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RESEARCH ARTICLE

QUANTITATIVE ANALYSIS OF TOTAL AND LIPID BOUND SIALIC ACID LEVEL IN ORAL POTENTIALLY MALIGNANT CONDITION AND ORAL SQUAMOUS CELL CARCINOMA-AS MARKER OF ORAL CANCER

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ABSTRACT

Background: Oral cancer is currently the most frequent cause of cancer-related deaths among Indian men, which is usually preceded by oral potentially malignant conditions (OPC) like leukoplakia and/or oral submucous brosis. The idea of screening and following patients with malignancy by blood-based tests is appealing from several points of view including its ease, economic advantage, non-invasiveness and possibility of repeated sampling.the purpose of the present study is to estimate serum levels of sialic acid, in the oralpotentially malignant conditions (OPC) along with the oral cancer patients and in healthy control group to evaluate their role in diagnosis and prognosis of oral cancer.

Aim: To compare and correlate serum level of total sialic acid (TSA) and lipid bound sialic acid (LSA) in oral Squamous cell carcinoma and potentially malignant condition with normal healthy subjects.

Methods: Total sialic acid (TSA) and Lipid bound sialic acid (LSA) determination was carried out in patients included in study along with 30 healthy individuals without any systemic disease. Total sialic acid (TSA) determination was carried out using the Thiobarbituric acid method. [10]Lipid bound sialic acid (LSA) determination was carried out using method described by Katopodis and coworkers.[11]

Clinical stage of the disease was determined as per American Joint Committee of Cancer (AJCC) norms.[60]Patients with Oral submucous fibrosis were included in the study as a potentially malignant condition and graded according to classification given by Khanna JN and Andrade NN (1995) [61]

Result: The increase in TSA and LSA in oral Squamous cell carcinoma patients(Group III) patients were statistically significant compared with control as well as with the patients with (Group II) oral submucous fibrosis ($p<0.001$). The increase in TSA and LSA were not significant in oral submucous fibrosis when compared with controls.

Conclusion: A significant increase in serum TSA & LSA levels in OSMF and oral cancer as compared to control group was noticed.

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INTRODUCTION

India, the most densely populated country with over a billion population, is unique in its extreme diversity in climate and culture. India is known for an extremely high incidence of head and neck cancer (especially oral and oropharyngeal) owing to the unique use of tobacco and its related products. (Mishra, 2009) Unless major action is taken, it is estimated that up to one billion people could die from tobacco use during the 21st century. (Lyndon Paul Abreu *et al*., 2010) Stage at diagnosis is the most important prognostic indicator for oral and oropharyngeal squamous cell carcinoma. If lesions are detected when they are small, localized, and treated expeditiously, survival rates of 70–90% can be achieved. (Morelatto *et al*., 2007) Unfortunately, almost half of the oral cancers are diagnosed at advanced stages (III orIV), with 5-year survival rates ranging from 20% to 50%, depending on tumour sites (Neville and Day, 2002; Warnakulasuriya, 2009). The majority of oral squamous cell carcinomas (OSCC) are preceded by visible changes of the oral mucosa. the terms pre-cancer', precursor lesions', pre-malignant', intra epithelial neoplasia' and potentially malignant' have been used in the international literature to broadly describe clinical presentations that may have a potential to become cancer. The latest WHO

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monograph on Head and Neck Tumours (2005) uses the term 'epithelial precursor lesions'. (Warnakulasuriya *et al*., 2007) Oral cancer is currently the most frequent cause of cancer-related deaths among Indian men, which is usually preceded by oral pre-cancerous conditions (OPC) like leucoplakia and/or oral submucous brosis. The idea of screening and following patients with malignancy by blood-based tests is appealing from several points of view including its ease, economic advantage, non-invasiveness and possibility of repeated sampling. In spite of the prolonged and detailed study of PMD, accurately predicting which patients or lesions will develop OSCC is impossible at present. (Seamus *et al*., 2008) Although scalpel biopsy conventionally con rms diagnosis, it is not problem-free despite the application of other techniques, like vital staining (the Toluidine Blue test'). As the scalpel biopsy is also invasive, this is of major importance in the follow-up of PML, often leading to patient refusal of repeated histological evaluation. In addition to these clinicopathological parameters, biomarkers are being intensively sought and validated for oral cancer. Knowledge of molecular alterations in various stages of oral tumorigenesis will greatly help in identifying putative biomarkers for early diagnosis and as novel targets for therapeutic intervention. (Muzafar A. Macha *et al*., 2010) Progress in molecular oncology has signi cantly advanced the knowledge on tumorogenesis; yet the practical applications of these genetic markers remain unresolved in detecting oral dysplasia. (Larsen *et al*., 2009) Neoplastic transformation of a variety of cell types is associated with changes in the composition of membrane glycoproteins (Dwivedi *et al*., 1990). Glycoproteins are complex proteins in which carbohydrates are linked covalently to asparagine or serine or threonine residues of polypeptides. The cell surface glycoproteins have been shown to play an important role in pinocytosis, differentiation, tumorigenesis, intracellular recognition and adhesion, as receptors for many hormones and viruses and as mediators of immunological specificity. Tumor associated carbohydrate changes have also been used in the diagnosis of human cancers (Dabelsteen, 1996). (Shanmugam Manoharan *et al*., 2009)

Aberrant glycosylations are the universal features of cancer. Glycoprotein's and glycolipids are important constituents of cell membrane; hence, they play an important role in malignancy. These glycoconjugates are released into the circulation through increased turn over, secretion, and / or shedding from malignant cells. (Sanjay *et al*., 2008) Usefulness of assay of serum glycoconjugates in early detection of cancer and in monitoring the progress of treatment, have been evaluated in previous studies. Earlier workers have reported elevated serum levels of total sialic acid (TSA), lipid-bound sialic acid (LSA), and TSA to total protein ratio, in various malignancies. Various studies have reported the signi cance of sialic acid as a tumor marker. (Sanjay *et al*., 2008) The signi cant elevations of serum sialic acid in oral cancer patients compared to precancerous conditions suggested the potential utility of this parameter in diagnosis as well as determining clinical stage of the malignant disease. (Sanjay *et al*., 2008) So the purpose of the present study was to estimate serum levels of sialic acid, in the oral pre-cancerous conditions (OPC) along with the oral cancer patients and in healthy control group to evaluate their role in diagnosis and prognosis of oral cancer.

MATERIALS AND METHODS

The present Study was conducted in the Department of Oral Pathology and Microbiology, in collaboration with Regional cancer hospital and Department of Biochemistry. By obtaining an informed consent, the patients were examined thoroughly and detailed case history proforma was recorded. The study conducted in 90 patients which were divided into three groups. The Present study included total 90 subjects with age range from 20-70 years, which were divided into three groups.

Group I- Control,

Group **II** -Oral submucous fibrosis and

Group III - Oral Squamous cell carcinoma of 30 subjects each

Blood samples were collected by venous arm puncture from each of the subjects and allowed to clot at room temperature followed by centrifugation at 3000 rpm for 10 minutes. To avoid possible diurnal variation the samples were drawn between 9 a.m. and 11 a.m. The serum samples were separated and stored at -20^0C until assayed.

Total sialic acid (TSA) determination was carried out using the Thiobarbituric acid method. (Aminoff, 1961)

Principle

After oxidation with Periodic acid followed by heating with Thiobarbituric acid, sialic acid develops non-fading chromophore with dimethyl sulfoxide. The colourintencity of the chromophore is in direct proportion to sialic acid contents.

Lipid bound sialic acid (LSA) determination was carried out using method described by Katopodis and coworkers. (Katopodis *et al*., 1982)

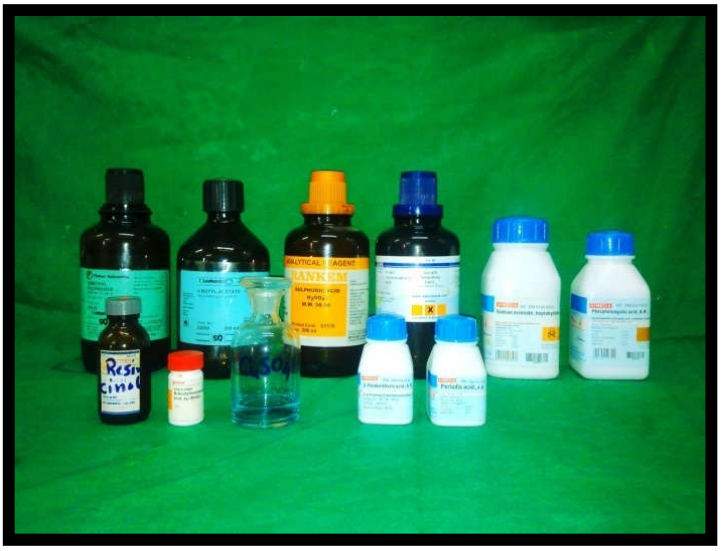
Principle

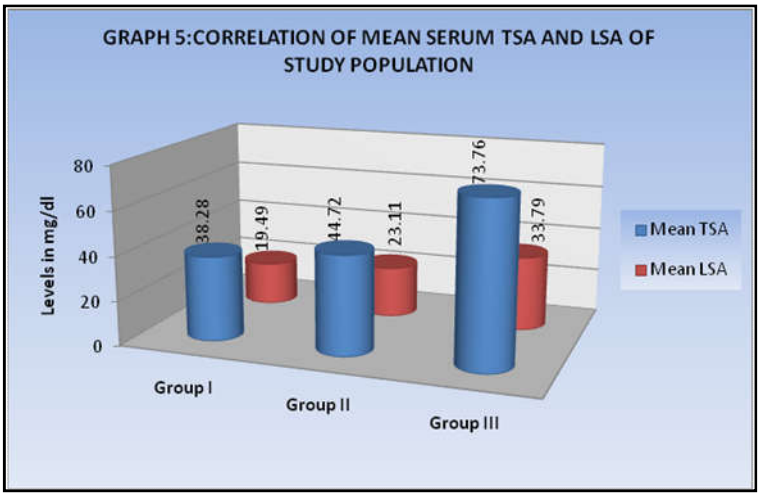
Serum gangliosides are treated with chloroform: methanol mixture and extracted in aqueous phase followed by precipitation with phosphotungastic acid. The precipitates, when boiled with resorcinol reagent, give blue colour which is directly proportional to the amount of lipid bound sialic acid present in serum. All the obtained data was collected tabulated and Statistical analysis was carried out by using SPSS 15.0 © (statistical package for social science) software. ANOVA (analysis of variance) AND POST HOC BONFERRONI TEST were applied for significance and variability of the values. All subjects were analyzed for Serum level of total sialic acid (TSA) and lipid bound sialic acid (LSA). The data was collected, tabulated and analyzed by SPSS 15.0 © (statistical package for social science) software. Various statistical tests were applied to determine if a statistically significant difference (level of 0.05) existed between the studied groups.

Table: Corelation of mean serum tsaandlsalevel in controls, oral submucous fibrosis and oral squamous cell carcinoma patients

Tumor marker	Groups	N	Mean	Std. Deviation	P value (ANOVA)	Group compared (Post Hoc Test)	P value
TSA	Group I	30	38.28	7.49	0.000	Group I&II	0.051
mg/dl	Group II	30	44.72	8.22		Group II&III	0.000*
	Group III	30	73.76	13.82		Group I&III	0.000*
LSA	Group I	30	19.49	3.29	0.000	Group I&II	0.442
mg/dl	Group II	30	23.11	3.34		Group II&III	0.000*
	Group III	30	33.79	7.10		Group I&III	0.000*

* P< 0.05(Anova and post hoc test bonferroni)





Corelation of meanserumtsa and lsa level in controls, oral submucous fibrosis and oral squamous cell carcinoma patients (Table 8 Graph 5)

Table shows comparison of total sialic acid (TSA) and lipid bound sialic acid (LSA) values between controls (Group I) patients with oral submucous fibrosis (Group II) and oral Squamous cell carcinoma patients (Group III). In this study, in Group I the mean serum TSA level was 38.28 mg/dl and mean serum LSA level was 19.49 mg/dl. In Group II the mean serum TSA level was 44.72 mg/dl and mean serum LSA level was 23.11mg/dl. While in Group III patients the mean serum TSA level was 73.76 mg/dl and mean serum LSA level was 33.79mg/dl. The increase in TSA and LSA in oral Squamous cell carcinoma patients(Group III) patients were statistically significant compared with control as well as with the patients with (Group II) oral submucous fibrosis ($p<0.001$). The increase in TSA and LSA were not significant in oral submucous fibrosis when compared with controls.

DISCUSSION

In the post-antibiotic era, oral cancer remains as one of the few life-threatening oral diseases in the western world. Oral cancer, globally, is the sixth most common cancer and is a major problem in regions where tobacco habits, in the form of chewing and / or smoking, with or without alcohol intake, are common. Its distribution and occurrence varies by age, ethnic group, culture and life style, and level of country development.

Worldwide, oral cancer has one of the lowest survival rates and poor prognosis remains unaffected despite recent therapeutic advances. Reducing diagnostic delay to achieve earlier detection is a cornerstone to improve survival. Thus, intervention strategies to minimize diagnostic delays resulting from patient factors and to identify groups at risk in different geographical areas seem to be necessary. (Gomez *et al*., 2010) Oral submucous brosis (OSF) is a high risk precancerous condition characterized by changes in the connective tissue bers of the lamina propria and deeper parts leading to sti ness of the mucosa and restricted mouth opening. OSF has been reported almost exclusively among Indians living in India and among other Asiatics, with a reported prevalence ranging up to 0.4% in Indian rural population. Epidemiological and in vitro experimental studies have shown that chewingareca nut (Areca catechu) is the major aetiological factor for OSMF. In an epidemiological study on oral cancer and precancerous lesions in rural Indian population, the malignant transformation rate of OSMF was 7.6% over a 17- year period.[65.] A dramatic increase in the incidence of oral cancer in the coming decades in India is being widely predicted (Babu *et al*, 1997; Gupta *et al*, 1998). (Rajalalitha and Vali, 2005) Stage at diagnosis is the most important prognostic indicator for oral and oropharyngeal squamous cell carcinoma. Unfortunately, approximately 50% of these cancers are identi ed late (stage III or IV) (1). If lesions are detected when they are small, localized, and treated expeditiously, survival rates of 70–90% can be achieved. (Morelatto *et al*., 2007) In spite of the prolonged and detailed study of PMD, accurately predicting which patients or lesions will develop OSCC is impossible at present. (Seamus S. Napier, 2008) Although previous studies have reported a high prevalence of oral abnormalities found during oral cancer screening programmes (5–15%), not every oral lesion can be evaluated by scalpel biopsy as it is a complex, time-consuming, expensive and invasive procedure. Another disadvantage is that the scalpel biopsy can be used for a limited number of sites, thus covering only small areas. Moreover, when faced with multiple lesions of similar appearance, there may be 'only one' with a malignant evolution, which could well be overlooked. This is not surprising, given that molecular changes consistent with early malignant changes can be scattered through and beyond a potentially malignant clinical lesion, therefore, emphasize the need for other tools for prediction of cancer development in susceptible lesions. (Roberto Navone *et al*., 2008)

The quest is ongoing for more reliable serum and/ or plasma markers for detecting and staging malignant disease and for evaluating various therapeutic approaches. Studies of malignant cells have revealed alterations in cell surfaces and membranes in terms of the sialic acid content of glycoproteins and glycolipid. (Mark C. plucinsky *et al*., 1986) Tumor marker levels in certain situations reflect Tumor burden in the body and hence can be used in staging, prognostication or prediction of response to therapy. Also detect recurrence of disease well before any clinical or radiological evidence of disease is apparent. (Sharma, 2009) Malignant cell surface glycoproteins and glycolipids have altered carbohydrate compositions that may contribute to aberrant cell-cell recognition, cell adhesion, antigenicity, and the invasiveness demonstrated by malignant cells. These glycoproteins and glycolipids can be released into the sera through increased turnover, secretion, and/or shedding and are of considerable interest for their potential diagnostic and prognostic value. Various studies have reported the significance of sialic acid as a tumor marker. Aberrant glycosylation processes in tumor cells contribute to the biosynthesis of certain oligosaccharides; hence, malignant or transformed cells contain increased sialic acid residues on their surfaces.Previous studies revealed elevated serum levels of TSA and LSA in malignancies of lung, breast, skin, colon, prostate, and bladder. Sialic acid levels also served to monitor treatment of cancer.Value of serum sialic acid as a tumor marker in OSCC was demonstrated by few workers. (Manjula Shantaram *et al*., 2009) Unlike tumor antigens, which are associated with a limited spectrum of tumors, increased sialic acid levels due to sialyltransferase activity and associated sialylglycoprotein production appear to be a common phenomenon of a variety of neoplastic cells. As a result, relatively nonspecific markers such ässialic acid may have useful clinical applications äs a cancer screen. (Shamberge, 1984)

Summary and Conclusion

Oral cavity cancer is currently the most frequent cause of cancer-related deaths, which is usually preceded by oral pre-cancerous lesions and conditions. However, it is essential to study marker levels in patients with oral precancer who are at a high risk of developing oral cancer. The present study was carried out to evaluate usefulness of serum Total Sialic Acid (TSA) and serum Lipid-Bound Sialic Acid (LSA) as markers of oral submucous fibrosis and oral cancer. Study consisted of 60 patients and 30 controls. There were 3 study groups, Group I controls Group II OSMF, and Group III oral cancer consisting of 30 patients each. Serum of all the patients in the control group and study groups were collected and stored at – 20 ^{0} C until analyzed. Levels of serum TSA and LSA were estimated spectrophotometrically. The mean serum levels of TSA and LSA obtained were 38.28 and 19.49 mg/dl for control group. For OSMF & Cancer group, the mean level of TSA was 44.72, & 73.76 mg/dl and for LSA the mean level was 23.11, 33.79 mg/dl respectively. There was a significant increase in serum TSA & LSA levels in OSMF and oral cancer as compared to control group. Serum TSA and LSA levels of Group III oral Squamous cell carcinoma also elevated significantly when compared with Group II (OSMF). Thus, it can be concluded that serum TSA and LSA can be used as biochemical markers adjuvant to other markers for oral precancerous lesions and early detection of oral cancer. Combined evaluation of these markers may be used as an adjunct to diagnosis, prognosis and staging of the precancerous lesions of the oral cavity. These parameters also have a potential utility in diagnosing as well as determining clinical stage of the malignant disease. The results also demonstrated that the assessment of TSA and LSA by simple, in-expensive and reproducible methods can provide significant clinical information about the extent of malignant disease and can differentiate between patients with oral precancer and oral cancer. The present study supports the earlier studies in the reliability of serum sialic acid as a tumor marker. The serum sialic acid may be used as a tumor marker for early diagnosis as well as to predict the prognosis of a malignancy since the

procedure is simple and non-invasive. However, a longitudinal study with a large sample is needed to assess the reliability of these parameters as specific tumor markers. It would be beneficial for the mankind if the concerned regulatory authorities implement the use of these biomarkers in routine clinical practice for early detection, prognosis, treatment planning and responsiveness to treatment for precancerous lesions and/or conditions and oral cancer.

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