Sialic Acid as a Biomarker of Oral Potentially Malignant Disorders and Oral Cancer

Abstract

Background: Carcinogenesis is a multistep process where a healthy cell has initially a precancerous stage and finally an early cancerous stage. The process of carcinogenesis can be divided into three stages of initiation, promotion, and progression. In this process, there is increased turnover, secretion, and/or shedding from malignant cells. Glycoproteins like sialic acid are expressed on the cell surface. In oral potentially malignant disorders (OPMDs) and oral cancer (OC), the sialic acid level is seen to increase due to high cell turnover and shedding of malignant cells which, in turn, results in the release of glycopolypeptides like sialic acid into circulation. Glycoproteins also form an important constituent of salivary mucins and hence due to the same mechanism, an increase in sialic acid level is also seen in saliva. Objective: The aim is to estimate serum and salivary sialic acid levels in healthy controls, patients with OPMDs and patients with OC. Materials and Methods: In this observational cross-sectional study, serum and salivary sialic acid levels were estimated in thirty healthy controls, thirty patients with OPMDs and thirty patients with OC. Results: Serum and salivary sialic acid levels obtained were subjected to statistical analysis. Post hoc Tukey test was used to compare the serum and salivary sialic acid levels of the two study groups to the control group. ANOVA test was used for the comparison of sialic acid levels between the groups. Pearson’s correlation coefficient was used to assess correlation (P < 0.05 was considered statistically significant). The mean serum and salivary sialic acid levels were increased significantly in subjects with OPMDs and OC when compared to healthy controls. Conclusion: This study highlights the high expression of sialic acid on outer cell membranes, due to the significant increase in subjects with OPMDs and OC when compared to healthy controls. A significant increase in sialic acid level is also seen in saliva. Hence, it can be stated that saliva can be used as a reliable, noninvasive tool in diagnosis and management of OPMDs and OC.

Keywords: Oral potentially malignant disorders, oral cancer, sialic acid

Introduction

The incidence of cancer in the head and neck region accounts for 30%–40% of all malignant tumors in India.[1] The oral cavity is the fourth common site for carcinoma after lungs, stomach, and liver in males and fifth common site in females after cervix, breast, stomach, and liver.[2] A total of 222,000 new cases in men and 90,000 new cases in women are diagnosed each year globally.[3] It was estimated that 4.9 million people died of tobacco-related illness in the year 2000, and by 2020s that figure will increase to 10 million deaths per year.[4]

Oral cancer (OC) is usually preceded by oral precancerous lesions and conditions such as oral leukoplakia and oral submucous fibrosis.[3] Prevention and early detection of such potentially malignant disorders (PMDs) have the potential of not only decreasing the incidence but also in improving the survival of those who develop OC.[5]

Various terminologies such as “precancerous” and “premalignant” were being used for oral lesions with a predisposition to malignant transformation but “potentially malignant” was preferred and furthermore, it has been recommended to abandon the traditional distinction between potentially malignant lesions and potentially malignant conditions and to use the term “PMDs” instead.[6]

Recognition and diagnosis of early superficial cancer of the oral cavity are the most difficult task. It is usually asymptomatic for a long time and by the time the patient seeks advice it would have invaded deeply, and hence prognosis becomes poor. With the threat of high...
recurrence rate and secondary metastasis, the clinical decision for a treatment plan and adjuvant therapy assumes importance.\(^7\) The present clinical approach for cancer diagnosis and management involves invasive and painful procedures. Therefore, a simple and noninvasive tumor marker is needed for early diagnosis as well as to monitor the progress during the treatment.

Tumor markers are substances specific for certain tumor or cancer cells and thus are important for diagnostic and prognostic purpose in OC patients.\(^3\) Substances changing quantitatively in the serum during tumor development are collectively called tumor marker or biochemical serum markers.\(^8\)

From over thirty acetylated derivatives of neuraminic acid, N-acetyl neuraminic acid referred to as sialic acid, is the most common in humans. They are attached to the nonreducing residue of the carbohydrate chains of glycoproteins and glycolipids. Among the glycoconjugates, sialic acid is present up to 30% in various glycoproteins.\(^9\) The cell coat is made up predominantly of sialic acid containing glycoproteins.\(^9\) Sialic acid plays an important role in cell-cell recognition, invasiveness, adhesiveness, and immunogenicity.\(^10\) The major structural component of cell surface and glycoproteins undergo alteration on the neoplastic transformation of the cell. One such transformation is increased levels of sialic acid on the cell surface. These glycoconjugates are released into the circulation through increased turnover, secretion and/or shedding from malignant cells thus leading to elevation of sialic acid levels in the blood.\(^11\) Neoplasms often have an increased concentration of sialic acid on the tumor cell surface and are shed or secreted by some of these cells which increase the concentration in blood.\(^11\)

Detection of cancer at an early stage is of utmost importance to decrease the morbidity and mortality of the disease. Noninvasive methods like analysis of saliva may provide a cost-effective approach for screening a large population. Salivary glycoproteins play an important role in the properties and functions of saliva; where sialic acid is a constituent of many of the salivary glycoproteins.\(^11\)

The outcome of the present study intends to assess the importance of sialic acid levels as an adjunctive diagnostic marker in head and neck cancer by evaluating serum and salivary total sialic acid levels in oral potentially malignant disorders (OPMDs) and OC.

**Materials and Methods**

The present observational cross-sectional study was conducted on subjects reporting to the outpatient department. The study comprised ninety subjects which included thirty healthy controls (Group I), thirty subjects diagnosed clinically and confirmed histopathologically with OPMDs (Group II) of which 14 were oral submucous fibrosis, ten were leukoplakia (speckled type/erythroplakia) and seven were lichen planus (erosive type) and 30 subjects diagnosed clinically and confirmed histopathologically with OC (Group III). Informed consent was obtained from all the subjects. Ethical clearance was obtained from the Ethical Committee of the Institute. Detailed case history was recorded along with thorough examination of the soft and hard tissues of the oral cavity. The study was conducted for 2 years.

All subjects included were in the age group of 20–70 years. Subjects in Group I were healthy without any oral or systemic diseases, who were not under any medications and do not have any oral adverse habits. In Group II and Group III, subjects with history of any systemic diseases such as diabetes mellitus, hypertension, endocrine disorders, and cardiovascular disorders, pregnant and lactating women, subjects diagnosed with malignancies in sites other than the oral cavity, subjects who are on any medications, subjects with any other oral mucosal lesions, subjects with OPMDs and OC but without any history of adverse oral habits were excluded.

All salivary samples were collected from the subjects using spit technique 2 h after the consumption of food. Unstimulated saliva was collected by asking the subject to spit in a sterile graduated container every minute for 5–8 min. The collected sample was centrifuged at 3000 rpm for 10 min, and the supernatant was collected and stored at ~20°C. Five milliliters of venous blood was collected from the antecubital vein with a syringe and placed in vials. Serum was then extracted and stored at a temperature of ~20°C in glass vials. Sialic acid was then estimated from the salivary and serum samples by diphenylamine method. A protein precipitate of the sample containing sialic acid was made to react with diphenylamine producing a purple color which was then quantitatively mentioned as a spectrophotometer of 530 nm.

The serum and salivary sialic acid levels obtained was then analyzed using SPSS version 17.0 (IBM) software. Post hoc Tukey test was used to compare the serum and salivary sialic acid levels of the two study groups to the control group. ANOVA test was used for the comparison of sialic acid levels between the groups. Pearson’s correlation coefficient was used to assess correlation. \(P < 0.05\) was considered statistically significant.

**Results**

The mean age in Group I was 46.37 years. Females comprised 23.3%, whereas males comprised 76.7%. The mean age in Group II was 40.83 years. Females comprised 20.0% of the cases, whereas males formed 80.0%. The mean age of the subjects in Group III was 56.23 years. 26.7% were females, whereas males comprised 73.3%.

When serum and salivary levels of sialic acid were compared between Group I and Group II, the difference was statistically highly significant \((P < 0.001)\). Similarly, when the serum and salivary levels of Group I was
compared with Group III, highly significant difference were obtained ($P < 0.001$). Statistical comparison between serum and salivary levels of group II and group III also showed highly significant differences ($P < 0.001$) [Tables 1 and 2].

The good positive correlation was observed between serum and salivary sialic acid levels in all the three groups [Table 3 and Graph 1].

**Discussion**

Tumor markers have a diverse set of applications from detection of cancer to their therapy. More attention has been paid to the tumor markers in the course of the search for possible causes of cancer and the need for a modality affording early diagnosis and follow-up of malignant states.

Tumor markers are substances specific for certain tumor or cancer cells and thus are important for diagnostic and prognostic purpose in OC patients. During the malignant transformation of cells, there may be either an upregulation or downregulation of the biochemical substances. With the development of new and sensitive techniques for measuring very minute quantities of biochemical substances, now it is possible to identify early malignant transformation of the cells. Substances changing quantitatively in the serum during tumor development are collectively called tumor marker or biochemical serum markers.

Cell surface glycoconjugates are considered to be important in relation to cancer because many of the altered properties of cancer cells are expressed at the cell surface. Glycoproteins and glycolipids are major constituents of the cell membrane. Altered glycosylation of glycolconjugates is one of the important molecular changes that accompany malignant transformation. Studies have shown alterations in cell surfaces and membranes in terms of the sialic acid content of glycoproteins and glycolipids.

Literature has shown sialic acid levels to vary in various other general diseases and in inflammatory conditions as well. Hence, in the present study, subjects without any of the systemic diseases were taken in study groups.

**Serum sialic acid levels**

In this study, the mean serum sialic acid level in healthy controls was 57.674 mg/dl, while in subjects with OPMDs it was significantly increased to 67.022 mg/dl. This was in accordance with studies conducted by Joshi and Patil, Taqī, Baxi et al., which said that the level of sialic acid increases with the degree of dysplasia seen in oral precancer, suggesting its association with malignant transformation and thus proved that elevation in sialic acid can give an early indication of a premalignant change. Increased levels of serum sialic acid were also seen in the study done by Sawhney et al.

In the current study, when the mean serum sialic acid level in healthy controls was compared with subjects with OC, it was significantly increased to 86.221 mg/dl. This was in accordance with a study done by Vallikkanthan et al., which stated that increase in serum sialic acid in OC is attributed to the proliferation of cells and subsequent increase in sialic acid content which is shed or secreted into the circulation. The present study was also in accordance with studies conducted by Joshi and Patil, Taqī and Rajpura et al., which suggested that elevations in sialic acid levels appeared to reflect the tumor burden.

**Salivary sialic acid levels**

There are only a few studies documented in the literature till date where salivary sialic acid has been studied. In the current study, the mean salivary sialic acid level in healthy controls was 40.373 mg/dl and showed a significant increase in subjects with OPMDs where the mean value was 57.562 mg/dl. This was consistent with the study conducted...
Table 3: Correlation of serum and salivary sialic acid levels

| Group                        | Saliva sialic acid | Pearson Correlation | Sig. (2-tailed) | N  |
|------------------------------|-------------------|---------------------|----------------|----|
| Control                      |                   | 0.525               | 0.003          | 30 |
| Serum sialic acid            |                   |                     |                |    |
| Sig. (2-tailed)              |                   |                     |                |    |
| Oral cancer                  |                   | 0.597               | 0.001          | 30 |
| Serum sialic acid            |                   |                     |                |    |
| Sig. (2-tailed)              |                   |                     |                |    |

by Bansal and Rath[23] where the salivary sialic acid levels were significantly increased in oral premalignancy when compared to healthy individuals. Sialic acid is a significant component in all salivary mucins. Sialic acid levels have been reported to correlate with stage of disease, tumor burden, the degree of metastasis and recurrence of disease and thus significant elevations in sialic acid levels in oral precancer patients suggested its potential utility as an adjutant in diagnosis and progression of the disease.

In the present study, the mean salivary sialic acid level in subjects with OC was significantly increased to 80.422 mg/dl when compared to the healthy subjects. This was in accordance with a study conducted by Shivashankara and Prabhu[23] where the salivary sialic acid levels were significantly increased in subjects with oral squamous cell carcinoma. Circulatory levels of sialic acid are proposed to reflect changes in the cell surface characteristics, adhesiveness and cellular invasiveness. Thus, it can be stated that salivary sialic acid could serve as a sensitive marker of oral squamous cell carcinoma. The current study also showed data consistent with a study done by Sanjay et al.[31] where sialic acid levels were significantly higher in oral squamous cell carcinoma patients when compared to normal healthy controls.

The good positive correlation was observed between serum and salivary sialic acid levels in all the three groups. In all the three groups, the Pearson's correlation coefficient between the levels of serum and salivary sialic acid was statistically significant. None of the studies have mentioned about the correlation between serum and salivary sialic acid levels of the study groups.

Conclusion

The present study highlights the high expression of sialic acid on outer cell membranes suggesting that sialic acid has a role in stabilizing molecules and membranes. Increased levels of sialic acid on the cell surface indicate alteration of the structural components and glycoproteins on the neoplastic transformation of the cell. There is increased cell turn over, secretion and/or shedding from malignant cells. This results in the release of glycoconjugates into the circulation thereby resulting in increased levels of sialic acid in the blood. Sialic acid forms an important constituent of many salivary glycoproteins and salivary mucins. Similar mechanism results in increased levels of sialic acid in saliva in OPMDs and OC. As significant increase is observed in the levels of sialic acid in serum and saliva with good positive correlation, this study suggests the use of sialic acid as a potential adjunctive biomarker for OPMDs and OC. Furthermore, since a good positive correlation was observed between serum and salivary sialic acid levels, saliva could be used as a reliable, noninvasive tool in diagnosis, monitoring and treatment of oral potentially malignant conditions and OC.

Limitations of this study are that smaller sample size was taken and posttreatment assessment of sialic acid levels was not estimated. Hence, further extensive studies may be required with larger samples along with pre- and post-treatment monitoring of the sialic acid levels in OPMDs and OC to establish the effectiveness of sialic acid as a potential biomarker in these conditions.

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Conflicts of interest

There are no conflicts of interest.
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