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## Vascular Endothelial Growth Factor in Serum and Saliva of Oral Lichen Planus and Oral Squamous Cell Carcinoma Patients

Farzaneh Agha-Hosseini, DDS.<sup>1</sup>, Iraj Mirzaii-Dizgah, Ph.D.<sup>2</sup>, Mina Mohebbian, DDS.<sup>3</sup>,  
Mohammad-Reza Sarookani, Ph.D.<sup>4</sup>

1- Full Prof. of Dental Research Center, Dental Research Institute, Tehran University of Medical Sciences & Department of Oral and Maxillo facial Medicine, Faculty of Dentistry, The Academy of Medical Sciences, Tehran, Iran (Corresponding author; aghahose@sina.tums.ac.ir)

2- Full Prof. of Department of Physiology, School of Medicine, Aja University of Medical Sciences, Tehran, Iran

3- Assistant Prof. of Oral and Maxillo facial Medicine, Faculty of Dentistry, Zanjan University of Medical Sciences, Zanjan, Iran

4- Full Prof. of Cell and Molecular Research Center, Qazvin University of Medical Sciences, Qazvin, Iran

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### Abstract

**Background:** Oral lichen planus (OLP) is considered as a potentially malignant disorder and vascular endothelial growth factor (VEGF) may play a key role in cancer development. The aim of this study was to compare serum and saliva VEGF among patients with OLP, oral squamous cell carcinoma (OSCC) and a healthy control group.

**Methods:** A cross sectional study was performed on 27 patients with OLP, 27 patients with OSCC and 27 healthy volunteers. The serum and saliva VEGF were assayed by ELISA method. Statistical analysis of ANOVA was used.

**Results:** The mean saliva flow rate and serum VEGF in OLP and OSCC patients were significantly lower compared to healthy control group ( $p < 0.05$ ), but there was no significant difference between OLP and OSCC patients. There was no significant difference in mean salivary VEGF among groups.

**Conclusion:** It seems that saliva VEGF may not be a good biomarker for OLP and OSCC.

### Introduction

Oral lichen planus (OLP) is a widespread, chronic, immunological mucocutaneous disease that commonly entails the oral mucosa (1). It is relatively prevalent, affecting about 1–2% of the adult population, primarily the middle-aged and elderly, and women more frequently than men (2). The different mechanisms hypothesized to be involved in the immunopathogenesis are antigen-specific cell-mediated immune response, non-specific mechanisms, autoimmune response, and humoral immunity (3). Several investigations

have estimated varying probability of the malignant potential of OLP, in general ranging from 0.04% to 1.74%. Consequently, many authors have come to the opinion that OLP is an actual pre-malignant lesion, and the World Health Organization (WHO) has categorized OLP as a potential malignant disorder (4). As yet, many salivary molecules have been suggested to diagnose and predict prognosis in oral squamous cell carcinoma (OSCC) patients. In this case, saliva can be obtained as a non-invasive diagnostic specimen.

Among the major endothelial cell specific stimulatory factors, vascular endothelial growth factor (VEGF) is considered as one of the most powerful mediators of angiogenesis (5), promoting endothelial cell migration toward a hypoxia area. It is a chimerical glycoprotein with a molecular weight of 34-45 KDa, consisting of two sub-units (6). The adult vasculature is mainly quiescent, and angiogenesis does not occur under normal circumstances. Hence, this process plays a part in physiological conditions like embryonic development and wound healing, and in pathological circumstances such as growth of cancer, and the development of chronic inflammatory diseases such as rheumatoid arthritis and psoriasis (6). VEGF and its receptors activate a phosphoinositide 3-kinase (PI3K) pathway involved in wound healing and endothelial cell survival (7).

Saliva is the first biological medium encountered by external materials taken into our bodies as part of food, drinks or inhaled volatile ingredients. During evolution, a wide variety of defense mechanisms have developed, and saliva is equipped with several such mechanisms, as immunological and enzymatic defense systems (8). Whole saliva appears to represent the environment the best, as it reflects the components of all salivary glands (9).

Overall, OLP is an important view of several aspects. It is a common disorder, after dental caries and gingivitis, in dental clinics. It is a chronic disorder and may be with the patient for a long time. Almost all of its forms are painful and a patient with OLP will suffer discomfort while eating, drinking, speaking and swallowing; even patients with the keratotic form claim unpleasant roughness in their mucous membrane (10). The ethiopathogenicity of OLP is not yet elucidated, therefore at the present time we have no cure for it. Lastly, its

predilection to malignancy and its pathogenicity is unclear. Therefore, studies are conducted to predict its probability for transformation. The focus is on its ethiopathogenicity, modalities of treatment or predicting its probability for transformation”.

The factors or agents which cause transformation of potential malignant disorders are not known, but can be divided into environmental and genetic; some of the genetic factors are known in OSCC, including VEGF.

Saliva is thought to be a mirror of the body and the diagnostic fluid of the future. As the advantage of using saliva as a biological specimen, it is used to aid in the diagnosis of diseases and assessment of the severity of some illnesses. Accordingly, in this study, we compared VEGF expression in saliva of OLP, OSCC and healthy control, to see whether VEGF is detectable in saliva of these patients, and whether saliva can be used as an appropriate matrix in clinical practice for detection of OLP and OSCC.

## Method

### Subjects

Our participants included twenty seven patients with OSCC (12 males / 15 females, aged 35-83 years, mean age 65 years), 17 low stages (1 and 2) and 10 high stages (3 and 4), who were recognized by biopsy and pathological examinations and referred to the Cancer Department of Hospital, Tehran University of Medical Sciences (TUMS). All patients had primary tumor without any treatment, surgery, radiation therapy or chemotherapy.

Twenty seven patients suffering from OLP (9 males / 18 females, aged 25-77 years, mean age 52 years) -10 reticular forms, 17 atrophic-erosive forms- were selected from the

Department of Oral and Maxillo facial Medicine, Faculty of Dentistry. The diagnosis of OLP was made based on the WHO diagnostic criteria for oral lichen planus (8). These criteria were on the basis of the clinical and histopathological properties. Clinical criteria were: presence of bilateral, more or less symmetrical lesions; presence of a lace-like network of slightly raised gray white lines (reticular pattern); erosive, atrophic, bullous and plaque-type lesions are only accepted as a subtype in the presence of reticular lesions elsewhere in the oral mucosa. Histopathological criteria were: presence of a well-defined band-like zone of cellular infiltration restricted to beneath the epithelium, consisting primarily of lymphocytes; signs of 'liquefaction degeneration' in the basal cell layer; absence of epithelial dysplasia. Every patient who had lichenoid lesions objectively related to exogenic agents such as dental materials, drugs or specific allergens was excluded (11).

The control group had 27 healthy volunteers (8 males / 19 females, aged 31-64 years, mean age 41.7 years) without any clinical sign of oral disease. Subjects with systemic diseases or who were taking any medication at the time of the study were excluded.

The protocol was approved by the ethics committee and informed consent was obtained from patients before commencing the study (Ethical code: 91-03-70-18883).

### Saliva and serum collection

Venous blood and saliva of each participant were gathered at the same time under resting conditions, in a quiet room between 9 a.m. and 12 p.m., and at least 90 minutes after the last intake of food or drink. Unstimulated saliva samples were collected without chewing movements through expectoration. A piece of standard-size paraffin was chewed by each

participant for pre-stimulation for 2 minutes, and then they were asked to expectorate the stimulated saliva present in the mouth for 5 minutes. Salivary flow rates were measured as ml/min.

Venipuncture was used to obtain blood specimens, which were collected in 15 ml glass vacuum tubes with clot activator, and allowed to clot. The serum and supernatants of the saliva were separated by centrifuging (3000 g, 10 min). The specimens were kept at -20°C promptly after serum and saliva collection until the determination of VEGF.

### Determination of VEGF concentration

Salivary and serum VEGF content were measured with a commercial Enzyme-Linked Immune-Sorbent Assay kit (Boster Immunoleader, Pleasanton, CA), following instructions provided by the manufacturer.

### Statistical analysis

Statistical analysis was performed using SPSS software. Data were analyzed by ANOVA followed by Tukey's post-hoc test. Data are expressed as mean  $\pm$  SEM for each group. Statistical significance was determined at  $P < 0.05$ .

### Results

There was no significant difference in sex among groups ( $P = 0.621$ ). However, the mean age of OSCC group was significantly higher than OLP and control groups ( $P < 0.05$ ).

A one-way ANOVA showed that there was a significant difference in stimulated ( $F = 5.768$ ,  $P = 0.005$ ) and unstimulated ( $F = 9.503$ ,  $P = 0.000$ ) saliva flow rate among groups (Table 1). Stimulated and unstimulated saliva flow rate were significantly low in both patients suffering from OLP and

OSCC than in healthy individuals. However, there was no significant difference between OLP and OSCC groups.

Serum VEGF was significantly different among groups ( $F= 3.797, P=0.027$ ) (Table 1). The mean serum VEGF in OLP and OSCC patients was significantly lower compared to control group, but there was not a significant difference between OLP and OSCC patients.

There were no significant differences in the mean stimulated ( $F= 2.014, P=0.140$ ) and unstimulated ( $F=2.114, P= 0.128$ ) saliva VEGF among OLP, OSCC and healthy control groups (Table 1).

There was no significant correlation between serum and saliva levels of VEGF with age of participants.

**Table 1.** Serum and saliva levels of VEGF and total protein in OLP, OSCC and healthy control groups

|  | Healthy control | OLP          | OSCC         |
|--|-----------------|--------------|--------------|
| Sex (Male/Female)                      | 9/18            | 9/18         | 12/15        |
| Age (mean (range))                     | 41.7 (31-64)    | 52 (25-77)*  | 65 (35-83)*# |
| Unstimulated saliva flow rate (mL/min) | 0.78 ± 0.09     | 0.43 ± 0.05* | 0.40 ± 0.06* |
| Stimulated saliva flow rate (mL/min)   | 0.91 ± 0.12     | 0.62 ± 0.07* | 0.50 ± 0.06* |
| Serum VEGF (ng/mL)                     | 0.29 ± 0.05     | 0.16 ± 0.02* | 0.20 ± 0.03* |
| Unstimulated saliva VEGF (ng/mL)       | 4.59 ± 0.57     | 3.26 ± 0.43  | 3.50 ± 0.46  |
| Stimulated saliva VEGF (ng/mL)         | 1.94 ± 0.20     | 1.60 ± 0.13  | 2.20 ± 0.28  |

Data are expressed as mean ± SEM, \*: Different from control group,  $P < 0.05$ , #: Different from OLP groups,  $p < 0.05$

## Discussion

OLP is a common non-infectious oral mucosal disorder among adult patients attending oral pathology and oral medicine clinics (7). Moreover, oral lesions are often refractory to conventional therapies compared to cutaneous lesions (12). Given the progress of some of the OLP cases to the OSCC, early diagnosis of the disease will be commendable. VEGF is a multifunctional vasoactive peptide with both “direct” and “indirect” angiogenic potential (13). The aim of this study was to measure the level of VEGF in serum, and stimulated and unstimulated saliva of OLP and OSCC patients compared to a healthy control group.

In the present study, the level of serum VEGF in OLP and OSCC patients was significantly lower than the healthy control group which is compatible with other reports (7,14) but it is in contrast with other studies (6,15,16). No significant differences

were seen in saliva VEGF between OLP, OSCC and healthy individuals in this study. It has been shown that the vascular endothelial growth factor receptor 2 expression in saliva is higher in OLP than in healthy individuals and higher in OSCC than in OLP (17).

Whereas the histopathological characteristics of OLP are defined as dense sub epithelial lympho-histiocytic infiltrate, enhanced numbers of intra-epithelial lymphocytes, and apoptotic basal keratinocytes, the trigger for keratinocytes apoptosis in OLP is not clear (18). Normally, increased VEGF expression under hypoxia promotes angiogenesis, endothelial cell migration, and survival. The lower VEGF expression under hypoxia is likely to impair epithelial repair, and induction of apoptosis through PI-3K pathway among OLP or OSCC patients (7). Research carried out in acute gastric injury revealed that blocking endogenous VEGF impacts with anti-

VEGF antibodies worsened mucosal injury, while administration of recombinant VEGF depleted the severity of mucosal injury (19). Growth factors are known to play a crucial role in wound repair. A large number of vulnerary growth factors are secreted in saliva, such as VEGF, epidermal growth factor (EGF), transforming growth factor- $\alpha$  and - $\beta$  (TNF $\alpha$ , TNF $\beta$ ), acidic and basic fibroblast growth factors, and insulin-like growth factors (IGF-1, IGF-2). The expression profile of these growth factors is significantly different between skin and oral mucosa, and may account for the distinction in the wound healing phenotype between the two tissues (13). Oral mucosal tissue repair proceeds through the classic stages of wound healing including hemostasis, inflammation, proliferation, tissue regeneration and resembling cutaneous wound healing. This orchestrated sequence of events culminates in reepithelialization and restoration of tissue homeostasis. However, unlike cutaneous wound healing, wounds in the oral mucosa have been recognized to heal more swiftly, in a regenerative fashion and with reduced inflammation (20). The mechanisms of this increased wound healing phenotype have not been totally crystallized, although saliva is known to be a fundamental determinant of oral homeostasis (21). This significance is revealed in clinically deficient disease states such as xerostomia due to medications, head and neck radiation, or autoimmune diseases including Sjögren's syndrome, which all end in an impaired wound healing phenotype (22). In the present study, the level of serum VEGF in OLP and OSCC patients was lower in comparison to the healthy control group. we know that there

is a decrease in VEGF level in the area of wound, as in a previous study investigating salivary VEGF in Recurrent Aphthous Stomatitis (RAS) patients, we found that the salivary VEGF is significantly lower in the acute phase of the disease than in the remission phase (23). Lower levels of salivary VEGF were correlated with impaired neovascularization and reepithelialization (13). Nonetheless, tumors will not grow beyond 1 to 2 mm<sup>3</sup> unless an intratumoral capillary network is formed (24). In all previous studies on OLP, patients either during examination (objective) or reporting themselves, complained (subjective) about dry mouth (or xerostomia) (8,12,25-28). In this study, we investigated that unstimulated and stimulated saliva flow rate was significantly low in OLP and OSCC patients with respect to healthy control. This finding is in line with Colquhoun et al. study (29). It has been shown that M3 receptors are lower in saliva of OLP patients (27). This may contribute to hyposalivation in OLP; therefore, further studies are necessary to detect its real etiopathogenicity.

### Conclusion

It seems that unlike serum, saliva VEGF may not be a good biomarker for OLP and OSCC.

### Conflict of Interest:

The authors of this manuscript certify that they have no conflict of interest.

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