Effect of Non-Surgical Periodontal Therapy on the Serum Sialic Acid Levels in Diabetic Patients with Periodontitis

Reshma Suresh[1,*], Perayil Jayachandran[1], Angel Fenol[1], Raja Biswas[2], Sajitha Krishnan[3], K. Aswini Kumar[4], Darshan Devang Divakar[5],Sajith Vellappally[6]

ABSTRACT

Sialic acid (SA), a family of acetylated derivatives of neuraminic acid, an acute phase reactant by itself. It usually occurs as a terminal component at the non-reducing end of carbohydrate chains of glycoproteins and glycolipids. SA participates in multiple physiological functions, such as cell-to-cell interactions, cell migration and proliferation. Diabetes mellitus (DM) is a chronic metabolic disorder characterized by rise in blood glucose level. Periodontitis is a chronic inflammatory disease of the periodontal tissue, leading to destruction of bone surrounding the tooth and ultimately tooth loss. There is a two way relationship between diabetes mellitus and periodontitis. Periodontitis is the sixth complication of diabetes along with retinopathy, nephropathy, neuropathy, macrovascular disease, and altered wound healing. Inflammatory mediators like interleukin-6 and tumor necrosis factor-alpha produced during periodontal inflammation can interfere with the actions of insulin receptors and worsen the glycemic control of diabetic patients.

Periodontitis is a major cause of tooth loss, affecting over 300 million people and bacteria associated with periodontitis are also linked with systemic problems like endocarditis, atherosclerosis. Recent work has highlighted a major role for the host sugar sialic acid in the biofilm physiology and host-pathogen interactions of T. forsithya, a key periodontal pathogen.

There exists a need for a biomarker, for early detection of disease evolution and more robust therapy efficacy measurements. Serum sialic acids were estimated in Indian population by diphenylamine method and Thiobarbituric acid method. The average values were 68 ± 2.6 mg percent by DPA method and 56 ± 5 mg percent by TBA (thiobarbituric acid assay) method. Age and sex showed no influence on serum sialic acid level. Objectives of the present study was to compare (TSSA) level in healthy subjects, subjects with (CMP) with and without (NIDDM) and its effect on non-surgical periodontal therapy.

In the present study, the participants were divided into three groups: Group A, B and C. Group A consists of systemically healthy subjects, Group B consists of subjects with (CMP) while Group C consists of subjects with (CMP) with (NIDDM) and results of this study indicated that, at baseline, there were significant differences between Group A, B and Group C with respect to all the clinical parameters, including (GI), (OHI-S), (PPD), (CAL), (TSSA) and (HbA1c) levels. Thus (TSSA) level could be considered as novel biomarker in the progression of periodontal disease and diabetic status. Periodontitis could be considered as a potential, modifiable, and independent risk factor for the development of diabetes. Early detection of elevated (TSSA) level may help in interpreting the progression of periodontitis, risk of development of diabetes mellitus in future and also to prevent complications.

KEYWORDS

total serum sialic acid; chronic periodontitis; diabetes mellitus; biomarker; non-surgicalperiodontal therapy; acute phase proteins

AUTHOR AFFILIATIONS

[1] Department of Periodontics, Amrita School of Dentistry, Amrita vishwa vidhyapeetham, Ponekara, Edapally, Cochin, India
[2] Nano Science and Molecular Biology, Amrita institute of medical science, Cochin, India
[3] Department of Biochemistry, Amrita Institute of Medical Science, Cochin, India
[4] Department of Prosthodontics, Amrita School of Dentistry, Cochin, India
[5] Dental Biomaterials Research Chair, Dental Health Department, College of Applied Medical Sciences, King Saud University, Riyadh, Saudi Arabia
[6] Division of Preventive Dentistry, Dental Health Department College of Applied Medical Science, King Saud University, Riyadh, Saudi Arabia
* Corresponding author: Department of Periodontics, Amrita School of Dentistry, Amrita vishwavidhyapeetham, Kochi, 682041, Kerala, India; e-mail: resh.doc53@gmail.com

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INTRODUCTION

Periodontitis is a chronic inflammatory disease affecting the periodontium and resulting in progressive attachment and alveolar bone loss (1). During the disease process, there is an increase in production of proinflammatory mediators like tumor necrosis factor–alpha [TNF-α], interleukin-6 [IL-6], interleukin-1beta [IL-1β], and interferon gamma [IF-γ]; and elevated levels of acute phase proteins like capsular reactive protein, C-reactive protein(CRP) and thus causing insulin resistance and apoptosis of pancreatic β cells.

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. It is characterized by a cascade of events like activation of acute phase proteins, release of proinflammatory cytokines. In 2010, it was estimated that 285 million people had been diagnosed with diabetes mellitus worldwide, with a prevalence of 6.4%. This is predicted to reach up to 439 million, a prevalence of 7.7%, by 2030 (2). Diabetes mellitus and periodontitis are chronic diseases which are highly prevalent in the world population and its association have been proved by many investigators. Periodontitis is considered as the sixth complication of diabetes mellitus (3). There exists a bidirectional relationship between diabetes mellitus and periodontitis and this fact is confirmed by many authors (4). Sialic acid (SA) belongs to a family of acetylated derivatives of neuraminic acid, an acute phase protein by itself, a nine carbon acidic monosaccharide that occur naturally at the end of sugar chains attached to the surface of cells and soluble proteins (5). An important function of host SA is to regulate innate immunity (6). The concentration of SA in human serum is abnormally high during tissue destruction, tissue proliferation, depolymerization or inflammation. Elevated level of serum SA has been seen in malignancy, diabetic mellitus, and coronary artery disease (7).

Acute phase inflammation has been suggested to be associated with infectious diseases such as periodontal diseases. SA occupies the interface between the host and pathogenic microorganisms. Micro-organisms incorporate SA into their cell surface, which helps them evade the innate immune response of the host. Removal of terminal SA either by neuraminidase (sialidase) enzyme of virulent bacteria or by inherited disorder of host endogenous neuraminidase from sialylated glycoprotein, could incorporate onto the surface of developing plaque which may play a role in its formation and cause destruction of host tissue. There exists a need for a biomarker, for early detection of disease evolution and more robust therapy efficacy measurements.

MATERIAL AND METHODS

Systemically healthy subjects without periodontitis, subjects with chronic moderate periodontitis (CMP) with and without Non-insulin dependent diabetes mellitus (NIDDM), with (HbA1c) ≤ 7 reporting to Out Patient Department of Periodontics, were selected. After obtaining the written informed consent and the ethical committee approval, the selected subjects were divided into three groups; Group A [Systemically healthy subjects without periodontitis, having Oral Hygiene Index – Simplified (OHI-S) score 0.8 to 1, Gingival Index (GI) score 0.1 to 1, with Probing pocket depth (PPD) not >3 mm and with no Clinical Attachment Loss (CAL)], Group B [Systemically healthy subjects with CMP as defined by CAL 3–4 mm, having OHI-S score 1.3 to 3, GI score 1.1 to 2, with PPD ≥3 to ≤5 mm, CAL ≥3 to ≤4 mm in 5 or more teeth were selected]. The groups A and B had the Random blood sugar (RBS) levels <140 mg/dl, and Group C [Subjects with CMP with NIDDM whose HbA1c levels were ≤7, having OHI-S score 1.3 to 6, GI score 1.1 to 3, PPD ≥3 to ≤5 mm and CAL ≥3 to ≤4 mm in 5 or more teeth, and the RBS level was >140 mg/dl]. All participants in the study were between 35 to 65 years with ≥20 natural teeth present at the time of study. Subjects with history of other systemic diseases, those who had used antibiotics in the preceding 6 months, pregnant ladies, lactating mothers, smokers, alcoholics, and those who had undergone periodontal therapy in the past six months were excluded from the study.

A total of 100 subjects were found to be eligible for participating in this comparative study. 30 subjects met the criteria for Group A, 35 subjects each, met the criteria for Group B and Group C. Of these 100 subjects, 60 subjects completed the study,20 in each group. The attrition of subjects was compensated by the initial intake of extra samples. For all the selected subjects, the periodontal clinical parameters like OHI-S, GI PPD, CAL were recorded by a single examiner at baseline,one,three and six months.The PPD and CAL were assessed using Williams graduated probe markings at six sites per tooth (mesio-buccal/labial, mid-buccal/labial, disto-buccal/labial, mesio-lingual/palatal, mid-lingual/palatal, disto-lingual/palatal) in all teeth, excluding third molars. (Fig. 1) To ensure reproducibility during examinations, a customized acrylic stent was used as a reference to determine site and angle of insertion of periodontal probe. Total serum sialic acid (TSSA), HbA1c levels were also evaluated at baseline, one, three and six months.

Four ml and two ml of peripheral venous blood was collected from antecubital vein of the study subjects and stored at –20 °C. Two ml blood in violet vacutainers was thawed for HbA1c level estimation whereas four ml blood stored in red vacutainers was centrifuged to separate serum and given for TSSA estimation using Ehrlich's method by trained nurses using a commercially available ion-exchange (HPLC) device (BIO-RAD D10) at Department of Nano medicine and Biochemistry.

All the selected subjects in the study had confirmed their glycemic level status by measuring their RBS level using glucometer (ONETOUCH Select Simple Blood Glucose Monitoring System) and their systemic conditions were evaluated by thorough medical history. All subjects had their RBS < 140 mg/dl except for subjects in group C. The weight of subjects in kilogram and height in meters were recorded and their Body Mass Index BMI was calculated. After recording the clinical parameters and collection of blood samples for TSSA and HbA1c estimation, all subjects received oral hygiene instructions and non-surgical periodontal therapy which included thorough supragingival scaling, performed using piezoelectric ultrasonic scaler, and root planing using gracey curettes. The clini-

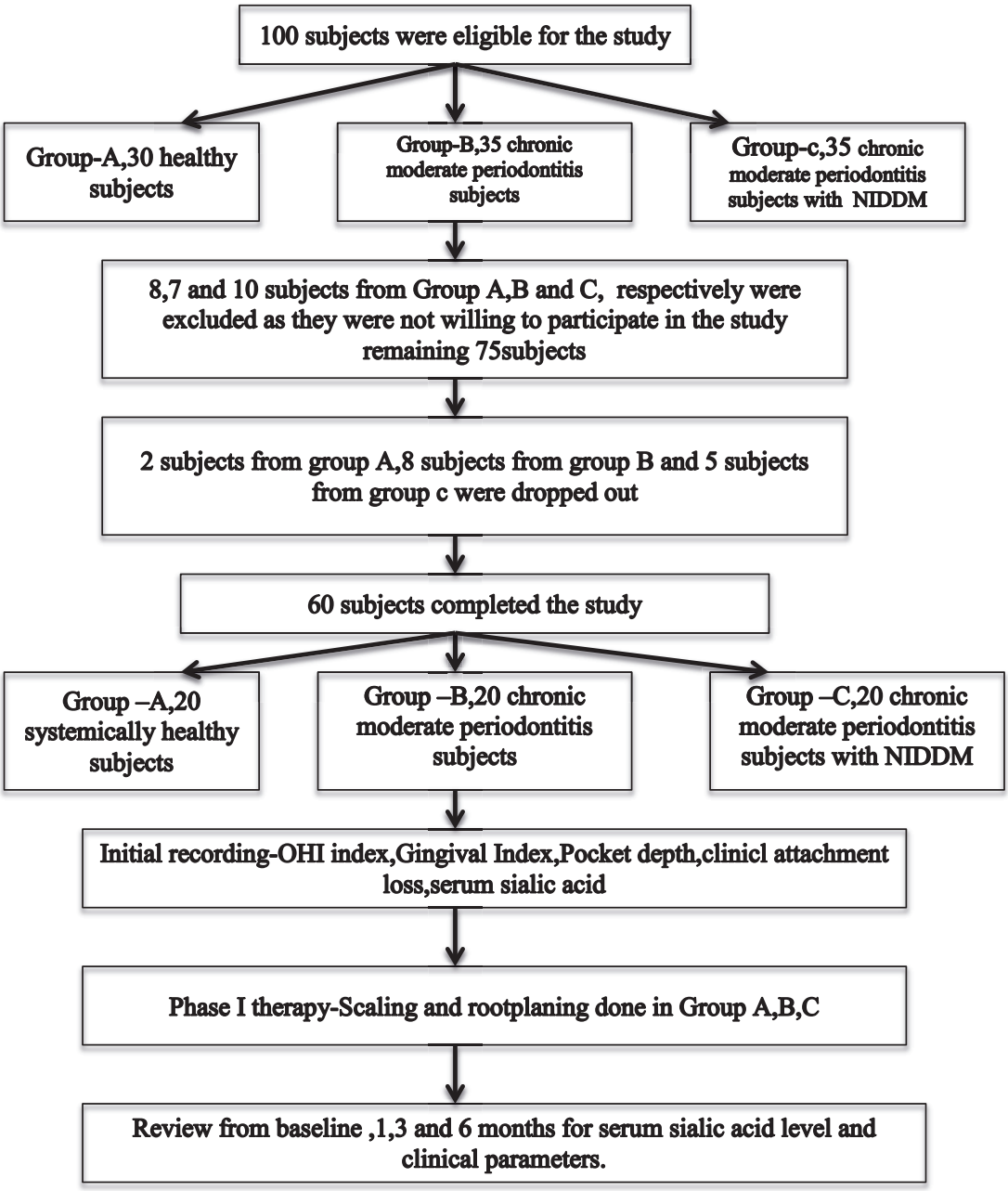

Fig. 1 Flowchart showing flow of patient selection and dropouts.

cal parameters which were recorded at the baseline were again evaluated during recall visits at one, three and six months intervals.

ESTIMATION OF TSSA

Preparation of standard solution: Sialic acid solution (standard):

The standard neuraminic acid (sigma) contained total of 25 mgs. Out of this, 12.5 mg was weighed and mixed with 5 ml of milli Q water to get 2.5 µg/µl of sialic acid. Samples of 200 µl were mixed with 400 µl of 0.2 N H_2SO_4 and incubated in dry bath at 80 OC for 1 hour. Then 1 ml of 10% Trichloroacetic acid (TCA) was added and mixed, after which the solution was centrifuged at 3000 rpm for 5 minutes. The supernatant (250 µl) was collected for analysis. It was diluted with 1ml of milli Q water and a further 250 µl of Ehrlich's reagent was added. The solution was boiled in the water bath for 30 minutes. The reaction was controlled by cooling the samples in an ice bath. Serum samples and standard samples were added in the range of 0, 40, 80 and 160 µl to the microtiter well and were subjected to Ehrlich's method for TSSA. Absorbance of the color was measured by a spectrometer at 650 nm. The pure sialic acid (2.5 µg/µl of tube) was used as the standard and 0.2N H_2SO_4-10% (TCA) was used as a blank. 40 µl of serum collected from each subject was taken as the test sample and quantification of TSSA in individual samples was performed in triplicates.

STATISTICAL ANALYSIS

Statistical tests were performed using the software Statistical Package for Social Sciences (SPSS), version 20.

The mean BMI, PPD, CAL, GI, OHI-S, HbA1c and TSSA levels were calculated per group. Intergroup comparisons of age and (BMI) were performed using ANOVA. Chi-square test was used for finding the association of TSSA with gender. Inter group and intra group comparison of parameters at baseline, one, three and six months recall interval were performed using ANOVA test and paired *t*-test. Independent sample *t*-test was used for intergroup comparison in Group B and C for PPD and CAL. Post hoc analysis was done using Bonferroni for multiple comparisons. Karl Pearson correlation was used to test correlation of clinical parameters, HbA1c levels with TSSA levels. The results were considered statistically significant when *p*-value was ≤0.05.

Tab. 1 Comparison of Gender, Age and BMI between Group A, B and C.

			Groups			P value
			A	B	C	
Gender	Males	count	10	16	14	
		% within gender	50%	61.5%	49.5%	0.201
	Females	count	10	14	16	
		% within gender	50%	38.5%	50.5%	
Age	Mean		48	44	47	0.132
	Standard deviation		7.13	7.20	5.50	
BMI (kg/m^2)	Mean		18.27	19.34	20.41	0.48
	Standard deviation		3.1	3.2	3.11	
n			20	20	20	

Baseline comparison of general characteristics.

RESULTS

Comparison of TSSA levels in systemically healthy subjects, subjects with CMP with and without NIDDM, and the effect of non-surgical periodontal therapy on TSSA levels in these groups and its influence on diabetic status were assessed in this study. Table 1 shows the inter group comparison of age, sex and BMI. There was no statistically significant difference between the groups with respect to age and sex, however, BMI showed a statistically significant difference between the groups ($p < 0.05$).

Table 3 shows intergroup correlation of BMI with TSSA levels indicating no correlation between BMI with TSSA levels between the groups ($p > 0.05$). Table 2 shows intergroup comparison of OHI-S and multivariate intergroup comparison of (OHI-S) at baseline and six months. At baseline, the mean OHI-S of Group A, B and Group C were 1.13 ± 0.15, 3.17 ± 0.64 and 3.51 ± 0.45 respectively. At six months recall, for Group A, Group B and group C the mean OHI-S were 0.47 ± 0.03, 0.90 ± 0.25, 0.96 ± 0.16 respectively. Inter group comparison using ANOVA test indicated that there was statistically significant difference in OHI-S between the groups at baseline ($p < 0.05$), but at the end of six months recall there was no statistically significant difference between the groups ($p > 0.05$) except between group C and the other two groups. Intra group comparison using paired *t*-test showed that for Group A there was no statistically significant difference in OHI-S at baseline and at the end of six months recall ($p > 0.05$), Group B showed a statistically significant difference in OHI-S at baseline and at the end of six months recall ($p < 0.05$). While Group

Tab. 2 Comparison of clinical parameters between Group A, B and C.

		Baseline	1 month	3 months	6 months	P value
OHI-S	Group A	1.13 ± 0.15	0.90 ± 0.10	0.63 ± 0.07	0.47 ± 0.03	0.000
	Group B	3.17 ± 0.64	2.25 ± 0.59	1.36 ± 0.50	0.90 ± 0.25	0.000
	Group C	3.51 ± 0.45*‡	2.67 ± 0.41	1.95 ± 0.41	0.96 ± 0.16*	0.000
Gingival index	Group A	0.80 ± 0.22	0.59 ± 0.15	0.48 ± 0.12	0.45 ± 0.10	0.000
	Group B	2.61 ± 0.36*	1.97 ± .30	1.23 ± 0.23	0.81 ± 0.22	0.000
	Group C	2.31 ± 0.39	1.73 ± 0.32	1.30 ± 0.23	0.90 ± 0.19*	0.000
Probing pocket depth	Group A	2.12 ± 0.78	2.76 ± 0.67	2.56 ± 0.64	2.49 ± 0.64	0.101
	Group B	6.57 ± 0.55	8.41 ± 4.76	6.89 ± 4.07	5.5 ± 2.09*	0.000
	Group C	6.99 ± 0.46 *‡	6.11 ± 0.47	5.68 ± 0.42	5.2 ± 0.50*	0.000
Clinical attachment level	Group A	2.12 ± 0.78	2.76 ± 0.67	2.56 ± 0.64	2.49 ± 0.64	0.103
	Group B	6.61 ± 0.77	8.42 ± 4.65	6.91 ± 4.11	5.6 ± 2.12*	0.000
	Group C	6.98 ± 0.48*‡	6.16 ± 0.49	5.69 ± 0.49	5.9 ± 0.32*	0.000
Total serum sialic acid level	Group A	66.85 ± 2.00	65.20 ± 1.82	63.55 ± 1.39	62.64 ± 1.29	0.000
	Group B	144.50 ± 22.2	115.8 ± 18.1	91.85 ± 13.95	70.05 ± 6.16*	0.000
	Group C	212.5 ± 29.9*‡	158.1 ± 27.5	113.15 ± 18.99	77.70 ± 8.54*	0.000
HbA1c levels	Group A	5.67 ± 0.27	–	4.86 ± 0.17	4.61 ± 0.41	0.000
	Group B	5.74 ± 0.19	–	5.34 ± 0.17	4.50 ± 0.30	0.000
	Group C	6.65 ± 0.42*	–	5.70 ± 0.44	5.26 ± 0.41	0.000

* Indicates highly significant when groups were compared.
*‡ Indicates highly significant when groups were compared (inter group).

Tab. 3 Correlation between Age, Gender and BMI with Serum sialic acid level.

			Group A	Group B	Group C
Total serum sialic acid	Age	Pearson correlation	0.203	0.209	0.211
		P value	0.062	0.068	0.069
	Gender	Pearson correlation	0.030	0.122	0.290
		P value	0.856	0.600	0.294
	BMI	Pearson correlation	0.156	0.264	0.213
		P value	0.071	0.059	0.173
	OHI-S	Pearson correlation	0.042	–	–
		P value	0.458*	–	–
	Probing pocket depth	Pearson correlation	–	0.039	–
		P value	–	0.465*	–
	Clinical attachment	Pearson correlation	–	–	0.034
		P value	–	–	0.432*
	Gingival index	Pearson correlation	–	0.032	0.037
		P value	–	0.456*	0.474*
	HbA1c levels	Pearson correlation	–	–	0.029
		P value	–	–	0.457*
	n	–	20	20	20

C showed a highly significant difference at six months ($p < 0.05$).

Multivariate intergroup comparison of GI at baseline and at six months was performed using ANOVA and is shown in Table 2. The test indicated that there was a statistically significant difference in GI between the groups at baseline ($p < 0.05$), but at the end of six months there was no statistically significant difference between the groups ($p > 0.05$), except for Group C which showed significant difference ($p < 0.05$). Intra group comparison using paired *t*-test showed that for Group A there was no statistically significant difference in GI at baseline and at the end of six months ($p > 0.05$), while both Group B and C showed a statistically significant difference in GI at baseline and at the end of six months ($p < 0.05$).

Table 2 shows intergroup comparison of PPD and CAL of Group B and Group C at baseline, one, and three and at the end of six months. At baseline, there was a statistically significant difference between the groups with respect to PPD and CAL ($p < 0.05$) with group C showing a greater PPD reduction and CAL gain. At six months, there was no statistically significant difference between the groups with respect to both the parameters ($p > 0.05$).

Intra group comparison of Group B using paired *t*-test indicated that there was statistically significant difference in (PPD) ($p < 0.05$) and (CAL) ($p < 0.05$) at baseline and at the end of six months. Intra group comparison of Group C using paired *t*-test indicated that there was statistically significant difference in (PPD) ($p < 0.05$) and (CAL) ($p < 0.05$) at baseline and at the end of six months.

Table 2 shows the comparison of TSSA at baseline, one, three and six months recall and the multivariate intergroup comparison of TSSA at baseline and at six months. Intra group comparison using paired *t*-test showed that all the groups had shown a significant reduction in the level of TSSA ($p < 0.05$). And group C has shown a highly significant difference from baseline to six months recall visit ($p < 0.05$). Intergroup comparison of groups A, B and C using ANOVA test showed that group C had shown a significant difference in TSSA levels when compared to other groups at six months recall ($p < 0.05$).

Table 2 shows the multivariate intergroup and intragroup comparison of (HbA1c) levels of groups A, B and C at baseline, one, three and six months recall. Intragroup analysis using paired t test showed that there was no significant difference in either of three groups ($p > 0.05$). Intergroup analysis of HbA1c using ANOVA showed that Group C had shown the significant difference at baseline when compared to other groups ($p < 0.05$). But there was no significant difference among the groups at six months recall visit ($p > 0.05$). Table 3 showed the positive correlation of TSSA level with periodontal clinical parameters like GI, OHI-S, PPD from baseline to six months ($p < 0.05$).

DISCUSSION

Oral-systemic disease interrelationship has become a major concern because oral infections and conditions may contribute to pathologic processes elsewhere in the body (8–11). There are evidences suggestive, that periodontal diseases may trigger potential systemic inflammations (10) and a source of elevated proinflammatory markers. Interventional studies showed the effects of periodontal treatment on serum inflammatory markers (11).

Objectives of the present study were to compare TSSA level in healthy subjects, subjects with CMP[1]with and without NIDDM and its effect on non-surgical periodontal therapy. SA is a protein–bound carbohydrate considered to be monosaccharide and occurs in combination with other monosaccharides like galactose, mannose, glucosamine, galactosamine (12). It exists as a group of

acetylated neuraminic acid, N-glycolylneuraminic acid and diacetylneuraminicacid. Only N-acetyl neuraminic acid has been isolated from human serum. Total SA is the combination of free SA and bound SA. The bound SA can be either a protein or lipid. Lipid associated sialic acid (LASA), is a marker of the acute-phase response (12). It is a predictor of several systemic disorders, cardiovascular events, rheumatoid arthritis, diabetes (13), and head and neck cancer patients (14). Elevated serum and urinary SA concentrations were associated with several risk factors for diabetic vascular disease: diabetes duration, (HbA1c) levels, Triglycerides (TGLS) and cholesterol concentrations, waist to hip ratio and hypertension (15). Total SA is present in biological fluids like saliva, serum, Gingival Crevicular Fluid (GCF), cerebrospinal fluid etc. The reason why we opted for TSSA is that it gives more realistic evidence of the disease progression, enough sample volume rather than multiple attempts for adequate sample volume contamination with other constituents as seen in (GCF) and saliva.

Non-enzymatic glycosylation of hemoglobin is not induced by inflammation, but rather results from hyperglycemia caused by insulin resistance, low insulin levels and impaired wound healing. Thus this could explain why subjects with periodontitis have high (HbA1c) levels. Vascular endothelium carries high levels of SA (16) and vascular damage leads to its increase in its circulation. A relationship between serum SA levels and microvascular complications has been observed before for microalbuminuria and clinical proteinuria in type 1 and type 2 diabetes mellitus (17).

In the present study, the participants were divided into three groups: Group A, B and C. Group A consists of systemically healthy subjects, Group B consists of subjects with CMP while Group C consists of subjects with CMP with NIDDM and results of this study indicated that, at baseline, there were significant differences between Group A, B and Group C with respect to all the clinical parameters, including GI, OHI-S, PPD, CAL, TSSA and HbA1c levels. The above results were in accordance with results obtained, in various studies performed by Davis G. et al., Jawazaly J. et al., Usman M. S. et al., where they found a significant association between elevated TSSA levels in oral epithelial cells and gingivitis (19) and NIDDM (20). According to Jawazaly G. (21) there exists a significant association between elevated levels of salivary LASA levels and periodontal diseases. The elevation in TSSA concentration could be attributed to elevated levels of sialidase enzyme activity in periodontal diseases (22). TSSA concentration increases rapidly following the inflammatory and injury process. According to Crook M. (23) and Yokoyama et al. (24) SA concentrations in the blood may be a useful marker of diabetic complications, but there had been no large scale studies examining the link between SA and complications in type 1 diabetes. According to Syed et al. (25) increase in circulating serum SA is an early manifestation of diabetic renal disease.

The three groups were similar in terms of gender and age, whereas there was a slight variation in terms of BMI. These results were in accordance with the study done by Singh R. and Ramraju B. (26), Usman et al. (20), Shivanandanayak et al. (15) that showed age, sex and duration of diabetes, degree of metabolic control had no influence over the TSSA level. Other contradictory studies are as follows, Crook M et al. (27) showed that serum SA was significantly higher in men with diabetic complications than those without any complications. SA concentration increases with age in both men and women and the results may be due to young age of the subjects (mean 32.5 years in the men, 33.3 years in the women) included. While the present study was done in higher range of age groups could explain the positive results obtained with respect to the elevated level of TSSA in type 2 diabetic patients.

In the present study, HbA1c level was taken to know the glycemic status because it reflects on chronic exposure of hemoglobin to blood glucose. And is not affected by blood glucose fluctuations on the day of assay. (HbA1c) is considered as a beneficial indicator of long-term homeostasis, reflecting an average blood glucose concentration for the past two to three months (29). In the absence of diabetes, non-enzymatic glycosylation of proteins can occur which is considered as a major disadvantage of this assay. Diagnosis of diabetes based on elevated (HbA1c) levels is not dependable. Only those subjects with (RBS) level <140 mg/dl were included in our study. (HbA1c) levels were measured in laboratory using ion exchange (HPLC) and was evaluated after non-surgical periodontal therapy and smokers were excluded from our study. Wolf et al. (36) showed that periodontitis is associated with a slight elevation in glycosylated hemoglobin in non-diabetic subjects. According to Rajan p et al. (30), (HbA1c) levels were slightly higher and statistically significant in chronic periodontitis than in healthy controls. To detect any change in (HbA1c) levels there should be at least a three-month interval from the baseline estimation. In this study reexamination was performed at two months interval for six months and this may be one of the reasons for not obtaining a significant difference in groups A, B and C. Intergroup comparison between groups A, B and C showed a significant difference at baseline ($p < 0.05$) (Table 2). The reason why there was no significant reduction in (HbA1c) levels after six months may be due to the fact that the subjects were under good diabetic control, and of moderate periodontitis (31). This is in contradictory to the results obtained by Guo H. (32) Jayachandran et al. (33) and Yun F. et al. (34) that showed a significant decrease in (HbA1c) levels after non-surgical periodontal therapy. According to Guo et al. (32) the selected subjects had no glycemic control during their reexamination and this may be reason for their results obtained.

The results of present study was in accordance with previous studies of Amitha et al. (30) Crook M. A. et al. (35) that showed elevation of TSSA level in chronic periodontitis and NIDDM subjects when compared to healthy subjects. According to previous authors serum SA could be considered for monitoring disease progression. Reevaluation of clinical parameters and SA level after giving periodontal therapy was not considered and exclusion of confounding factors demographic characteristics were its limitations.

In the present study after SRP, there was no significant change in GI, OHI-S, PPD, TSSA and (HbA1c) levels of healthy subjects without any systemic diseases Group A, while there was significant reduction in GI, OHI-S, PPD,

CAL and TSSA levels in CMP subjects with and without NIDDM Group B and C after non-surgical periodontal therapy. These findings were in accordance with the results of many previous studies (37, 38) which showed the effectiveness of SRP in improving the periodontal status of subjects with periodontitis. In Group C, the (HbA1c) levels of the subjects were not significantly reduced after SRP. These results indicate the possibility that the slight reduction in the HbA1c levels in Group C after six months could be due to an improvement in their periodontal status brought about by SRP. The mean TSSA levels at the end of six months for Group A, B and Group C were 62.64 ± 1.29 mg/dl, 70.05 ± 6.16 mg/dl and 77.70 ± 8.54mg/dl respectively and it was in accordance with previous studies (39, 40). At the end of six months, there was statistically significant difference in TSSA levels of Group B and Group C ($p < 0.001$) (Table 2). The significance was more when Group C was compared with Group A ($p < 0.05$). This indicated that even though there was a significant reduction in TSSA levels in subjects with periodontitis with and without NIDDM after SRP, the clinical parameters never came down to the level as that of healthy subjects.

Several studies have investigated the effect of periodontal therapy on the glycemic control of diabetes patients. Faria-Almedia R. et al. (41) reported that SRP alone significantly reduced (HbA1c) levels in diabetics. According to Crook M. (35) total (SA) concentration in the blood may be useful marker of diabetic complications, but the efficacy of non-surgical periodontal therapy was not considered. SRP did not result in the complete elimination of periodontal pockets and the complete gain in CAL and TSSA level in CMP subjects with and without NIDDM (Group B and Group C) and this is due to the fact that SRP alone cannot eliminate tissue-invading periodontal pathogens (42).

In our study, the significant reduction in clinical parameters, including OHI-S, GI, PPD, CAL and (HbA1c) levels, clearly indicates that periodontal inflammation was reduced after SRP, which could possibly lead to a decrease in TSSA levels. The role of TSSA in systemically healthy subjects, subjects with CMP with and without NIDDM is well understood from the present study. Proper oral hygiene measures also played a very important role in maintaining the periodontal status by reduction in periodontal clinical parameters, so as in TSSA level and glycemic status after non-surgical periodontal therapy.

Strength of this study include adhering to strict inclusion and exclusion criteria, use of ion-exchange (HPLC) for estimation of (HbA1c) levels, Ehrlich's method of estimation of TSSA levels and monitoring the RBS levels of all subjects for confirming their non-diabetic status instead of relying on medical history alone. Only those with RBS levels <140 mg/dl were included in the study. Since subjects were recruited directly from Outpatient Department, we were not able to record the Fasting Blood Sugar (FBS) levels which would have been a more reliable measurement than RBS levels.

Limitations of this study includes lack of microbiological analysis and its correlation with TSSA level, possible laboratory errors due to manual method of estimation, small sample size, measuring only RBS to confirm the glycemic status of the participants, not evaluating serum and/or GCF levels of pro-inflammatory mediators such as TNF-α, IL-1β, IL-6, IF-γ and CRP, and the failure to adjust for confounding factors like race/ethnicity. Future studies and clinical trials; better technique sensitive method for estimation of TSSA level has to be carried out to overcome these limitations.

CONCLUSION

Based on the results obtained from this Non-Randomized Clinical Trial, it can be concluded that, TSSA levels do decrease after Non-surgical periodontal therapy in chronic moderate periodontitis patients with and without NIDDM, we may conclude that TSSA could be considered as a novel biomarker in progression of periodontal disease and diabetic status. Periodontitis could be considered as a potential, modifiable, and independent risk factor for the development of diabetes. Early detection of elevated TSSA level may help in interpreting the progression of periodontitis, risk of development of diabetes mellitus in future and also to prevent complications. In the future, more randomized controlled clinical trials with larger sample sizes, and with strict adjustments of confounding factors, such as race/ethnicity and smoking status, are needed to confirm the findings of this study. However, periodontology must explain a multitude of unclear or insufficiently clear phenomena which will be a priority of science in forthcoming years.

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