

Comparative Evaluation of EGF in Oral Lichen Planus and Oral Squamous Cell Carcinoma

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Abstract- Oral lichen planus (OLP) is classified as a potential malignant disorder, and epidermal growth factor (EGF) may play a key role in cancer development. The aim of this study was to compare serum and saliva EGF among patients with OLP and oral squamous cell carcinoma (OSCC). A cross-sectional study was performed on 27 patients with OLP (10 reticular and 17 atrophic-erosive forms), 27 patients with OSCC and 27 healthy control group. The study was conducted at the Cancer Department, Clinic of Oral Medicine, Tehran University of Medical Sciences. The serum and saliva EGF were assayed by ELISA method. Statistical analysis of ANOVA was used. The mean serum EGF in OLP and OSCC patients was significantly lower compared to healthy control group ($P < 0.05$), but no significant difference was observed between OLP and OSCC patients. There was no significant difference in mean salivary EGF among groups. As serum EGF levels appear to be statistically similar in OLP and OSCC, it seems that EGF might play a role in the pathogenesis of OLP and its cancerization.

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Introduction

Lichen planus is a common chronic dermatologic disease which affects the oral mucosa. About 1-2% of the population are reported to have oral lichen planus (OLP) (1,2), and the number of afflicted men is about half that of afflicted women in the population in the 30-60 years age range (1,3). Cutaneous lesions are mostly self-limiting, but oral lesions are chronic, and result in significant morbidity (4-6). They are refractory to control and do not usually react to conventional therapies (6,7). In this common disorder, auto-cytotoxic T lymphocytes trigger apoptosis of epithelial cells, leading to chronic inflammation (2). Very little is known about this disease, though it was first described in 1869. At the present time, OLP is actually considered a potentially malignant disorder (8).

The most frequent malignant tumor in the oral cavity and the eighth among all cancers is oral squamous cell

carcinoma (OSCC) (9). The sixth decade of life is the peak time for this disease, and its variants comprise 90-95% of all oral cancers. A persistent mass, nodule or indurated ulcer would be the typical presentation of OSCC, which also can develop from precancerous lesions or normal epithelium. Half of patients suffer a recurrence of the disease after treatment, 80% within 2 years, and the rest within 4 years (10).

Cancer epidemiologists have identified daily diet, smoking, and infection/inflammation as factors which cause cancer. An unquestionable factor in carcinogenesis is infection/ inflammation. For example, the viruses of chronic hepatitis tend to increase liver cancers; infection with papillomavirus increases anogenital cancers, inhaling asbestos and other irritants increases the risk of lung cancers, even for people who are not smokers. Ulcerative colitis and Crohn's' and other autoimmune diseases are typical examples of tissue-inflammation-associated carcinogenesis (11).

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Epidermal Growth Factor (EGF) is a 53-aminoacid polypeptide which is primarily taken from mouse salivary glands. Saliva is a potential source of EGF in oral milieu since the synthesis of EGF is basically done in salivary glands in humans although it is the responsibility of kidneys to produce EGF systematically. Based on the evidence, salivary EGF is a cytoprotective factor against injuries that contributes to the maintenance of the integrity of gastrointestinal (GI) mucosa, such as the oral cavity mucosa membrane and the protection of oral mucosa from different injurious substances (12).

Saliva is known as a diagnostic fluid. It has specific benefits in comparison with serum, since individuals with modest training can collect it noninvasively; in addition, analysis of saliva has shown that it has comparable values of different biochemical and immunological parameters as those parameters are routinely evaluated in blood (13).

This study was to investigate biomarkers which are considered as high-risk factors in oral cancer such as EGF in OLP patients. We compared this factor in OSCC as a positive control, the healthy subjects as a negative control. Also, we measured and compared the salivary and serum levels of total protein and salivary flow rate in patients with OLP, OSCC, and healthy participants. In fact, this purpose was planned to understand better the role of factors affecting the malignant transformation of this common disorder.

Materials and Methods

Participants

A total of 27 patients with OSCC who were diagnosed by biopsy and pathological examinations (12 male / 15 female, aged 35-83 years, mean age 65 years) referred to the Cancer Department of Imam Khomeini Hospital, Tehran University of Medical Sciences (TUMS), were selected. All patients had primary tumor without any treatment, surgery, radiation therapy or chemotherapy.

Twenty seven patients (9 male / 18 female, aged 25-77 years, mean age 52 years), with OLP -10 reticular forms, 17 atrophic-erosive forms- were selected from the Department of Oral Medicine, Faculty of Dentistry, TUMS. Inclusion criteria consisted of: clinical diagnosis of lichen planus (the presence of bilateral lesions, reticular lesions elsewhere in the mouth and well-defined band-like zones of inflammatory infiltration confined to the superficial part of connective tissue), consisting mainly of mature lymphocytes-vacuolar

alteration of basal layer of the epithelium, and identified through histopathological examination. Histological sign of dysplasia, lichenoid drug reaction, drug consumption in the past month, pregnancy, breastfeeding and any kind of localized or systemic disease were exclusion criteria. Patients who had lesions adjacent to an amalgam filling were also excluded from the study (6,10,14,15). The control group included 27 healthy volunteers (19 male / 8 female, aged 31-64 years, mean age 41.7 years) without any clinical sign of oral disease. Participants 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 of Tehran University of Medical Sciences, Iran and patient informed consent was obtained before commencing the study.

Saliva and serum collection

Venous blood and saliva of each participant were collected 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 two minutes, and then they were asked to expectorate the stimulated saliva present in the mouth for five 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 determining of EGF and total protein.

Determination of EGF and total protein concentrations

Salivary and serum EGF content were measured with a commercial Enzyme-Linked Immune-Sorbent Assay kit (Boster's human EGF ELISA Kit), following instructions provided by the manufacturer. Saliva samples were analyzed for total protein by the method of Bradford, with Bovine albumin as standard. EGF concentration was also expressed as a fraction of salivary total protein content (normalized EGF = EGF/TP ng/mg).

Statistical analysis

Statistical analysis was performed using SPSS software. Data were analyzed by ANOVA followed by

Tukey's posthoc test. Data are expressed as mean \pm SEM for each group. Statistical significance was determined at $P < 0.05$.

Results

A one-way ANOVA showed that there was a significant difference in serum EGF among groups [$F =$

12.06, $P = 0.0001$] (Table 1). The mean serum EGF in OLP and OSCC patients was significantly lower compared to control group, but there was no a significant difference between OLP and OSCC patients.

There were no significant differences in the mean stimulated [$F = 0.79$, $P = 0.47$] and unstimulated [$F = 0.88$, $P = 0.423$] saliva EGF among OLP, OSCC and healthy control groups (Table 1).

Table 1. Serum and saliva levels of EGF and total protein in OLP, OSCC patients and healthy control

	Healthy control	OLP	OSCC
Serum EGF (ng/mL)	0.39 \pm 0.04	0.18 \pm 0.03*	0.16 \pm 0.03*
Unstimulated saliva EGF (ng/mL)	4.74 \pm 0.44	5.04 \pm 0.42	4.30 \pm 0.41
Unstimulated saliva total protein (mg/mL)	0.91 \pm 0.07	0.93 \pm 0.10	1.63 \pm 0.17*#
Normalized unstimulated saliva EGF (ng/mg)	5.56 \pm 0.54	6.11 \pm 0.53	3.42 \pm 0.43*#
Stimulated saliva EGF (ng/mL)	2.69 \pm 0.37	2.08 \pm 0.22	2.44 \pm 0.36
Normalized stimulated saliva EGF (mg/mL)	0.69 \pm 0.07	0.62 \pm 0.05	1.39 \pm 0.19*#
Stimulated saliva normalized EGF (ng/mg)	4.15 \pm 0.53	3.44 \pm 0.29*	2.41 \pm 0.44*

Data are expressed as mean \pm SEM. *Different from control group and # different from OLP group, $P < 0.05$

There were significant differences in the mean stimulated [$F = 12.24$, $P = 0.0001$] and unstimulated [$F = 11.73$, $P = 0.0001$] saliva total protein among groups (Table 1). The mean stimulated and unstimulated saliva total protein were significantly higher in OSCC patients than both OLP and control group. However, no significant difference was observed between OLP and control groups.

There were significant differences in normalized unstimulated [$F = 8.11$, $P = 0.0001$] and stimulated [$F = 4.01$, $P = 0.021$] saliva EGF levels among groups (Table

1). Normalized unstimulated saliva EGF level was significantly higher in the patients suffering OSCC than OLP and healthy control groups, but there was no significant difference between OLP and control groups. Normalized stimulated saliva EGF was significantly lower in OSCC group than both OLP and control groups and also low in OLP group than controls.

There were no significant differences in serum and saliva levels of EGF among OLP groups (reticular and erosive forms) and OSCC groups (low and high stages) [$F = 0.17$, $P = 0.919$] (Table 2).

Table 2. Serum and saliva levels of EGF and total protein in patients suffering OLP (from reticular and erosive forms) and OSCC (stages 1, 2 and stages 3,4).

	OLP (Reticular)	OLP (Erosive)	OSCC (low stages)	OSCC (high stages)
Serum EGF (ng/mL)	0.21 \pm 0.5	0.16 \pm 0.04	0.16 \pm 0.04	0.17 \pm 0.06
Unstimulated saliva EGF (ng/mL)	5.10 \pm 0.63	5.00 \pm 0.58	4.66 \pm 0.59	3.69 \pm 0.48
Unstimulated saliva total protein (mg/mL)	0.91 \pm 0.14	0.94 \pm 0.14	1.58 \pm 0.24	1.72 \pm 0.22*#
Normalized unstimulated saliva EGF (ng/mg)	6.20 \pm 0.85	6.06 \pm 0.69	3.91 \pm 0.57	2.59 \pm 0.57*#
Stimulated saliva EGF (ng/mL)	2.33 \pm 0.35	1.93 \pm 0.28	2.54 \pm 0.55	2.27 \pm 0.30
Stimulated saliva's total protein (mg/mL)	0.53 \pm 0.06	0.68 \pm 0.08	1.25 \pm 0.24*	1.63 \pm 0.30*#
Normalized stimulated saliva EGF (ng/mg)	3.93 \pm 0.38	3.18 \pm 0.39	2.83 \pm 0.68	1.75 \pm 0.34*

Data are expressed as mean \pm SEM

*Different from reticular and # Different from Erosive, $P < 0.05$

There were significant differences in unstimulated [$F = 4.21$, $P = 0.01$] and stimulated [$F = 5.79$, $P = 0.002$] saliva levels of total protein among OLP groups (reticular and erosive forms) and OSCC groups (low and high stages) (Table 2). Total protein was significantly higher in the patients suffering OSCC in high stage than both reticular and erosive forms of OLP in stimulated and unstimulated saliva; and it was higher in low stage of OSCC than reticular form of OLP in stimulated saliva.

There were no significant differences in saliva's total protein between reticular and erosive forms of OLP and also between low and high stages of OSCC.

There were significant differences in unstimulated [$F = 5.82$, $P = 0.002$] and stimulated [$F = 2.67$, $P = 0.043$] saliva normalized EGF levels among reticular and erosive forms of OLP and low and high stages of OSCC groups (Table 2). Saliva normalized EGF level was significantly higher in the patients suffering high stage

of OSCC than a reticular form of OLP, but only unstimulated saliva normalized EGF was higher in high stages of OSCC than an erosive form of OLP. However, there were no significant differences in saliva normalized EGF between reticular and erosive forms of OLP and also between low and high stages of OSCC.

Discussion

OLP is a relatively common disorder with an unknown cause. As the first reported case of a squamous cell carcinoma developing from a mucosal lichen planus, the real odds of such transformation are a matter of discussion. Thus, several studies have shown the different probability of the malignant potential of OLP between 0.04% and 1.74%. Hence, many authors have accepted the idea that OLP is an actual pre-malignant lesion. However, the underlying mechanisms initiating development of cancer in OLP lesions are not understood. EGF is considered as high-risk factors in oral cancer. In this study serum and saliva level of EGF was investigated and we found that serum EGF level was low in OSCC and OLP than the control group. Furthermore, there were no differences in terms of serum EGF between OLP and OSCC and their subgroups.

EGF receptor is implicated in activating pathways increasing cellular proliferation, survival, migration and differentiation in most epithelial tissues, fibroblasts and endothelial cells, so the biological activities of EGF depends upon its binding to its receptor (12). EGF binding with receptor stimulates intrinsic tyrosine kinase activity that brings about the phosphorylation of the receptor, generating a signal that consequently changes gene regulation (16). In more than 80% of OSCC, EGFR (erb B1) is overexpressed. The overexpression of EGFR had an association with tumor invasion and metastasis, refractory to standard therapies (hormonal therapy, chemotherapy, and radiation) and diminished patient survival (17).

A number of biological processes inside and outside of the oral cavity can be attributed to EGF (16), entailing the proliferation and differentiation of epithelial and mesenchymal cells. Furthermore, EGF plays a pivotal role, not only in maintaining the homeostasis of epithelial cells but also in inflammation and immunological responses as well (8). In mice, the submandibular gland is the only proven tissue in which EGF is found, and, in humans, EGF has been isolated from submandibular gland and other tissues, in addition to saliva, serum and other fluids (18).

Current results showed that the level of serum EGF in descending order was: healthy control group, a reticular form of OLP, low stages of OSCC, an erosive form of OLP and high stages of OSCC. These results confirm that as serum EGF decreases, clinical signs of disease increases, and erosive form was closer to malignancy than reticular form.

Saliva plays a fundamental role in the protection and maintenance of the integrity of the upper part of the gastrointestinal mucous membrane (16). The diagnosis and treatment of OSCC have progressed, however little has been revealed on the significance of the oral environment and saliva in the development of this cancer (12). The volume of saliva and growth factors including EGF play a crucial role (16). Salivary EGF systematically influences the skin and greatly improves wound healing. Salivary EGF levels are low in patients with risk factors for cancer development (smoking, alcohol) and in those suffering from head and neck neoplasm (19).

The normalized unstimulated saliva EGF levels in OLP patients and healthy control group were significantly higher than OSCC patients, while in Dumbrigue *et al.*, study, the difference between normalized unstimulated saliva EGF levels in healthy control group and patients suffering from head and neck cancer was not statistically significant (19).

In the present study, stimulated and unstimulated saliva total protein was significantly lower in OLP patients and healthy control than in OSCC patients. The levels in ascending order are healthy control group, a reticular form of OLP, an erosive form of OLP, low stages of OSCC and high stages of OSCC. The finding may result from the wound and cellular shedding which usually exist in OSCC and erosive OLP, and this may also be the result of the increasing biomarkers such as tumor suppressors, oncogenes and proto-oncogenes in OSCC. Similar to the current study, Shpitzer *et al.*, concluded that unstimulated saliva total protein levels in OSCC patients were significantly higher than healthy controls (20).

In this study, stimulated saliva's total protein in OLP patients and control group was significantly lower than OSCC patients. In OSCC patients, it was higher in high stages OSCC group than the low stages group and in OLP patients; it was higher in erosive type than reticular type. The interpretation previously mentioned about unstimulated saliva total protein can also be considered here.

As serum EGF levels appear to be statistically similar in OLP and OSCC, it seems that EGF might play

a role in the pathogenesis of OLP and its cancerization.

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