

## ORIGINAL ARTICLE

# Total Sialic Acid (TSA) Level as a Tumor Marker in the Diagnosis of Oral Cancer

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### ABSTRACT:

**Objective:** To estimate serum Total Sialic Acid (TSA) levels in different grades of oral squamous cell carcinoma and to assess its utility as a tumor marker in this cancer.

**Materials and Methods:** This study was conducted in 68 adult subjects equally divided into two groups, healthy individuals and patients with oral squamous cell cancer. Under aseptic precautions venous blood was drawn and serum was separated. Estimation of serum total sialic acid level was done according to Spectrophotometric method of Plucinsky. Statistical analysis was carried out by using SPSS 19.

**Results:** Total subjects in the study included were 58.82% males and 41.17% females. Mean age of oral cancer patients was  $48.05 \pm 8.82$  years. There was significant male predominance with  $P < 0.05$ . Oral cancer was most common in Tobacco + Chaliya + Areca nut and Gutka + pan groups. Mean serum total sialic acid (TSA) level in control group was  $60.2 \pm 4.27$  mg/dl, whereas it was  $99.1 \pm 18.30$  mg/dl in oral cancer group. It was significantly increased in oral cancer group when compared to control group with  $P$  value  $< 0.01$ . There was progressive elevation in mean serum TSA level in oral squamous cell carcinoma.

**Conclusion:** Estimation of serum total sialic acid level (TSA) in different grades of oral squamous cell carcinoma showed positive relation with stage of malignancy, specifically with the tumor burden. It can be used as a diagnostic biomarker in oral squamous cell cancers.

**Keywords:** Oral cancer, Squamous cell carcinoma, Different grades, Serum total sialic acid, Tumor marker.

### INTRODUCTION:

Worldwide, the oral cancer accounts for 2%–4% of all cancers. The prevalence of oral cancer is higher, reaching to 10% of all cancers in Pakistan.<sup>1,2,3</sup> More than 95% of carcinomas of oral cavity are of squamous cell type in nature.<sup>4,5,6</sup> Oral cancer is a major cause of morbidity and mortality in Southeast Asian countries because more

than 600 million people chew areca nut and its products like pan, gutka, panmasala etc. worldwide and 85% of these live in Southeast Asian countries.<sup>7,8</sup> Therefore oral cancer is a major health problem and cause of death in developing countries. Other etiological factors are; Tobacco and lime chewing; Tobacco related habits, smoking, Alcohol consumption, Nutritional deficiencies, exposure to Sunlight and other miscellaneous factors.<sup>9,10,11,12</sup> Early detection of lesions in the oral cavity is very important because there are more chances of treatment outcomes which will reduce the rate of morbidity and mortality.<sup>13</sup> In this respect tumor markers are very important because they help in the screening, diagnosis and prognosis in monitoring the response of the disease to the treatment.<sup>14,15,16</sup>

Tumor markers are naturally occurring or modified molecules and can be measured in serum, plasma and other body fluids like saliva.<sup>17,18</sup> In presence of cancer their concentration may be changed. Substances changing quantitatively in the serum during tumorigenesis are collectively called tumor markers or biomarkers.<sup>19</sup> Actually, a biomarker is synthesized by the tumor and released into circulation in large quantities.<sup>20,21</sup> In oral cancer various biomarkers have been studied and one of such markers is Sialic Acid (SA). Sialic acid is an acetylated derivative of neuraminic acid. It is attached to the non-reducing residue of the carbohydrate chain of glycoproteins and glycolipids.<sup>22,23,24,25</sup> SA is a glycoprotein component of cell membrane which is synthesized in liver and it exists in conjugated form on the external surface of cell membrane.<sup>26</sup> Altered glycosylation of glycoconjugate is one of the important molecular changes in the malignant transformation.<sup>27,28,29</sup> Considering the high prevalence of oral malignancy in Pakistan, present study was conducted to evaluate serum TSA levels in different grades of oral cancer patients and also to validate its importance as a tumor marker in oral cancer patients.

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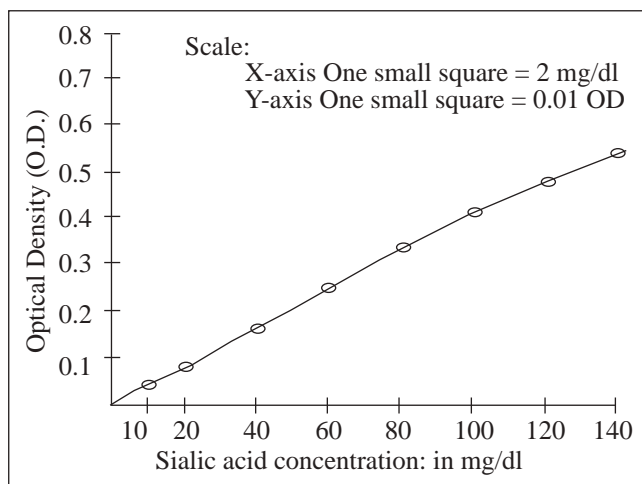
**MATERIALS AND METHODS:**

After approval from BASR of Karachi University, present study was conducted in the department of Biochemistry BMSI-JPMC. A total of 68 adult subjects were included in the study, 34 diagnosed cases of oral cancer from the clinical oncology ward of Jinnah Postgraduate Medical Centre, Karachi, and 34 healthy subjects were taken from general population, for comparison. Out of 34 diagnosed cases 23, 11 and 01 were of well differentiated squamous cell carcinoma (WDSCC), moderately differentiated squamous cell carcinoma (MDSCC) and poorly differentiated squamous cell carcinoma (PDSCC) respectively. Subjects having diabetes mellitus, hepatobiliary disorders, depression, premalignant neoplasms, renal disorders, cardiovascular disorders and other malignancies were excluded from the study.

Measurement of Total sialic acid (TSA): Serum total sialic acid level was determined by the Spectrophotometric method of Plucinsky as mentioned by Joshi and Kadam<sup>19,21</sup>. 20µl of serum was diluted with 980 µl distilled water. After treatment with resorcinol reagent the blue chromophore was extracted by butyl acetate/n-butanol (85:15) (v/v) and determined spectrophotometrically at 580nm and sialic acid was determined by the use of standard curve of N-acetyl neuraminic acid (Figure 1).

**Statistical analysis:** The data is expressed as mean ± SD. The statistical significance of the results was analyzed using a student's t test, Chi-square and Anova. Values of P<0.01 were considered as significant.

Figure: 1  
Standard curve of sialic acid



**RESULTS:**

Total subjects in the study included 58.82% males and 41.17% females. The mean age of oral cancer patients was 48.05 ± 8.82 years. These values indicate that individuals above 40 years are at high risk for oral cancer. The sex ratio was evaluated by Chi-square test, which was significant (P<0.05) showing the male predominance in oral cancer, this could be because the habits like tobacco chewing, smoking alcohol consum-

ption are more common in males.

Details of habits of oral cancer patients are mentioned (Table 1). Oral cancer patients were using tobacco in one or the other way. Oral cancer was most common in Tobacco+ Chaliya + Areca nut and Gutka+pan groups.

Table: 1  
Habits of oral cancer patients

Habits	Number of patients (n)
Tobacco + Pan with lime	03
Tobacco + gutka	04
Tobacco + Chaliya + Areca nut	06
Naswar + Pan	04
Gutka + pan	06
Beeri + Hucka	05
Cigarette + Naswar	05
Naswar+Gutkha	01

The mean serum total sialic acid (TSA) level in control group was 60.2 ± 4.27 mg/dl, whereas it was 99.1 ± 18.30 mg/dl in oral cancer group. It was significantly increased in oral cancer group when compared to control with P value < 0.01. It means that serum TSA level should be considered along with other traditional diagnostic tools for the accurate diagnosis of oral cancer (Table 2). Mean serum total sialic acid (TSA) level in WDSCC group was 80.8 ± 19.65 mg/dl, whereas it was 96.0 ± 15.86 mg/dl and 105.4 ± 22.3 mg/dl in MDSCC and PDSCC group respectively. Difference in values of mean serum TSA levels was statistically significant between WDSCC, MDSCC and PDSCC in oral cancer with P value < 0.01 (Table 3). The significant elevation of mean serum TSA levels in oral cancer patients was also noted when compared to controls with P value < 0.01. However, the difference between cases of MDSCC and WDSCC was again significant, which proves the role of serum TSA as an ideal biomarker of oral cancer. Because of the independent clinical significance of various tumor markers their serum concentrations are incorporated in clinical grading of the malignancies.

Table: 2  
Comparison of serum TSA levels among case and control groups

Study groups	Mean serum TSA	SD	P-Value
Control group	60.2	4.27	0.001
Oral cancer group	99.1	18.30	

Table:3  
Comparison of mean serum TSA levels in various histopathological grades of OSCC patients

OSCC patients	No	Mean TSA (mg/dl)	SD	P-Value
WDSCC	22	80.8	19.65	0.001
MDSCC	11	96.0	15.86	0.001
PDSCC	01	105.4	22.03	0.001

ANOVA utilized.(p<0.01) statistically significant

**DISCUSSION:**

Recently, tumor markers are receiving more attention in early detection as well as predicting prognosis of the lesion<sup>1</sup>. In the last few decades, considerable research efforts have been focused on defining the changes in cell surface membrane molecules in the neoplastic transformation, particularly the cell surface glycoproteins which contribute for malignant transformation of a cell. Among these glyco-conjugates, sialic acid is present up to 30% in various glycoproteins<sup>19</sup>.

With reference to oral squamous cell carcinoma, many workers have found significantly elevated levels of mean serum TSA as compared to healthy subjects.<sup>30</sup> They have also noticed increased levels of TSA when correlated with the different grades of oral squamous cell carcinoma. Present study has also shown the comparison of mean serum TSA levels with different grades of oral cancer<sup>5</sup>. We have also correlated TSA level with histopathologic grading of tumor. Histopathologic grading of oral squamous cell carcinoma (OSCC) was done according to the degree of differentiation as per Broder's classification as mentioned by Joshi<sup>19</sup>. The TSA levels according to histopathologic grading<sup>31</sup> in present study are evaluated. There is significant rise of TSA level with the advancing stage of tumor. This means that TSA level is directly proportional to the tumor burden. The values we found are closely related to those of Taqi<sup>16</sup> and Rajpura.<sup>29</sup> When mean serum TSA levels were mutually compared in our study between the WDSCC and MDSCC it showed significantly increased levels of mean serum TSA in MDSCC but when mean serum TSA levels were compared between MDSCC and PDSCC, again we found significantly the increased levels of mean serum TSA in PDSCC as compared to the MDSCC which were statistically significant with p value < 0.01. Yet not any previous study has given such correlation. The possible reason could be subjective variation between histopathologic grading and only one case of PDSCC which was studied. Tumor burden might be the cause of higher values of serum TSA level in MDSCC and PDSCC when compared with WDSCC. This statement is in agreement with Joshi<sup>19</sup> but it is against the Vora.<sup>5</sup> The present study also suggests strong correlation between habits of tobacco chewing/betel nut chewing/smoking<sup>32,33,34</sup> with increased levels of mean serum total sialic acid. This finding was inconsistent with Greenberg,<sup>4</sup> Kadam<sup>21</sup> and Kurtul.<sup>25</sup>

**CONCLUSION:**

Estimation of serum total Sialic acid (TSA) level in oral squamous cell carcinoma, is suggestive of a positive relation between TSA level and stage of malignancy, specifically with the tumor burden. Serum total Sialic acid level can be used as an adjunctive diagnostic marker as well as an early indicator of oral cancer. Future studies with larger sample size on this aspect of Sialic acid should be explored by the researchers.

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