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Research Article

Study of Salivary and Epithelial Cell Glycoproteins and Sialic Acid as Biomarkers in Oral Submucous Fibrosis and Oral Squamous Cell Carcinoma- A Proposed Hypothesis of Potential Pathogenesis during Malignant Transformation

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Abstract

Background: In the development of carcinoma, a healthy cell adapts, alters and strives to endure before transforming into malignancy by modulating the cell surface molecules. This study was conducted to estimate and correlate total carbohydrates (TC), total proteins (TP) and total sialic acid (TSA) levels in saliva and exfoliative cells of oral submucous fibrosis (OSF) and oral squamous cell carcinoma (OSCC).

Methodology: The TC, TP and TSA levels were biochemically estimated and statistically analyzed in saliva and exfoliated cells of 15 patients each of OSF and OSCC. In each patient two sites were evaluated, the lesional tissue (test) as well as an apparently normal tissue (control), thus having similar genetic and epi-genetic environment.

Result: Evaluation of saliva showed an increase in the mean values of glycoprotein levels from OSF to OSCC with significant difference in TC ($p = 0.033$) and TSA ($p = 0.000$). Evaluation of exfoliated cells showed an increase in the mean values of glycoproteins in lesional cells of OSCC as compared to OSF, with statistical significance in TSA ($p = 0.002$) levels. The mean values of TP ($p = 0.000$) and TSA ($p = 0.019$) were found to be significantly different between the non-lesional sites of OSF and OSCC.

Conclusion: The present study confirms that aberrant glycosylation precedes and occurs ubiquitously in cells participating in the disease process. The findings suggest that glycoproteins and sialic acid may be sensitive biomarkers useful in predicting malignant transformation, either de novo or from a pre-existing OSF.

Keywords: Exfoliative Cells; Glycoproteins; Oral Submucous Fibrosis; Oral Squamous Cell Carcinoma; Sialic Acid

Introduction

The oral mucosa maintains its structural and functional integrity under the influence of precise, yet comprehensive information from the environment in the form of growth and differentiation molecules, apoptotic signals, microbial and dietary components, or deleterious substances like tobacco and betel nut, resulting in an uncontrolled proliferation or cell death [1]. The perception of oral carcinoma developing in a previously existing, detectable but non-invasive potentially malignant disorder (PMD) is well established [2]. The overall annual incidence rate of PMDs in Indian subcontinent is 0.6 - 30.2/1000 [3,4].

Oral submucous fibrosis (OSF) is one of the commonly developing PMDs, described as a chronic, progressive debilitating condition of the oral mucosa caused due to consumption of areca-nut,

largely restricted to South-east Asia [5-7]. OSF is 19-times more prone to evolve into oral squamous cell carcinoma (OSCC) with a malignant transformation rate of 2.3 - 7.6% in India, annually approximating to 0.5% [5,7-9].

OSCC accounts for more than 90% of oral malignancies and 3% of all first time diagnosed cases [5]. In contrast to other authors, Guo., et al. noted that OSCC originating from OSF occur more frequently in younger males with an increased invasiveness and metastatic potential [10,11].

Oral carcinogenesis is a cellular disease, and in the event of their progression through preceding pre-neoplastic stages, large cellular transformations develop due to alterations in glycoprotein synthesis and their arrangement [12-14]. Glycosylation processes undergo abrupt and rapid changes at various phases of development and oncogenesis [15]. The micro-heterogeneity of glycoproteins is enhanced due to under-expression, over-expression or neo-expression of these moieties, resulting in increased glycan branching [16].

Aberrant sialylation of the terminal glycan sugar, sialic acid (Sias), occur in cancer cells, characteristically associated with adhesiveness, immunogenicity, invasiveness and metastatic potential of tumor [4,13]. These altered glycoproteins are discharged into circulation as a result of increased turn-over, secretion and/or shedding from malignant cells [17].

The cell membrane changes occurring in tumorigenesis can be assimilated with improved precision by assessing them at the cellular level itself. To the best of our knowledge, this is the first study wherein, glycoprotein and Sias are evaluated using exfoliative cytology such that the study and control group of each sample comprises of same patient with similar genotypic and phenotypic composition.

Purpose of this Study

The purpose of this study is to quantitate glycoprotein and Sias levels in saliva and exfoliative cells of OSF and OSCC patients to give enhanced insight into the concept of field cancerization and oncogenic potential by understanding the basic pathology that underlies each alteration.

Materials and Methods

Source of the subjects

This prospective, hospital-based cross-sectional clinical study was undertaken by selecting the patients visiting Department of Oral Medicine and Radiology at S.D.M. College of Dental Sciences and Hospital, Dharwad, after obtaining approval from the Institutional Review Board (IRB No. 2014/P/OP/22). The study consisted of 30 cases selected by random sampling method, comprising of 15 OSF and 15 OSCC patients in the study group.

Sample collection

5 ml of whole saliva specimen was collected by "drooling method" in a wide-bore sterile container to prevent incorporation of mucin and food debris by expectoration. Exfoliative cytology was performed using a sterile cement spatula to acquire epithelial cells from two different sites in each patient (Photograph 1). In an OSF patient, the 'lesional site' (L_{OSF}) included the most affected area in the oral cavity, while the 'least affected site' (LA_{OSF}) was represented by minimal changes. Similarly, the 'lesional site' (L_{OSCC}) in OSCC patient represented the area with carcinomatous changes, while the 'non-lesional site' (NL_{OSCC}) consisted of the apparently normal mucosa, usually the buccal mucosa. Thus, each patient presented with the test as well as its control, providing a similar genetic and epi-genetic milieu. This offers similar discrepancies, if any, in the estimation of glycoproteins and Sias from two distinct clinical presentations.

Photograph 1: The photograph depicts the method of performing exfoliative cytology from lesional (A) and non-lesional (least affected in OSF) (B) sites in OSCC patient using a sterile cement spatula.

Adequate cells were scrapped and transferred to sterile Eppendorf tubes containing 2 ml of phosphate buffer saline (PBS; pH = 7.4). All the samples, saliva and exfoliated cells, were stored in deep freezer at -80ºC until further analysis. Thus, from each patient, the samples collected included:

OSF patient = Saliva + Exfoliated cells from L_{OSF} and LA_{OSF}
OSCC patient = Saliva + Exfoliated cells from L_{OSCC} and NL_{OSCC}

Sample processing and biochemical analysis

The collected samples were subjected to centrifugation at 1000g for 5 minutes for cell washing to form cell deposit and a supernatant consisting of debris, thus providing debris-free study samples of reduced viscosity, allowing accurate and reproducible analysis. Cell lysis buffer was added to cell deposit and the assortment was centrifuged at 1000g for 10 minutes. A supernatant consisting of cytosol and cell pellet sediment consisting of cell membrane constituents were obtained. The supernatant was discarded and the cell pellet sediment was homogenized to subject it for spectrophotometric estimation of total carbohydrates (TC) by Phenol-Sulphuric acid (PSA) method, total proteins (TP) by Bicinchoninic acid (BCA) method, and total sialic acid levels (TSA) by Diphenylamine (DPA) method using Shimadzu UV170.

The levels of the TC, TP and TSA levels were expressed as mean ± standard deviation (S.D.). Statistical analysis was done using one-way ANOVA-Tukey test and Mann-Whitney U test to observe statistical significant difference ($p < 0.05$). Receiver Operator Characteristic (ROC) curve was obtained.

Results and Observations

The mean value of TC, TP and TSA levels (Table 1) in saliva of OSCC patients was found to be more than that of OSF patients. The TSA/TP ratios (Table 1) were found to be higher in OSCC as compared to OSF in saliva as well as exfoliated cells. The mean values in exfoliated cells varied as below (Table 1):

		Saliva		Lesional		Least affected/Non-lesional	
		OSF	OSCC	L_{OSF}	L_{OSCC}	LA_{OSF}	NL_{OSCC}
TC (mg/ml)	Mean ± SD	91.24 ± 28.16	142.70 ± 71.74	20.76 ± 7.10	26.61 ± 19.40	22.11 ± 9.19	19.78 ± 7.30
	p-value	.033*		.775		.367	
TP (mg/ml)	Mean ± SD	2.05 ± 0.87	2.91 ± 1.71	0.63 ± 0.28	0.77 ± 0.40	0.57 ± 0.19	0.25 ± 0.17
	p-value	.137		.305		.000*	
TSA (µg/ml)	Mean ± SD	3.81 ± 1.85	7.33 ± 2.83	5.42 ± 0.84	6.75 ± 1.16	6.33 ± 1.13	6.86 ± 0.78
	p-value	.000*		.002*		.019*	
TSA/TP		0.00186	0.00252	0.00860	0.00877	0.0111	0.0274

Table 1: The mean TC, TP and TSA levels in saliva and exfoliated cells of OSF and OSCC patients and their pair-wise comparison using Mann-Whitney U test.

L: Lesional Site; LA: Least Affected Site (OSF); NL: Non-lesional Site (OSCC).

*: Indicates statistical significance.

Mean TC levels: $NL_{OSCC} < L_{OSF} < LA_{OSF} < L_{OSCC}$.
Mean TP levels: $NL_{OSCC} < LA_{OSF} < L_{OSF} < L_{OSCC}$.
Mean TSA levels: $L_{OSF} < LA_{OSF} < L_{OSCC} < NL_{OSCC}$.

On the basis of Kolmogorov-Smirnov and Shapiro-Wilk tests of normality, the mean values of TC, TP and TSA in saliva of OSF were subjected to one-way ANOVA and Tukey test for statistical analysis which showed significant difference ($p = 0.000$) between the mean levels of TC and TP, and TC and TSA.

A statistical significant difference ($p = 0.000$) was found between the mean values of TC, TP and TSA in the saliva of OSCC patients and the exfoliated cells of OSF and OSCC patients using Mann-Whitney U test.

In OSF, the mean values of TC ($p = 0.000$), TP ($p = 0.000$) and TSA ($p = 0.011$) were found to be significantly higher in saliva as compared to L_{OSF}. The mean of TSA ($p = 0.010$) was significantly higher in L_{OSF} than LA_{OSF} (Table 2).

In OSCC, a statistically significant difference was present in the mean of TC ($p = 0.000$) and TP ($p = 0.000$) between saliva and L_{OSCC}, while only the mean TP levels ($p = 0.000$) were found to be significantly higher in the L_{OSCC} compared to NL_{OSCC} (Table 3).

Parameter	Saliva	L_{OSF}	p-value	L_{OSF}	LA_{OSF}	p-value
TC (mg/ml)	91.24	20.76	.000*	20.76	22.11	.806
TP (mg/ml)	2.05	0.63	.000*	0.63	0.57	.412
TSA (µg/ml)	3.81	5.42	.011*	5.42	6.33	.010*

Table 2: Pair-wise comparison of the parameters within samples in OSF using Mann-Whitney U test.

*: Indicates statistical significance

Parameter	Saliva	L_{OSCC}	p-value	L_{OSCC}	NL_{OSCC}	p-value
TC (mg/ml)	142.70	26.61	.000*	26.61	19.78	.187
TP (mg/ml)	2.91	0.77	.000*	0.77	0.25	.000*
TSA (µg/ml)	7.33	6.75	.870	6.75	6.86	.806

Table 3: Pair-wise comparison of the parameters within samples in OSCC using Mann-Whitney U test.

*: Indicates statistical significance.

On comparison between OSF and OSCC, the salivary TC ($p = 0.033$) was significantly higher in OSCC than OSF patients. The levels of TP ($p = 0.000$) were significantly higher in the LA_{OSF} as compared to NL_{OSCC}. In contrast, the TSA levels were significantly higher in the saliva ($p = 0.000$), L_{OSCC} ($p = 0.002$) and NL_{OSCC} ($p = 0.019$) as compared to corresponding OSF levels (Table 1). ROC curve was plotted on the basis of mean TC, TP and TSA levels and the cut-off values were obtained to distinguish the stages of disease progression from OSF to OSCC.

Discussion

According to Theodore L. Steck, "the structure lies at the heart of function" and hence cell membrane components exhibit significant functions for maintaining the integrity of the cell and tissue. The plasma membrane barrier between the inner and outer part of the cell harbors immunological and chemical uniqueness along with receptors for definite structural and functional signal molecules [18,19].

The cell surface glycans, present in the form of glycoproteins and glycolipids, are concerned with cell-cell adhesion, non-adhesion, cell-substrate and cell-extracellular matrix interactions [20-22]. Glycoproteins, produced by glycosylation and catalyzed by sequence of enzymes, exhibit increased molecular microheterogeneity and antigenic properties because of multiple oligosaccharide side-chains [16,23,24].

Sias are acidic monosaccharides located classically as terminal branches of glycans, thus capping the antigenic side-chains of glycans and enabling the cells to be proclaimed as being "self" [25,26]. However, in contrast to operating as an 'anti-recognition agent,' Sias also represent as biological recognition epitopes for a number of molecules [25].

During development, the cells adhere, associate, dissociate and migrate to re-associate elsewhere, thus playing a vital role in embryonic growth and differentiation [22]. Therefore, terminal sugars control biological behavior of the cell and consequently, modifications in surface glycans may reflect changes in cell behavior during development, differentiation and oncogenesis [20-22]. Aberrant glycosylation is a well-established observation in the process of oncogenetic development. Since 1985, until 2012, only twelve glycosylation-based serum biomarkers for the malignancies of colon, lung, pancreas, liver, thyroid, ovary, prostate and breast have been approved by FDA [27].

In view of the fact that saliva comes in direct contact with oral cancerous tissue and exfoliative cytology provides direct examination of cancerous cell contents, saliva and exfoliative cytology were employed as investigating media for estimating glycosylation-related markers.

Cancer is a social disease at a cellular level, and many symptoms are manifested by the cell [18]. The alteration of cell surface is accompanied by appearance of larger surface glycopeptides, independent of the histogenetic origin [28]. Shedding is an important feature of normal protein turn-over occurring continuously in the growing as well as cancer cells [29].

Cancer cells approach the cell structural characteristics of undifferentiated, rapidly growing cells or embryonic cells [18]. Increased membrane turn-over and enhanced cell proliferation have been suggested following transformation [30]. This may result in raised serum and salivary glycoconjugate levels.

Adding evidence to existing data, our study also found significant elevation in the salivary levels of glycoconjugates and Sias in OSCCs as compared to OSF (Table 1), thereby suggesting that alterations in cell surface glycoconjugates may be responsible for malignant transformation. Comparable studies have been performed to estimate the total glycoprotein and Sias levels in serum and saliva of healthy controls, PMDs and OSCC patients. The authors have observed similar changes in the serum levels of glycoconjugates with progressive rise in levels from controls to PMDs to OSCCs [17,31-34].

Alterations in TP levels may be present and hence, the ratio of TSA to TP levels was calculated to normalize the presence of any such variations [33]. The value of the ratio was highest in NL_{OSCC} followed by LA_{OSF} (Table 1). This may be justified by the explanation that amount of TP may show higher gradual increase as compared to that in TSA to facilitate invasion into the adjacent areas. Raval., *et al.* have quoted TSA/TP to be the best tested markers [33].

ROC curve analysis showed that with respect to TC, the optimal cut-off value to predict malignant transformation was calculated to be 14.735 mg/ml with 93.3% sensitivity. The most favorable cut-off value to expect precancerous transformation was observed to be 13.815 mg/ml with 100% sensitivity.

An optimal cut-off value of 0.367 mg/ml of TP levels was obtained to anticipate the transformation of non-lesional mucosa to PMD with sensitivity of 93.3% and specificity of 87.6%.

TSA was found to predict the conversion of non-lesional mucosa into PMD with a cut-off value of 7.091 µg/ml showing a low sensitivity but specificity of 86.7%.

A proposed hypothesis

The cell surface changes were investigated by subjecting the exfoliative cells from two distinct regions in the same patient for biochemical analysis. The study of cellular changes occurring in two different sites of the same oral environment in a patient permits improved understanding of a well-recognized phenomenon called “field cancerization”. No similar studies on exfoliative cytology were available in the existing literature, and hence we could not relate our results with any data. Thus on the basis of results acquired, a hypothesis has been proposed below to understand the underlying mechanism that may exist in various stages of disease initiation, development and progression (Figures 1A-1D).

Figure 1A: The cell surface of a normal healthy cell with normal and proportionate distribution of glycoproteins and Sias.

Figure 1B: Increased oligosaccharide branching with corresponding augmentation of Sias is present. As a protective mechanism, Sias shed and expose the oligosaccharide chains, thus conferring the “eat me” signals to macrophages to induce cell death. Hence, in the initial stages of OSF, an increase in TC and TP is evident. TSA levels, as explained further on, are slighter than normal healthy cells due to increased shedding albeit increased oligosaccharide branching.

Figure 1C: As the disease progresses, the cell surface molecules display excessive modulations conferring signals to the host immunity of abnormal behavior. The host immunity releases cytokines from the adjacent, comparable normal cell, or the underlying mesenchymal cells, or the host immune cells to perturb the undisciplined process and curb the malignant transformation. These cytokines cause increased shedding of Sias as well as provide the apoptotic signals to the dysfunctional cell to avert further tissue damage. This result in decrease in the TC and TSA levels, however increased TP levels are evident due to increased apoptosis and tissue destruction secondary to cytokines.

Figure 1: The figure depicts the proposed pathogenesis of role of the cell surface carbohydrates, proteins and sialic acid during the various stages of OSF and its progression to malignant transformation.

Figure 1D: On one occasion when the cell transforms and shows malignant changes, it displays irregular increased oligosaccharide branching to facilitate uncoordinated cell communication with increased Sias, masquerading the cancer cell from host immunity. Nonetheless, the cancer cell, in the fear of being destroyed by host, starts producing excessive and disproportionate Sias to decorate their cell surface for enhanced protection. The inconsistent Sias, in enlarged quantities, outweigh the capacity of the cell and consequently, are shed in the adjacent medium, saliva being the medium in case of oral cancers. Thus, irrespective of the excessive amplification in surface Sias, they are always a lesser amount when compared to a normal cell. The cancer cell, in order to proceed and destruct the adjacent tissue releases large amounts of proteases which aid them in local invasion. This causes an increased TP and TC levels with an increased TSA as compared to OSF but not the healthy cell.

The normal cell surface is decorated with a complex of oligosaccharide chains with their terminal end-sugars, predominantly the sialic acids (Sias). Once the injurious substances, which in our case are tobacco and betel-quid products, contact the cell surface, a cascade of signaling may occur to provide information for protection against these hazardous components.

Our results showed TP levels to be increased in the order of $NL_{OSCC} < LA_{OSF} < L_{OSF} < L_{OSCC}$. A normal cell is in a continuous state of equilibrium. In the initial stages, the cells may exhibit the consequences of chemical injury by undergoing apoptosis or necrosis. As stages enhance with a continuous chemical insult, a continuous turn-over of the cell membrane structures or its lysis result, and cells exhibit necrotic or apoptotic changes in response to the inflammatory cytokines (OSF cells exhibit apoptosis, a feature which is lost during malignant transformation). Once the malignant process has set in, the rate of apoptosis decreases, and instead the tumor cell undergoes uncontrolled proliferation. However, the ma-

lignant cells, in order to invade the surrounding structures, exhibit an amplified quantity of cell surface proteolytic enzymes, namely proteases, to cause peri-cellular and extra-cellular matrix destruction. Thus, a gradual increase in the TP levels is observed as a normal cell progresses through the sequential stages of OSF and finally during malignant transformation.

According to our results, TC levels increased in the order of $NL_{OSCC} < L_{OSF} < LA_{OSF} < L_{OSCC}$.

Altered carbohydrates do not display a direct role in the proliferation defect of malignant cells, but favor the process by breakdown of the intercellular control mechanism by modulating the cellular recognition and communication function. A cell may initiate exhibiting certain large variations in the amount and relative composition of oligosaccharides, secondary to cell insult, by undergoing increased branching which are tolerant to genetic or inhibitor-induced modifications [35]. As the amount of aberrations increase in course of time, causing loss of contact inhibition, another characteristic trait of cancer cells, the host may attempt to downgrade this risk of undisciplined growth by recruiting cytokines. Accordingly, just before a malignant change sets in, the carbohydrate levels diminish as compared to the initial stages of OSF. Nevertheless, in a fully transformed cell, dysregulated cellular communication serving an uncontrollable proliferation is witnessed.

Therefore, in a healthy non-lesional cell, the carbohydrate levels were found to be less as compared to the cells showing certain premalignant changes. The decrease in mean TC levels of LOSF compared to LAOSF may be contributed to the impact of host immune cells to prevent malignant transformation by curbing the action of carbohydrates, which are found to abet the occurrence of carcinomatous changes by modulating intercellular control mechanisms. In a cancer cell, however, large amount of carbohydrate modifications may occur resulting in an uncontrolled proliferation.

In accordance with Smets., et al. we believe that 'changes at the genetic level must become expressed in cellular sites and organelles crucial to the control of normal proliferation and cell behavior' and that 'by the virtue of the position and anomeric configuration of the glycosidic units and in the occurrence of branch points, the can theoretically carry information that by far exceeds that contained in the linear structures of nucleic acids, which depends only on the number of different monomeric units and their sequence. Therefore, surface carbohydrates have the potential to be important determinants of the chemical identity of the cell' [18].

The mean TSA levels were found to increase in the order of $L_{OSF} < LA_{OSF} < L_{OSCC} < NL_{OSCC}$ in our patients. Increased levels of sialyltransferase, an enzyme essential for sialylations of glycans, are found in the dividing and transforming cells. Sias, incorporated on the cell surfaces, inhibits cell death by preventing apoptosis or immune attack. This occurs so because it imparts a "self" tag to the cells.

The same mechanism is adopted by malignant cells. They illustrate increased sialylation to conceal their immunogenic sites to prevent complement-mediated reaction, and impair the cell binding and killing by lymphocytes and macrophages by increasing the negative charge on the membrane [36].

A normal, healthy cell is enveloped with millions of units of Sias to shield the antigenic sites, which if exposed confer the "eat me" signals to macrophages. After the initial insult by the chemical contaminants, an increase in the terminal capping by Sias follow the amplified oligosaccharide branching. Nevertheless as a protective mechanism, the affected cells may shed their Sias to permit the event of apoptosis to occur, as seen in early OSF. At later stages of disease progression, just before transformation, the host response sets in and strives to restrain the neoplastic change to occur in the damaged cell. The inflammatory cytokines may lead to the increased shedding of Sias to facilitate cell death, thus causing least TSA levels in OSF as compared to its initial stages.

The malignant transformation, once initiated, aims to build an environment which would promote its survival and progression to invade the normal tissues. To enable this, the cancer cell may begin to produce copious quantities of Sias to shield itself from the host immunity. However, a cell has a specific cell surface capacity to possess the Sias. Due to over-sialylation, these aberrant Sias may overgrow the threshold capacity of the cell surface and start shedding by the action of membrane-bound sialidases to allow the further accommodation of continuously producing Sias. The shedding of Sias may as a result, also be contributed to the cytokines released by the immune cells who necessitate an exposed oligosaccharide chain to cause death of the cancer cell. In addition to masking the antigenic sites, the Sias possess anti-oxidative properties, which in the neoplastic stage are improvised due to its huge quantities and hence, may prevent the effects of ROS produced by the extensive interaction between the neoplastic cells and its micro-environment.

These findings, as described above, can be interpreted from our results. Just ahead of malignant transformation, the host immunity is at its paramount to prevent this transformation to take place. Hence, the levels of Sias are least in the lesional area of OSF. The continuous turn-over and shedding by the action of sialidases result in slightly lower levels of Sias in the cancer cells than those present in the normal-appearing mucosa.

The increase or decrease in the amount of Sias does not detect the glycosylation differences. They, rather signify the defect that has occurred in the chemical composition of these glycosylated molecules. Example: Tumor-produced HCG contains fewer amounts of Sias as compared to the normal hormone. Decreased sialylation corresponds to incomplete glycosylation.

As a consequence, it is imperative on our part to recognize the array of underlying mechanisms resulting in either an upraise or a downfall in the levels of these molecules.

On the basis of this rationale, we understand that a healthy cell unexposed to a deleterious environment displays normal cell surface molecules. Once modified by increased oligosaccharide branching to acclimatize to the surrounding harmful environment, the body tries to eliminate such cells by sending apoptotic signals or necrosis. This takes place by shedding of Sias by the cell to expose the oligosaccharide side-chains. However, the cells continue to modulate themselves, befitting "survival of the fittest," until they transform malignantly. Just before complete transformation takes place, the host strives its best to prevent the malignant change from occurring by releasing host cytokines to enhance the shedding of Sias and consequently, cell death. Once transformed, the inherent trait of a cancer cell is to proliferate intractably by abnormal oligosaccharide branching causing abnormal intercellular communication and invade the adjacent stroma and its structures by causing its destruction. This occurs by release of numerous cell surface proteases, aiding invasion. In order to protect itself from being recognized as abnormal, it loads itself with Sias and thus, continues host destruction.

The salivary TC levels showed significant difference in OSF and OSCC patients. This supports the concept of altered composition responsible for poorly modulated cell communication. A highly significant difference was observed in the TP levels between the NL_{OSCC} and $LA_{OSF.}$ This may suggest that during the initial stages of precancerous conversion, protein levels differ due to cell and host protective mechanisms that ensue to prevent further damage by causing increased cell death. With respect to TSA levels, significant difference was observed between saliva, lesional and, the least affected and non-lesional sites of OSF and OSCC. This advocates that TSA may be a better tumor marker in the process of carcinomatous transformation, both at salivary and cellular levels. However, from the above results, TP levels may prove better diagnostic marker to assess the development of PMD (Table 1).

Comparison of the mean values between saliva and exfoliative cells showed high statistical significance with respect to TC, TP and TSA between saliva and L_{OSF} (Table 2). However, only TSA levels significantly differed between L_{OSF} and LA_{OSF} (Table 2). Thus, investigation of Sias may prove useful in distinguishing an OSF-affected site with increased predilection for higher aberrations. The significant elevation of glycoproteins in precancer patients may be attributed to increased predominance in malignant transformation of the cell [33].

In OSCC patients, significant difference was observed in TC and TP levels between saliva and L_{OSCC} (Table 3). However, with respect to TSA levels, direct relationship exists between TSA levels in tumor cell and that in saliva such that, with an increase in the TSA levels in the cancer cell, an increased shedding occurs which ultimately results in increased salivary TSA levels, thus generating a statistically small difference. The TP levels were found to be significantly higher in L_{OSCC} than NL_{OSCC}, justifying the variation in rate of apoptosis and activity of proteases between the two cells (Table 3).

Additional studies are encouraged in this field of glycobiology to reinforce this hypothesis and further enlighten the unidentified mechanisms that underlie malignant transformation.

Conclusion

According to our results, we conclude that highly significant difference is observed in the TC, TP and TSA levels in saliva and exfoliative cells of OSF and OSCC. The variations in their levels at different stages of disease progression establish that definite and distinct changes occur in surface glycoproteins during premalignant and malignant transformation. The literature shows variations in the values of salivary biomarkers, which are present in very low concentrations, thus signifying the execution of standardized methods with high sensitivity.

The hazardous substances primarily contact the cell surface molecules, which may then modulate themselves to adapt to the deleterious environment for survival, thus supporting the concept of field cancerization. The direct analysis of the cell surface glycoproteins and their corresponding levels in saliva enhance our understanding of basic mechanism that every fundamental molecule may pursue to survive the premalignant and malignant transformation in host environment.

Further studies are encouraged in this field to establish a standardized biomarker in determining the stage of progression of the disease process. This would establish early detection, diverse treatment options, better prognosis and increased survival and improved quality of life.

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Disclosure

The authors have declared no conflicts of interest.

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