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Clinical Relevance of Serum Total Sialic Acid In Oral Leukoplakia and Oral Squamous Cell Carcinoma - A Randomized Study.

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ABSTRACT

Background: *An important molecular change that accompanies malignant transformation is altered glycosylation of glycoconjugates. Total Sialic acid is a glycoconjugate, which forms the terminal epitope of carbohydrates. It is thought to be important in determining the surface properties of cells. It has been implicated in cellular invasiveness, adhesiveness and immunogenicity. In this background, this study was aimed to investigate the clinical relevance and direct correlation of circulatory levels of serum Total Sialic acid for early diagnosis and management of patients with potentially malignant disorder and cancer of oral cavity.*

Method: *Serum Total Sialic acid levels (TSA) were evaluated from 25 each untreated patients of Oral Leukoplakia(OLP), Oral Squamous Cell Carcinoma (OSCC) and 25 age and sex matched healthy controls (HC) using the simplified quick method by G Sydow and measured Spectrophotometrically at 525nm.*

Results: *Statistical analysis showed Significant elevated Serum TSA (P<0.001) in untreated OSCC patients as compared to untreated OLP patients and HC. Analysis of variants (ANOVA) documented significant difference in the mean TSA levels in the 3 groups; OSCC, OLP and HC (P<0.001). Bonferroni's test acknowledged significant difference between HC & OLP group; HC group & OSCC group; and OLP group & OSCC group with respect to the mean TSA levels (P<0.001).*

Conclusion: *The data revealed an ascending order of serum TSA levels from HC to OLP to OSCC, suggesting the potential utility of this parameter in initial diagnosis of OLP &OSCC.*

KEYWORDS : Glycoprotein, Sialic Acid, Potentially malignant disorders, Oral cancer, Spectrophotometry.

Introduction:

Globally, Oral Cancer (OC) is the sixth cause of Cancer related morbidity and mortality.[1] This serious health problem accounts for extensive disfigurement, loss of function, behavioral changes, financial and social adversity.[2] Despite improvement in imaging and therapy, the survival rates for these patients have not changed considerably for many years.[3]

OC is usually preceded in many cases by potentially malignant disorders (PMD) like OLP, which is attributed to different types of tobacco usage.[4] Clinical, epidemiological and laboratory studies suggest direct etiological relationship with prolonged use of tobacco with OC.[1] Therefore the early diagnosis of OSCC would improve survival and quality of life, avoiding the mutilation that physician often have to make to save patients lives. The search for biological marker that could predict the changes in the pre-malignant Lesion would be useful in detecting patients with high risk for malignancy.[4]

Glycoproteins and gylcolipids forms the major constituents of cells. They have been implicated in cellular invasiveness, adhesiveness and immunogenicity[4]. TSA consists of Glycoproteins and Gylcolipids bound Sialic acids. Sialic acids (N-acetyl neuraminic acid, NANA) frequently occupy the terminal, non-reducing position on membrane glycoproteins.[5] The presence of Sialic acid at or near the terminal position underlies its importance in determining chemical, biological diversity and characteristic of cell surface and secreted glycoproteins.[6] These glycoconjugates are released in to circulation due to increased turnover or secretions, shedding from malignant cells[5] and in many pathological conditions.[4]

Being non-invasiveness, economic advantage, possibility of repeated sampling and above all its ease in repeated sampling had made the blood based screening test most acceptable in patients with PMD and malignancy. Therefore, the present study was an attempt to investigate the serum levels of Glycoconjugate–the TSA in patients with OLP and OSCC for its early diagnosis.

Materials and methods:

The present Randomized study with convenience sampling was carried out in the Department of Oral Medicine and Radiology, Vokkaligara Sanga Dental College and Hospital and Kempegowda Institute of Medical Sciences at Bengaluru after obtaining Institutional Ethical clearance and consent from all patients.

Total of 75 subjects were made into 3 groups. Group I-OLP patients (Pathologic controls. Age range between 20-70 years); Group II- OSCC (Age range between 30-75 years). Group III-HC (Age and sex matched for comparison of results). All the patients were evaluated from General Physician for having no systemic illness in recent past. Diagnosis of OLP and OSCC was based on clinical, radiologic and Histopathologic reports. Clinical stage of OLP was determined as per Pindborg JJ et al and Schepman KP et al. And of OSCC by American Joint committee on Cancer.

5ml of Blood samples was collected from all 50 patients and 25 controls from anticubital vein under aseptic condition in a sterile bulb between 9-11am on every occasion to avoid diurnal variation. Serum was separated and stored at -4^{0}C until analysed. Serum TSA was estimated through a simplified quick method of G.Sydow.

First, Stock standard solution and working standard solution were prepared. TSA levels from standard curve (Graph 1) were obtained. Then using patient serum the OD values were plotted and the corresponding TSA levels were calculated.

The principle of chemical reaction is; free Sialic acid in serum reacts with Paradimethyl aminobenzaldehyde (Ehrlich's Reagent) to form a pink colored solution. The absorbance of the color developed in the sample at 525nm is proportional to the total Sialic acid concentration in the serum.

Estimation of standards of TSA (concentration gradient):

Stock standard solution: 25 mg of TSA powder was dissolved in 10 ml of deionized water and transferred to a 25 ml volumetric flask. Water was added to make up the volume up to 25ml. (concentration: 1mg/ml).

Working standard solutions: 100mg%, 80mg%, 60mg%, 40mg%, 20mg% and blank solutions (0mg %) was prepared from stock standard solution. From each of these solutions, 0.5ml of solution was taken individually and Simplied quick method of G Sydow was performed.

Procedure: To 0.5 ml of standard solution, 2.0 ml of 5% Perchloric Acid was added and incubated for 5 min at 100^{o}C. After cooling, this was centrifuged at 2500 rpm for 4 minutes. To 1.0 ml of this clear supernatant 0.2 ml of Ehrlich's reagents was added.Then heated for 15 min at 100^{o}C. Again the mixture was cooled and 1.0 ml of water

was added. Optical density was measured at 525 nm against reagent blanks in spectrophotometer. The OD values were plotted in a graph paper against TSA concentrations of the standard solution to obtain a linear curve.

Calculation: The serum (0.5 ml) collected from all 50 cases and 25 controls were subjected to similar treatment. The OD values were plotted on the standard graph (curve) (Graph 1) to obtain the serum levels of TSA in controls and cases.

Statistical analysis: ANOVA (Analysis of variants) between three groups showed a significant difference in the mean TSA levels ($P<0.001$) (Table 1). Bonferroni's test between three Groups with multiple comparison inferred a significant difference between HC group & OLP group; HC group & OSCC group; and OLP group & OSCC group with respect to the mean TSA levels ($P<0.001$)(Table 2). The mean TSA is found to be more in SCC group compared to OLP and HC group (Graph 2) and this difference is statistically significant. The mean TSA in OLP group is higher than HC group and this difference is also statistically significant.

Results:

In our comparative study, 84% (n=7) of OLP patients were between the age group of 20-30 years with 84% (n=21) of male predominance. 6% of OSCC patients were between the age group of 41-50 years with almost equal gender predilection. In HC subjects, 60% (n=15) were males and 40% (n=10) were females.

Habits distributions between three Groups showed 100% (n=25) of OLP patients with deleterious oral habits. 43% (n=20) of OLP and 57 % (n=20) of OSCC patients had predominantly smokeless (chewing) tobacco habit. HC subjects did not had any deleterious oral habits.

52% (n=23) of OLP and 32% (n=14) of OSCC showed Buccal mucosa as the site of disease predilection. 96 % (n=24) of OLP patients outnumbered in clinical staging I.

68% (n=17) of OSCC patients outnumbered in clinical staging III with 52% (n=13) of T2 Tumor size and 80% (n=20) with N1 Nodal.

The Serum TSA levels were estimated in all the three Groups and the arithmetic mean along with Standard deviation was calculated.OLP Group showed the mean of 76.18 mg/dl with the standard deviation of 17.08 mg/dl; the interval of mean was between 69.13 to 83.23 mg/dl, with the minimum of 45.00 mg/dl to a maximum of 107.00 mg/dl. OSCC Group showed the mean of 95.68 mg/dl with the standard deviation of 18.45mg/dl; the interval of mean was between 88.06 to 103.30 mg/dl, with the minimum of 54.00 mg/dl to a maximum of 125.00 mg/dl. HC Group showed the mean of 60.22 mg/dl with the standard deviation of 4.92mg/dl; the interval of mean between 58.19 to 62.25 mg/dl, with the minimum of 51.50mg/dl to a maximum of 69 mg/dl. Our study showed the ascending order of Serum TSA levels in HC, OLP and OSCC.

Discussion:

Carcinoma of the oral cavity is one of the most frequent malignant tumors worldwide, with major predominance in South-East Asia and India.[7,8] Among the oral tumors, 90% are OSCC, which arises from the mucosal lining. This high incidence of OC in India is due to the widespread habits of tobacco chewing, smoking and alcohol.[8,25,26,27]

OLP is the most common PMD of the oral mucosa[9] that frequently precedes OC.[10,11] They both have similar etiologic factors[6] and can be induced and promoted by tobacco.[10, 11] Our every case of OLP and OSCC had the habit of tobacco usage except one case of OSCC which did not had any habit. The age and gender distribution for OLP mainly depends on geographic variation and tobacco usage.[12, 13] In our study though the age distribution was between 20-70 years in OLP cases, there was an incidence of tobacco usage in 15 year old male patient.[12] The site of predilection for OLP being buccal mucosa and then commissure of lip in different studies[14] correlates with our study. 96 % of OLP cases was in stage 1 clinical stage (n=24).

The mean age of 43 years in OSCC correlated with other study.[5] Almost equal predilection in OSCC for males and females in our study was contradictory to the sex predilection in India which in turn depends on the usage of tobacco.[1, 5, 12, 7, 8] In India, the buccal mucosa was the primary site for cancer development as against the tongue and the floor of the mouth in Western countries, which may be due to the habit of keeping the betel-quid and tobacco in contact with the cheek for a long time[1,14,8] which was similar to our study. Majority of OSCC patients were in Stage III clinical staging with T2 tumor size including Lymph node involvement in all the cases. This may be due to lack of awareness as majority of the cases are poorly educated and are from rural background.[4]

Substances like Glycoproteins and Gylcolipids forms major constituents of cell membrane and hence, cell-surface Glycoconjugates are important in malignancy.[6] During carcinogenesis, any intracellular micro-environmental change may lead to alteration in the cell surface membranes constituents, releases certain molecules in the blood which are called Tumor markers. They are specific for certain tumor or cancer cells and thus could be of appreciable diagnostic and prognostic value in cancer patients.[15]

The Glycoconjugate, serum Total Sialic acid is one of the Tumor markers. Sialic acid is a Glycoprotein and has negative charge. The negative charge is due to the presence of carboxyl group enable the Sialic acid to mediate in transport of positively charged molecules, cell to cell and cell-matrix interactions, confirmation of membrane glycoproteins and masking antigenic sites of receptors.[1]

In our present study, the idea of screening patients with OLP and OSCC by blood test using simplified quick methodology by G Sydow was appealing from several points, including its ease in technical procedures, less time consuming, economic advantage, non-invasiveness, and possibility of repeated sampling. The values obtained by this method were within the range which was noticed in different studies with different methods in different untreated malignancies and potentially malignant disorders.[4, 5,6,17]

The interval of mean was between 69.13 to 83.23 mg/dl in OLP patients, between 88.06 to 103.30 mg/dl in OSCC patients and between 58.19 to 62.25 mg/dl in HC subjects. The mean TSA was found to be more in OSCC group compared to OLP and HC group. The mean TSA in OLP group was higher than HC subjects which was statistically significant.

The rise in the level of Sialic acid was also noticed in both smoking and smokeless tobacco habits indicating its harmful effects.[18] The changes in serum TSA was noticed in different systemic diseases like diabetes mellitus, Ischemic heart disease, and different bone disorders like Pyogenic arthritis, Rheumatoid arthritis, malignant bone tumors.[19, 20, 21, 22, 23] The patients in our study did not have any systemic disease. So, the increase in the TSA in both OLP and OSCC is due to increased turnover, secretion, loss of adhesiveness and or shedding from OLP and OSCC cells.

Serum TSA can be used for initial diagnosis, monitoring therapy of PMD and malignant lesions and even in recurrence of malignancy.[1,4,5,6,15,16,24] Recently contemporary studies have been carried out to include Sialic acid as a specific Tumor marker in the initial diagnosis of PMD and OSCC. [28, 29]

Conclusions:

OSCC, a demoralizing disease is becoming equal predilection for men and women with direct association related with Tobacco usage. Our results suggested that there is progressively ascending order of serum TSA in HC to OLP to OSCC that could be of great value in early assessment of OLP & OSCC. The results also demonstrated the evaluation of serum TSA by simplest, non-invasive, less time consuming, cost effective and reproducible method that can provide significant clinical information about differentiating the patients between normal, OLP and OSCC which help in early diagnosis of OLP and OSCC in turn reducing the potentiality of mortality and morbidity. But further studies are required with the same methodology of simplified quick method by G. Sydow for estimation of TSA to confirm the clinical usefulness of serum TSA as an early diagnostic tumor marker.

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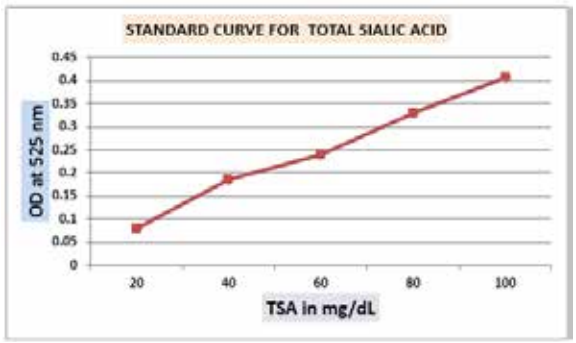
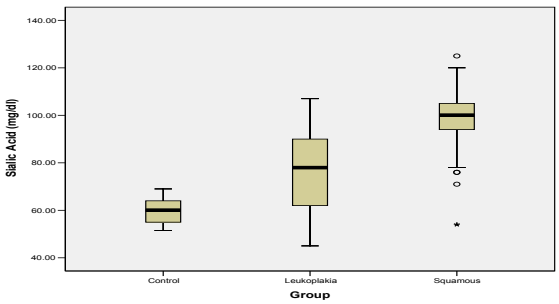
Figure legends:



Graph 1: Standard curve for Total Sialic Acid



Graph 2: Serum Total Sialic acid levels in HC, OLP, and OSCC

ANOVA

Sialic Acid (mg/dl)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	15769.860	2	7884.930	36.043	.000
Within Groups	15750.920	72	218.763		
Total	31520.780	74			

Table 10: Statistical analysis by ANOVA

Multiple Comparisons

Dependent Variable: Sialic Acid (mg/dl)

Bonferroni

(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Control	Leukoplakia	-15.96000*	4.18342	.001	-26.2144	-5.7056
	Squamous	-35.46000*	4.18342	.000	-45.7144	-25.2056
Leukoplakia	Control	15.96000*	4.18342	.001	5.7056	26.2144
	Squamous	-19.50000*	4.18342	.000	-29.7544	-9.2456
Squamous	Control	35.46000*	4.18342	.000	25.2056	45.7144
	Leukoplakia	19.50000*	4.18342	.000	9.2456	29.7544

*. The mean difference is significant at the .05 level.

Table 11: Multiple Comparisons by Bonferroni Test

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