

Clinical Evaluation of Sialic Acid in Head and Neck Squamous Cell Carcinoma Patients and Tobacco Chewers or Smokers with no Cancer

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ABSTRACT

Sialic acid plays a significant role in cancer due to increased sialylation and sialyltransferase activity. Patients with cancer have been reported to have significant elevations of serum Total Sialic Acid (TSA) and Lipid Bound Sialic Acid (LBSA) levels as compared to control persons. The present study was carried out to evaluate sialic acid levels in control, non-cancer smokers or tobacco chewers and Head and Neck Squamous cell carcinoma (HNSCC) cancer patients. Blood samples were obtained from the histopathologically diagnosed HNSCC patients, healthy controls and those persons who were either smokers or tobacco chewers with no oral cancer. Serum TSA and LBSA were measured spectrophotometrically. Serum TSA and LBSA levels were significantly elevated in HNSCC patients compared to healthy control with $P < 0.0001$. These levels were also significantly increased in individuals who were smokers or tobacco chewers with no cancer compared to healthy control ($P < 0.0001$). Our results found significant elevation of serum sialic acid levels in HNSCC patients and in smokers and tobacco chewers with no cancer as compared to control individuals. These findings suggested role of tobacco in biochemical changes during the malignant transformation. These results also indicate that these parameters can be utilized in diagnosis of the HNSCC.


Keywords: Sialic acid, Head and neck squamous cell carcinoma, spectrophotometer.

INTRODUCTION

In recent years, the field of glycobiology has emerged due to its relevant discoveries in biological as well as medical field with special reference to cancer. ¹ Glycans have gained importance due to its role in fundamental mechanisms of malignant transformation of the cells and various cellular mechanisms that includes cell signaling, tumor cell invasion and metastasis and immune modulation. ²

They are ubiquitously present in all biological systems on the cell surface as a complex array of sugar chains which are mainly conjugated to proteins and lipids. Sialic acid is one class of sugar which is located at the outermost end of glycan chains of all cell types. ³ Sialoglycans are involved in regulation of glycoprotein and glycolipid structure, stability, trafficking and they are mediators of wide variety of physiological and pathological processes. Sialic acid plays a significant role in cancer due to



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increased sialylation and sialyltransferase activity found in some cancer cell.⁴

Aberrant glycosylation in cell membrane occurs due to newly activated glycosyl transferases during tumorigenesis. Malignant transformation of cell shows the alteration in structural components and glycoproteins causing the increase in sialic acid levels on the cell surface. Glycoprotein and glycolipid bound sialic acid together makes total sialic acid (TSA) and glycolipid bound sialic acid is termed as lipid bound sialic acid (LBSA). These glycoconjugates enters the circulation due to increased production, secretion and/or shedding from malignant cells resulting in increased levels of sialic acid in blood.^{4,5,6,7} Various studies have shown the alteration in sialic acid levels in different cancers. Patients with oral cancer were reported to have significant elevations of serum levels of TSA and LBSA compared to control persons.^{8,9}

India harbors about 57.5% of global head and neck cancer cases, for both sexes.¹⁰ The generous use of tobacco in various forms is the most common risk factor for HNSCC, with oral cancer being the most common in Indian population. Various carcinogens present in tobacco may have a role in certain biochemical and molecular changes during malignant transformation. If these alterations are detected well before the appearance of any physical changes associated with cancer, the chances of prevention of the cancer can increase.

The current investigation was aimed at determining the biochemical alterations in serum TSA and LBSA levels in non cancer smoker or tobacco chewers and its comparison with oral cancer patients and healthy controls.

MATERIALS AND METHODS

Patients

The patients who were histologically diagnosed to have HNSCC, specifically oral cancer were included in the study. The study was approved by Ethical Committee of Smt. V. R. Desai Cancer Research centre, Rajkot. The informed consent was sought from the patients. The patient information was obtained through questionnaire that included demographic (age, gender, marital status, education,

occupation, geographic location) and clinical data (family history of cancer, tobacco and alcohol consumption, nutritional status). The information of signs and symptoms of their oral lesion, its duration were also taken.

Control

The persons having no history of oral cancer and are otherwise normal were selected for case-control study. They gave informed consent for the study. Sociodemographic information was obtained through questionnaire. The other group of persons were selected who were tobacco chewers or smokers but do not have show any signs of cancer.

Sample

The venous blood samples were collected from the patients as well as both controls in plain vials. The serum was separated and stored at -80°C until use.

METHOD

Assays

Estimation of total sialic acid

Total sialic acid content was estimated from serum sample using a periodate–thiobarbituric acid method. 100 µl serum was hydrolyzed with equal amount of 1N H₂SO₄, at 80°C for 1 hour. This will release bound sialic acid in serum. The proteins were then precipitated with 1.0 ml of 10% trichloroacetic acid. 0.025N periodic acid was mixed with the supernatant and incubated at 37°C for 30 min. By addition of 2% sodium arsenite the reaction was terminated. After adding 6% thiobarbituric acid, the mixture was kept in boiling water bath for 7.5 min. Adding dimethyl sulphoxide increases the stability of the chromophore. The absorbance was read using spectrophotometer against blank at 549 and 532 nm to overcome any interference from 2-deoxy-D-ribose.

Estimation of Lipid bound sialic acid

Estimation of lipid bound sialic acid from serum was estimated using the method suggested by Katopodis *et al.*¹¹ 50µl serum extraction was done with chloroform–methanol (2:1 v/v) at 4°C. The extract was separated with 0.5 ml of distilled water. Phosphotungstic acid was used to precipitate the aqueous layer. The precipitates were then

resuspended in 1 ml of distilled water. LBSA in suspension was determined by resorcinol reagent.

$TSA/LBSA \text{ (mg/dl)} = \frac{\text{O.D. of sample}}{\text{O.D. of std. X Conc. of std. / Volume of test}} \times 100$

The amount of serum TSA and LBSA were calculated using standard curves which was obtained by various known concentration of N-acetyl Neuraminic Acid

The following formula was used for calculation of TSA and LBSA.

Table 1: Mean levels of TSA and LBSA in study groups

Study groups	LBSA mg/dl Mean± SD	TSA mg/dl Mean± SD
Healthy Control (23)	23.3±3.58	52.15±4.40
Non-cancer Tobacco chewers/smokers (21)	31.29±2.20	61.54±2.77
HNSCC patients (21)	43.23±3.88	80.86±5.25

RESULTS

We included 23 healthy controls, 21 tobacco chewers or smokers without any cancer history and 21 oral cancer patients. Table I shows the mean levels of TSA and LBSA among the study groups. The mean concentrations of TSA and LBSA were increased in oral cancer patients in comparison with controls as well as non-cancer tobacco chewers/smokers. The mean levels of serum TSA and LBSA were compared statistically by independent (unpaired) student t test and One Way ANOVA test using Graph Pad prism 7.03 software. The difference between healthy controls and HNSCC patients, HNSCC and non-cancer tobacco chewers/smokers and Healthy controls and non-cancer tobacco chewers/smokers were statistically significant by individual t test with p value <0.0001 for each pair

Table 2: Comparison of study groups by unpaired t test

Test	Group	t-value	p-value
LBSA	Healthy control	8.802	P<0.0001
	Non Cancer Tobacco chewers/smokers		
	Non Cancer Tobacco chewers/smokers	12.26	
	HNSCC patients		
TSA	Healthy control	17.72	P<0.0001
	HNSCC patients		
	Healthy control	8.372	
	Non Cancer Tobacco chewers/smokers		
	Non Cancer Tobacco chewers/smokers	14.91	
	HNSCC patients		
	Healthy control	19.72	
	HNSCC patients		

Table 3: Comparison of study groups by One Way ANOVA test

Test	Study Group	F –value	p-value
LBSA	Healthy control	200.1	P<0.0001
	Non Cancer Tobacco chewers/smokers		
	HNSCC patients		
TSA	Healthy control	255.2	P<0.0001
	Non Cancer Tobacco chewers/smokers		
	HNSCC patients		

(Table II). The One Way ANOVA was carried out to compare the difference in the levels of TSA and LBSA among the study groups (Table III and Figure I, II) and the significant difference was found between the various study groups (p value <0.0001).

DISCUSSION

The carbohydrate molecules in glycolipids and glycoproteins are often changed during malignant transformation. Altered glycosylation of glycoconjugates plays important role in malignant

transformation. Spontaneous discharge of abnormal sialic acid rich glycoproteins and glycolipids is responsible for elevated levels of sialic acid in cancer patients. They have significant diagnostic and prognostic value.^{1, 4, 12} The aberrant structure of terminal sialic acid and its up regulation is considered as a hallmark of cancer.^{13, 14, 15}

Comparison of alterations in sialic acid levels between oral precancer and oral cancer have been done in many studies. Increased levels of LSA and protein bound hexose in oral precancer

Comparison of serum LBSA among study group

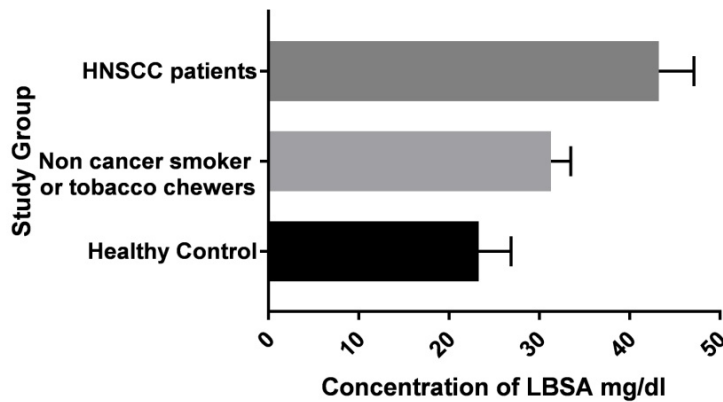


Fig. 1: Comparison of serum Lipid Bound Sialic Acid (LBSA) levels between Healthy control, Non cancer smoker or tobacco chewers and HNSCC patients

Comparison of serum TSA among study group

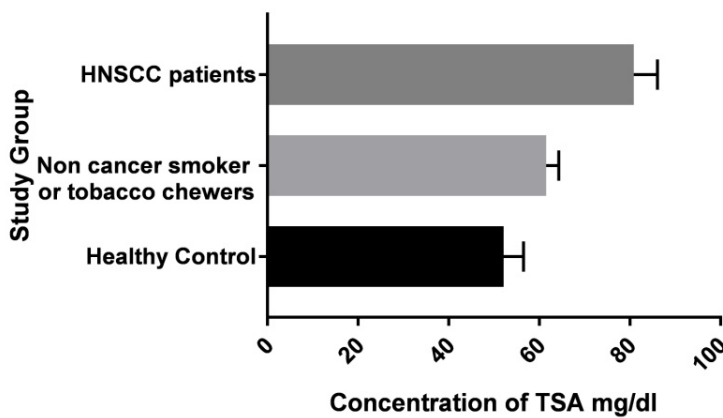


Fig. 2: Comparison of serum total sialic acid (TSA) levels between healthy control, Non cancer smoker or tobacco chewers and HNSCC Patients

and high levels of TSA and LBSA were found in oral cancer,^{16,17,18} while the significant increase in TSA and LBSA levels in both precancer and oral cancer were reported compared to healthy controls.⁹ A study showed the increase in serum TSA in oral precancer and oral cancer compared to control individuals.¹⁹

Significantly increased levels of serum TSA and LBSA were reported in oral precancer patients compared to control group in one study.^{20, 21} The study also found the statistically significant difference in serum TSA and LBSA levels between untreated oral cancer and precancer group. This indicates that serum TSA and LBSA can be used to distinguish between oral cancer and precancer.

Biochemical changes in glycoproteins begin at an early stage of tumorigenesis. Elevated TSA and LSA values are indicative of a premalignant change. Through constant monitoring of these parameters malignancy can be detected at an early stage.²² This surely signifies that cell surface glycoconjugates are altered during early malignant transformation.²³

Measurement of serum sialic acid in cancer patients can serve as a diagnostic and prognostic marker in malignant diseases. While comparing the serum TSA and LBSA with other tumor markers like ferritin, carcinoembryonic antigen and neuron specific enolase, it was found that TSA, LBSA and TSA/total proteins are better in monitoring the disease extent and anticancer therapy. Sialic acid can be a useful biomarker for monitoring of response to chemotherapy of those cancers where tumor markers are unavailable.²⁴ Sialic acids promote tumorigenesis and enhance tumor progression at multiple levels by facilitating escape from apoptosis, formation of metastasis, and resistance to therapy.

Most of the previous study compared the serum TSA and LBSA between oral cancer, pre-cancer and healthy control.^{25, 26} The present study included the oral cancer, healthy control and non-cancer smokers or tobacco chewers who have no sign of any cancer in oral cavity. This study reported the significant elevation in serum TSA and LBSA levels in HNSCC patients compared to healthy control. This study is consistent with earlier studies.^{17, 26, 27,28,29} Further sialic acid levels were

significantly increased in those persons who are either smokers or chewers of tobacco, but do not have cancer yet, compared to healthy individuals. The rise in glycoprotein components in these individuals surely indicates that tobacco usage is responsible for early biochemical changes in sialic acid and altered surface carbohydrate composition resulting into aberrant cell-cell recognition leading to malignant transformation of the cell. The high levels of serum TSA and LBSA in individuals who are habituated to tobacco chewing or smoking without any apparent lesions in mouth can be considered as sign for future neoplastic changes. This group should be closely monitored over a period of time to check any malignant transformation. The serum TSA and LBSA analysis can be done in these individuals if they have refrained from the use of tobacco to ensure the effect of tobacco on serum glycoconjugates.

Limitations of this study are smaller sample size and patients after the treatment were not included in our study. Thus, larger sample size including pre and post treatment patients for close monitoring of sialic acid should be used for considering the usefulness of sialic acid a potential biomarker in oral cancer.

CONCLUSION

The present study concluded that evaluation of serum TSA and LBSA can differentiate between oral cancer and healthy individual. The study also indicated that tobacco chewing and smoking can lead to abnormal glycosylation in the cell membrane that is responsible for elevated levels of sialic acid. Serum TSA and LBSA can be considered as useful biomarkers for assessing the malignant transformation, its spread and invasiveness. Further large scale study may be carried out to show the association of these factors with the extent of malignancy, metastasis and prognosis of the disease.

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