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Research Article

SERUM AND SALIVARY SIALIC ACID AND L-FUCOSE AS PROGNOSTIC MARKERS IN POTENTIALLY MALIGNANT DISORDERS AND ORAL CANCER

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ABSTRACT

Background: Aberrant glycosylation is the universal feature of cancer and components of various glycoconjugates such as Sialic acid and L-fucose, are found to rise in various malignancies. The objective of this study was to evaluate the serum and salivary sialic acid and L-fucose in oral precancerous states and oral squamous cell carcinoma in order to investigate the possibility of using these as diagnostic markers.

Patients and Methods: The study included 85 subjects, who were grouped as control (30), precancer patients (25) and oral squamous cell carcinoma patients (30). Serum and unstimulated whole saliva was collected from subjects of all groups and sialic acid and L-fucose estimation was done using spectrophotometry. The results were tabulated and analyzed statistically.

Results: The mean serum sialic acid in normal, precancer and oral cancer group was 7.515, 19.620 and 55.235 mg/dl respectively while the levels of salivary sialic acid were 1.5113, 2.3302 and 9.0304 mg/dl respectively. The mean serum L-fucose was 3.3181, 19.1792 and 30.4897 mg/dl in normal, precancer and oral cancer group respectively and mean salivary L-fucose was 2.9363, 7.0258 and 11.6636 mg/dl respectively. A very highly significant rise ($P<0.005$) in serum and salivary sialic acid and L-fucose was observed in study subjects compared to control.

Conclusion: The present study showed a significant and gradual increase in serum and salivary sialic acid and L-fucose from control to precancer to squamous cell carcinoma. From this study we can suggest that these glycoconguate can be used as a reliable biomarker. As these glycoconguates are observed in saliva in detectable quantity, saliva can be used as a diagnostic medium for screening and early detection of oral cancer.

Keywords: Saliva, Serum, Sialic Acid, Fucose, Cancer, Premalignancy.

INTRODUCTION

Oral cancer remains one of the major causes of deaths accounting for nearly 50-70% of total cancer mortality and is a matter of great concern as 80,000 new cases of oral cancer are reported each year. Detection of oral cancer at an early stage is of utmost importance to decrease the morbidity and mortality of the disease. Apart from conventional biopsy, other methods like analysis of serum and saliva may provide a cost effective approach for screening as well as post therapeutic monitoring[1,2]. Despite rapid advances in multimodality therapy, the morbidity and mortality rates of this devastating disease have not improved in decades. Early detection of oral cancer is the most effective way to improve survival[3]. The treatment planning of oral cancer is mainly based on the tumour, node and metastasis (TNM) classification and histopathological diagnosis. These methods are subjective and often lack sensitivity to detect the disease in early stages. Furthermore, these methods do not reflect the aggressiveness of tumours, prognosis and response to therapy. Therefore, there is an urgent need to develop tumour markers to: identify high risk individuals, improve cancer detection in early stages, predict disease outcome and response to therapy[4,5].

Tumour markers are biochemical substances elaborated by tumour cells either due to the cause or effect of malignant process. These markers can be normal endogenous products

that are produced at a greater rate in cancer cells or the products of newly switched on genes that remained quiescent in the normal cells. A tumour marker produced by the tumour and, when present in significant amounts, indicates the presence of a cancer. They may be present as intracellular substances in tissues or may be released into the circulation and appear in serum. Continuing search for suitable tumour markers in serum, tissue and body fluids during neoplastic process is of clinical value in the management of patients with various malignancies[6].

As neoplastic changes are expressed at the cell surface, altered surface characteristics are essential for the abnormal growth and behaviour of malignant cells. Substances like glycoproteins and glycolipids are major constituents of cell membrane, and hence, cell-surface glycoconjugates are important in malignancy. Glycoproteins are usually defined as protein-carbohydrate complexes in which oligosaccharides and/or polysaccharides are joined by covalent linkage to specific amino acids of proteins. The carbohydrate portion contains aminosugars (glucosamine, galactosamine, or sialic acid) and hexoses (galactose, mannose or fucose)[7].

N-acetylneuraminic acid (referred to as sialic acid, SA) is a negatively charged nine carbon monosaccharide commonly attached by a glycosidic linkage to the non-reducing residues of the carbohydrate chains of glycoproteins and glycolipids[8]. Sialic acid is thought to be important in determining the surface properties of cells and has been implicated in cellular invasiveness, adhesiveness, and immunogenicity. These glycoconjugates are released into the circulation through increased turnover, secretion, and/or shedding from malignant cells[9].

Fucose is a monosaccharide that is a common component of many N and O-linked glycans and glycolipids produced by mammalian cells. Fucosylation of glycoproteins (the addition of L-fucose at the terminal end of the oligosaccharide chain) is one of the most important features that mediate several specific biologic functions. It has been documented that tumour cells modulate their surface by increasing fucosylation levels to escape recognition, which contribute to several abnormal characteristics of tumour cells, such as decreased adhesion and uncontrolled tumour growth. Several studies have suggested that monitoring serum/tissue fucose levels could be a promising approach for the early detection, diagnosis, and prognosis of various cancer types[9,10].

The significant correlation between total sialic acid (TSA), lipid associated sialic acid (LASA) and fucose in cancer patients indicated that they could be used as diagnostic criteria. Studies have shown that changes of serum sialic acid and fucose levels in cancer patients correlate well with reduction in tumour mass and recurrence and metastasis of the disease and has been considered as a valuable tumour marker in monitoring the clinical status of the carcinoma patient[9,10].

Many studies have been done to co-relate the levels of serum sialic acid and L-fucose and oral cancers. Surprisingly, almost no studies examined tumour markers in the saliva of oral squamous cell carcinoma (OSCC) patients. Such an examination might be of great benefit because of the direct contact between the oral cancer lesion and saliva[11]. Whole saliva can be collected non-invasively and by individuals with limited training. Thus, this study aims to estimate serum and salivary sialic acid and L-fucose in patients with oral precancerous states and carcinomas to evaluate their role in diagnosis and prognosis of the above diseases.

MATERIALS AND METHODS

Patients with oral precancerous lesions and conditions and oral carcinoma, visiting the Department of Oral Medicine and Radiology and nearby cancer centers during a period of 1.5 years were chosen for the study. The selection of the subjects for the study was done, based on the case history and clinical examination. Similarly, age and sex matched healthy volunteers without tobacco related oral habits or oral lesions were included in the control group. Thus the selected individuals were categorized in 3 groups:

A. Patients with clinically and histopathologically diagnosed with potentially malignant disorders of the oral cavity.

B. Patients with clinically and histopathologically diagnosed oral cancer.

C. Healthy individuals with good oral hygiene status without history of tobacco related oral habits and free of oral mucosal diseases.

Method of collecting blood sample: After obtaining an informed consent five ml of peripheral blood sample was collected from each subject with disposable syringes under aseptic conditions through venipuncture. Blood was collected and serum was separated by centrifugation at 3000rpm for 15 minutes. The samples thus collected were stored at -80°C until analyzed for sialic acid and L-fucose.

Method of collecting saliva sample: 1.5 to 2 ml of unstimulated whole saliva also was collected under resting condition during the hours 10AM-12AM, 2 hours after the subject's usual breakfast time, according to the method of Navazesh. [11] Saliva sample was centrifuged to remove unwanted particulates. The supernatant was separated and stored at -80°C until biochemical analysis was done.

1. **Estimation of sialic acid:** Estimation of sialic acid was estimated by the method of Yao et al.[12]In presence of acidic medium, sialic acid react with ninhydrin to form coloured product and can be measured colorimetrically at 470nm. The following reagents were used i) Acidic ninhydrin reagent: 250mg ninhydrin was dissolved in 6ml glacial acetic acid and 4ml concentrated sulphuric acid, by thorough vortexing for 30 minutes ii) N-acetyl neuraminic acid (NANA) standard: 10mg NANA was dissolved in 100ml distilled water.

Procedure: 0.1ml saliva was added to 0.9ml normal saline to make it 1ml and to this 4ml ethyl alcohol was added, mixed and centrifuged at 3000rpm for 30 minutes. The precipitate obtained was dissolved in 1ml distilled water, to this 1ml of glacial acetic acid and 1ml of acid ninhydrin reagent (freshly prepared) was added. This tube was labeled as "protein-bound sialic acid". 1ml supernatant from (1) was taken; to this 1ml glacial acetic acid and 1ml acidic ninhydrin reagent were added. This tube was labeled as "free sialic acid". Both the tubes were kept in boiling water bath for 10 minutes, cooled under tap water and absorbance was read at 470nm. NANA standards ranging in concentration from 20-1000µg/ml were

run simultaneously. The optical density value of sample was compared with that of standards at various concentrations S1, S2, S3, S4 and S5, and calculated accordingly. The concentration of sialic acid in each sample was expressed in mg/dl.

2. **Estimation of l-fucose:** L-fucose was estimated according to the method of Winzler [13] Fucose is a methylpentose present in glycoproteins. It can be assayed by dissolving ethanol precipitated proteins of serum in alkali, heating with sulphuric acid, and determining the color after the addition of cysteine. The color produced by hexoses under these conditions is corrected by determining absorbance at two wavelengths. The following reagents were used a) Ethyl alcohol – 95% b) Sulphuric acid water mixture (freshly prepared): 60ml of concentrated sulphuric acid was added carefully to 10ml of water and kept refrigerated until use. c)Cysteine reagent, 3% (W/V): 3g of cysteine hydrochloride was dissolved in water and made up to 100ml d) Sodium hydroxide, 0.1N: 400mg of sodium hydroxide was dissolved in water and made up to 100ml e) Stock fucose solution, 0.2% (W/V): 200mg of fucose was dissolved in water and made up to 100ml f) Working standard, 2mg/100ml (freshly prepared): 0.1ml of stock solution was diluted to 10ml with water.

Procedure: For each serum sample two 15 x 150 mm test tubes were prepared. One was marked 'serum-blank' and the other 'test'. 0.1ml of serum and 5ml of 95% ethyl alcohol were added to each tube and mixed on a Vortex mixer. The tubes were centrifuged at 1500rpm for 15 minutes, the supernatant was decanted and the precipitate was suspended in 5ml of 95% ethyl alcohol. It was re-centrifuged for 15 minutes and the supernatant was completely decanted. The precipitate was dissolved in 1ml of 0.1N NaOH. The 'reagent blank' and 'standard' tubes were prepared by adding 1ml of water and 1ml of working standard to appropriately marked 15 x 150mm test tubes. All the test tubes were placed in a rack in an ice water bath. 4.5ml ice cold sulphuric acid-water mixture was added to each tube. All the test tubes were mixed on a vortex mixer and transferred to an already boiling water bath and heated for exactly 3 minutes. Then they were cooled in tap water. 0.1ml of cysteine reagent was added to the 'reagent blank', 'standard' and 'test'. 0.1ml of water was added to the 'serum-blank'. They were immediately mixed on a Vortex mixer. After 60 minutes, the solutions were transferred to appropriate cuvettes and the absorbance was read at 400nm and 430nm in spectrophotometer set at zero with the 'reagent blank'.

RESULTS

The present study included 85 subjects, who were grouped as oral squamous cell carcinoma (30), oral precancer group (25) and normal control group (30). Serum and salivary samples were collected from all these subjects and were analyzed for sialic acid and L-fucose. The biochemical values of this study were subjected to statistical analysis to specify the statistical correlation between the groups and various parameters. Kruskal-Wallis test, Mann Whitney U test and Wilcoxon test were used to compare and correlate different parameters in subgroups.

The total sample size was 85 out of which 64 (75%) were males and 21(25%) were females. Out of 30 oral squamous cell carcinoma cases, 23 were males (77%) and 7 were females (23%).In oral precancer group, out of 25 cases, 23 were males (92%) and 2 were females (8%). In control group, out of 30 cases, 18 were males (60%) and 12 cases were females (40%) (Table 1)

Table 1: Male and Female Ratio in Study Groups

Sex	SCC	Precancer	Control	Total
Male	23 77%	23 92%	18 60%	64 75%
Female	7 23%	2 8%	12 40%	21 25%
Total	30 100%	25 100%	30 100%	85 100%

The age of the subjects in the OSCC group ranged from 25 to 75 years with a mean of 51 years. The age of the subjects in oral precancer group ranges from 18 years to 60 years with a mean age of 35 years. Age matched subjects with a mean age of 33 years were included in the control group (Table 2).

Table 2: Mean Ages in the Study Groups

Study group	Age range (in years)	Mean age (in years)
OSCC	25-75	51
Oral precancer	18 – 60	35
Control	21 – 52	33

Biochemical analysis of serum sialic acid was carried using spectrophotometric method described by Yao et al with some modifications. Values were recorded for all three groups including normal, precancer and OSCC groups. The serum sialic acid in normal group ranged from 1.0 to 27.5 mg/dl with mean value of 7.515 mg/dl and SD 6.8638. The serum sialic acid in precancer group ranged from 1.8 to 53.1 mg/dl with mean value of 19.620 mg/dl and SD 11.8366. The serum

sialic acid in OSCC group ranged from 18.6 to 168.9 mg/dl with mean value of 55.235 mg/dl and SD 32.5069. The difference in serum sialic acid in all the study groups was found to be very highly significant (P<0.0005). (Table- 3)

Table 3: Comparison of Serum Sialic Acid in Study Groups Analysed by Kruskal Wallis Test using SPSS Version 17

Serum sialic acid	N	Mean	Standard Deviation	Minimum	Maximum	Median	Inter-quartile Range	P
Normal	30	7.515	6.8638	1.0	27.5	4.890	7.8	<0.0005
Precancer	25	19.620	11.8366	1.8	53.1	19.300	17.5	
Oral cancer	30	55.235	32.5069	18.6	168.9	57.900	38.3	

When comparison of the result was done between the groups using Mann Whitney test, the difference in levels was found to be very highly significant (P<0.0005) between the normal and precancer group, normal and OSCC group and between the precancer and OSCC group. (Table-4)

Table 4: Comparison of Serum Sialic Acid among Study Groups Analysed by Mann Whitney Test using SPSS Version 17

Group	Mean	Standard Deviation	Median	Inter-quartile range	P
Normal Precancer	7.515 19.620	6.8638 11.8366	4.890 19.300	7.8 17.5	<0.0005
Normal OSCC	7.515 55.235	6.8638 32.5069	4.890 57.900	7.8 38.3	<0.0005
Precancer OSCC	19.620 55.235	11.8366 32.5069	19.300 57.900	17.5 38.3	<0.0005

Biochemical analysis of salivary sialic acid was carried using spectrophotometric method described by Yao et al with some modifications. Values were recorded for all three groups including normal, precancer and OSCC groups. The salivary sialic acid in normal group ranged from 0.20 to 7.30 mg/dl with mean value of 1.5113 mg/dl and SD 1.49423. The salivary sialic acid in precancer group ranged from 0.73 to 6.50 mg/dl with mean value of 2.3302 mg/dl and SD 1.31440. The salivary sialic acid in OSCC group ranged from 0.81 to 20.61 mg/dl with mean value of 9.0304 mg/dl and SD 6.21734. The difference in salivary sialic acid in all the study groups was found to be very highly significant (P<0.0005). (Table-5)

Table 5: Comparison of Salivary Sialic Acid in Study Groups Analysed by Kruskal Wallis Test Using SPSS Version 17

Salivary sialic acid	N	Mean	Standard Deviation	Minimum	Maximum	Median	Inter-quartile Range	P
Normal	30	1.5113	1.49423	.20	7.30	.9150	1.74	<0.0005
Precancer	25	2.3302	1.31440	.73	6.50	2.0400	1.22	
Oral cancer	30	9.0304	6.21734	.81	20.61	7.3400	9.09	

When comparison of the result was done between the groups using Mann Whitney test, the difference in levels was found to be highly significant (P=0.005) between the normal and precancer groupand very highly significant (P<0.0005) between normal and OSCC group and between precancer and OSCC group. (Table-6)

Table 6: Comparison of Salivary Sialic Acid among Study Groups Analysed by Mann Whitney Test Using SPSS Version 17

Group	Mean	Std. Deviation	Median	Interquartile range	P
Normal Precancer	1.5113 2.3302	1.49423 1.31440	0.9150 2.0400	1.74 1.22	=0.005
Normal OSCC	1.5113 9.0304	1.49423 6.2134	0.9150 7.3400	1.74 9.09	<0.0005
Precancer OSCC	2.3302 9.0304	1.31440 6.2134	2.0400 7.3400	1.22 9.09	<0.0005

The mean value of salivary sialic acid in normal group is 1.5113 mg/dl and serum fucose is 7.515 mg/dl. In precancer group, the mean value of salivary sialic acid is 2.3302 mg/dl and serum sialic acid is 19.260 mg/dl. In oral cancer group, the mean salivary sialic acid is 9.0304 mg/dl and serum sialic acid is 55.235 mg/dl. On comparison of serum and salivary sialic acid using Wilcoxon test in various groups, very highly significant (P<0.0005) difference was obtained in all the study groups. (Table-7)

Table 7: Comparison of Serum And Salivary Sialic Acid using Wilcoxon test

Group	Sialic acid	Mean	Standard Deviation	P
Normal	saliva serum	1.5113 7.515	1.49423 6.8638	<0.0005
Precancer	saliva serum	2.3302 19.620	1.31440 11.8366	<0.0005
Oral cancer	saliva serum	9.0304 55.235	6.21734 32.5069	<0.0005

Biochemical analysis of serum L-fucose was carried using spectrophotometric method described by Winzler with some modifications. Values were recorded for all three groups including normal, precancer and OSCC groups. The serum L-fucose in normal group ranged from 0.11to 17.40 mg/dl with mean value of 3.3181 mg/dl and SD 2.31129. The serum L-fucose in precancer group ranged from 11.17 to 18.46 mg/dl with mean value of 19.1792 mg/dl and SD 5.87947. The serum L- fucose in OSCC group ranged from 3.38 to 30.60 mg/dl with mean value of 30.4897 mg/dl and SD 18.01458. The difference in serum L-fucose in all the study groups was found to be very highly significant (P<0.0005). (Table-8)

Table 8: Comparison of Serum L-Fucose in Study Groups Analysed by Kruskal Wallis Test Using SPSS Version 17

Serum L-fucose	N	Mean	Standard Deviation	Minimum	Maximum	Median	Inter-quartile Range	P
Normal	30	3.3181	2.31129	.11	17.40	2.8850	2.92	<0.0005
Precancer	25	19.1792	5.87947	11.17	18.46	18.3000	8.50	
Oral cancer	30	30.4897	18.01458	3.38	30.60	27.4400	12.75	

When comparison of the result was done between the groups using Mann Whitney test, the difference in levels was found to be very highly significant (P<0.0005) between the normal and precancer group, normal and OSCC group and highly significant between the precancer and OSCC group (P=0.001). (Table-9)

Table 9: Comparison of Serum L-Fucose among Study Groups Analysed by Mann Whitney Test Using SPSS Version 17

Group	Mean	Standard Deviation	Median	Inter-quartile range	P
Normal Precancer	3.3181 19.1792	2.31129 5.87947	2.8850 18.3000	2.92 8.50	<0.0005
Normal OSCC	3.3181 30.4897	2.31129 18.01458	2.8850 27.4400	2.92 12.75	<0.0005
Precancer OSCC	19.1792 30.4897	5.87947 18.01458	18.3000 27.4400	8.50 12.75	=0.001

Biochemical analysis of salivary L – fucose was carried using spectrophotometric method described by Winzler with some modifications. Values were recorded for all three groups including normal, precancer and OSCC groups. The salivary L- fucose in normal group ranged from 0.38 to 17.40 mg/dl with mean value of 2.9363 mg/dl and SD 3.43755. The salivary L- fucose in precancer group ranged from 0.11 to 18.46 mg/dl with mean value of 7.0258 mg/dl and SD 3.67886. The salivary L- fucose in OSCC group ranged from 0.11 to 30.60 mg/dl with mean value of 11.6636 mg/dl and SD 7.07835. The difference in salivary L-fucose in all the study groups was found to be very highly significant (P<0.0005). (Table-10)

When comparison of the result was done between the groups using Mann Whitney test, the difference in levels was found to be very highly significant (P<0.0005) between the normal and precancer group and between normal and OSCC group. The difference was highly significant (P=0.006) between the precancer and OSCC group. (Table-11)

The mean value of salivary fucose in normal group is 2.9363 mg/dl and serum L-fucose is 3.3181 mg/dl. In precancer group, the mean value of salivary L-fucose is 7.0258 mg/dl and serum L-fucose is 19.1792 mg/dl. In oral cancer group, the mean salivary L-fucose is 11.6636 mg/dl and serum L-fucose is 30.4897 mg/dl. On comparison of serum and salivary L-fucose in various groups using Wilcoxon test, no significance was found (p=0.382) in the normal group. In the precancer and oral cancer group, high significance was found (p<0.005). (Table–12)

Table-10: Comparison of Salivary L-Fucose in Study Groups Analysed by Kruskal Wallis Test Using SPSS Version 17

Salivary L- fucose	N	Mean	Standard Deviation	Minimum	Maximum	Median	Inter-quartile Range	P
Normal	30	2.9363	3.43755	.38	17.40	1.3850	2.57	<0.0005
Precancer	25	7.0258	3.67886	.11	18.46	7.8200	4.23	
Oral cancer	30	11.6636	7.07835	.11	30.60	12.4000	9.47	

Table 11: Comparison of Salivary L-Fucose among Study Groups Analysed by Mann Whitney Test using SPSS Version 17

Group	Mean	Standard Deviation	Median	Inter-quartile range	P
Normal	2.9363	3.43755	1.3850	2.57	
Precancer	7.0258	3.67886	7.8200	4.23	<0.0005
Normal	2.9363	3.43755	1.3850	2.57	
OSCC	11.6636	7.07835	12.4000	9.47	<0.0005
Precancer	7.0258	3.67886	7.8200	4.23	
OSCC	11.6636	7.07835	12.4000	9.47	=0.006

Table 12: Comparison of Serum and Salivary L-Fucose using Wilcoxon test

Group	L-fucose	Mean	Standard Deviation	P
Normal	saliva	2.9363	3.43755	.382 ns*
	serum	3.3181	2.31129	
Precancer	saliva	7.0258	3.67886	
	serum	19.1792	5.87947	<.0005
Oral cancer	saliva	11.6636	7.07835	
	serum	30.4897	18.01458	<.0005

***not significant**

DISCUSSION

Tumour markers are a major part of the secondary prevention (detection) efforts. There are major logistic and economic constraints which could be overcome if a simple laboratory test could be devised that would indicate the presence of cancer-with a high degree of specificity and sensitivity-before there is metastasis. [11, 13]

Aberrant glycosylation is the universal feature of cancer. Glycoproteins 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 turnover, secretion and/or shedding from malignant cells. Increased level of different components of glycoproteins have been associated with different types of malignancies, for example higher serum sialic acid values have been observed in patients with malignant melanomas, breast cancer, cancer of stomach, gall bladder, colorectal cancer, endometrial cancer, laryngeal carcinoma, and oral cancer. [14] Likewise higher serum fucose level have been found in breast cancer, colorectal carcinoma, cervical cancer, lung cancer, brain tumours, and oral cancer. There is paucity of studies using saliva as a diagnostic fluid for oral cancer. Biochemical analysis of saliva is not been evaluated in detail in routine clinical laboratories. This prompted us to evaluate the levels of serum sialic acid and fucose as well as salivary sialic acid and fucose and correlate them. We found that serum as well as saliva levels of sialic acid and fucose increases in oral precancer states and oral cancer compared to the normal healthy state. [14]

Total sialic acid is a major constituent of glycoproteins and has been studied by several investigators and elevation of total sialic acid level in the serum of cancer patients has been reported. [15] In oral squamous cell carcinoma, many studies have reported increased levels of serum sialic acid and few studies were conducted in both precancer and oral cancer patients. [16]

In our study, the serum sialic acid in normal, precancer and oral cancer group was 7.515 mg/dl, 19.620 mg/dl and 55.235 mg/dl respectively. Results of these studies are consistent with our study, where increased levels of mean serum total sialic acid values were found among oral precancer states and oral cancer patients when compared to control group.

The mean serum sialic acid in the precancer and oral cancer group was found to be 47.74 mg/dl and 54.78 mg/dl respectively in the study done by Baxi et al [17]. In the study done by Delphine et al [18], the mean serum sialic acid in the normal and oral cancer group was 82.1 mg/dl and 114 mg/dl respectively. In the study done by Raval et al [19] also, the mean values of serum sialic acid in normal, precancer and oral cancer was 44.62 mg/dl, 52.28 mg/dl and 63.20 mg/dl respectively. Values obtained in these studies are higher than the values in our study. Variation in the values can be explained to be related to the different methods followed for the estimation of serum sialic acid in these studies and in our study.

Studies have also shown that the serum sialic acid levels rises with the clinical stage of the oral cancer but the study done by Xing et al [20] suggested the usefulness of serum sialic acid as an adjunct in clinical staging is limited. Serum sialic acid also correlated with tumour size and lymph node metastasis. Moreover, in the study done by Kimura et al [21] sialic acid was found to be strongly associated with survival rate and hence the authors concluded that serum sialic acid is useful as prognostic factor. Serum sialic acid has been shown to reduce after the treatment of oral cancer and thus can also be used as an adjunct for treatment monitoring as shown by many studies.

On estimation of salivary sialic acid in our study, the mean value in normal, precancer and oral cancer was found to be 1.5113, 2.3302 and 9.0304 mg/dl respectively. To compare our results, only few studies are available in the literature. Salivary sialic acid has been shown to rise in individuals with various types of cancer in a study by Koc L et al. [22] The mean salivary sialic acid in their study was 185mg/dl. This value is higher than the value obtained in our study which can be explained based on the fact that in our study only oral cancer patients were included whereas in the study done by Koc et al various types of cancers were included. Sanjay PR et al [23] and Markopoulos AK et al [24] estimated free and protein bound salivary sialic acid in OSCC patients. The mean value of free and protein bound sialic acid in oral cancer was 4.3mg/dl and

3.02mg/dl respectively. Their findings are consistent with our results where significant increase in the levels of salivary sialic acid was found among oral cancer and oral precancer patients. In these studies, comparison of sialic acid in different grades of OSCC was also done and significant difference was found between well differentiated and poorly differentiated OSCC.

Serum fucose has been shown to be elevated in various malignancies, including breast cancer, colorectal cancer, cervical cancer and brain tumours. Serum fucose level has been shown to rise in oral squamous cell carcinoma in few studies done. Estimation of serum fucose and its correlation with potentially malignant disorders is done by only two researchers. Therefore, we have included estimation of serum fucose as one of the parameters our study. Mean serum fucose in normal, precancer and oral cancer group in our study was 3.3181, 19.1792 and 30.4897 mg/dl respectively. The rise of serum L-fucose from normal to precancer and oral cancer was found to be highly significant (P<0.0005) on statistical analysis. In the study done by Parwani et al [23] , the mean serum L-fucose in normal and OSCC group was 5.323 and 15.34 mg/dl respectively. The variation in values in our study can be explained on the basis of difference in the method followed for the estimation. All the studies done on OSCC patients have shown a significant rise (P<0.001) of serum fucose in oral cancer patients compared to the control group which is consistent with our findings. Studies done on potentially malignant disorders also have shown that serum fucose levels rise in precancer states as well. These results are also consistent with our findings where significant rise was observed in precancer states compared to the control group.

Serum fucose level has been shown to be a measure of the tumour spread and the serum sialic acid as an index of acute phase response. Serum fucose has been shown to correlate with the clinical stage of OSCC in many studies. On comparison of serum sialic acid and serum fucose levels post treatment, Taneja et al [24] concluded that serum sialic acid is a better prognostic marker malignancy. According to Shashikanth et al [25], serum fucose is a better marker than serum sialic acid as in their study, fucose levels correlated well with the clinical stage of OSCC whereas sialic acid did not.

No study in our knowledge has been published regarding the salivary L-fucose in oral cancer and precancer states. In our study it was found that the salivary L-fucose increases in the precancer and oral cancer patients as compared to the normal healthy individuals and the mean values obtained in normal, precancer and oral cancer group were 2.9363, 7.0258 and 11.6636 mg/dl. The elevation of salivary L-fucose from normal to precancer and oral cancer was found to be highly significant (P<0.0005).

In our study, the comparison between serum and salivary sialic acid and L-fucose was done. This comparison, in our knowledge, has not been done in any studies done previously. The mean serum and salivary sialic acid in normal group was 7.515 and 1.5113 mg/dl respectively. In precancer group, the mean serum and salivary sialic acid was 19.620 and 2.3302 mg/dl respectively. The value of mean serum and salivary sialic acid in the oral cancer group was 55.235 and 9.0304 mg/dl respectively. On statistical analysis, it was found that the sialic acid in serum was significantly higher than in saliva (P<0.0005) in all the three groups. The mean values of serum and salivary L-fucose were also compared in all the groups. In normal group, the mean serum and salivary L-fucose was 3.3181 and 2.9363 mg/dl respectively. In precancer group, the mean serum and salivary L-fucose was 19.1792 and 7.028 mg/dl respectively. The mean serum and salivary L-fucose in oral cancer group was 30.4897 and 11.6636 mg/dl respectively. Highly significant difference (P<0.0005) was found in the serum and salivary L-fucose in the precancer and oral cancer groups on statistical analysis but in the normal group, the difference was not significant (P=0.382).

A major drawback to the use of saliva as a diagnostic fluid has been the notion that informative analytes are generally present in lower amounts in saliva than in serum. For this reason, the difference in the values of sialic acid and L-fucose in serum and saliva can be explained. Though the sialic acid and fucose values are less in saliva than serum, they are present in detectable amount in saliva and significantly rise in precancer and oral cancer. Thus, salivary sialic acid and L-fucose can be used as a biomarker for screening purposes for oral cancer. There are compelling reasons to use saliva as a diagnostic fluid to monitor health and diseases. As a clinical medium, saliva has many advantages over serum. Saliva is easy to collect, store and ship and can be obtained at low cost in sufficient quantities for analysis. For patients, the non-invasive collecting techniques dramatically reduce anxiety and discomfort and simplify procurement of repeated samples for longitudinal monitoring over time. For professionals, saliva collection is safer than venepuncture, which could expose health care providers to HIV or hepatitis virus. Saliva is also easier to handle for diagnostic procedures since it does not clot, lessening the manipulations required. Saliva-based diagnostics are therefore less invasive, less expensive and present less risk to both the patient and the provider than current methodologies. [26 -30]

Although utmost care was taken in conducting this study, further research is recommended to re-confirm our findings with more samples and better techniques available for the estimation of the sialic acid and L-fucose.

Our study throws light on the promising future of saliva to be used as a diagnostic medium. Salivary cancer diagnostics is in its infancy and the salivary literature is a mixture of apples and oranges. There is necessity for additional research and more efforts to overcome the multitude of barriers. Field of salivary diagnostics offers scope for further research for early diagnosis, treatment monitoring and determining prognosis of this dreaded disease.

CONCLUSION

Elevated levels of serum and salivary sialic acid and L-fucose in oral precancer and cancer indicate its importance as a biomarker and may have a potential for using for early diagnosis. As these biomarkers were present in detectable amount in saliva, saliva can be used as a diagnostic medium and it provides a more convenient and non-invasive alternative to blood investigation.

REFERENCES

1. Shah M, Telang S, Raval G, Shah P, Patel PS. Serum fucosylation changes in oral cancer and oral precancerous conditions: alpha-L-fucosidase as a marker. Cancer 2008; 113(2): 336-46.
2. Kemal M, James D, Klee GG. Use and limitation of serum and total and lipid bound sialic acid concentration as markers for colorectal cancers. Cancer 1985; 55(2): 404-409.
3. Ralhan Ranju. Diagnostic potential of genomic and proteomic signatures in oral cancer. Int J Hum Genet 2007; 7(1): 57-66.
4. Rao VR, Krishnamoorthy L, Kumaraswamy SV, Ramaswamy G. Circulating levels in serum total sialic acid, lipid associated sialic acid and fucose in precancerous lesions and cancer of the oral cavity Cancer Detect Prev 1998; 22(3): 237-40.
5. Goodarzi MT, Shafiei M, Nomani H, Shahriarahmadi A. Relationship between total and lipid-bound serum sialic acid and some tumour markers. Iran J Med Sci 2005; 30(3):124-127.
6. Nagler R, Bahar G, Shpitzer T, Feinmesser R. Concomitant analysis of salivary tumor markers—a new diagnostic tool for oral cancer. Clin Cancer Res 2006; 12(13): 3979-84.
7. Harvey HA, Lipton A , White D and Davidson E. Glycoproteins and Human Cancer:II. Correlation between Circulating Level and Disease Status. Cancer 1981; 47: 324-327.
8. Nonda Katopodis,Yashar Hirshaut, Nancy L. Geller and C. Chester Stock. Lipid-associated sialic acid test for the detection of human cancer. Cancer Res 1982; 42: 5270-5275.
9. Shamberger RJ. Serum sialic acid in normals and in cancer patients. J Clin Chem Clin Biochem 1984; 22(10): 647-51.
10. Plucinsky MC, Riley WM, Prorok JJ and Alhadeff JA. Total and lipid-associated serum sialic acid levels in cancer patients with different primary sites and differing degrees of metastatic involvement. Cancer 1986; 58: 2680-2685.
11. Navazesh M. Methods for collecting saliva. Ann NY Acad Sci 1993; 20:72.
12. Yao K, Ubuta T, Masuoka N, Kinuta M, Ikeda T. Direct determination of bound sialic acids in sialoglycoproteins by acidic ninhydrin. Anal Biochem 1989; 179(2): 332-5.
13. Winzler RJ. In: Methods of biochemical analysis. Glick D, editor. New York:Interscience Publishers Inc; 1955. pp. 279–377.
14. Kossowska B, Ferens-Sieczkowska M, Gancarz R, Jankowska R. Fucosylation of serum glycoproteins in lung cancer patients. Clin Chem Lab Med 2005; 43(4):361-9.
15. Upadhya S, Upadhya S, Prabhu K.S. Serum glycoconjugates and ceruloplasmin in cancer of uterine cervix. Indian J Clin Biochem 2002; 17(1): 20-24.
16. Dreyfuss AI, Clark JR and Andersen JW. Lipid-associated sialic acid, squamous cell carcinoma antigen, carcinoembryonic antigen, and lactic dehydrogenase levels as tumor markers in squamous cell carcinoma of the head and neck. Cancer 1992; 70: 2499-2503.
17. Bina R. Baxi, Prabhudas S. Patel, Siddharth G. Adhvaryu and Promod K. Dayal. Usefulness of serum glycoconjugates in precancerous and cancerous diseases of the oral cavity. Cancer 1991; 67: 135-140.
18. Silvia WD, Vasudevan DM and Prabhu KS. Evaluation of serum glycoproteins in oral carcinoma. Indian J Clin Biochem 2001;16(1): 113-115
19. Raval GN, Patel DD, Parekh LJ, Patel JB, Shah MH, Patel PS. Evaluation of serum sialic acid, sialyltransferase and sialoproteins in oral cavity cancer. Oral Dis 2003; 9:119–128
20. Xing RD, Wang ZS, Li CQ, Tang QY, Jiang CB, Zhang YZ. Total sialic acid as a tumor marker for oral cancer. Int J Biol Markers 1994; 9(4):239-42.
21. Kimura Y, Fujieda S, Takabayashi T, Tanaka T. Conventional tumor markers are prognostic indicators in patients with head and neck squamous cell carcinoma. Cancer Lett 2000; 155(2): 163-8.
22. Koç L, Yarat A, Emekli N, Serdengeçti S, Berkarda B. Salivary sialic acid and cancer. J Marmara Univ Dent Fac 1996;2(2-3):523-6.
23. Sanjay PR, Hallikeri K, Shivashankara AR. Evaluation of salivary sialic acid, total protein, and total sugar in oral cancer: A preliminary report. Indian J Dent Res 2008;19(4):288-291.
24. Markopoulos A K, Michailidou E Z, Tzimagiorgis G. Salivary markers for oral cancer detection. Open Dent J 2010; 4: 172-178.
25. Parwani RN, Parwani SR. Quantitative evaluation of serum fucose in oral squamous cell carcinoma patients. J Cancer Res Ther 2011;7 (2):143-147
26. Taneja N, Bathi RJ, Parveen S, Bhat K. Serum glycoproteins as prognosticator in Oral Cancer Patients-A follow up study. Int J Oral Med Sci 2009; 8: 74–82.
27. Shashikanth MC, Rao BB. Study of serum fucose and serum sialic acid levels in oral squamous cell carcinomia. Indian J Dent Res. 1994; 5(4): 119-24.
28. Miller SM. Saliva testing—a nontraditional diagnostic tool. Clin Lab Sci 1994;7:39-44.
29. Bathi RJ, Nandimath K, Kannan N, Shetty P.Evaluation of glycoproteins as prognosticators in head and neck malignancy. Indian J Dent Res. 2001 Apr-Jun; 12(2): 93-9.
30. Rajpura KB, Patel PS, Chawda JG, Shah RM. Clinical significance of total and lipid bound sialic acid levels in oral pre-cancerous conditions and oral cancer. J Oral Pathol Med. 2005; 34(5): 263-7.

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