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Total sialic acid, total protein and total sugar levels in serum and saliva of oral squamous cell carcinoma patients: A case control study

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ABSTRACT

Background: Detection of cancer at an early stage is of utmost importance to decrease the morbidity and mortality of the disease. Apart from the conventional biopsy, non-invasive methods like analysis of serum and saliva may provide cost-effective approach for screening a large population. Tumor markers are a major part of secondary prevention and thus, the detection of malignancies. The aim of this study was to evaluate total sialic acid (TSA), total protein and total sugar (TS) in serum and saliva of oral squamous cell carcinoma (OSCC) and controls to assess their role as a diagnostic marker.

Materials and Methods: Unstimulated whole saliva and sera were collected from 40 squamous cell carcinoma patients and 20 controls. Serum and salivary TSA, total protein and TS estimation was carried out. This was correlated with clinical stages and histopathological grades of OSCC. The data obtained was analyzed statistically using Chi-square test, ANOVA and Student's *t*-test with SPSS statistical software.

Results: A highly significant rise in the salivary sialic acid, serum sialic acid and serum protein was noted in OSCC subjects compared to controls. Salivary protein, serum and salivary sugar did not show any significance. Furthermore, serum and salivary sialic acid levels were found to be significantly increased with increasing level of histopathological grading.

Conclusion: The present study showed a significant increase in serum sialic acid, salivary sialic acid and serum protein from control to OSCC and suggests that these markers may be reliable in diagnosis and predicting prognosis.

Key Words: Saliva, serum, oral squamous cell carcinoma, total protein, total sialic acid, total sugar

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INTRODUCTION

Despite half century of intensive efforts throughout the world, cancer still remains an enigma. The incidence of head and neck cancer accounts for 30-40% of all malignant tumors in India.[1] Head and neck squamous cell carcinoma ranks 6th world-wide for cancer-related mortality, with an estimated 500,000 new cases diagnosed yearly.[2]

Recognition and diagnosis of early superficial cancer of the oral cavity is the most difficult task. It is usually asymptomatic for a long time and by the time the patient seeks advice, it would have invaded deeply and prognosis becomes poor. With the threat of high recurrence rate and secondary metastasis, the clinical decision for treatment plan and adjuvant therapy assumes importance.[3] Hence a reliable diagnostic and prognostic marker's need is felt.

In order to be clinically significant, any biochemical substance must be associated with tumor cells and must

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be present in appreciable quantity in tissue or fluid, where their concentration can be related to underlying tumor burden. Further it should be ascertained that the biochemical change are as a result of the cancer and has not occurred independently or prior to signs of cancer developed. The biochemical changes should correlate with the progression of the disease.[4] These biochemical substances are termed as tumor markers. A tumor marker is any substance that can be related to the presence or progress of a tumor. Classically a marker is synthesized by the tumor and released into circulation or expressed (at the cell surface) in larger quantities by malignant cells, than by their counterparts.[5]

Sialic acid is a protein-bound monosaccharide which occurs in combination with other mono-saccharides like galactose, mannose, glucosamine and fucose.[6] It is a constituent of many proteins in saliva and is mainly present in salivary mucin. Its level is studied for the evaluation of synthesis and secretion of glycoprotein.[7] It has been known to increase in patients with various malignancies and also in conditions like acute inflammation, high fever and rheumatoid arthritis.[6]

Aberrant glycosylations are the universal feature of cancer and the levels of these glycoconjugates increases as the cancer advances. Studies have shown that changes of serum sialic acid levels in cancer patients correlate well with reduction in tumor mass, recurrence and metastasis and has been considered as a valuable tumor marker in monitoring the clinical status of the carcinoma patient.[7]

Total sugar (TS) and total protein are the routine tests which are performed in patients suspected of any pathology and therefore any significant changes in them, if proved, can aid in early diagnosis. Majority of the previous studies have been focused on serum sialic acid and very few on salivary parameters. Saliva can serve as a valuable tool as it can be collected non-invasively and easily. Therefore, this study was undertaken to estimate salivary levels of total sialic acid (TSA), total protein, TS in oral squamous cell carcinoma (OSCC) patients and compare them with serum levels to aid in assessing their role as diagnostic marker for early detection of cancer and monitoring the progression of the disease.

MATERIALS AND METHODS

The present retrospective case control study was carried out at National institute of occupational health, Ahmedabad, India. It comprised a total of 60 subjects, which included 40 clinically and histopathologically diagnosed cases of OSCC and 20 healthy age and sex matched controls. Informed consent was obtained from all the subjects and ethical approval was obtained from the local ethical committee of the institute. The OSCC patients were categorized clinically according to the American joint Committee on Cancer-Tumor, lymph nodes, metastasis staging system[8] and histopathologically into well differentiated, moderately differentiated and poorly differentiated squamous cell carcinoma according to Border's criteria [Table 1].[9]

Serum and whole unstimulated saliva samples were collected from all the subjects to analyze TSA, total protein and TS levels. Saliva collection procedure was carried out between 10 am and 12 pm, 2 h after the subject's usual breakfast time to ensure minimum diurnal variation in composition. Prior to the collection, subjects were instructed to rinse the mouth thoroughly with distilled water and to void the mouth of saliva. Saliva was allowed to accumulate in the floor of the mouth and the subjects were asked to spit it out into sterile plastic containers making a volume of approximately 2-3 ml. Venous sample was collected from cubital vein under aseptic precautions. Both the saliva and blood samples were centrifuged at 3000 rpm for 15 min. The supernatant was taken for the estimation of TSA, total proteins and TS levels. Serum and salivary TSA level was determined by the method as described by Skoza and Mohos,[10] total proteins were estimated by Biuret method,[11] TS was estimated by Phenol-Sulfuric acid method.[12]

All the values of biochemical parameters so obtained were tabulated and analyzed statistically using SPSS 11 software. All the values were expressed as mean ± SD and two-tailed Chi-square test was used

Table 1: Details of oral squamous cell carcinoma patients

Age range	Sex distribution	Clinical staging of patients	Histopathological grading of patients
45-60 years	No. of males: 31	Stage I: 10	Well differentiated: 22
		Stage II: 16	Moderately differentiated: 10
	No. of females: 09	Stage III: 11	Poorly differentiated: 08
		Stage IV: 03	

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to assess the association between two parameters. Pearson's coefficient of correlation "*r*" was used to assess correlation. $P < 0.05$ was considered statistically significant.

RESULTS

Serum and salivary TSA and serum total protein were statistically significantly higher in OSCC patients as compared to controls. However, there was no statistically significant difference between other parameters analyzed [Table 2].

Comparison of serum and salivary TSA, total protein and TS with clinical stages of OSCC showed no statistically significant correlation [Table 3].

Correlation of serum and salivary TSA, total protein and TS with histopathological grades of OSCC showed the serum TSA was significantly higher in Poorly differentiated squamous cell carcinoma (PDSCC) as compared to Moderately differentiated squamous cell carcinoma (MDSCC) and Well differentiated squamous cell carcinoma (WDSCC). Furthermore, the salivary TSA was significantly higher in PDSCC as compared to MDSCC. However, the other parameters did not show any statistically significant correlation [Table 4].

Table 2: Comparison of serum and salivary TSA, TP and TS between OSCC and controls

	OSCC	Controls	*P* value
Serum			
TSA[#]	88.997±6.89	59.492±12.8397	0.000
TP[$]	7.48±0.5321	6.770±0.4092	0.000
TS*	139.2±212.2976	97.250±15.1618	0.383
Salivary			
TSA[#]	102.12±15.3856	40.941±5.7722	0.000
TP*	1.68±0.19	1.63±0.15	0.322
TS*	2.497±0.5521	2.63±0.3339	0.311

[#]TSA: Total sialic acid; [$]TP: Total protein; *TS: Total sugar; OSCC: Oral squamous cell carcinoma

There was a significant correlation in serum and salivary TS in the controls. The other parameters did not show any significant correlation [Table 5].

DISCUSSION

The aim of the present study was to analyze serum and salivary TSA, total protein and TS in OSCC patients and correlate these parameters with those of healthy controls and with different clinical stages and histopathological grades of OSCC.

In the present study, the serum TSA level was significantly higher in OSCC patients as compared to controls, similar to the observations of several other authors.[13-21] It has been demonstrated that sialic acid

Table 4: Comparison of serum and salivary TSA, TP and TS with histopathological grades of OSCC

	WDSCC (*n*=22)	MDSCC (*n*=10)	PDSCC (*n*=8)	*P* value
Serum				
TSA	79.249±14.359	84.468±25.96	88.064±9.3128	0.001
TP	7.545±0.5431	7.562±0.6696	7.27±0.3529	0.362
TS	164.5±286.35	111.625±18.255	105.6±14.5846	0.716
Salivary				
TSA	101.124±4.6035	102.118±7.3147	104.313±6.68	0.015
TP	1.661±0.1491	1.783±0.3267	1.6590.1470	0.299
TS	2.441±0.6246	2.7±0.4840	2.46±0.4274	0.52

TSA: Total sialic acid; TP: Total protein; TS: Total sugar; WDSCC: Well differentiated squamous cell carcinoma; MDSCC: Moderately differentiated squamous cell carcinoma; OSCC: Oral squamous cell carcinoma

Table 5: Correlation of serum and salivary TSA, TP and TS in OSCC and controls

	OSCC		Controls	
	r value	*P* value	*r* value	*P* value
TSA	0.296	0.064	−0.25	0.917
TP	0.203	0.209	−0.008	0.975
TS	0.154	0.344	0.547	0.013

OSCC: Oral squamous cell carcinoma; TSA: Total sialic acid; TP: Total protein; TS: Total sugar

Table 3: Comparison of serum and salivary TSA, TP and TS with clinical stages of OSCC

	Stage I (*n*=10)	Stage II (*n*=16)	Stage III (*n*=11)	Stage IV (*n*=3)	*P* value
Serum					
TSA	84.27±8.37	86.61±14.73	81.22±16.68	89.6±18.47	0.165
TP	7.64±0.47	7.38±0.30	7.42±0.55	7.73±0.86	0.634
TS	103.43±13.56	104.0±15.37	106.79±17.78	119.0±11.79	0.559
Salivary					
TSA	99.05±11.17	101.47±9.72	103.57±6.50	104.32±8.06	0.244
TP	1.63±0.119	1.66±0.218	1.71±0.22	1.64±0.026	0.785
TS	2.15±0.276	2.65±0.53	2.55±0.59	2.56±0.64	0.346

TSA: Total sialic acid; TP: Total protein; TS: Total sugar; OSCC: Oral squamous cell carcinoma

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increases at the tumor cell surface, so the increase in their serum levels may be related to their increased release through increased turnover, secretion, and shedding.[13-21] The mean value of serum total protein too was significantly higher in OSCC patients than in controls. The serum TS level also was higher in OSCC patients than in controls, but was not statistically significant. No studies are reported in the literature estimating serum total protein and sugar in OSCC and hence the results of our study can't be compared with any.

The mean value of salivary sialic acid was significantly higher in OSCC as compared to the controls in the present study. Similar results were also observed by Koç *et al*.[22] This may be explained on the basis that some of the tumor associated carbohydrate changes are not restricted only to the cell surface membranes but may also occur due to secreted mucin.[7] Salivary total protein did not show any significant increase in OSCC as compared to the controls. Krasteva *et al*.[23] and Sanjay *et al*.[24] showed increased values in OSCC as compared to controls. The cause and significance of this difference is not clear, these salivary proteins are non-specific and their exact significance and role in malignancy is not clearly understood.[24] Salivary TS levels did not show any significant difference in OSCC patients as compared to the controls. However, Sanjay *et al*. reported a significantly higher level in OSCC patients than in controls.[24] The reason for this difference in observations can't be exactly figured out but may be due to variations in the methodology of both studies.

In the present study, the mean value of serum TSA, total protein and TS did not show any significant correlation with the clinical stages of OSCC. However, Xing *et al*.[14] and Rajpura *et al*.[19] found a significant correlation between serum TSA and clinical stages of OSCC. They explained that the elevations in the levels of sialic acid appeared to reflect tumor burden and metastasis.[19] However, Xing *et al*. further inferred that sialic acid does not appear to be helpful in the diagnosis of early disease or as an adjunct tool to tumor staging based on clinical examination.[13] No studies are reported in the literature comparing serum total protein and sugar with clinical stages of OSCC.

The mean value of salivary TSA correlated well with the clinical stages of OSCC; however, this was not the case for salivary total protein and salivary TS. No studies are reported in the literature comparing these parameters.

The mean value of serum and salivary TSA was significantly higher in PDSCC followed by MDSCC and WDSCC and this finding for serum sialic acid is consistent with the results obtained by Rao *et al*.[15] This may be due to tumor differentiation and increased shedding of the malignant cells into the circulation as a result of metastasis.[15] However, Rajpura *et al*. did not find any significant correlation between these parameters.[19] Mean values of serum and salivary total protein and TS did not correlate with the histopathological grades of differentiation. Studies have not been reported in the literature for comparison of salivary sialic acid, serum and salivary total protein and TS with histopathological grades of OSCC.

The serum TSA, total protein and TS did not show any significant correlation with salivary TSA, total protein and TS in OSCC. In controls, the serum TSA and total protein showed a negative correlation with salivary TSA and total protein which was not significant while TS showed significant correlation. None of the studies have been reported in the literature for correlation of serum and salivary TSA, total protein and sugar in OSCC and controls.

CONCLUSION

The present study supports the earlier studies in the reliability of serum sialic acid as a tumor marker. Further our observations show that the salivary 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. Very few studies have been done on salivary sialic acid estimation and its use as a diagnostic tool would go a long way in early prognosis of squamous cell carcinoma. However, a longitudinal study with a large sample is needed to assess the reliability of these parameters as specific tumor markers.

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