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Predictive Role of Total and Lipid Bound Sialic Acid in Oral Precancerous Condition

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Author`s Contribution

[1,2]Substantial contributions to the conception or design of the work; or the acquisition, [4,6]Active participation in active methodology, [2,3]analysis, or interpretation of data for the work, [5]Drafting the work or revising it critically for important intellectual content

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ABSTRACT

Objective: To determine the role of total sialic acid (TSA) and lipid bound sialic acid (LBSA) as the early predictors / indicators of oral cancer and precancerous in study subjects.

Methodology: A cross-sectional study was conducted at the Department of Biochemistry, Basic Medical Sciences Institute, in collaboration with the clinical oncology ward of Jinnah Postgraduate Medical Centre, Karachi from June 2019 to December 2019. Participants were categorized into three groups: oral cancer (Group A), oral precancer (Group B), and healthy controls (Group C). Blood samples (8 ml) were collected for biochemical analysis of TSA and LBSA levels. Serum proteins were measured using the Biuret reaction method, while TSA and LBSA were quantified via a colorimetric method involving sialic acid's reaction with resorcinol, producing a pink chromosphere measured at 580 nm using a spectrophotometer. Statistical analysis was performed using SPSS.

Results: In the Pre-cancer group, TSA levels were significantly higher (78.7 ± 16.62 mg/dl) compared to the Control group (60.2 ± 4.27 mg/dl), (p-0.001). Similarly, LBSA levels were also elevated in the Pre-cancer group (22.7 ± 2.06 mg/dl) compared to Controls (19.8 ± 3.18 mg/dl), (p-0.001). The TP levels were markedly higher in the Pre-cancer group (11.3 ± 2.37 g/dl) compared to the Control group (6.2 ± 0.61 g/dl), (p-0.001). Conversely, the TSA/TP ratio was lower in the Pre-cancer group (7.4 ± 2.72) compared to Controls (9.6 ± 1.26), (p-0.001).

Conclusion: The TSA and LBSA levels were observed significantly elevated in the pre-cancer group, while the TSA/TP ratio was notably lower and the findings indicating that the TSA and LBSA may serve as potential biomarkers for the early detection of oral pre-cancerous conditions, highlighting their predictive role in the progression towards oral cancer.

Keywords: Oral precancer, early predictor, TSA, LBSA.

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Introduction

Oral and oropharyngeal cancers rank as the sixth most prevalent malignancies worldwide.[1] Each year, it is estimated that more than 400,000 new cases of oral cancer are diagnosed globally, with approximately two-thirds of these cases occurring in Asian nations, including Indonesia, Sri Lanka, India, Bangladesh and Pakistan.[1,2] Oral submucous fibrosis (OSF) is a long-standing condition strongly associated with the risk of developing malignancy.[3,4] Classified as a precancerous disorder, it carries a significant potential to transform into oral

cancer. The condition is primarily linked to the habitual use of betel nuts, which play a key role in its pathogenesis.[3] Oral cancer has the highest incidence rates in Pakistan and India, although it is the second most common cancer in Pakistan. Particularly oral cancer ranks as the third most common cancer among males and the fourth among females.[5,6] Across the world, oral cancer (OC) is recognized as one of the most debilitating and devastating diseases. The etiology of oral cancer is primarily associated with the use of tobacco, whether through smoking or chewing, and the consumption of alcohol. However, several other contributing factors have been identified that play a role in its development. These include infections with human papillomavirus (HPV) and Candida, nutritional deficiencies such as iron deficiency, exposure to radiation, compromised immune function, environmental carcinogens, and genetic alterations involving tumor-suppressor genes.[7,8]

Oral cancer encompasses a group of malignancies that can develop in various regions of the oral cavity, including the tongue, lips, and floor of the mouth. While some oral squamous cell carcinomas (OSCCs) emerge from seemingly normal mucosa, others are preceded by identifiable premalignant lesions, such as erythroplakia and leukoplakia, which serve as early warning signs of potential malignant transformation.[9] It is the major cause of fear, morbidity and mortality all over the world. Despite significant advancements in tumor surgery and multimodal treatment strategies, the prognosis for oral cancer remains relatively unfavorable. One major contributing factor is that symptoms indicating the presence of carcinoma often become apparent only when the tumor has already progressed to an advanced stage, limiting the effectiveness of therapeutic interventions. Therefore, based on the current data, it is imperative to develop and investigate diagnostic techniques that enable the rapid detection of oral cancer. Early and swift diagnosis can significantly improve treatment outcomes by allowing interventions to occur before the tumor progresses to an advanced stage.[10]

When discussing the diagnosis of cancer and precancerous conditions, biopsy remains the gold standard. However, the procedure is both time-consuming and invasive, which can be a challenge for patients. To address these limitations, this study explores the use of salivary and serum sialic acid levels as a less invasive diagnostic approach. This method offers the potential for quicker, more patient-friendly detection of cancer and precancerous lesions, aiming to complement or, in some cases, provide an alternative to traditional biopsy techniques.[10] Tumor markers are substances that are specific to certain tumor or cancer cells, making them valuable tools for both diagnostic and prognostic purposes in patients with oral cancer.[11,12] These markers include substances whose levels in the serum undergo quantitative changes during tumor development and are collectively referred to as tumor markers or biochemical serum markers.[11] There was a positive correlation has been observed between serum levels of Total Sialic Acid (TSA) and Lipid-Bound Sialic Acid (LSA).[13] Changes in serum proteins at the terminal end of α-2-6 sialic acid have been linked to variations in the levels of different forms of sialic acid and the enzyme sialyltransferase. In cases of malignancy, these levels are significantly elevated compared to healthy controls, highlighting their potential as biomarkers for cancer diagnosis and progression monitoring.[13]

This study is particularly important for developing countries, including Pakistan, due to the significant prevalence of oral precancerous diseases, which necessitates the adoption of novel diagnostic techniques to reduce the burden of late-stage malignancy presentations. By confirming the diagnostic role of TSA and LSA in the oral precancerous condition, current study aims to support the development of non-invasive diagnostic methods, enhance accessibility, reduce diagnostic delays, and improve patient outcomes within the local population.

Methodology

This cross-sectional was carried out in the Department of Biochemistry, Basic Medical Sciences Institute, in collaboration with the clinical oncology ward of Jinnah Post Graduate Medical Centre Karachi. Study approval was taken from Institutional Review Board (IRB) (ethical committee BMSI) of JPMC Karachi (Ref. No. F.1-2/BMSI-E.COMT/018/JPMC). A written consent was taken from every subject. The study subjects were divided into three groups as 34 cases in group A (diagnosed cases of oral cancer), 34 cases in group B (diagnosed cases of oral precancerous) and 34 cases (healthy subjects). All the diagnosed patients with oral cancer and precancer, aged 14 years or above, both genders and habitual for tobacco chewing and smoking were included. All the diagnosed cases of nephropathy, diagnosed cases of malignancies other than oral cancer, known cases of diabetes mellitus, chronic liver disorders, cardiovascular disorders and severe depression were excluded. The subjects were divided into three groups.

Group "A" was comprised of 34 diagnosed cases of oral cancer, Group "B" 34 subjects of oral precancer and Group "C" 34 healthy subjects. A total of 8 ml of venous blood was collected from each study subject from antecuboidal vein under all aseptic measures. 02 ml blood was separated for hematological parameters and was analyzed as soon as possible. The remaining 6 ml of blood was transferred to a proper container, allowed to clot at 37°C and was centrifuged at 3000 rpm for 15 minutes. The serum was separated and transferred to a proper container and kept at -70°C till analysis. The serum was analyzed for biochemical variables. Serum Total Sialic Acid (TSA) and Lipid-Bound Sialic Acid (LBSA) were estimated using a colorimetric method. Serum TSA reacts with resorcinol reagent to produce a pink color, with absorbance measured at 580 nm. For preparation, sialic acid powder was dissolved and standardized with N-acetyl neuraminic acid. Blood samples were collected, centrifuged to isolate serum, and stored at -70°C. For TSA determination, samples were mixed with resorcinol reagent, boiled, cooled, and extracted with butyl acetate/methanol before reading absorbance. LBSA was measured by extracting sialolipids from serum with chloroform-methanol, followed by phosphotungstic acid precipitation, resuspension, and further reaction with resorcinol reagent. Concentrations were calculated using a standard curve, with results expressed in mg/dl. Results were analyzed statistically by the appropriate SPSS tool.

Results

The cancer group had a higher proportion of males (67.6%) compared to the precancer (44.1%) and control groups (50%). Most cancer cases were found in older age groups, with 35.3% aged 55 and above, while younger participants under 25 were more common in the control group (17.6%). Although gender and age differences were statistically in significant (p=>0.05). (Table I)

Table I: Age groups and gender of the cases. (n=102)

	Controls (n=34)	Pre cancer (n=34)	Cancer (n=34)	P-value
Gender				
Male	17 (50.0%)	15 (44.1%)	23 (67.6%	0.128
Female	17 (50.0%)	19 (55.9%)	11 (32.4%)	
Age in years				
Under 25	6 (17.6%)	2 (5.9%)	2 (5.9%)	0.057
25-34	9 (26.5%)	8 (23.5%)	4 (11.8%)	
35-44	11 (32.4%)	7 (20.6%)	8 (23.5%)	
45-54	7 (20.6%)	8 (23.5%)	8 (23.5%)	
55 & above	1 (2.9%)	9 (26.5%)	12 (35.3%)	

TSA and LBSA levels were significantly higher in the precancer group (78.7 mg/dl and 22.7 mg/dl) compared to the control group (60.2 mg/dl and 19.8 mg/dl), (p-0.001) for both. Total protein levels were also higher in the precancer group (11.3 g/dl vs. 6.2 g/dl), and the TSA/TP ratio was lower in the precancer group (7.4 vs. 9.6) (p-0.001). (Table II)

Table II: Comparison of total sialic acid (TSA) & lipid bound sialic acid in controls and pre cancer.

Bio-chemical parameter	Controls (n=34) (Mean ± S.D)	Pre cancer (n=34) (Mean ± S.D)	P-value
TSA (mg/dl)	60.2 ± 4.27	78.7 ± 16.62	0.001
LBSA (mg/dl)	19.8 ± 3.18	22.7 ± 2.06	0.001
TP (g/dl)	6.2 ± 0.61	11.3 ± 2.37	0.001
TSA/TP ratio	9.6 ± 1.26	7.4 ± 2.72	0.001

The study found significant differences between the control and oral cancer groups in TSA, TP, and TSA/TP ratio. TSA and TP levels were significantly higher in the cancer group, while the TSA/TP ratio was lower. However, LBSA levels showed no significant difference between the two groups. (Table III)

Table III: Comparison of bio-chemical parameters in controls and oral cancer groups.

Bio-chemical parameter	Controls (n=34) (Mean ± S.D)	Cancer (n=34) (Mean ± S.D)	P-value
TSA (mg/dl)	60.2 ± 4.27	99.1 ± 18.30	0.001
LBSA (mg/dl)	19.8 ± 3.18	21.5 ± 7.23	0.207
TP (g/dl)	6.2 ± 0.61	16.8 ± 2.34	0.001
TSA/TP ratio	9.6 ± 1.26	6.0 ± 1.50	0.001

The comparison between precancer and cancer groups showed significant differences in TSA, TP, and TSA/TP ratio, with higher TSA and TP levels and a lower TSA/TP ratio in the cancer group. However, LBSA levels did not differ significantly between the two groups as shown in table IV.

Table IV: Comparison of Bio-chemical parameters in Pre cancer and Cancer groups.

Bio-chemical parameter	Pre cancer (n=34) (Mean ± S.D)	Cancer (n=34) (Mean ± S.D)	P-value
TSA (mg/dl)	78.7 ± 16.62	99.1 ± 18.30	0.001
LBSA (mg/dl)	22.7 ± 2.06	21.5 ± 7.23	0.386
TP (g/dl)	11.3 ± 2.37	16.8 ± 2.34	0.001
TSA/TP ratio	7.4 ± 2.72	6.0 ± 1.50	0.011

Discussion

Oral cancer is a severe and life-threatening condition with rising incidence rates and persistently low survival outcomes over the past two to three decades.[6,13] This study investigates the potential of non-invasive serum

biomarkers TSA**,** LBSA**,** TP and the TSA/TP ratio to predict oral precancerous conditions such as leukoplakia, erythroplakia, and submucous fibrosis by comparing these biomarkers in cases with oral precancer, oral cancer, and compared to healthy controls. In this study, the overall mean age was 42.79 ± 4.12 years, with males being more common in the cancer group (67.6%) compared to the pre-cancer group (44.1%). Participants aged 55 and older were predominantly in the cancer group (35.3%), followed by the pre-cancer group (26.5%) and controls (2.9%), while younger individuals (under 25) were mostly in the control group (17.6%). No significant differences in gender or age distribution were observed ($p > 0.05$). These findings reflect with the observations of Binns et al.[14] In the oral cancer case group, 23 (67.6%) were males and 11 (32.4%) were females, with a mean age of 48.05 ± 8.82 years. This indicates a higher prevalence of oral cancer in males, which could be attributed to greater exposure to risk factors compared to females, as reported in similar studies such as Falaki et al[15]. Furthermore, the age distribution findings for the oral cancer group are consistent with the results of Ahmed et al[16] and Kinra et al.[17] The higher incidence of oral cancer in males compared to females may result from lifestyle differences and increased susceptibility to environmental and behavioral risk factors.

This study found that mean serum Total Sialic Acid (TSA) and Lipid-Bound Sialic Acid (LBSA) levels were significantly elevated in oral precancer and cancer groups compared to the control group ($p < 0.01$). These findings suggest that increased TSA and LBSA levels can serve as early indicators of premalignant changes. Routine monitoring of these biomarkers in at-risk patients could enable the detection of malignant transformation at an early and more treatable stage. Additionally, TSA and LBSA levels were significantly higher in oral cancer compared to oral precancer, highlighting their potential utility in distinguishing between these conditions. These findings align with those reported by Taqi SA et al[12] as the TSA and LSA levels were significantly higher in oral precancer patients compared to the controls ($P < 0.001$).

Furthermore, these levels showed a notable increase in oral cancer patients when compared to both oral precancer and control groups ($P < 0.001$). Mahdi NR et al[17] also indicated that serum levels of TSA and LBSA were significantly elevated in cancer patients compared to healthy controls ($P < 0.001$), with the highest levels observed in patients with cancers of the ethmoid and frontal sinuses. Consistently Sonone A et al[18] revealed significantly elevated levels of TSA and LBSA in patients with LP and OSCC compared to the healthy control group. The rise in TSA and LBSA levels in LP can serve as an early indicator of disease onset.

Moreover, these biomarkers provide a means to distinguish between different grades of OSCC. Therefore, serum TSA and LBSA hold potential as valuable diagnostic and prognostic tools in clinical practice.[18] The increased TSA and LBSA levels in oral precancer and cancer may be attributed to the higher tumor burden and the ability of tumor cells to secrete large amounts of glyco-conjugates, which play a role in tumor genesis. Furthermore, elevated LBSA levels in oral precancer could be due to early dysplastic changes and hyper keratinization of tissue, marking the initial stages of malignancy.

In this study it has been observed that the mean total sialic acid/total protein (TSA/TP) ratio levels were 7.4 ± 2.72 mg/g and 6.0 ± 1.50 mg/g in oral precancer and cancer respectively which were significantly decreased when compared to control group i.e. 9.6 ± 1.26 mg/g. There was decreased value of TSA /TP ratio from control to squamous cell carcinoma and oral precancer patients as compared with controls which is significantly high with p value <0.01. The decreased values were decreasing from normal controls to oral precancer and cancer groups respectively. These findings were in accordance to analysis by Durgawale P et al[19] where they demonstrated a highly significant increase ($p < 0.001$) in TSA levels and the TSA/TP ratio among cancer patients, with values of 98.41 ± 13.5 mg/dl and 15.31 ± 2.57 mg/g, respectively, while the findings were inconsistent according to Sonone A et al[18] and Hamalatha et al.[20] The findings of the present study confirm that serum TSA, TP, and LBSA levels are effective non-invasive biomarkers for diagnosing oral precancer and cancer. The progressive increase in these biomarkers observed across different types of oral precancer and cancer compared to the control group highlights their reliability, accuracy, and practicality in clinical settings. Previous studies have also reported elevated TSA and LBSA levels in case groups compared to controls, reinforcing their association with oral precancer and cancer. Similar observations on the correlation between these biomarkers and the severity of histopathological changes have been documented by other studies.[11,21,22] The diagnostic accuracy of serum TSA, TP, and LBSA levels improves progressively with advancing stages and grades of oral precancer and cancer.

These biomarkers serve as early predictors and demonstrate better performance compared to other non-invasive indices commonly used in routine clinical practice for detecting oral precancer and cancer. However, due to several study limitations and some controversies with previously published studies, further large-scale studies are recommended to validate the findings.

Conclusion

Though biopsy remains the gold standard for diagnosing and grading oral precancer and cancer, it is an invasive procedure with associated risks and costs. In contrast, serum TSA and LBSA offer a non-invasive, cost-effective, and reliable alternative. These biomarkers are simple to measure, accurate, and serve as early predictors for identifying oral precancerous and cancerous conditions, making them a valuable tool in clinical practice.

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