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Salivary mucin 4 levels in subjects with oral potentially malignant disorders and oral squamous cell carcinoma

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ABSTRACT

Aims: Oral cancer remains a substantial global health burden. Oral squamous cell carcinoma (OSCC) is a multi-step process characterized by invasive cancer and metastasis. Mucin 4 (MUC4) has been studied for its differential expression in cancer. The current study aimed to evaluate salivary MUC4 levels in subjects with oral potentially malignant disorders (OPMD) and OSCC.

Methods: This case-control, cross-sectional study evaluated salivary MUC4 levels in healthy subjects (Group 1), OPMD patients (Group 2), and OSCC patients (Group 3). Saliva was collected from the subjects 1 h before food consumption using the spit method, and MUC4 levels were analyzed using an Enzyme-linked immunosorbent assay.

Results: The study included 26 controls (group 1, age, mean±SD: 42.9±7.2, males: 50%), 26 subjects with OPMD (group 2, age, mean±SD: 46.2±7.5, males: 73.1%), and 26 subjects with OSCC (group 3, age, mean±SD: 57.2±6.2, males: 65.4%). MUC4 levels were significantly higher in patients with OPMD (6.20±3.07 ng/dL) and OSCC (7.87±4.30 ng/dL) than in controls (4.22±2.05 ng/dL) (p=0.001). Group 3 had significantly higher salivary MUC4 levels than group 1. OSCC with TNM stage 4a had higher salivary MUC4 levels (8.53±4.15 ng/dL), followed by TNM stage 3 (7.49±4.93 ng/dL) and TNM stage 2 (6.33±2.89 ng/dL).

Conclusions: Salivary MUC4 levels were significantly higher in patients with OPMD and OSCC. This study showed that MUC4 may play a role in the diagnosis of OPMD and OSCC.

Introduction

Oral cancer is a non-homogeneous group of cancers of the head and neck region, including neoplasms affecting the oral cavity (1). In South Asia, approximately 90% of oral cancers develop from pre-existing oral potentially malignant disorders (OPMDs), including leukoplakia, oral submucous fibrosis (OSMF), and erythroplakia (2). Although the oral cavity can be easily examined, oral squamous cell carcinoma (OSCC) is routinely diagnosed in advanced stages. Common reasons for this include ignorance and neglect of the changes occurring in the oral mucosa (3). Saliva is a potential source of biomarkers because of its diverse composition. Because it is in close contact with oral lesions like carcinoma, locally expressed molecules may reflect signs of tumorigenesis and malignant transformation

(4). Saliva collection is a non-invasive procedure that is cost-effective compared with other investigations and is therefore an ideal non-invasive medium for diagnosing OSCC (4).

Salivary mucins play an important role in innate immunity by promoting bacterial colony aggregation and clearance in the oral cavity. There are approximately 22 types of clinically recognized mucins, of which mucin 1 and mucin 4 (MUC4) are associated with OSCC (5). Mucins are classified based on their transmembrane domain, which directly influences their attachment to the plasma membrane. MUC4 is a membrane-bound mucin that has been studied extensively in several types of cancer and many systemic diseases (5). Various authors have demonstrated the expression of MUC4 in normal epithelia like cornea and conjunctiva, lacrimal gland, salivary gland secretory epithelium, upper aerodigestive tract, gastrointestinal tract, breast, endocervix, and vagina. MUC4 is also found in several body fluids such as blood, saliva, tears, and breast milk. Abnormal expression of MUC4 has been noted in several human cancers, including breast, lung, pancreas, salivary gland, oral mucosa, esophagus, and cervix (5,6). To date, there has been limited research into the relationship between MUC4 expression in OPMD and OSCC. The current study examined salivary MUC4 levels in subjects with OPMD and OSCC using enzyme-linked immunosorbent assay (ELISA).

Methods

Study population and recruitment

A total of 78 subjects aged >30 years who were reporting to the department of oral medicine and radiology between November 2019 and February 2021 were included in the study after obtaining informed consent. The subjects were categorized into three groups of 26 each: Group 1 consisted of randomly selected healthy controls, Group 2 consisted of subjects diagnosed clinically with OPMD, and Group 3 consisted of subjects diagnosed clinically and histopathologically with OSCC. Clinical TNM staging was performed for OSCC cases. Subjects with a history of any systemic complications, suffering from any major illness or malignancy other than OSCC, and those under treatment for OPMD or OSCC were excluded.

Saliva samples were obtained from the subjects one hour before food consumption using the spit technique. Salivary samples included oral fluid from major and minor salivary glands. Samples were subjected to centrifugation at 3000 rpm for 20 min and stored at -20 °C. Salivary MUC4 levels were estimated using an ELISA (Human MUC4 ELISA Kit, Immunoconcept India Pvt Ltd).

Statistical Analysis

The data obtained were computed, and statistical analysis was performed using IBM Statistical Package for the Social Sciences (SPSS) Statistics for Windows, version 24.00 (IBM Corp., Armonk, NY, USA). The Kolmogorov-Smirnov test was used to test the normality of the data distribution. Categorical variables are presented as frequencies and percentages, and continuous variables are presented as means and standard deviations. Because the data followed a normal distribution, data analysis was performed using one-way ANOVA. The mean difference between the groups was calculated, and the association was assessed using Tukey's post-hoc multiple comparison test. P value <0.05 was considered statistically significant.

Results

Demographic characteristics

In the control group, subjects were aged between 30 and 70 years, with a mean age of 42.9±7.2 years and 50% were men. In the OPMD group, the age range was from 30 to 70 years, with a mean age of 46.2±7.5 years and 73.1% were men (Table 1). Among the OPMDs, 13 cases were OSMF (50%), 10 cases were leukoplakia (38%), and 3 cases were erosive lichen planus (12%). In the OSCC group, the age range was 30 to 70 years, with a mean of 57.2±6.2 years and 65.4% were men.

Salivary MUC4 levels in groups

The mean salivary MUC4 levels in the control, OPMD, and OSCC groups were 4.22±2.05 ng/dL, 6.20±3.07 ng/dL, and 7.87±4.30 ng/dL, respectively. The OSCC group had higher salivary MUC4 levels, followed by the OPMD and control groups. There was a significant difference (p=0.001) in salivary MUC4 levels between the three groups using ANOVA. Post-hoc results revealed that the OSCC group had significantly higher (p<0.001) salivary MUC4 (7.87±4.30 ng/dL) than the controls (4.22±2.05 ng/dL). There were no significant differences in salivary MUC4 between the OPMD group and the control and OSCC groups (p=0.083 and p=0.176, respectively) (Table 2).

Salivary MUC4 levels according to OSCC stages

In the OSCC group, 12 (46.2%) patients presented with stage 4a, 11 (42.3%) with stage 3, and 3 (11.5%) with stage 2 OSCC. Stage 4a had higher salivary MUC4 levels (8.53±4.15 ng/dL), followed by stage 3 (7.49±4.93 ng/dL) and stage 2 (6.33±2.89 ng/dL). The difference in salivary MUC4 levels among the three OSCC stages was not significant (p=0.706).

Table 1. Age and gender distribution in the groups

	Age, years, mean±SD	Men, n (%)	Women, n (%)
Control group	42.9±7.2	13 (50.0)	13 (50.0)
OPMD group	46.2±7.5	19 (73.1)	7 (26.9)
OSCC group	57.2±6.2	17 (65.4)	9 (34.6)

SD: Standard deviation, n: Number, OPMD: Oral potentially malignant disorder, OSCC: Oral squamous cell carcinoma

Table 2. Intergroup comparison using Tukey's post-hoc test

	Control vs. OPMD	OPMD and OSCC	Controls vs. OSCC
Mean difference	-1.97	-1.63	-3.61
P value	0.083	0.176	**<0.001***

*p<0.05 was considered as statistically significant.
OPMD: Oral potentially malignant disorder, OSCC: Oral squamous cell carcinoma

Discussion

Oral carcinogenesis is multifactorial and occurs when the squamous epithelium is affected by various genetic alterations. Various cellular, inflammatory, biochemical, and hematological changes occur during carcinogenesis and have been well documented in the literature (3). Numerous tumor markers can be used to identify malignant transformation (7). Mucins are expressed by epithelial cells (8). High molecular weight proteins are responsible for various biological functions, such as growth, differentiation, and cell signaling (5,9). Various clinically significant mucins are documented in the literature, including mucin 1, 5AC, 5B, 6, 7, 16, and MUC4 (10).

The normal and pathophysiological functions of MUC4 have been extensively studied. MUC4 is commonly expressed in the respiratory epithelium and body fluids like saliva, tears, and breast milk. It has also been identified in carcinomas like OSCC, laryngeal carcinoma, adenocarcinoma of the lungs, and pancreatic carcinoma (5,11). Cancer cells utilize mucin for proliferation, survival, invasion, and the avoidance of innate immunity (11). Immunohistochemical studies have revealed that aberrant MUC4 expression is associated with increased tumor aggressiveness and poor outcomes in many carcinomas. However, in OSCC, the role of MUC4 is not yet clear (12). Saliva sampling is a noninvasive, readily available method with high compliance in all populations, and it is increasingly being used as an alternative to blood sampling (4). The current study assessed salivary MUC4 levels in patients with OPMD and OSCC.

The mean age of the OPMD group was 46.23±7.47 years. Similar to the results reported by Hosagadde et al. (13), who reported a mean age of 42.39 years among patients with OPMDs. In the current study, the mean age of patients diagnosed with OSCC was 57.5±6.22 years. Baykul et al. (14) conducted a study in which the mean age of patients with oral cancer was 57±19 years. In general, OSCC has been reported in individuals older than 60 years of age who have tobacco habits. Decreased age-related immunological surveillance and the cumulative effects of tobacco cause alterations at the molecular level, leading to malignant transformation (15). In recent years, there has been a greater tendency for OPMDs to occur in younger individuals, probably due to increased substance abuse (13).

In our study, OSCC and OPMD were predominantly diagnosed in male patients. Male predominance was previously reported by Hosagadde et al. (13) and Iype et al. (16), and it can be attributed to lifestyle factors or habits such as smoking and alcohol consumption, which are more prevalent in males.

In the present study, OSMF was the most common OPMD, followed by leukoplakia and erosive lichen planus. According to Hosagadde et al. (13), leukoplakia is the most frequently reported form of OPMD. However, studies in Karnataka, India, have stated that OSMF is more prevalent than leukoplakia, which could be attributed to the high betel quid consumption in the region (15,17).

The predominant stage of OSCC in our study was stage 4a, followed by stage 3 and stage 2. This is similar to the study by Oliveira et al. (18), in which 82.1% of patients were diagnosed with stage 3 and 4 OSCC. Delayed diagnosis of OSCC is a major cause of high mortality and morbidity (19). This can be due to various factors, such as inadequate access to healthcare services and poor socioeconomic status, resulting in delays in seeking medical attention (19). Inadequate awareness, poor socioeconomic status, and delayed access to healthcare facilities may be responsible for the higher OSCC stage of our study subjects.

In the current study, the mean salivary MUC4 level in the control group was 4.22±2.05 ng/dL. Similar findings were demonstrated by Lundmark et al. (20), who reported a mean salivary MUC4 level of the control group was 4.5 ng/ml. Mucins are associated with the renewal and differentiation of epithelial cells, signaling, and adhesion (5). Altered mucin levels lead to abnormal cellular function and possible malignancy (5). In our study, salivary MUC4 levels were increased compared with the normal levels in patients with OPMD. Narashiman et al. (6) evaluated MUC4 expression in leukoplakia cases and correlated it to different grades of dysplasia. They found that MUC4 expression increased steadily from mild to severe dysplasia (6). Abidullah et al. (21) reported that MUC4 expression increased from mild to severe dysplasia in cases with oral epithelial dysplasia. This increase in MUC4 expression according to dysplasia grade indicates a role in the malignant transformation of OPMD.

The mean salivary MUC4 level in the OSCC group was the highest among the three groups. MUC4 overexpression in OSCC sends continuous growth signals, causing uncontrolled cell proliferation (6). Regarding the correlation of salivary MUC4

levels in the three groups, higher levels were observed in the OSCC group, followed by the OPMD and control groups. A statistically significant intergroup difference was detected regarding the mean salivary MUC4 level. Although the control group was correlated with the OSCC group, the difference in salivary MUC4 levels was statistically significant. However, no significant difference in salivary MUC4 levels was observed between the OPMD and control and OSCC groups. Narashiman et al. (6) compared MUC4 levels between oral leukoplakia and OSCC and observed higher MUC4 expression in OSCC samples, followed by leukoplakia. In a systematic review and meta-analysis, Normando et al. (22) identified MUC4 as a protein marker of malignant oral leukoplakia. Since MUC4 levels in our study increased from OPMD to OSCC, MUC4 may help predict the conversion of OPMD to OSCC.

The current study demonstrated that salivary MUC4 levels were higher in stage 4a OSCC, followed by stage 3 and stage 2. No statistically significant association of salivary MUC4 was found among different stages of OSCC. These observations were similar to the study by Hamada et al. (12), which showed that MUC4 was expressed in patients with OSCC and was significantly correlated with greater tumor stage, nodal metastasis, advanced tumor stage, and cancer cell invasion. MUC4 expression in OSCC has been associated with increased invasion, tumor progression, nodal metastasis, and decreased survival (12). MUC4 has also been correlated with local recurrence and lymph node metastasis following treatment (12). Therefore, high MUC4 expression is considered a poor prognostic factor in patients with OSCC (12).

MUC4 may attain novel functions in malignant cells because of its aberrant expression and biochemical and cellular changes, leading to the interaction of MUC4 with cyto-architecturally segregated proteins (22). MUC4 colocalizes and interacts with human epidermal growth factor receptor 2 (HER2) in pancreatic cancer cells, leading to their activation (23). Multiple ligands can bind, causing activation of specific receptors (23). Once HER2 receptors are activated, cascades of intracellular signaling are initiated leading to cell proliferation, angiogenesis, metastasis, apoptosis inhibition, and other events leading to cancer development (23). Kohli et al. (11) and Abidullah et al. (21) found that MUC4 is overexpressed in well-differentiated OSCC, and this expression decreases in moderately and poorly differentiated OSCC. Similarly, in esophageal carcinoma, MUC4 expression decreases with the differentiation of the lesion (24). However, this parameter was not evaluated in the current study; thus, we were unable to determine any correlation.

Hamada et al. (12) reported the presence of MUC4 in premalignant and malignant epithelium but not in normal squamous epithelium. Thus, it could serve as a future diagnostic biomarker for the initial detection of malignant changes. The current study noted a significant increase in salivary MUC4 levels in patients with OSCC and OPMD. These findings indicate that MUC4 is an adjunctive salivary biomarker in OSCC and OPMD.

The current study had a few limitations, such as a smaller sample size of 26 subjects in each group. This result may have been insufficient to explore the diagnostic role of salivary MUC4 levels in OPMD and OSCC.

Conclusion

The current study showed significantly higher salivary MUC4 levels in patients with OSCC and OPMD than in healthy controls. On intergroup comparison, salivary MUC4 levels were significantly increased in the OSCC group compared with the control group. Our findings emphasize the role of salivary MUC4 in OSCC. Further studies are required to explore the diagnostic and prognostic role of salivary MUC4 levels in patients with OPMD and OSCC.

Ethics

Ethics Committee Approval: This case-control, cross-sectional study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Ethical Clearance Committee AB Shetty Memorial Institute of Dental Sciences (ABSMIDS) (decision no: EC/35/2019, dated: 10.10.2019).

Informed Consent: Consent form was filled out by all participants.

Footnotes

Authorship Contributions

Surgical and Medical Practices: V.P., V.A., S.B., S.N.K., S.H., Concept: V.P., V.A., S.B., S.N.K., S.H., M.F.A., Design: V.P., V.A., S.B., S.N.K., S.H., M.F.A., Data Collection or Processing: V.P., V.A., S.B., S.N.K., S.H., M.F.A., Analysis or Interpretation: V.P., V.A., S.B., S.H., M.F.A., Literature Search: V.P., V.A., S.B., S.H., M.F.A., Writing: V.P., V.A., S.B., S.N.K., S.H., M.F.A.

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