878        |

### Table 3. Level of salivary flow rate

| flow rate (ml/min) | N  | Minimum | Maximum | Mean    | Std. Deviation |
|--------------------|----|---------|---------|---------|----------------|
| case               |    |         |         |         |                |
| Unstimulated       | 33 | 0.10    | 0.80    | 0.3103  | 0.14077        |
| Stimulated         | 33 | 0.16    | 1.00    | 0.4345  | 0.19100        |
| control            |    |         |         |         |                |
| Unstimulated       | 33 | 0.20    | 1.00    | 0.4336  | 0.19762        |
| Stimulated         | 33 | 0.22    | 1.00    | 0.6009  | 0.21109        |