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Original Research

Estimation Of Sialic Acid Level In Oral Squamous Cell Carcinoma And Potential Malignent Disorder

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ABSTRACT

Introduction: Tumor markers are substances, which change quantitatively in serum during tumor development. The sialic acid is a member of acylated derivatives of neuraminic acid family (2-keto-5-amino-3,5-dideoxy-n-nonulosonic acid), which are widely distributed in nature. The objective of this study was to evaluate and compare the serum sialic acid level in oral squamous cell carcinoma and potential malignant disorder and to assess their role as a diagnostic marker. **Method:** Serum sialic acid estimation was done by resorcinol method modified by Plucinsky et al. Colored complex intensity was read at 580nm in 50 patients with oral squamous cell carcinoma, 60 patients with potential malignant disorder (30 Oral Submucous Fibrosis and 30 Leukoplakia), and 50 age & sex matched disease-free controls. The results were tabulated and analyzed statistically. **Results:** Significant increase in serum sialic acid levels were observed in oral squamous cell carcinoma patients as compared to those with potential malignant disorder and controls. In the present study, we found significant increase in serum sialic acid levels in oral squamous cell carcinoma patients as compared to leukoplakia and those with Oral Submucous Fibrosis. Also significant increase in serum sialic acid levels in Oral Submucous Fibrosis patients as compared to Leukoplakia. **Conclusion:** From these results, it seems that evaluation of serum sialic acid levels may be useful as one of the battery of tests in assessment of oral carcinoma and potential malignant disorder.

Introduction

Cancer is now one of the five main causes of death, in all societies.[1]It is not only responsible for significant mortality but also causes physical, mental, financial and sociologic mutilation to a person. Each year in India an estimated total of 700,000 - 900,000 new cancer cases are diagnosed.[2]The term 'Oral Cancer' encompasses all malignancies that originate in the oral tissues. Over 90% of oral cancer lesions are squamous cell carcinomas.[3] It represents the third most common form of malignancy in the developing countries while in developed countries it is the eighth most common.[4]According to World health organization report, in India out of ten cases of cancer, four are oral cancers and it is the sixth most common cause of death.[5]

Majority of Oral carcinomas are preceded by precancers. This concept of a two-step process of cancer development in the oral mucosa, that is., the initial presence of a precursor (pre-malignant, pre-cancerous) lesion subsequently developing into cancer, is well-established.[6]Oral Precancer is defined as "Intermediate clinical state with increased cancer risk". Pre-cancers can be classified into two broad

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groups:-Precancerous lesions and Precancerous condition**.** Precancerous lesion is defined as "morphologically altered tissue in which oral cancer is more likely to occur than in its apparently normal counterpart", example- leukoplakia, erythroplakia, actinic cheilitis etc. Precancerous condition is defined as a "generalized state associated with significantly increased risk of cancer", e.g. Oral Submucous Fibrosis (OSMF), Plummer Vinson syndrome, Lichen Planus, Xeroderma Pigmentosum, Dyskeratosis Congenita, etc.[7]

Various changes occur in the body in presence of any type of cancer because the tumor cells produce certain types of chemical mediators into blood like oncofetal proteins (alpha-fetoprotein, carcinoembryonic antigen), B-protein, enzymes (example- Lactate Dehydrogenase), B_2M and sialic acid. In the presence of any kind of tumor, the levels of these substances will change. These substances are called tumor marker.

In suspicious lesions or precancer, the use of tumor marker will help to define rate of transformation into cancer, in treatment planning and prognosis of disease. Currently, tumor markers have been introduced for early detection of malignancy. These markers have wide range of potential applications; for screening purpose, diagnosis, prognosis, and monitoring the response to treatment. Identification of an ideal tumor marker can offer an exciting opportunity for early detection of such lesions. Therefore, study of tumor markers has become a focal point of research in oncology.

In the carcinomas of oral cavity, various serum markers have been studied: these include oncofetal proteins (alpha-fetoprotein, CEA), B-protein and enzymes (Lactosedehydrogenase). In addition to the markers already studied, several tumor makers with clinical promise need further evaluation.[8] such tumor marker is sialic acid.

The sialic acid is a member of acylated derivatives of neuraminic acid family (2-keto-5-amino-3,5-dideoxy-n-nonulosonic acid), which are widely distributed in nature. These are generally components of the oligosaccharide units in mucins, glycoproteins, gangliosides, milk oligosaccharides, and certain microbial polymers.[9]

Elevation of sialic acid is seen in different diseases like cirrhosis of liver, nephrotic syndrome, rheumatoid arthritis, γ myeloma, β Myeloma, macroglobulinaemia, leukaemia, chronic tuberculosis infection, and amyloidosis[10] and certain type of cancer like breast cancer, colon cancer, lung cancer, leukemia, lymphoma, Hodgkin's disease, and malignant melanoma.[11]

Studies have shown that changes of serum sialic acid levels in cancer patients correlate well with reduction in tumor mass, its recurrence, and metastasis and has been considered as a valuable tumor marker in monitoring the clinical status of the carcinoma patient.

The present study attempts to correlate the serum levels of sialic acid in oral precancer and oral squamous cell carcinoma and to evaluate the role of the same as a biochemical parameter for screening purposes.

Material and method:

Patients and controls: Serum was obtained from 50 untreated, clinically evident oral cancer patients, proved by clinical and histopathological evidence: 60 patients with potential malignant disorder (30 leukoplakia and 30 OSMF) but no evidence of invasion: and 50 age and sex matched controls.

Subjects who had taken treatment for any Precancerous lesions/conditions or Oral cancer and

Subjects suffering from any systemic diseases like diabetes, cardiac diseases, renal diseases, liver diseases and other malignancies, subjects who are taking antioxidants/multivitamin preparations were excluded from the study.

Patients were further divided into three groups- Group A: 50 healthy individuals, 38 males and 12 females. Group B: 60 potential malignant disorder, 51 males and nine females. Group B further divided into two groups, Group Ba: 30 patients with Luekoplakia and Group Bb: 30 patients with OSMF. Group C: 50 oral carcinoma patients, 40 males and ten females.

Methods of sample collection

Ethical clearance was obtained from ethical clearance committee of the institute and the hospital. All the patients fulfilling the above criteria were informed about the study being conducted and only those who agreed were enrolled in the study. All the enrolled subjects were then interviewed using clinical examination tools and recorded in a case history Performa.

After obtaining consent from the patient, five millilitre of venous blood samples were collected by venipuncture of the median cubital vein under aseptic precautions. The blood samples were allowed to clot for 1 hour and then centrifuged at 3000 rotations per minute(rpm) for ten minutes to provide serum. This serum was preserved in a frozen state at -20 degree until the analysis.

Sialic acid

Principle: sialic acid estimation was done by resorcinol method modify by Plucinsky et al.[12] colored complex whose intensity is read at 580nm.

Total sialic acid values were determined as follows:

20 microlitre (µl) of sera and 980 µl of water were placed into 13 X 100 mm. To each assay tube, TSA was added in one ml of resorcinol reagent prepared daily as follows: ten ml of two% (weight/volume) stock resorcinol in water, 9.75 ml of water, 0.25 ml of 0.1 M Copper sulphate, brought to a final volume of 100 ml with concentrated Hydrochloric acid. Each tube was capped, vortexed, and placed in a 100°C boiling water bath for exactly 15 minutes followed by 10 minutes in an ice bath.

Two milliliters of butyl acetate/n-butanol (85: 15, v/v) was added to each tube, and the tubes were vortexed and centrifuged at room temperature for ten minutes at 3000 rpm. The extracted chromophore was read on a systronics double beam spectrophotometer 2203 at 580 nm (against a standard curve of 0-110 µ***g*** of N-acetylneuraminic acid (NANA) (CHEMPURE Chemical Co., Mumbai ,India**),** prepared by treating standard NANA tubes as TSA assay tubes.

The calculation for sialic acid is:

$$\text{Concentration of unknown}\left(\frac{\text{mg NANA}}{\text{100 ml. serum}}\right) = \frac{\text{absorbance of unknown}}{\text{absorbance of standard}} X\ 80\ X\ f$$

Calculations

Where ***f*** is the correction factor which is necessary to apply because of the interference caused principally by the hexoses. This correction is minor when our extraction solvent mixture is used, ***f***, under the conditions of our procedure being 0.95.

Thus the observations obtained by these methods were tabulated and statistically analysed By **STUDENT 't' TEST and Tukey's HSD test.**

Result:

The mean serum Sialic acid levels in Group A was 86.45 ± 7.28 mg/dl. It was significantly elevated when compared to Group C in which it was 55.91 ± 4.83 mg/dl. It is statistically significant (p value <0.0001). {Table I, Graph I }

PARAMETERS	GROUP	n	Mean ± SD	p-Value	Result
SIALIC ACID (mg/dl)	Group A	50	86.46 ± 7.29	<0.0001	Significant
	Group B	60	56.64 ± 9.48		
	Group C	50	55.91 ± 4.83		

Table I- Sialic acid levels in study and controls groups

PARAMETERS	GROUP	n	Mean ± SD	p-Value	Result
SIALIC ACID (mg/dl)	Group C	50	55.91 ± 4.83	<0.0001	Significant
	Group A	50	86.46 ± 7.29		
	Leukoplakia	30	64.02 ± 6.98		

TABLE II: Sialic acid levels in group a, group c and Leukoplakia

The mean serum Sialic acid levels in Group B (56.64 ± 9.48 mg/dl) was not significantly increased when compared to Group C (55.91 ± 4.83 mg/dl). It is not statistically significant (p value <0.8).{Table II , Graph II}

The mean serum Sialic acid levels in Group A (86.45 ± 7.28 mg/dl) was significantly increased when compared to Group B (56.64 ± 9.48 mg/dl). It is statistically significant (p value <0.0001). {Table II, Graph II }

The mean serum Sialic acid levels in group A was 86.45 ± 7.28 mg/dl which was significantly increased when compared to Group Ba in which it was 64.01 ± 6.97 mg/dl. It is statistically significant (p value <0.0001). {Table II, Graph II }

The mean serum Sialic acid levels in Group A group was 86.45 ± 7.28 mg/dl which was significantly increased when compared to Group Bb in which it was 49.26 ± 4.66 mg/dl. It is statistically significant (p value <0.0001). {Table III)

PARAMETERS	GROUP	N	Mean ± SD	p-Value	Result
SIALIC ACID (mg/dl)	Group C	50	55.91 ± 4.83	<0.0001	Significant
	Group A	50	86.46 ± 7.29		
	OSMF	30	49.27 ± 4.67		

TABLE III: Sialic acid levels in group a, group c and osmf

PARAMETERS	GROUP	Mean ± SD	n	p-Value	Result
SIALIC ACID (mg/dl)	OSMF	49.27 ± 4.67	30	<0.0001	Significant
	LEUKOPLAKIA	64.02 ± 6.98	30		

TABLE IV: Sialic acid levels in osmf and leukoplakia

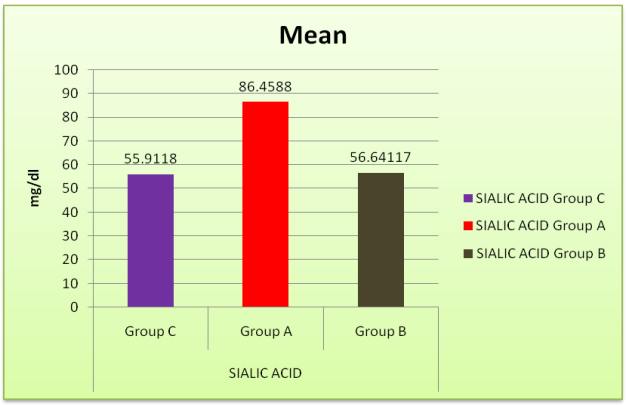
The mean serum Sialic acid levels in Group Ba (64.01 ± 6.97 mg/dl) was significantly increased when compared to Group C (55.91 ± 4.83 mg/dl). It is statistically significant (p value <0.0001). {Table II , Graph II}

The mean serum Sialic acid levels in Group Bb (49.26 ± 4.66 mg/dl) was decreased as compared to Group C (55.91 ± 4.83 mg/dl) but it was not statistically significant (p value <0.5). {Table III, Graph III }

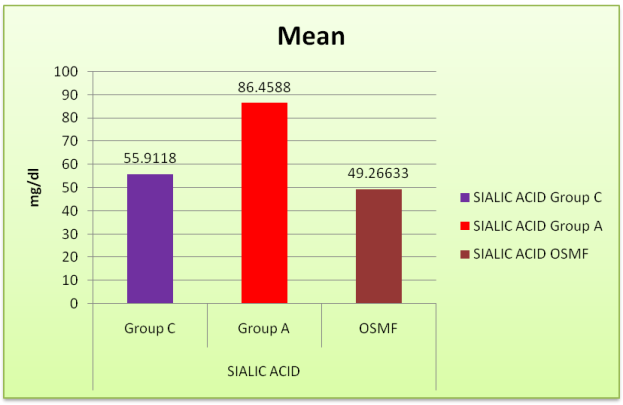
The mean serum Sialic acid levels in Group Ba (64.01 ± 6.97 mg/dl) was significantly increased as compared to Group Bb (49.26 ± 4.66 mg/dl). It is statistically significant (p value <0.0001). {TABLE 4 , Graph4 }

Discussion:

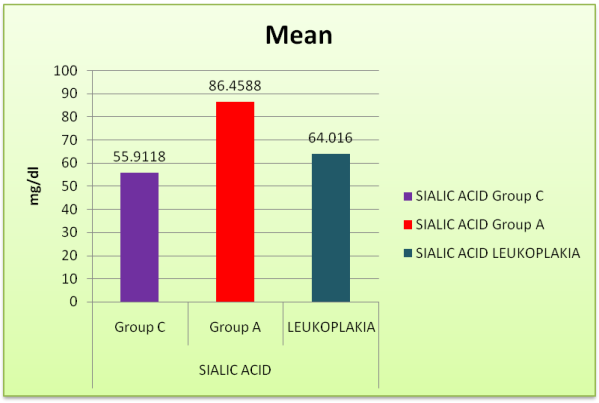
Tumor markers are substances that are produced either by the tumor itself or by the body in response to the presence of cancer or certain benign (noncancerous) conditions that can aid in the diagnosis of cancer and in the assessment of tumor burden. Tumor markers can often be detected in higher than normal amounts in the



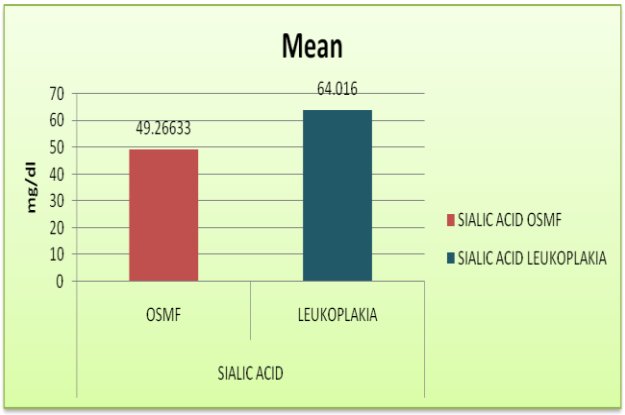
GRAPH I: Mean value of sialic acid in study and controls groups



GRAPH III: Mean value of sialic acid in group a, group c and osmf



GRAPH II: Mean value of sialic acid in group a, group c and leukoplakia



GRAPH IV: Mean value of sialic acid in osmf and leukoplakia

blood, urine, or body tissues of patients with certain types of cancer. Estimation of tumor marker level can be useful when used along with radiographs or other tests in the detection and diagnosis of certain types of cancer.[10] In the present study, we estimated the levels of sialic acid in oral cancer and precancer patients and compared it with the levels of normal healthy, deleterious habit free individuals.

In the present study the level of sialic acid in oral cancer patients were increased when compared with controls which is in accordance with few studies, reported in the literature.[9, 13, 14, 15-18] The level of sialic acid in different stages of oral cancer were marginally increased but it was not statistically significant. Sialic levels were not significantly increased in Oral Precancer group when compared to controls. Similar findings were also found in one study[14] but it differed with a certain studies which were conducted in Vadodara (Gujarat), and Mumbai (Maharashtra) where significant increase in sialic acid in precancer group were observed.[17,18]

Sialic acid plays an important role in cell to cell recognition, invasiveness, adhesiveness, and immunogenecity. The major structural component of cell surface is glycoproteins. This glycoprotein undergoes alteration on neoplastic transformation of cell. Such alteration leads to an elevation in the level of sialic acid on the cell surface. These glycoconjugates are released into the circulation through increased turnover, secretion, and shedding from malignant cells leading to elevation of sialic acid

levels in blood.[18] Another hypothesis is that various cell-membrane changes, *e.g.,* modified glycoproteins and glycolipids, changes in surface enzymes, and other phenotypic changes, have been associated with malignant transformation of a cell. The biochemical changes in cell-surface glycoproteins and glycolipids that take place during malignant transformation.[14] Elevations found in sialic acid levels in cancer patients might also be due to selective increase in existing specific sialylated sequence or a tumor associated *de novo* synthesis of specific sialylated sequence.[19]

In the present study the level of sialic acid in leukoplakia patients were significantly increased when compared with controls which is comparable to a study reported in the literature.[70] The sialic acid levels were slightly decreased in OSMF patients when compared to controls which was not statistically significant.

In present study the level of sialic acid in oral cancer patients were significantly increased when compared with to leukoplakia which is comparable to a study reported in the literature.[17] Sialic acid levels were significantly increased in oral cancer patients when compared with OSMF patients which was comparable to a study reported in the literature. [17]

In the present study the level of sialic acid in leukoplakia patients were significantly increased when compared with OSMF patients which differs from a study reported in literature, which reports no significant elevation in the sialic acid levels of OSMF and leukoplakia patients.[17]

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 present study provided valuable preliminary data in early detection of oral cancer and oral precancer and also will help in early treatment planning and hence for longer survival of the patient. The significant high level of total sialic acid in this study suggests that these parameters could be used as a diagnostic marker along with other investigations. On the basis of present study we concluded that the levels of sialic acid were increased in oral cancer and precancer, however further comprehensive studies involving large sample size are required to confirm the clinical usefulness of serum sialic acid as a biochemical parameter.

References:

1. Shah JP, Johnson NW, Batsakis JG. Oral Cancer. 2003. Martin Duniz. London, UK.

2. Gupta PC, Ray CS. Tobacco related cancer - its impaction the health economy. Health Administrator. ;17(1): 85-92.

3. S.EL-MOFTY. Early detection of oral cancer. *Egypt. J. Oral Maxillofac. Surg. 2010;1: 25- 31.*

4. Doshi N, Shah S, Patel K, Jhabuawala M. Histological grading of oral cancer: a comparison of different systems and their relation to lymph node metastasis. National journal of community medicine .2011 ;2(1): 136-142.

5. Basnaker M, Kumar G.S. P53 Gene Expression in oral smears of tobacco chewers. Int J Dent Clinics. 2010 Jan-March; 2(1):3-17.

6. Antony G, Sreenevasan BS et al. Potentially malignant disorders of oral cavity. Oral & Maxillofacial Pathology Journal. 2011; 2(1):95-100.

7. R Rajendran. Benign and Malignant Tumors of the oral cavity in: R Rajendran, B Sivapathasundhram (eds.) Shafer's text book of Oral Pathology 5th edi. New Delhi, India: Elsevier publication 2005: 122,123,136.

8. Reddy SB, Reddy MB, Shyam NDVN. Tumour Markers in Oral Neoplasia. IJDA.2010; 2(1):103-14.

9. Raval GN, Patel DD, Parekh LJ, Patel JB, Shah MH, Patel PS. Evaluation of serum sialic acid, sialyltransferase and sialoproteins in oral cavity cancer. oral diseases. 2003;9:119-28.

10. Carter A and Martin NH. Serum sialic acid levels in health and disease. J. clin. Path. 1962; 15:69-72.

11. Dnistrian AM and Schwartz MK. Plasma Lipid-bound Sialic Acid and Carcinoembryonic Antigen in Cancer Patients. CLIN. CHEM. 1981;27(10): 1737-39.

12. Plucinsky MC, Riley WM, Prorok JJ, AND Alhadeff JA. Total and Lipid-Associated Serum Sialic Acid levels in Cancer Patients with Different Primary Sites and Differing Degrees of Metastatic Involvement. Cancer. 1986;58:2680-85.

13. Kadam CY, Katkam RV, Suryakar AN, Kumbar KM, Kadam DP. Biochemical markers in oral cancer. Biomedical Research 2011; 22 (1): 76-80.

14. Baxi BR, Patel PS, Adhvaryu SG, and Dayal PK. Usefulness of Serum Glycoconjugates in Precancerous and Cancerous Diseases of the Oral Cavity. Cancer. 1991; 67:135-40.

15. Sanjay PR, Kaveri Hallikeri, Shivashankara AR. Evaluation of salivary sialic acid, total protein, and total sugar in oral cancer: A preliminary report. Indian J Dent Res. 2008; 19(4): 288-91.

16. Taneja N, Bathi RJ, Parveen S, Bhat K. Serum Glycoproteins as Prognosticator in Oral Cancer Patients-A Follow up Study. Int J Oral-Med Sci. 2009;8(2): 74-82.

17. Manjiri Joshi, Ranjitkumar Patil. Estimation and comparative study of serum total sialic acid levels as tumor markers in oral cancer and precancer. Journal of Cancer Research and Therapeutics. 2010; 6(3): 263-66.

18. Taqi SA. Clinical evaluation of total and lipid bound sialic acid levels in oral precancer and oral cancer. Indian Journal of Medical and Paediatric Oncology. 2012;33(1): 36-41.

19. Raval G.N., Parekh L.J., Patel D.D., Jha F.P., Sainger R.N., Patel P.S. Clinical usefulness of alterations in sialic acid, sialyltransferase and sialoproteins in breast cancer. Indian Journal of Clinical Biochemistry. 2004; 19 (2): 60-71.