Estimation of salivary sialic acid levels as a prognostic marker in patients with oral leukoplakia - A case control study

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Abstract

Background: Oral leukoplakia (OL) is a potentially malignant disorder seen in tobacco users. Salivary biomarkers such as salivary sialic acid which is significantly high in OL and oral cancer serves as a potent biomarker for early diagnosis thereby improving prognosis.

Aim: To estimate and compare salivary sialic acid levels in tobacco users with and without OL.

Methods: The study included 172 tobacco users reporting to Private Dental Teaching Hospital, aged between 20 and 70 years, selected according to inclusion and exclusion criteria and divided into two groups. Study group included 76 patients with OL and control group, 96 patients without OL. Unstimulated whole saliva was collected and salivary sialic acid levels were estimated and the results were subjected to statistical analysis (unpaired students t-test).

Results: The mean range of salivary sialic acid in controls was 1.898 ± 0.289 µmol/L and cases was 2.680 ± 0.189 µmol/L. Salivary sialic levels are high in cases when compared with controls with a statistically significant \( P < 0.001^* \).

Conclusion: The results of our study clearly indicate that salivary sialic acid levels are significantly raised in patients with OL when compared with patients without OL. With the observations of our study salivary sialic acid proves to be a sensitive, specific, and cost-effective biomarker for OL.

Keywords

Oral cancer, oral leukoplakia, oral potentially malignant disorder, sialodiagnosis, salivary sialic acid

Introduction

Oral cancer is the sixth most common cancer of head and neck region worldwide.[1] In India, about 30-40% of all malignant tumors arises from head and neck region.[4] Worldwide, head- and neck-related squamous cell carcinoma hold 6th place with emergence of 5,00,000 new cases diagnosed every year.[3] In most clinical scenario, the occurrence of oral cancer is preceded by oral potentially malignant disorders (PMDs).[4] The WHO (2005) referred to all clinical presentations that carry a risk of cancer under the term “PMD” to reflect their widespread anatomical distribution and has defined PMDs “as the risk of malignancy being present in a lesion or condition either during the time of initial diagnosis or at a future date.” International literature reports that oral PMDs are major threats for oral cancer accounting for increased risk of malignant transformation to oral squamous cell carcinoma (OSCC). Oral PMDs include oral leukoplakia (OL), oral submucous fibrosis, oral lichen planus, dyskeratosis congenita, actinic cheilosis, and keratoacanthoma of which OL and erythroplakia carry highest potential to transform into a frank carcinoma.[5,6]

OL is defined as white plaques of questionable risk having excluded (other) known diseases or disorders that carry no increased risk for cancer.[6] The incidence and the prevalence of OL varies according to the region and habit of individuals. Several risk factors play a key role in malignant transformation of OL which includes age, gender, tobacco habits, homogeneity, size of the lesion, oral subsite, degree of epithelial dysplasia, salivary biomarkers and microflora of oral mucosa.[7]

The prevalence of OL ranges from 0.2% to 5%, with Sweden (3.6%), Germany (1.6%), Holland (1.4%), and India (0.2-4.9%) showing conspicuous regional differences.[6] Van der Waal (2009) reported that annual malignant transformation of OL into OSCC ranges between 1% and 2%.[6] Epithelial dysplasia is considered to be one of the most important criteria in malignant transformation.[10]
OL has an association with several risk factors including salivary biomarkers which alters the biological macromolecules and micromolecules. Tobacco confronts the saliva at first place; hence, salivary enzymes are directly involved in pathogenesis of oral cancer.\textsuperscript{[11,12]} The use of salivary enzymes in determining the risk of oral cancer may be more pertinent in early detection and diagnosis of PMDs and oral cancer. The advantages of saliodiagnosis are that it is non-invasive and much facile to perform.\textsuperscript{[13]} The sensitivity of a biomarker increases when it is linked directly with the tumor progression even before the occurrence of cancer.\textsuperscript{[14,15]}

Sialic acid (N-acetylneuraminic acid) is a protein-bound monosaccharide with nine carbons. It is seen mostly in salivary mucin.\textsuperscript{[16]} Sialic acid is seen associated with cellular invasiveness, adhesiveness, and immunogenicity. Increased glycoproteins in the circulation is caused due to increased turnover, secretion, and shedding from malignant cells.\textsuperscript{[17]} The classic feature of cancer is aberrant glycosylations. Studies have reported that changes in levels of sialic acid are correlated with clinical status of cancer lesions.\textsuperscript{[18]}

There are numerous studies on serum and salivary biomarker levels in literature but very few researches on salivary sialic acid levels.\textsuperscript{[19]} Hence, we undertook this study to estimate the salivary sialic acid levels to assess the prognostic value in tobacco users with and without OL.

Materials and Methods

The study included 172 tobacco users who reported to the Department of Oral Medicine as outpatients. Clearance from the Institutional review board and Institutional ethical committee was obtained. Informed consent from the participants was obtained before the start of the study. A comprehensive habit history was obtained. Oral mucosa was examined for the presence of localized or extensive white patch with slight elevation, fissures, wrinkled, or corrugated appearance or a mixed red and white lesion in which keratotic white nodules or patches are seen over an atrophic erythematous background or the presence of thick white lesions with papillary surface. Selection of patients with OL was based on the exclusion and inclusion criteria.

Patients with habits such as smoking tobacco (beedi, cigarette) and smokeless tobacco (tobacco, betel leaf, areca nut, and slaked lime) and both smoking and smokeless tobacco habits with OL were included in the case group and those without OL included in the control group. Patients suffering from systemic or other oral diseases and those who were consuming long-term medications were excluded. Among the 172 tobacco users 76 patients (44%) were found with OL and 96 patients (56%) without OL.

Patients with OL were staged according to OLEP staging system of leukoplakia given by Van der Waal et al.\textsuperscript{[20]}

For collection of salivary sample, patients were instructed to rinse the mouth thoroughly with deionized water before the collection of saliva. Approximately 2 ml of unstimulated whole salivary samples were collected between 8 am and 12 pm according to the guidelines given by Navazesh.\textsuperscript{[21]} The plastic container was placed in an ice carrier box and transferred to the laboratory and was centrifuged at 3000 rpm for 15 min. The protein-bound sialic acid in saliva was measured by thiobarbituric acid (TBA) method.\textsuperscript{[22]}

Trichloroacetic acid precipitates the proteins present in saliva. The protein-bound sialic acid is released by sulfuric acid (\(H_2SO_4\)) and reacts with TBA to form TBA-sialic acid complex. On boiling in water bath, this gives a pink. This color is further extracted using acid-butanol mixture and then, measured at 549 nm spectrophotometrically.

Statistical analysis

Statistical analysis was performed using SPSS software (version 19) for windows. Standard deviation and mean values was computed for each parameter in control and case group. The results were tabulated and statistical significance was evaluated using unpaired Student’s \(t\)-test. Results were considered significant when \(P < 0.001\).

Results

In our study, the mean age between male and female was found to be in fourth decade (Table 1). The age distribution and gender distribution among the participants are given (Table 2 and Graph 1). The prevalence of tobacco habits either smoking or chewing was more among males (84%) than in females (16%) (Graph 2).

The salivary sialic acid levels were estimated in both groups and the arithmetic mean along with standard deviation was calculated. The mean range of salivary sialic acid levels was seen higher in patients with OL with a significant \(P < 0.001^*\) (Table 3).

Table 1: Age distribution among case and control

<table>
<thead>
<tr>
<th>Gender</th>
<th>Number of individuals</th>
<th>Average age (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>144</td>
<td>43.83</td>
</tr>
<tr>
<td>Female</td>
<td>28</td>
<td>46.82</td>
</tr>
<tr>
<td>Total</td>
<td>172</td>
<td>44.32</td>
</tr>
</tbody>
</table>

Graph 1: Gender distribution of individuals
Discussion

Sialic acid has a pivotal role in transformation of cells as at early tumorigenesis alteration of glycoproteins are evident with an increase in activity of glycosyltransferases causing overexpression of terminal glycans involving sialic acid. Increased sialylation masks the malignant cells and saves them from host immune systems such as lymphocytes and macrophages. Tumor tissue further shows increased sialylation which is reflected as elevated levels of sialic acid in serum, saliva and other body fluids. This feature makes sialic acid a significant biomarker in oral premalignant and malignant lesions.[23,24]

Hemalatha et al. evaluated salivary sialic acid in patients with different clinico pathological stages of OL and OSCC and reported that there was significant increase in the levels of the sialic acid (free and protein bound) levels in patients with OSCC and patients with OL.[25] Similarly, Vajaria et al. reported on salivary sialylation changes where the levels of sialic acid were increased in precancerous and oral cancer.[26] Sanjay et al. reported a higher level of salivary total protein, total sugar, and sialic acid in OSCC when compared to normal healthy individuals. According to their study, Salivary sialic acid levels were significantly elevated in well differentiated squamous cell carcinomas than in moderately differentiated squamous cell carcinomas.[27] According to Arduino et al., the levels of salivary sialic acid increases as the degree of dysplasia increases. This explains the association between sialic acid, progression of the lesion, and malignant transformation.[28] These studies suggest that the salivary sialic acid is a sensitive marker in oral PMD and oral cancer.

In our study, there was raise in levels of salivary sialic acid with a mean value of 1.898 ± 0.289 µmol/L in tobacco users without leukoplakia. The elevated levels of salivary sialic acid in tobacco users without lesions are due to positive correlation between oxidative stress and protein-bound sialic acid levels. Authors justify that increased exposure to tobacco increases oxidative stress thereby increasing the levels of total sialic acid in tobacco users.[29]

In comparison, our study results showed higher salivary sialic acid levels in tobacco users with leukoplakia which was 2.680 ± 0.189 µmol/L. This can be used as a predictor of mucosa progressing to potentially malignant diseases. The results were statistically significant with P < 0.001.[30]

When comparing our study results with the previous researches, we had similar results with elevated levels of salivary sialic acid in both groups with marked difference between the case and control groups. In previous studies, authors had compared the levels of sialic acid either amongst patients with oral cancer, OL with normal individuals. The key point in our study is the elevated levels of salivary sialic acid in tobacco users without lesions, which by itself serves as a prognostic marker to identify tobacco users who have potential to develop OL. Estimating the levels of salivary sialic acid can be used in initial diagnosis, monitor the progression of potentially malignant lesion and in evaluation of recurrence.

However, our study had some limitations such as small sample size and though salivary sialic acid is a reliable marker it is known to be raised in other conditions such as acute inflammation, tuberculosis, diabetes mellitus, and rheumatoid arthritis though these conditions were excluded in our study to improve the accuracy of the study estimation of other salivary biomarkers addition to sialic acid can be used.

Conclusion

Our study aimed at estimating the levels of salivary sialic acid in tobacco users with and without OL. The results showed elevated levels of salivary sialic acid in both groups with marked elevation in patients with OL. Since OL has a higher risk of malignant transformation, it is mandatory to diagnose OL at a much earlier stage. In such instances, estimation of salivary sialic acid can be performed to arrive at an accurate diagnosis which may greatly improve the treatment and prognosis.

References

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