Comparison in Total Sialic acid levels between control and cancer patients visiting Teaching hospital, as a basis for oral cancer diagnosis

Dahal S[1], Agrawal NK[1], Shrestha L[2].

[1] Lecturer, Department of Dentistry, Institute of Medicine, Tribhuvan University of Teaching Hospital, [2] PG student, Institute of Medicine, Maharajgunj

Correspondence: Dr. Samarika Dahal

Email: dr.samarika@gmail.com

Abstract

Introduction: Oral squamous cell carcinoma is the most common malignant neoplasm in the head and neck region and remains one of the major causes of worldwide deaths. Recognition and diagnosis of oral cancer at an early stage can reduce morbidity and mortality. Oral cancer screening in a large population can be done non-invasively by using sialic acid in saliva as a biological marker. The aim of this study was to estimate the total sialic acid (TSA) level in three categories of populations: normal healthy controls, tobacco users with a premalignant lesion like leukoplakia, and tobacco users who developed oral squamous cell carcinoma (OSCC).

Methods: A total of 45 subjects were involved in the study. Saliva was collected from three groups and total sialic acid was estimated. The data obtained was analyzed statistically using ANOVA and Tukey HSD test.

Results: A comparison of total salivary sialic acid levels in three groups revealed a significant rise in the level of TSA in OSCC group when compared to control group. The difference in the mean level of TSA of precancerous group was also significant when compared to control group.

Conclusion: The present study showed a significant rise in the mean level of total salivary sialic acid from control group (51.57 mg%) to precancerous group (74.64 mg%) to OSCC group (104.30 mg%), and suggests that it can be used as a reliable tool in oral cancer screening as the technique is non-invasive and inexpensive.

Keywords: oral cancer, salivary markers, total sialic acid.

Introduction

Oral squamous cell carcinoma is the most common malignant neoplasm in the head and neck region and remains one of the major causes of worldwide deaths due to the ability of cancer cells to metastasis to form secondaries and also due to their propensity to recur[1].

Recognition and diagnosis at an initial stage of cancer of the oral cavity is the most difficult task. It is usually asymptomatic for a long time and by the time the patient seeks advice, it has invaded deeply making prognosis poor. With the threat of high recurrence rate and secondary metastasis, the clinical decision for treatment plan and adjuvant therapy assumes importance. Hence a reliable diagnostic and prognostic marker need is felt. Unfortunately most of these markers are not always present in the patient with neoplasia and positive values are not tumor specific[2].

Biological markers are substances used to monitor cancer, predict the therapeutic response and prognosis of cancer, and sometimes even in the diagnosis of cancer. These markers, known as tumour markers, are naturally occurring or modified molecules that can be measured in serum, plasma, or other body fluids and their concentrations change in the presence of cancer[3]. Saliva is increasingly emerging

as a preferred, non-invasive tool for the diagnosis of cancer or potentially malignant conditions as many of the salivary constituents are altered in the presence of cancer. Studies of malignant cells have revealed alterations in the sialic acid content of the glycoproteins and the glycolipids of their cell surfaces[4].

The present study aimed to estimate total sialic acid (TSA) levels in saliva with a view to determine the feasibility of using a similar assay as a marker for malignant change. The data thus obtained may help in predicting the transition of oral mucosa through various stages of premalignancy to oral cancer in persons who use tobacco.

Methods

The present study was conducted in Tribhuvan University Teaching Hospital, Kathmandu, Nepal. The study group consisted of 45 patients selected in accordance with the inclusion and exclusion criteria and were sampled according to the below-mentioned three categories by convenience sampling technique. The categories were healthy control group, precancer group and oral squamous cell carcinoma (OSCC) group. The Ethical clearance was taken from Nepal Health Research Council committee prior to the study.

A complete case history was taken, and clinical findings of each patient were recorded, after a thorough examination of the oral cavity using mouth mirror and probe.

Subjects were explained about the study, and informed consent was taken. Then patients were asked to rinse the mouth to remove all food debris and were asked to expel saliva without force into a sterile labeled container until 2 ml of saliva was collected. The saliva was transferred to the lab in an ice carrier box for biochemical analysis.

For the assay of salivary sialic acid, thiobarbituric acid method was used in our study.

Saliva 0.1 ml and 0.8 ml of 0.06N H_2SO_4, were pipetted into 10 ×100 mm tubes and mixed well. Each tube was covered with a cap and heated in a boiling water bath for 10 minutes .The tubes were then cooled in cold tap water. A 5-g/dL solution of sodium wolfromate was added to each tube, mixed well, and centrifuged at 3000rpm.The clear salivary supernatant was placed into another tube marked sample. The sample, a standard and a blank (containing 0.1 ml 0.06 N H_2SO_4) were treated with 0.2mL of potassium periodate for 30 minutes in a water bath at 37^{o}C. The excess periodate was then reduced with 0.2ml of the sodium arsenite. One milliliter of the thiobarbituric acid reagent was then added and each tube was covered and heated in a boiling water bath for 8 minutes. The colored solutions were then cooled in cold tap water and shaken with 2.5 ml of the ethylene glycol monomethyl ether. The readings were read in spectrophotometer at 549 nm.

Results

The present study included 45 subjects, which were grouped as oral squamous cell carcinoma (15), Precancer (15) and control group (15). All these subjects were analyzed for salivary total sialic acid.

Table 1 shows mean value of three different groups along with standard deviation of the values. Control group had a mean value of salivary total sialic acid to be 51.57 mg%, and SD was 2.79. Mean value of salivary total sialic acid in Precancer was 74.64 mg%, and SD was 6.43. Similarly, mean value of salivary total sialic acid in Oral Squamous Cell Carcinoma (OSCC) was 104.30 mg%, and SD was 4.25.

Table 2 shows statistical analysis by ANOVA. ANOVA test was used to compare the mean values of sialic acid obtained in each group. The test showed that the difference in the mean values of sialic acid was statistically significant.

Table 3 shows statistical anlayisis by Tukey HSD test. OSCC and precancer group showed high significance in salivary total sialic acid when compared to control group and to each other. When Tukey HSD test was used, it was noted that there was high statistical difference between control group and precancer group at significance level of $p< 0.05$. Similarly, OSCC group also showed high level of significance when compared to control group at $p<0.05$. Even, OSCC group also showed high level of significance when compared to values of precancer group at a significance level of $p<0.05$.

Table1 Mean and Standard deviation values of salivary sialic acid in three groups

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Control	15	51.5787	2.79546	.72179	50.0306	53.1267	48.32	57.21
Pre cancer	15	74.6400	6.43763	1.66219	71.0750	78.2050	61.21	84.01
OSCC	15	104.3013	4.25065	1.09751	101.9474	106.6553	98.99	115.01
Total	45	76.8400	22.30932	3.32568	70.1375	83.5425	48.32	115.01

Table 2 ANOVA test

ANOVA					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	20956.497	2	10478.248	466.905	.000
Within Groups	942.561	42	22.442		
Total	21899.058	44			

Table 3: Tuskey HSD test for multiple comparisons between the groups

Multiple Comparisons						
Tukey HSD						
(I) Patient groups	**(J) Patient groups**	**Mean Difference (I-J)**	**Std. Error**	**Sig.**	**95% Confidence Interval**	
					Lower Bound	Upper Bound
Control	Pre cancer	-23.06133*	1.72981	.000	-27.2639	-18.8588
	OSCC	-52.72267*	1.72981	.000	-56.9252	-48.5201
Pre cancer	Control	23.06133*	1.72981	.000	18.8588	27.2639
	OSCC	-29.66133*	1.72981	.000	-33.8639	-25.4588
OSCC	Control	52.72267*	1.72981	.000	48.5201	56.9252
	Pre cancer	29.66133*	1.72981	.000	25.4588	33.8639

*. The mean difference is significant at the 0.05 level.

Discussion

During malignant transformation of cells, there may be either an upregualtion or down regulation 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. Such biochemical substances are referred to as tumor markers[5]. Neoplasms often have an increased concentration of TSA on the tumor cell surface, and sialoglycoproteins are shed or secreted by some of these cells, which increases the concentration in blood or saliva[6]. Furthermore, cancer cells have been related with an increased activity of sialytransferase, leading to a higher amount of TSA on the cell surface, thus increasing the plasma or salivary concentration[7,8]. TSA

concentrations have been reported to be related not only to diagnosis, but also to staging, prognosis, and detection of early recurrence[6].

Salivary sialic acid is an important structural component of glycoproteins, playing a part in enhancing bacterial agglutination. There is not much literature on the study of salivary sialic acid role in oral cancer like squamous cell carcinoma and premalignant lesion like leukoplakia. In our study, increased levels of mean salivary sialic acid values were found among oral cancer patients when compared to control group (51.57 mg %). Mean total salivary sialic acid found in OSCC patients was 104.30 mg %. The level of significance was ($p<0.001$). The findings of our study were similar to the study done by Koc L et al[9]. They found that lung cancer patients had a mean sialic acid level of 185 +/- 22.8 mg /dl and 6.2 +/- 3.72 mg/dl in control, and the difference between the two groups was significant ($p<0.0001$). A similar study was done by Shivashankara and Prabhu[11] to evaluate the status of sialic acid in oral squamous cell carcinoma. They concluded that OSCC patients had higher salivary levels of total proteins, free sialic acid, protein-bound sialic acid when compared to healthy controls.

In our study, the mean total salivary sialic acid in precancer group was found to be 74.64 mg %. When mean sialic acid level of precancer group was compared with control group, the level of significance was very high in our study, where $p<.001$ was noted as given in Table 2. The results are similar to the findings of study done by Dahal et al[10]. They had correlated levels of free sialic acid and protein bound sialic acid of saliva from healthy control group and patients with premalignant lesions. They had concluded that the difference between the mean level of sialic acid was significant in both the groups. The level of significance for protein bound sialic acid was $p<0.001$ and for free sialic acid was $p<0.01$.

Conclusion

Identification of reliable biological tumor markers or substance associated with neoplasia that can be used for the detection, staging and evaluation has been the goal of many investigators. The study aimed at evaluation of total salivary sialic acid in oral squamous cell carcinoma and precancer group. The significant high level of salivary sialic acid in this study suggests that these parameters could be used as a diagnostic and prognostic marker of oral malignancy, thus helping in early treatment and longer survival of the patients.

Conflict of interest: None declared.

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