A Study Of Protein Oxidation And Alterations In The Saliva Of Oral Squamous Cell Carcinoma

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Abstract: Oral Squamous Cell Carcinoma (OSCC) of Oral Cavity is a common malignant tumor of themouth that typically affects elderly men and women. It is more aggressive than conventional squamous cell carcinoma affecting other body regions. Our study consisted of 115 OSCC patients which were diagnosedclinically and histopathological. According to histological findings, oral squamous cell carcinoma (OSCC) is graded into three distinct categories, by Border's grading system. The severity of OSCC by the grading system is according to descending order. The control population consisted of 45 normal, apparently healthy, age and sex matched subjects. The total protein content in saliva of OSCC patients were significantly increases compare to healthy controls. The total protein content values were also estimated and were found to be increased with the severity of the disease The values are statistically significant.
Keywords- biomarkers, Oral cancer, OSCC, Protein, salivary diagnostic.

I. Introduction

Cancer is the second leading cause of death worldwide. Oral cancer (mouth cancer) is a sub-type of head and neck cancer, which is any cancerous tissue growth located in the oral cavity [1]. There are several types of oral cancers, but around 90% are oral squamous cell carcinoma (OSCC) originating in the tissues that line in the mouth and lips [2]. According to histological findings, oral squamous cell carcinomas (OSCC) are graded into three distinct categories, by Border's grading system [3].

Grade 1: Well differentiated oral squamous cell carcinoma (WD OSCC)
Grade 2: Moderately differentiated oral squamous cell carcinoma (MD OSCC)
Grade 3: Poorly differentiated oral squamous cell carcinoma (PD OSCC)

The severity of OSCC by the grading system is according to descending order. Saliva is considered a mirror of body health and is composed of variety of analyzes from systemic sources that reach the oral cavity through various pathways. Saliva constitutes both organic and inorganic components which generally change in small quantities that vary with changes in flow.The organic constituents are made up of proteins which include mucins and proline rich proteins which have lubricating properties, amylase and lipase with digestive properties, proteins such as sialoperoxidase, lysozyme [4], lactoferrins, chitinase, cystatins, histatins, defensins, salivary leukocyte proteinase inhibitors, calprotectin, peroxidase, acid phosphatase, chromogranin A, sialin, agglutinin which have anti-microbial properties [5]. Other proteins include statherin, mucoproteins and glycoproteins. The Saliva has been found to contain over a thousand proteins that are thought to be involved in a wide range of biological functions [6]. Detecting changes in the salivary concentrations of these molecules has allowed the detection of oral and systemic diseases. The multifarious components within the saliva not only protect the integrity of oral tissues, but also provide clues to various local and systemic conditions and diseases. These salivary components are constantly being explored as markers of various diseases and to monitor general health.

II. Materials And Methods

The study population consisted of 115 OSCC patients. OSCC was diagnosed clinically and histopathological during their visit to the Maitri college of dentistry and research center Durg. The control population consisted of 45 normal, apparently healthy, age and sex matched subjects. All the subjects were informed about the procedure and study was carried out after their consent.

Histopathological assessment:

Formalin fixed, paraffin embedded sections were prepared from biopsies taken from the OSCC group. On histopathological examinations, these were subdivided based on Broder's histopathological grading into well differentiated (65 cases), moderately differentiated (35 cases), and poorly differentiated (15 case) OSCC.

Unstimulated whole saliva samples were collected, after the histopathological conformation of the clinical diagnosis of OSCC, between 10 a.m. and 12 p.m., two hours after the subject's usual breakfast time. The subjects were asked to rinse the mouth with distilled water thoroughly to remove any food debris and then to spit into a sterile small plastic container. Once the saliva (2 ml) was collected, the plastic container was placed in ice carrier box and transferred to the laboratory for biochemical analysis [7]. Saliva sample was taken for analysis of total Proteins contents and for SDS-PAGE i.e. Lamlee method [8]. Salivary total proteins were estimated by the method of Lowry et al [9].

Statistical analysis:

The data were analyzed using the 'NTSYS software' (version 3.2). The value of biochemical parameters was expressed as mean ± SD, the levels of significance were determined by employing student's t test.

III. Results And Discussion

In this study 160 individuals of whom, 45 were healthy controls and 115 were OSCC patients. OSCC patients are histologically differentiated into three categories that is, well differentiated OSCC(wd), moderately differentiated OSCC(md) and poorly differentiated OSCC(pd). The numbers of well differentiated OSCC patients are 65, moderately differentiated OSCC are 35 and poorly differentiated OSCC patients are 15. Out of 115 patients, 76 are male and 39 are female patients. The ages of patients were in between 33-70 and median age is 52.

The total protein content in saliva of OSCC patients were significantly increases compare to healthy controls.
The total protein content values increase with the severity of the disease that is - wd OSCC > md OSCC > pd OSCC {Table no.1 and Graph-1}. The values are statistically significant.

**Table 1: Total Protein (mg/dl) content in saliva of Control & OSCC patient.**

	Total protein content(mg/dl)
Control (n=45)	93.8±6.4
Wdoscc (n=65)	115±4.9
Mdoscc (n=35)	141.7±11.6
Pdoscc (n=15)	187.1±16.3

Graph 1: Total Protein(mg/dl) content in saliva of Control & OSCC patient.
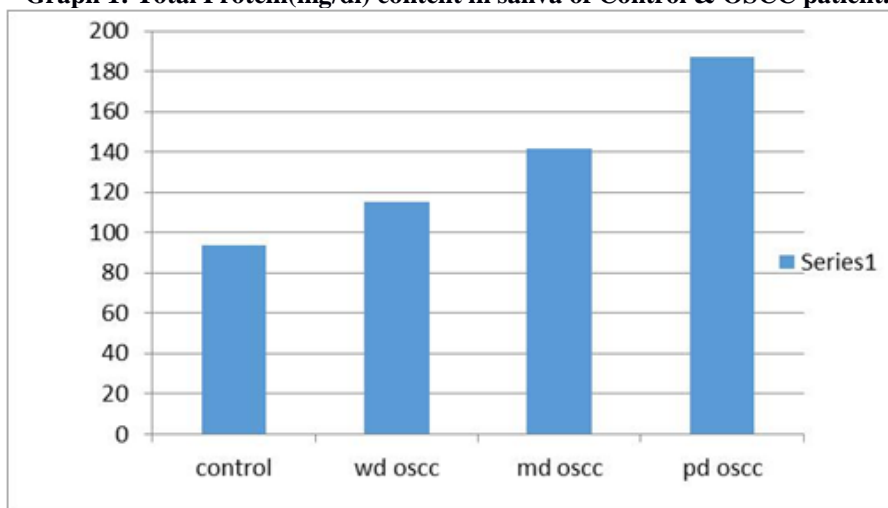
The result of SDS-PAGE shows that the alteration of proteins in OSCC patients compare to healthy controls. These are presented in Fig-1 and Table-2

Fig.1: SDS-PAGE Electrophoresis
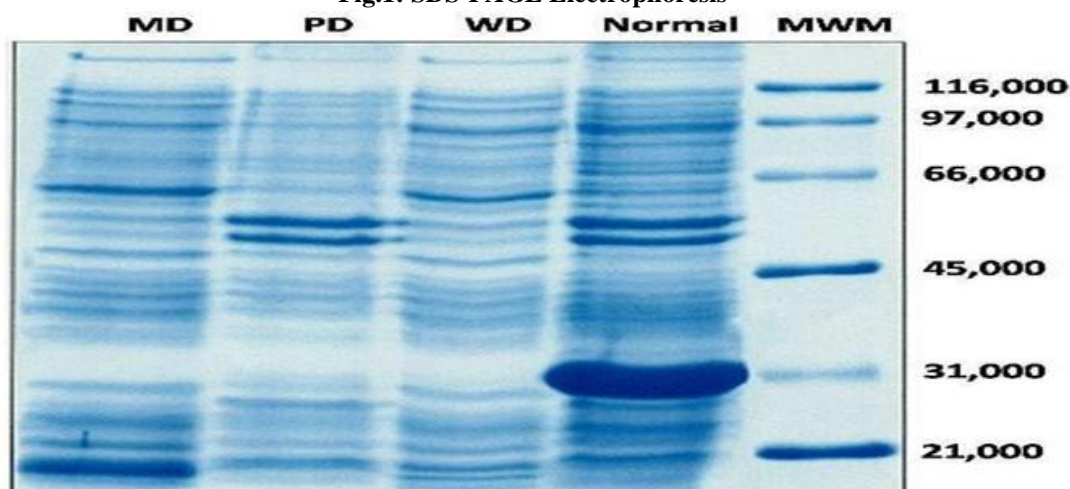
Table 2: Protein alterations in saliva of OSCC

Mol.wt	Prot-assession no.	Name of protein	Normal	Wdoscc	Mdoscc	Pdoscc
18229	P62937	Peptidyl-prolylcis-trans isomerase A	Present	Absent	Absent	Present
30521	Q13162	Peroxiredoxin-4	Present	Absent	Absent	Absent
28750	P12004	Proliferating cell nuclear antigen	Present	Absent	Absent	Absent
30011	P40261	Nicotinamide N-methyl transferase	Present	Present	Present	Absent
20146	P02511	Alpha-crystallin B chain	Absent	Present	Present	Present
33469	Q01105	Protein SET	Present	Present	Present	Absent
51589	P02533	Keratin, type1, cytoskeletal 14	Present	Present	Absent	Absent
60157	Q64133	Amine oxidase A	Present	Absent	Absent	Absent

According to MWM (molecular weight marker), there is alterations in many salivary proteins of OSCC. Peptidyl-prolylcis-trans isomerase- A, Peroxiredoxin-4, Proliferating cell nuclear antigen and Amine oxidase- A are present in healthy control but totally absent in OSCC patients. Alpha-crystallin B chain is absent in healthy control but present in OSCC patients.

IV. Discussion

Increasing interest has developed in using saliva to diagnose systemic diseases because of its simplicity in collection. The collection of saliva is relatively safe, noninvasive, inexpensive to sample and may be collected repeatedly with minimal discomfort to the patient; thereby, rendering saliva as a very desirable diagnostic model. More importantly, saliva contains constituents that are frequently altered in the presence of systemic diseases. As a result of these significant characteristics, finding biomarkers in saliva for the detection of serious systemic illnesses, such as cancer, is of great interest for most researchers [10]. There are only a few studies in the literature concerning the use of saliva to detect malignancies remote from the oral cavity. These reports deal with the identification and quantification of cancer related proteins in saliva that were previously discovered to be present in cancer tissue supernatants or elevated in the serum of diagnosed cancer patients. The importance of

this study is that we can use saliva for detection of disease of oral malignancy and pre-malignancy (conditions) because of anatomical proximity to the lesions [11].

Only a few studies were done on salivary protein analysis of different stage of OSCC. We have performed an estimation of total protein content and SDS-PAGE analysis from saliva sample of healthy controls and different stages of OSCC patients. With this analysis, we further analyzed the role of proteins in the biological process and cellular organization, which can generate a new insight into systemic biology in carcinogenesis [12]. We observed elevated levels of total protein content in the saliva of patients compared to those of healthy controls. We have also found significantly higher levels of total protein content in MD OSCC patients compared to that of WD OSCC and in WD OSCC patients compared to that of PD OSCC. This suggests correlation of elevated levels of total protein content to the progression of OSCC. This significant correlation has also made an attempt to the role of salivary biochemical parameter as marker of OSCC. Increased levels of salivary total protein content are observed in some studies [13].

Our most significant finding was that several proteins altered in between healthy controls and OSCC patients. The proteins which are altered as follows - Peptidyl-prolylcis-trans isomerase A, Peroxiredoxin-4, Proliferating cell nuclear antigen, Nicotinamid N-methyl transferase, Alpha-crystallin B chain, Protein SET, Keratin, type1, cytoskeletal 14 and Amine Oxidase A [14].

Peptidyl-prolylcis-trans isomerase A is also known as Prolylisomerase. It is an enzyme found inboth prokaryotes and eukaryotes that interconverts the cis and trans isomers of peptide bonds with the amino acid proline.Peptidyl-prolylcis-trans isomerases (PPI) catalyze cis-trans isomerization of imide bonds inpeptides and proteins. This enzyme have involvement in protein folding, and organization of PPI-containing receptors and membrane channels.Peptidyl-prolyl *cis-trans* isomerase (PPIase) catalyzes the *cis-trans* isomerization of prolineimidic peptide bonds in oligopeptides and has been shown to accelerate therefolding of several proteins in vitro [15].

Such activity has been detected in yeast, insects and *Escherichia coli* as well as in mammals, and it is thought to be essential for protein folding during protein synthesis in the cell [16]. In our study, Peptidyl-prolylcis-trans isomerase-A enzyme is present in control, pdoscc and absent in mdoscc, wdoscc. This indicates that in normal cellular activity it is express normally and in moderate oscc condition it is absent but in severe condition its activity increases.

Peroxiredoxin-4 is a protein that in humans is encoded by the*PRDX4*gene. The protein encoded by this gene isan antioxidant enzyme and belongs to the peroxiredoxin family [17]. The protein is localized to the cytoplasm. Peroxidases of the peroxiredoxin family reduce hydrogen peroxide and alkyl hydro peroxides to water and alcohol with the use of reducing equivalents derived from thiol-containing donor molecules. Peroxiredoxin-4protects cells from oxidative stress is analyzed in the studies. In our study, Peroxiredoxin-4 is present in controls but totally absent in oscc patients. This concluded the correlation in between Peroxiredoxin-4 andoxidative stress in oscc patients.

Proliferating cell nuclear antigen (PCNA) is a DNA clamp that acts as a processivity factor for DNA**polymerase δ** ineukaryotic cellsand is essential for replication.The most extensively investigated protein clamp in eukaryotes is the DNA polymerase processivity factor proliferating cell nuclear antigen (PCNA). PCNA is a central protein in both DNA replication and repair. PCNA is essential not only for DNA replication but also for several forms of DNA repair, including nucleotide excision repair (NER), the major pathway by which cells remove DNA damage introduced by UV light and a variety of chemical carcinogens [18]. Proliferating cell nuclear antigen (PCNA) is a key factor in DNA replication and cell cycle regulation is analyzed in many research [19].

In our study, PCNA is present in controls whereas absent in oscc patients. This shows that PCNA is involved in DNA replication and cell cycle regulation. Alpha-crystallin B chain is a protein that in humans is encoded bythe CRYAB gene. This is a proteins of vertebrate eye lens and maintains the transparency and refractive index of the lens. Elevated expression of Alpha-B crystallin occurs in many neurological diseases. In our study, Alpha-crystallin B chain protein is absent in controls while present in oscc patients. This concluded that CRYAB gene expressed in other tissues in oscc condition.

V. Conclusion

Saliva samples are collected, after the histopathological conformation of the clinical diagnosis of OSCC, these salivary components are constantly being explored as markers of various diseases and to monitor general health. Varying levels of differentially expressed proteins were possibly involved in the malignant transformation of oral cancer. This study shows that the proteins may play an important role in malignant transformation and is an example of a systematic biology study, in which functional proteomics were constructed to help to explain mechanistic aspects and potential involvement of proteins. The total protein content values increase with the severity of the disease that is OSCC. The values are statistically significant. Our

results provide new insights into the pathogenesis of oral cancer. These differentially expressed proteins may have utility as useful bio-markers of OSCC.

Acknowledgement

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