EMERGING ROLE OF SIALIC ACID: A NEW POTENTIAL MARKER OF TOBACCO ABUSE

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Abstract

Background: Tobacco smoking is one of the known risk factors for developing oral cancer. Sialic acid is a glycoprotein that is considered as an important constituent of cell membrane. Any alteration to this glycoprotein can leads to malignant transformation. It has been well established by the previous studies that there is an elevation in salivary sialic acid even before development of clinical symptoms in patients. The purpose of this study is to evaluate the role of salivary sialic acid as a screening tool for the prediction of potentially malignant as well as malignant lesions in smokers.

Materials and Methods: Eighty subjects were included in this study; they were divided into the two groups consisting of 40 participants. Group 1 –Healthy individuals with good oral hygiene and no habit of smoking. Group II- Individuals who are tobacco smokers, with a frequency of at least 5 cigarettes/day. Unstimulated saliva was collected using the spitting method directly into a sterile container. Biochemical analysis of saliva was done using ninhydrine reagent for the estimation of total Salivary sialic acid levels

Results: A statistically significant increase in salivary sialic acid level were noted in subjects with habit of smoking when compared to controls.

Conclusions: In conclusion, the significant raise of salivary SA in individual with tobacco smoking could be an indicator of early biochemical changes denoting malignant transformation of the cell.

Keywords: - salivary sialic acid, tobacco smoking, oral cancer, potentially malignant lesions.

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INTRODUCTION

Tobacco use is acknowledged as a serious global public health issue, as well as a significant risk factor for developing potentially malignant disorders and oral cancer. There are more than 60 carcinogens in cigarette smoke. Among these, tobacco specific nitrosamines (such as 4-(methylnitrosamino) -1-(3-pyridyl)-1-butanone (NNK) and N′-nitrosonornicotine (NNN)), polycyclic aromatic hydrocarbons (such as benzo[a]pyrene) and aromatic amines (such as 4-aminobiphenyl) seem to have an important role as causes of cancer. During inhalation of cigarette smoke saliva is the first biological medium encountered.[1] Saliva can be used as a non-invasive, uncomplicated diagnostic tool for disease screening. [2] Early diagnosis being the key to the treatment of oral cancer, remarkable efforts are being made to develop reliable and sensitive biochemical tests to predict OSCC.

Tobacco abuse remains a pressing global health issue, causing a significant burden on individuals and societies alike. The detrimental effects of tobacco consumption are well-documented, with smoking being linked to a wide range of diseases, including various types of cancer, cardiovascular disorders, and respiratory conditions. Consequently, the identification of reliable markers to detect tobacco abuse has become a crucial area of research. In recent years, emerging evidence has highlighted the potential role of sialic acid as a novel biomarker for tobacco abuse[1]. Sialic acid, a key component of glycoproteins and glycolipids, is involved in numerous physiological processes and has been implicated in various disease states. This introduction provides an overview of the emerging role of sialic acid as a potential marker of tobacco abuse, discussing its underlying mechanisms and the implications for public health interventions. By elucidating the connection between sialic acid and tobacco abuse, this research aims to contribute to the development of effective strategies for tobacco cessation and the prevention of associated health risks[2].

Cell membrane contains oligosaccharide such as glycoproteins (GPs), glycolipids (GLs) etc and sialic acids (SAs) is one of its terminal sugar components. They are thought to be important in determining the surface properties of cells and has been implicated in cellular invasiveness, adhesiveness, and immunogenicity[3]. By measuring the sialic acid levels, we may predict development of cancer. Even though many studies reported alteration of salivary sialic acid in malignancy but not much in smoking. This study aims to evaluate the role of salivary sialic acid as a screening tool for the prediction of potentially malignant and malignant lesions in smokers.

MATERIALS AND METHODS

A cross sectional study was carried out to assess and compare salivary sialic acid levels among tobacco smokers and non –smokers. Study subjects were randomly selected from the out patients of Malabar dental college and research center over a period of six months after obtaining institutional ethical clearance. A total of 80 subjects participated in the study, were categorized into two groups with 40 participants each.

Inclusion Criteria

Individuals aged between 25-65 years who consented to participate in the study were grouped as Group 1 – Healthy male subjects with good oral hygiene and no habit of tobacco smoking. Group II- Healthy male subjects with tobacco smoking with a frequency of at least 5 cigarettes/day.

Exclusion Criteria

Tobacco users in the form of smokeless tobacco were excluded from the study. Subjects with systemic disorders including cardiovascular disease, hypertension, diabetes mellitus, asthma, salivary gland disorders etc., and those who are under medications that can influence the salivary flow were excluded from the study as it can influence the body's levels of sialic acid. Subjects with periodontitis, pre-malignancy and malignancy were also excluded from the study.

Saliva collection

To reduce possible confounding effect of circadian rhythms in salivary flow rate, saliva samples collection was carried out in the morning. The participants were refrained from eating, drinking, and brushing their teeth for one hour before the sampling. Unstimulated saliva was collected using spitting method directly into a sterile container .Salivary sialic acid levels were estimated based on the reaction of sialic acid with Ninhydrin reagent in acidic medium using the UV-spectrophotometer. The absorbance of blue-colored complex was measured at 470 nm.[4]

RESULTS

The estimation of mean salivary sialic acid levels two groups was tabulated and shown in Table 1 and Figure 1 & 2. Data entry was done using Microsoft Excel (2007) and later exported to IBM SPSS version 25.0 (IBM SPSS version 25.0 Armonk, NY: IBM Corp.) for analysis. Among smokers, the maximum value was 68 mg/100ml while it was

only 25mg/100ml among the non-smoking group. Similarly, the minimum value in the smoker group was 29 mg/100ml as compared to 14 mg/100ml in non -smoker group.

Table 1: Mean salivary sialic acid levels in smokers and non-smokers

Groups	N	Mean	Standard deviation	Maximum value	Minimum value
Smokers	30	46.73	8.785	68	29
Nonsmokers	30	19.33	3.262	25	14
N=Subjects in each group					

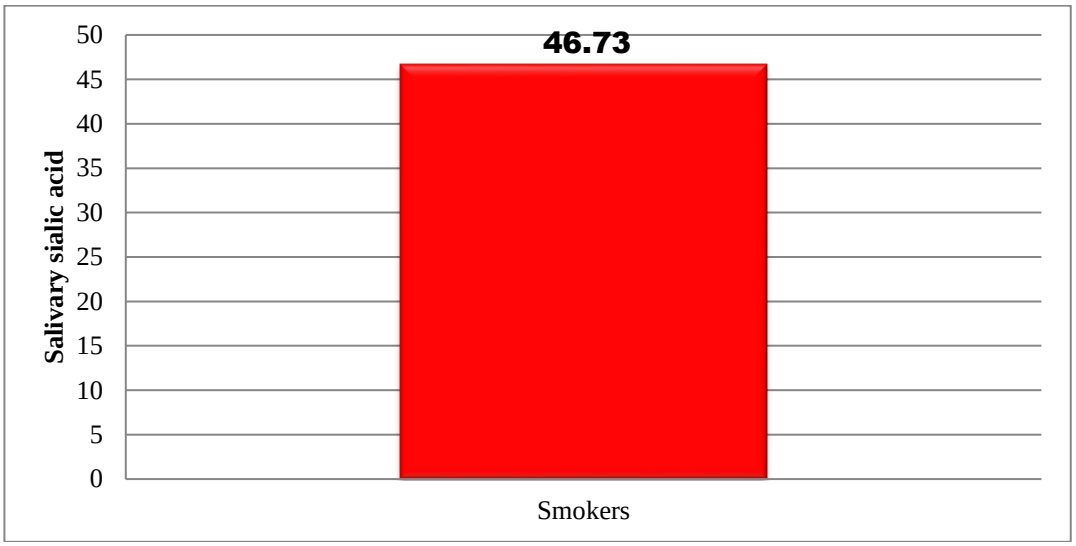

Figure 1: Mean salivary sialic acid level in smokers were 46.7

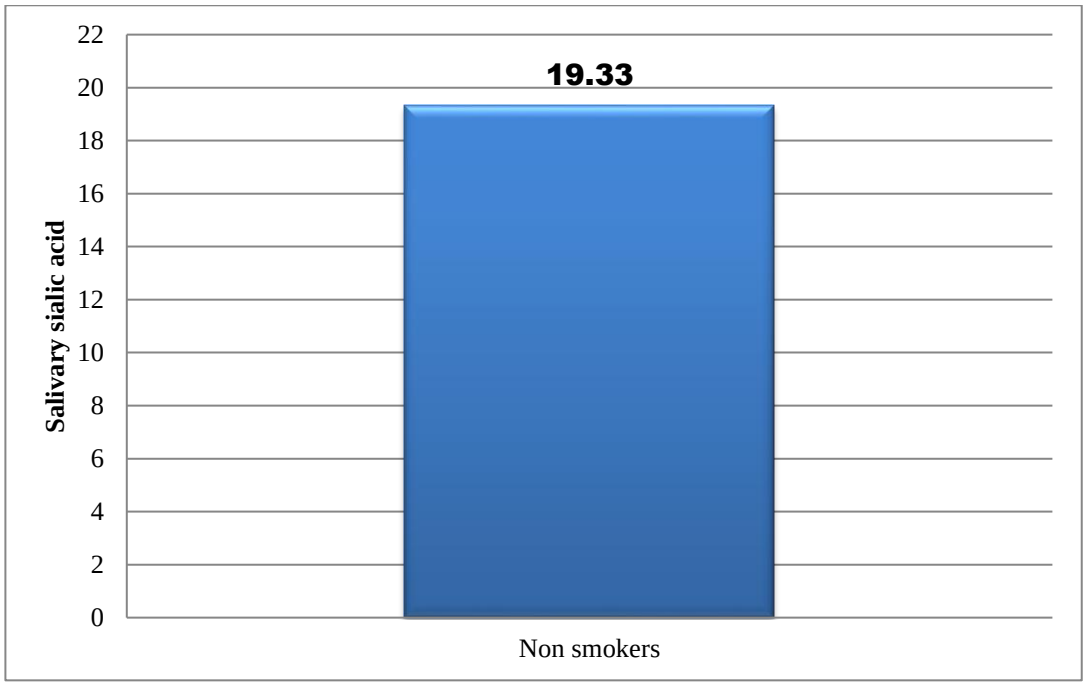

Figure 2: Mean salivary sialic acid level in nonsmokers were 19.33

Table 1 show the mean salivary sialic acid levels among smokers and non -smokers. Among smokers, the maximum value was 68 mg/100ml while it was only 25mg/100ml among the non-smoking group. Similarly, the minimum value in the smoker group was 29 mg/100ml as compared to 14 mg/100ml in non -smoker group.

The mean salivary sialic acid levels among two groups were compared and shown in Table 2 & Figure 3. An independent t-test was used to compare the mean salivary sialic acid between the groups. Difference in salivary sialic acid in both groups were found to be statistically significant ($p<0.001$) with smokers (46.73 ± 8.785 mg/100ml)

having higher mean salivary sialic acid levels than non-smokers (19.33 ± 3.262 mg/100ml).

Table 2: Comparison of mean salivary sialic acid levels between smokers and nonsmokers

	Groups	Mean±SD	Mean difference	t value	P value	95% CI of difference	
						Lower bound	Upper bound
Salivary sialic acid levels	Smokers	46.73 ± 8.785	27.400	16.015	<0.001**	30.825	23.975
	Non smokers	19.33 ± 3.262					
**Highly significant; SD= Standard deviation; CI= Confidence interval							

Table 2: The mean salivary sialic acid levels among smokers and non-smokers were compared using independent t-test. Difference in salivary sialic acid in both groups were found to be statistically significant (p<0.001) with smokers (46.73 ± 8.785 mg/100ml) having higher mean salivary sialic acid levels than non-smokers (19.33 ± 3.262 mg/100ml).

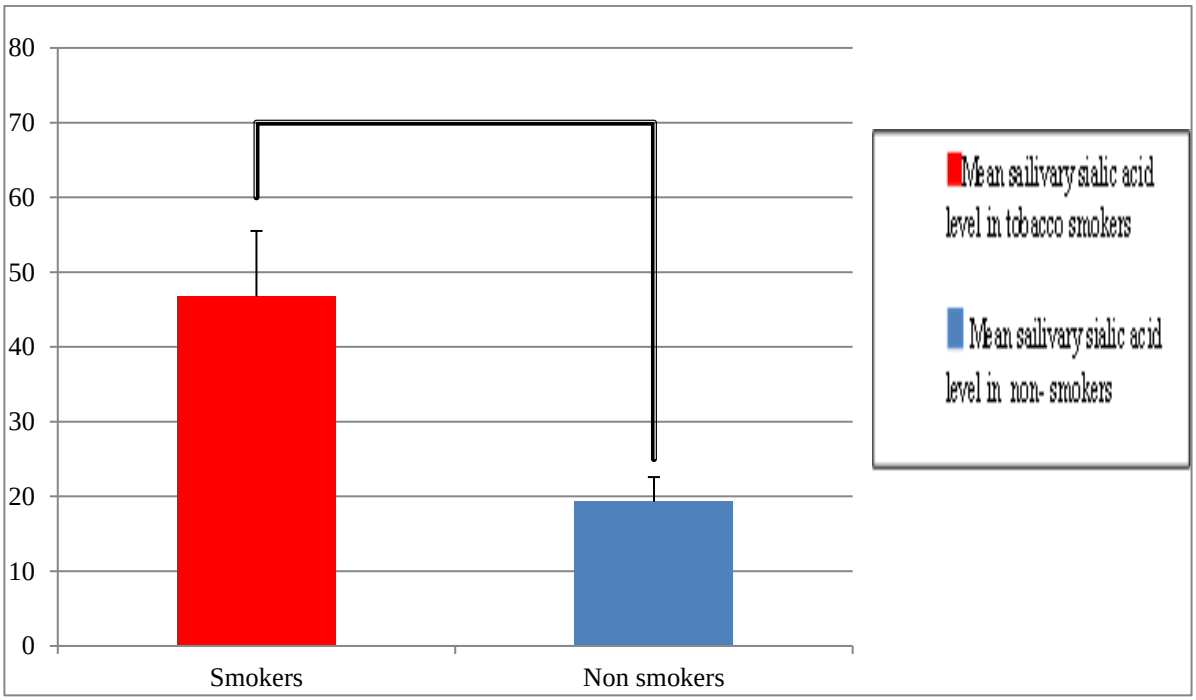

Figure 3: Comparison of mean total salivary sialic acid in smokers and nonsmokers. The Difference in salivary sialic acid in both groups were found to be statistically significant (p<0.001)

DISCUSSION:

Oral cancer is associated with multiple risk factors and high mortality rates, substantially contributes to the global cancer burden despite being highly preventable. Tobacco Smoking has a significant epidemiological correlation with oral cancer and plays an important role in its occurrence and development. Smokers are 7 to 10 times more prone to develop oral cancer and 3 times more likely to develop a second primary cancer than non-smokers.[5] Tobacco can cause epigenetic alteration of oral epithelial cells, inhibit multiple systemic immune functions of the host, and through its toxic metabolites cause oxidative stress on tissues to induce tissue damage.[6]

Consumption of tobacco can in turn lead to disturbance in the epithelial cells which might result in increased turnover rates and secretions from cells. Aberrant glycosylations are the universal feature of cancer and the levels of these glycoconjugates increases as the cancer advances. Glycoproteins like sialic acid are expressed on the cell surface. It is a protein-bound monosaccharide which occurs in combination with other mono-saccharides like galactose, mannose, glucosamine and fucose in saliva.[7] The term 'Sialic Acid', was derived from the Greek word “Sialos” meaning “Saliva” and was first introduced by Swedish biochemist, Gunnar Blix in 1952.[8] In periodontal lesions, oral potentially malignant disorders (OPMDs) and oral cancer (OC), the sialic acid level may increase due to high cell turnover and

shedding of cells which, in turn, may result in the release of glycoproteins like sialic acid into body fluids. Glycoproteins also form an important constituent of salivary mucins and hence due to the same mechanism, an increase in sialic acid level may also be observed in saliva [9].

Previous studies have identified that the serum sialic acid as a specific tumor marker. Due to the factors such as being inexpensive, non-invasive (basically an ultrafiltrate of blood), and simple to handle, a saliva-based study was done to determine the salivary sialic acid. [2] Alterations in the composition of salivary proteins can disturb the ecological balance, which triggers the development of disease processes[10]. Therefore, monitoring the salivary sialic acid levels could be a promising approach for the early diagnosis of OPMD and prognosis of OC. Our study was designed to check and compare the total sialic acid levels of saliva between smokers and non – smokers.

In our study, the mean salivary SA level of control group was 19.33 ± 3.262 mg/100ml which is in accordance with studies conducted by Kumar et al [11.] They assessed the salivary levels of SA in obese patients with/without chronic periodontitis (CP). Individuals with non -obesity and healthy gingiva were taken as control group and the mean value observed was 38.04 ± 3.28mg/dl. But in many other studies the mean salivary SA was found to be high. In a study conducted by Ancy R *et al.*, (2021)[12], the mean salivary sialic acid level among individuals with no tobacco exposure was 3.06 ± 6.00 mg/100ml. Oktay *et al.*[13] investigated the changes in SA levels in saliva of patients having both periodontitis and cardiovascular disease and the mean value of 5.05 ± 1.49mg/dl were detected in healthy controls. Taking the values of above studies in to consideration, we came to a conclusion that, there is no standardized value for salivary SA, it varies from individuals to individuals and groups to groups. But in all these studies, the salivary SA in healthy individuals were much less compared to that of the study population.

Angata & Varki[14] stated that sialic acid plays an important role in various physiological as well as pathological conditions. There was a negative corelation between the age and SA, which agrees with Kuyatt and Baum[15] who found a significant age-related decrease in the sialic acid level. Recent reports shows that sialic acid altered during pregnancy, menstruation and menopause due to changes in steroid hormones level.[16]

Mollashahi *et al.*,[17] compared SA levels in different forms of tobacco, significantly higher sialic acid levels were observed in the smokeless tobacco users (paan, maras powder) (39.57 ± 26.58 mg/L, 75.52 ± 6.86 μg/mL) and smokers (62.60 ± 3.91 μg/mL) than individuals with no tobacco exposure (38.39 ± 28.55mg/L, 51.60 ± 3.51 μg/mL). There was a significant upsurge in the Salivary sialic acid levels in smokers than non-smokers, they had included participants with >20 cigarette consumption per day and this could be the reason for very high values. The results are in accordance with our study, were we found the mean salivary sialic acid levels of tobacco smokers to be (46.73 ± 8.785 mg/100ml). In a similar study by Ancy R et al,[12] the mean salivary sialic acid levels among smokers were found to be 8.52 ± 9.21 mg/dL. Their study population included females also and years of tobacco exposure was considered whereas our study involved only male participants and exposure period was not taken in to consideration. Male subjects may show higher levels of sialic acid due to gender difference in tobacco associated habits.[18]

The comparison of mean of salivary sialic acid levels between smokers and non -smokers were done using independent samples t test. A mean difference of 27.400 were found between the groups. The difference in salivary sialic acid in both groups were found to be statistically significant ($p<0.001$) Sanjay et al[19] found that an increased level of salivary sialic acid in patients with OSCC and in oral potentially malignant disorders when compared to that of healthy individuals. An increased concentration of sialic acids in tumor cells, and its secretion by some of these cells, increases its concentration in the blood and saliva.[17]. Our study revealed that, higher variation in salivary sialic acid level among the individuals with the habit of smoking compared with non-smokers, suggesting that the carcinogenic chemicals present in the tobacco-related products cause cellular alterations.

Nicotine in tobacco causes oxidative stress in cells. The level of oxidative stress is directly proportional to its concentration, higher the nicotine content, higher the oxidative stress. [20] According to a report by Goswami et al.[21], there are significant associations between protein-bound sialic acid and oxidative stress Therefore, increased salivary sialic acid is justified in the individuals with tobacco smoking. Thus, higher levels of salivary sialic acid can be associated with diseases related to smoking tobacco such as periodontal lesions, premalignant conditions as

well as squamous cell carcinoma as SA is an indicator of oxidative stress and tissue changes.
Cardiovascular diseases are mainly caused by atherosclerosis and it has been documented that sialic acid has a role in the pathogenesis of atherosclerosis. Gopaul et al [22] reported that sialic acid levels are elevated in cardiovascular disease and also in people with tobacco habits. An increased level of salivary SA is reportedly a reflector of the risk of cardiovascular diseases in smokers.
There were few deficits of our study, one of which was not considering the duration of smoking and fewer number of participants (40 smokers). Salivary SA levels are also increased in many other conditions such as inflammatory conditions, cardiovascular diseases, and diabetes. Tobacco use cannot be merely impugned for the elevation of SA which constitute limitations of this study. Further studies on a larger sample can be used in future as a non-invasive method for forecasting oral cancers.

CONCLUSION

In conclusion, this study aimed to assess the role of salivary sialic acid (SA) as a screening tool for predicting potentially malignant and malignant lesions in smokers. The study included 80 subjects, divided into two groups: Group 1 consisted of healthy individuals with good oral hygiene and no smoking habit, while Group II consisted of tobacco smokers consuming at least 5 cigarettes per day.

Unstimulated saliva samples were collected from all participants using the spitting method and analyzed biochemically for total salivary sialic acid levels using ninhydrine reagent. The results revealed a statistically significant increase in salivary sialic acid levels among individuals with a smoking habit compared to the control group.

Based on these findings, it can be concluded that the elevated levels of salivary sialic acid in individuals who smoke tobacco may serve as an early indicator of biochemical changes associated with malignant cell transformation. This suggests that salivary sialic acid could potentially be utilized as a screening tool for the early detection of oral potentially malignant and malignant lesions in smokers.

Further research and larger-scale studies are warranted to validate these findings and explore the potential clinical applications of salivary sialic acid as a screening biomarker. Nonetheless, this study provides valuable insights into the potential use of salivary biomarkers for early detection and monitoring of oral lesions in high-risk populations, such as tobacco smokers.

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