

## **SALIVARY INSULIN LIKE GROWTH FACTOR BINDING PROTEIN-3 AND TRANSFERRIN LEVELS IN PATIENTS WITH ORAL SQUAMOUS CELL CARCINOMA VERSUS ORAL LICHEN PLANUS**

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### **ABSTRACT**

**Objective:** To gather preliminary data concerning local salivary levels of insulin like growth factor binding protein-3 (IGFBP-3) and its binding protein transferrin (Tf) in patients suffering from oral squamous cell carcinoma (OSCC) and oral lichen planus (OLP) compared to normal controls and to correlate it with clinical picture in OLp and histologic grading in OSCC.

**Materials and methods:** Salivary samples from 40 patients suffering from OSCC (20 poorly and 20 moderately differentiated), 40 patients suffering from OLP (clinically evaluated using REU scoring system) and 40 controls were collected and TF and IGFbP-3 levels were estimated using enzyme linked immunosorbent assay (ELISA), for statistical analysis, ANOVA followed by Tukey's post hoc and Pearson correlation tests were used.

**Results:** Significantly higher salivary levels of IGFBP-3 and Tf were recorded in different histological grades of SCC in comparison to Olp. Control group recorded the lowest significant values compared to OLP and OSCC ( $p < 0.0001$ ). A weak positive correlation was recorded between levels of Tf and IGFBP-3 in all groups. As well, a weak positive correlation between IGFBP-3 and Tf levels with REU in OLP and histological grades in OSCC was shown, except for the correlation between TF and OSCC histologic grading that was strong positive.

**Conclusions:** IGFBP-3 and Tf seems to play a role in pathogenesis of both OSCC and Olp, and could be considered as a reliable marker in diagnosis of OSCC and OLP, However, further studies are required .

### **INTRODUCTION**

OSCC is the most frequent cancer of the head and neck district, it accounts for more than 90% all oral neoplasms. It has a poor prognosis and the

global burden of cancer continues to increase largely because of aging, population growth and increasing adoption of smoking, alcohol drinking, physical inactivity, and unhealthy diet, therefore, primary

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prevention of oral cancer includes avoidance of those behaviors, whereas, secondary prevention is associated with early diagnosis and close monitoring of precancerous conditions<sup>(1,2)</sup>. The WHO classifies OLP as a premalignant disease with unspecified malignant transformation risk and suggests that OLP patients should be under close clinical observation<sup>(3)</sup>. Three clinical forms of OLP are described: reticular type; atrophic or erythematous type; and erosive type, the last one is considered to have the most premalignant potential. OLP is considered a T-cell mediated autoimmune chronic inflammatory disease in which cytotoxic CD8+ T-cells trigger the oral epithelial cells apoptosis, especially, basal cells<sup>(4,5)</sup>.

Studies have implicated Insulin like growth factor (IGF-I), and its binding protein- 3 in cancer development. IGF-I plays an essential role in regulating somatic growth, cell proliferation and differentiation via the IGF receptor I (IGF-IR), which allows IGF-I to exert its mitogenic effect on both normal and cancer cells<sup>(6)</sup>. The IGFbps are six high affinity proteins that bind IGF, increase its half-life and inhibit or potentiate its binding to its receptor. IGFBP-3 is mainly derived from hepatic Kupffer cells and is the most abundant in serum, over 90% of circulating IGF-I is complexed with IGFBP-3. IGFBP-3 normally inhibits the mitogenic action of IGF-I by preventing it from binding to its receptor; however, under certain circumstances - that is not fully determined-, this binding can enhance the activity of IGF-I by protecting it from degradation. IGFBP-3 modulate cell functions by both IGF-dependent mechanisms, which affect IGF1R signaling, and IGF-independent mechanisms, which do not involve changes in IGF1R signaling<sup>(7,8)</sup>.

IGFBP-3 possesses both growth-inhibitory and -potentiating effects on cells that are independent of IGF action and are mediated through specific IGFBP-3 binding proteins/receptors located at the cell membrane, cytosol, or nuclear compartments

and in the extracellular matrix that points to its function within major signaling pathways, Although the exact mechanism by which this occurs is not totally clear<sup>(8,9)</sup>. IGFbp-3 which can function as a tumor suppressor and is downregulated in some cancer tissues, nevertheless shows overexpression in association with markers of poor prognosis in many tumor types. A consistent evidence for an association between serum IGFBP-3 levels and either the risk of cancer development or cancer prognosis for several types of cancer is lacking, since the animal studies of IGFBP-3 overexpression and deletion are inconsistent and model endocrine/ autocrine, effects of IGFBP-3 did not help to define the contribution of circulating IGFBP-3<sup>(8)</sup>. Marimuthu et al., reported the over-expression of IGFBP-3 in 70% of Head and neck squamous cell carcinoma tissue samples<sup>(10)</sup>, on the other hand, IGFBP-3 was found to increase apoptosis and decrease the survival of breast cancer and other cells that are exposed to certain agents such DNA damaging agents<sup>(11,12)</sup>.

Transferrin has been identified as one of the IGFBP-3 binding proteins. Tf is a plasma protein that is synthesized mainly by hepatocytes and plays a central role in transferring iron around the body to sites where it is needed. Tf synthesis and storage are regulated by iron levels and nutritional status, Tf transports Fe<sup>++</sup> into the interior of the cell by transmembrane receptor mediated endocytosis (TfR1-TfR2) expressed on the surface of many cells<sup>(9,13)</sup>. Other tissues expressing Tf includes metastatic melanoma and human breast cancer cell lines, Tf has been detected in various body fluids including saliva<sup>(14,15)</sup>.

Tf is one of the growth factors that has been implicated in growth and differentiation activities that are independent of the iron -binding role. Apo- Tf preparation have been shown to possess growth - promoting effect. Tf possesses paracrine and autocrine effect, this paracrine effect has been taken advantage by some cancer cell lines such as human

colon tumor cells promoting their proliferation and metastasis<sup>(16)</sup>. Over expression of the transferrin receptor (CD71) has been reported in several cancers including lung, pancreas and breast<sup>(17,18)</sup>. However, other data showed that induction of apoptosis is one of TF multiple effects, that has not been fully determined<sup>(19)</sup>. The validity of Tf/IGFBP-3 binding through multiple independent in -vitro methods has been confirmed, and a physiologically significant consequence of this binding on cell proliferation and apoptosis was demonstrated<sup>(8,13)</sup>.

Salivary biomarkers for various malignant and potentially malignant diseases have become a subject of strong research interest. It has many advantages over serum as it is easily collected, less traumatic, readily available, easy to handle, plus being cost effective and safe<sup>(20)</sup>. Limited studies are available on salivary Tf expression in OSCC, Dowling et al., reported Tf levels were significantly increased in saliva of patients with head and neck SCC compared to controls<sup>(21)</sup> and Koc et al., showed that, these levels decreased significantly during radiotherapy treatment<sup>(22)</sup>. As for its expression in OLP, no data is available, but Mattson et al., showed that tissue biopsies of chronic graft versus host disease (GVHD) and OLP had significantly increased number of lymphocytes with transferrin receptors compared with the pre-transplant and control groups<sup>(23)</sup>.

Many studies have examined the relationships between circulating IGFBP-3 concentrations and cancer risk or prognosis, but a consistent association has not been found. It was suggested that local tissue levels of IGFBP-3 may be useful as diagnostic or prognostic markers<sup>(8,9)</sup>. Moreover, to the best of our knowledge, Salivary levels of IGFBP-3 and its binding protein Tf in patients suffering from OSCC or OLP have never been investigated before. Thus, the aim of the present study was to assess levels of IGFBP-3 and Tf in saliva of patients suffering from OSCC, OLP and correlate it to clinical parameters

in OLP and histologic grading in OSCC as compared to healthy controls, this is to evaluate its feasibility as a salivary biomarker for disease detection, plus shedding some light on the possible role for the Tf/IGFBP-3 binding system in the pathogenesis of these important oral conditions.

## SUBJECTS AND METHODS

Study subjects were 120 age and gender matched, randomly selected and enrolled after signing an informed consent. The study protocol was approved by the Al-Azhar University Institutional Review Board. OSCC and OLP patients were selected from the outpatients clinic of the Oral Medicine Department of Al Azhar and MSA Universities, Faculty of Dentistry, during the period of June 2015 to May 2016. The control participants were selected from visitors and staff of the faculties. A detailed medical history of each subject was obtained according to the questionnaire of the modified Cornell Medical Index<sup>(24)</sup>.

### Subjects groups

Subjects were divided into 3 groups, the first group included 40 patients suffering from OSCC (20 patients with moderately differentiated and 20 patients with poorly differentiated OSCC) based on established criteria<sup>(25)</sup>. The second group included 40 patients suffering from OLP with multiple visible symptomatic lesions diagnosed on the basis of the clinical and pathological characterizations according to the modified WHO criteria of OLP<sup>(26)</sup>, where as group 3 included 40 healthy control subjects who were systemically free and not suffering from any oral or systemic illness.

### Criteria of patient selection

For all groups, patients who had oral lichenoid lesions due to graft versus host disease, lupus erythematosus or hepatitis C were excluded. As well, patients with Sjögren's syndrome or previous radiotherapy or chemotherapy to the head and

neck region in the past 6 months prior to sample collection were excluded because of expected difficulty in obtaining saliva from these patients. For OSCC group, subjects previously diagnosed with OLP were excluded. For OLp group, only non smokers or ex-smokers were recruited because smoking is a known risk factors for OSCC and the risk for OSCC in ex-smokers was reported to be the same as for non-smokers<sup>(27)</sup>. Subjects previously diagnosed with oral epithelial dysplasia or OSCC were excluded. As well, alcohol consumers were excluded as alcohol consumption is a risk factor for OscC and was shown to affects levels of IFGBp-3 expression<sup>(28)</sup>.

Full dermatological and oral examination was carried out by a qualified clinicians (NE and HH), for clinical evaluation of the olp patients the reliable, easy to use REU scoring system was used (Table 1) <sup>(29)</sup>, the mouth is divided into 10 regions (lip, left cheek, right cheek, dorsal tongue, ventral tongue, floor of mouth, hard palate, soft palate, maxillary gingival and mandibular gingival), The signs and symptoms were scored. pain scores were not recorded as REU correlates with numerical rating scale for pain<sup>(30)</sup>.

TABLE (1) Scores of REU signs for oral lichen planus

Clinical signs	Score of signs
0 = none, 1 = white streaks or patches	-Reticulate / plaque-type (R)
0 = none, 1 = lesions <100mm <sup>2</sup> , 2 = lesions 100mm <sup>2</sup> to 300mm <sup>2</sup> , 3 = lesions > 300mm <sup>2</sup>	-Congestive / atrophic (E)
0 = none, 1 = lesions <100mm <sup>2</sup> , 2 = lesions 100mm <sup>2</sup> to 300mm <sup>2</sup> , 3 = lesions > 300mm <sup>2</sup>	-Ulceration (U)

$$Total = \sum R + \sum E \times 1.5 + \sum U \times 2$$

### Saliva sample collection

Whole unstimulated saliva (WUS) samples were collected early in the morning, between 6-9 am, under resting condition in a quiet room. Subjects were asked to brush their teeth and rinse their mouth with clean water before they went to sleep the day before sample collection and refrain from eating or drinking, for at least 1/2 h prior to saliva collection.

A water mouth rinse was administered prior to saliva sample collection. Each individual expectorated < 5 ml of saliva into a sterile centrifuge tube <sup>(31,32)</sup>.

### Saliva sample processing

Immediately after the saliva sample was collected, it was centrifuged at 2600g for 20 minutes at 4°C. After centrifugation, the supernatant was separated from the pellet. Three proteinase inhibitors were added to 1 mL of the supernatant: 1\_μL of aprotinin (10 mg/mL) (Sigma, St. Louis, MO), 3\_μL of sodium orthovanadate (Na<sub>3</sub>VO<sub>4</sub>, 400 mmol/L) (Sigma), and 10\_μL of phenyl methyl sulfonyl fluoride (PMSF, 10 mg/mL) (Sigma). The samples were then stored at -80°C until future use. All samples were analyzed within 6 months after the samples were collected and processed.

### Detection of IGFBP-3 and Tf levels in saliva samples

The levels of Tf and IGFBP-3 in saliva was measured using ELISA kits, for Tf, *Assaypro, GmbH, Immunbiologie Biochemie, Produkte und Systeme, Germany* and for IGFBP-3, *Quantikine R&D systems, Inc, USA*. were used according to the manufactures instructions. The results were expressed as concentrations in ng/ml.

### Statistical analysis

Tested values were presented as mean and standard deviation (SD) values. Data were explored for normality using Kolmogorov-Smirnov test of

normality. The results of Kolmogorov-Smirnov test indicated that most of data were normally distributed (parametric data), so ANOVA test was used to compare between the three groups, followed by Tukey's post hoc test, when ANOVA revealed a significant difference. Pearson correlation test was used to study the correlation between different parameters. Chi square test was used to compare the gender distribution.

The significance level was set at  $p \leq 0.05$ . Statistical analysis was performed with SPSS 16.0 (Statistical Package for Scientific Studies, SPSS, Inc., Chicago, IL, USA) for Windows.

## RESULTS

A total of 124 saliva samples were collected during the study period. Four samples showed salivary IGFbP-3 levels below the minimum detection level and were excluded from analysis. OSCC group included 25 male and 15 females with mean age 49.41 ys; the Olp group included 17 male and 23 female, mean age: 43.83 ys, whereas group 3 included 21 males and 19 females, mean age: 43.8 ys. Age and gender distribution of all groups are presented in table 2. A higher mean age was noted in the SCC group, but the difference didn't reach

the level of statistical significance ( $p= 0.0727$ ). Moreover, chi square test revealed no significant difference in gender distribution ( $p=0.3372$ ), (Table 2).

Of OLP patients, 28 patients had only oral lesions, and 12 patients (30%) had both oral and skin lesions. Common sites for oral lesions of Olp patients were buccal mucosa and gingiva/alveolar ridge area, all Olp patients were clinically presenting areas of atrophy, white patches as well as ulcerations, the duration of the disease ranged from 6 months to 9 years with a history of remissions and exacerbations. Of OSCC patients, 25 patients had OSCC in the tongue, the rest had OSCC in the palate, lip or in the buccal mucosa, all lesions were clinically presented as fungating ulcerative mass.

Regarding the salivary levels of transferrin and IGBP-3, significantly higher levels were recorded in different histological grades of SCC in comparison to OLP, whereas control showed the least mean value ( $p<0.0001$ ). Tukey's post revealed a significant difference between control OLP and SCC, as well as between OLP and different grades of SCC (Table 3). Moreover, there was a significant difference between moderately or poorly differentiated SCC (Table 3).

TABLE (2) Age and gender distribution in different groups

Clinical Parameter		Control	OLP	Mod. diff. SCC	Poorly diff SCC	SCC (both grades)
Age(ys)	Mean $\pm$ SD	43.8 $\pm$ 7.98	43.83 $\pm$ 10.99	52.46 $\pm$ 6.54	56.66 $\pm$ 14.43	49.41 $\pm$ 15.03
	F value	2.82				
	P value (ANOVA)	0.0727 <sup>ns</sup>				
Gender	Males: No(%)	21(52.5)	17 (42.5)	13 (65)	12 (60)	25 (62.5)
	Females: No(%)	19 (47.5)	23 (57.5)	7 (35)	8 (40)	15 (37.5)
	Chi square	4.545				
	P value	0.3372 <sup>ns</sup>				

*ns=non-significant at P<0.05*

TABLE (3) Salivary levels of Transferrin and IGFBP-3 in different groups and significance of the difference (ANOVA test)

Salivary levels(ng/ml)		Control	OLP	Mod. diff. SCC	Poorly diff SCC	SCC (both grades)
Transferrin	Mean ±SD	3.33 <sup>d</sup> ± 0.24	13.91 <sup>c</sup> ± 2.23	17.05 <sup>a</sup> ±0.99	21.1 <sup>b</sup> ± 1.24	18 <sup>a</sup> ±2.08
	F	147.05				
	P	<0.0001*				
IGFBP-3	Mean ±SD	1215.3 <sup>c</sup> ±428.92	2533.45 <sup>b</sup> ±290.35	4744.74 <sup>a</sup> ±907.11	5756.57 <sup>a</sup> ±952.33	4982.82 <sup>a</sup> ±991.42
	F	57.67				
	P	<0.0001*				

\*significant at P<0.05

Tukey's post hoc test: means with different superscript letters are significantly different.

Pearson correlation test revealed a weakly positive correlation between salivary levels of transferrin & IGFBP-3 levels in control, OLP and SCC (Table 4). A weakly positive correlation between transferrin salivary levels and REU in OLP was reported. However, a strong positive correlation was found between transferrin salivary levels and SCC histological grades (Table 5)

TABLE (4) Correlation between transferrin & IGFBP-3 salivary levels (Pearson Correlation test)

	R	R2	Significance
Control	0.2243	0.0503	Weak positive
OLP	0.321	0.103	Weak positive
SCC	0.3977	0.1582	Weak positive

TABLE (5) Correlation between transferrin salivary levels and histological parameters (Pearson Correlation test)

	R	R2	Significance
REU in OLP	0.0196	0.0004	Weak positive
Grade of SCC	0.8669	0.7515	Strong positive

Pearson correlation test revealed a weakly positive correlation between IGFBP-3 salivary levels and REU in OLP. Moreover, a weak positive correlation was found between IGFBP-3 salivary levels and SCC histological grades (table 6).

TABLE (6) Correlation between IGFBP-3 salivary levels and clinical parameters (Pearson Correlation test)

	R	R2	Significance
REU in OLP	0.1009	0.0102	Weak positive
Grade of SCC	0.4462	0.1991	Weak positive

## DISCUSSION

Egypt is considered as one of the highest countries in the overall incidence rates of oral cancer in the Middle East region<sup>(33)</sup>. OI is a chronic inflammatory disease with exactly unknown etiology and is considered to be a relatively common precursor of oral cancer<sup>(34)</sup>.

Oral malignant and premalignant lesions are such lesions where saliva examination can show the greatest benefit because of its direct contact with oral lesions and its contents of fallen cells in oral cavity, these reasons-plus others- make saliva an excellent media for investigations regarding oral lesions. In the present study Wus was, although stimulated saliva can be collected with higher amounts, its contents could be a little bit altered<sup>(35,36)</sup>.

In the present study, there were a significant differences between the three studied groups where the highest levels were in the OSCC group ( $4982.82 \pm 991.42$  ng/ml), and the lowest mean levels were recorded in the control group ( $1215.3 \pm 428.92$  ng/ml), a non significant difference was reported between the poorly and moderately differentiated OSCC groups. The elevated levels of IGfbp-3 in OSCC in the present study agrees with previous data where IGFBP-3 protein levels are reported to be increased in many types of cancers such as lung cancer, melanoma and in breast cancer tissue<sup>(37-39)</sup>.

IGFBP-3 modulates the interaction between IGFs and the type I IGF-R. In this capacity, IGFBP-3 may be potentiating to growth (by presenting IGFs to the IGF-R in a controlled fashion and preventing down-regulation of the IGF-R) or growth- inhibitory (by preventing IGF binding to the IGF-R)<sup>(8,9,40)</sup>. The significantly elevated salivary levels of IGfbp-3 in OI patients compared to controls ( $2533.45 \pm 290.35$  and  $1215.3 \pm 428.92$  ng/ml resp.) reported in our study could be explained by the growth inhibitory effects of IGfbp-3 via IGF-independent mechanisms. Cell death by apoptosis, is a hallmark in OI pathogenesis and is defined as proceeding through

either an intrinsic pathway, initiated by intracellular stimuli such as oxidative stress or DNA damage, or an extrinsic pathway, initiated by extracellular stress signals. These mechanisms require the activation of different proteases, including caspase-8 for death receptor-dependent extrinsic apoptosis and caspase-9 for caspase-dependent intrinsic apoptosis<sup>(41)</sup>. IGFBP-3 is inducible by the tumor suppressor p53 and has been demonstrated to induce apoptosis either directly or by potentiating other agents that activate the intrinsic pathway, such as ceramide, chemotherapeutics and irradiation<sup>(11,42,43)</sup>, Apoptosis induced by IGFBP-3 involves the activation of both caspase-8 and -9<sup>(44)</sup>. At least two unrelated proteins (LRP1 and TMEM219) have been designated as receptors for IGFBP-3, the latter in the presence of IGFBP-3 induces caspase-8-dependent apoptosis, that has been shown to be involved in pathogenesis of OI<sup>(45,46)</sup>.

In recent years, the dysregulation of autophagy-associated genes and proteins has been recognized and intensely investigated as it increases the susceptibility to diverse diseases, including inflammation, autoimmune disorders, and cancer<sup>(47,48)</sup>. An intracellular strong interaction between IGFBP-3 and heat shock protein GRP78 was shown to promote autophagy<sup>(49)</sup>. Recently, Tan et al., reported that dysregulation of T cell autophagy might be involved in pathogenic immune response of OI and may be correlated with clinical patterns<sup>(50)</sup>. Another possible mechanism that could justify the high levels of IGFBP-3 in saliva of OI and at the same time can point to a possible role in its malignant transformation is that IGFBP-3 could activate Smad2 and Smad3 phosphorylation<sup>(51)</sup>. One step in malignant development is epithelial mesenchymal transition (EMT) induced by transforming growth factor- $\beta$  (TGF- $\beta$ ), which encounters the Smad proteins as mediators for its signaling. Significantly higher expression of Smad3 in oral lichen planus compared to normal oral mucosa was recently reported<sup>(52)</sup>. In the present study, a weak positive relation was reported between IGFBP-3 levels with both histologic grading in OSSC and REU in OI.

Tf and Tf R plays a crucial role in the cellular uptake of iron that is essential for the rapidly growing cells as DNA synthesis, electron transport and mutagenic signaling pathways are all an iron dependent processes. The present study showed elevated levels of salivary transferrin in all of OSCC patients ( $18.89 \pm 1.6$  ng/ml) and these levels were significantly higher compared to OIP and control groups, this is in agreement with previous studies that correlated over-expression of Tf and TFR II with OSCC<sup>(21,22,53)</sup>, Tf and TFR C are expressed more abundantly in cancer cells than in their normal tissue counterparts as they require more iron to maintain their high cell proliferation rates<sup>(14,54)</sup>. Moreover, Tf deprivation from cells is associated with elevated indices of apoptosis. It was shown that this anti-apoptotic proliferative effect is mediated through alteration in cytokine expression (ex. IL-10 and TNF- $\alpha$ ). Tf or Tf-derived glycans (Tf-Gly) effectively protected nonfractionated human marrow cells against apoptosis induced by  $\gamma$ -irradiation, TNF- $\alpha$ , and agonistic anti-Fas mAb (19,55). Growth and aggressiveness of malignancies are also Tf-dependent; Tf-R number is positively correlated with tumor growth rate and metastasis<sup>(56)</sup>. That is why therapeutic agents have been introduced for treatment of cancers that targeted TFRs in two ways: 1) for the delivery of therapeutic molecules into malignant cells or 2) to block the natural function of the receptor leading directly to cancer cell death<sup>(57)</sup>.

The present study demonstrated a significant rise in Tf levels in saliva of OLP patients compared to controls ( $13.91 \pm 2.23$  and  $3.33 \pm 0.24$  ng/ml respectively). Tf has been found to play an important role in T cell activation and proliferation, in development of T-cell driven experimental autoimmune encephalomyelitis, Saksida et al. Proved that Tf exerted immunomodulatory effects on T cells by downregulating IL-2 in CD3<sup>+</sup> T cells<sup>(58)</sup>. IL-2 is a key growth and death factor for antigen-activated T lymphocytes and is essential for maintenance of self-tolerance<sup>(59)</sup>. With the lack

of sufficient data about the possible role of Tf in pathogenesis of OLP, the previous scenario of Tf effect on CD3<sup>+</sup> T cells and IL-2 could be applied as a hypothesis in pathogenesis of OLP, but indeed further studies are needed.

Like IGFbP-3, the pro-apoptotic effect of Tf could justify its elevated levels in saliva of OLP patients. In a subpopulation of marrow mononuclear cells expressing CD56 (NK cells), Tf caused enhanced apoptosis<sup>(19)</sup>. Tf has been found to activate apoptosis through the production of reactive oxygen species and activation of transcription of nuclear factor  $\kappa$ B<sup>(60)</sup>. Oxidative stresses are major features in pathogenesis of both OLP and cancer<sup>(61)</sup>. In the present study, a weak positive correlation was reported between salivary Tf levels and REU in OLP, but a strong positive relation existed between Tf levels and degree of differentiation in OSCC, this is in accordance with earlier data showing that the level of Tf is dependent on the levels of differentiation<sup>(53)</sup>.

The affinity of Tf-IGFBP-3 binding to Tf was previously shown to be dose-responsive and of a magnitude similar to that of IGF-I (13). A weak positive relation between salivary levels of IGFBP-3 and Tf in all tested groups was reported in our study. Results of the present work indicated that salivary IGFBP-3 and Tf would be a potential biomarker for detecting OSCC development in OLP patients. However, further study including a group of OLP patients with presence of OSCC will be needed to confirm this inference.

Finally, our data points to the physiologically significant ramifications of Tf/IGFBP-3 interactions on the modulation of cell proliferation and apoptosis. It was shown that Tf and IGFBP-3 are both growth-potentiating in BLSM, but the cocubation of these proteins in BLSM-conditioned media resulted in a dramatic reduction in growth. IGFBP-3 may affect cell growth through several Tf- and Tf-R-dependent mechanisms, Presentation of IGF-I to the cell surface by IGFBP-3 may indirectly influence growth

by regulating Tf-R density at the cell surface, and Tf/IGFBP-3 binding may directly interfere with each ligand from binding its own natural receptor, depending on the nutritional and iron status of the tissue or organism -Olp has been associated with decreased serum iron levels<sup>(62)</sup>, Whether these Tf/IGFBP-3 interactions occur predominantly at the cell surface or in the cytosolic or nuclear compartments is unknown as other IGFBP-3 binding proteins remain to be discovered<sup>(9,13)</sup>. The key to resolving the seemingly dichotomous roles of Tf and IGFBP-3 and bringing them closer to clinical applications no doubt lies in a better understanding of their biochemical and cellular functions, supported by extensive preclinical modeling especially for OLP, of which knowledge about possible causes and nature is still very limited.

## CONCLUSION

Our preliminary results showed that IGbP-3 and TF levels are elevated in saliva of Olp as well as OSCC, with a positive correlation between these levels and clinical features of Olp and histologic grading in OSCC suggesting that they could serve as a marker for disease detection and that they play a role in the pathogenesis of both diseases, but further biochemical and cellular localization studies are required .

## ACKNOWLEDGEMENTS

No external funding, apart from the support of Al-Azhar and MSA Universities, was available for this study.

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