

Estimation of Salivary and Serum Total Sialic Acid Levels in Periodontal Health and Disease

SUREKHA R RATHOD¹, FAROOQUE KHAN², ABHAY P KOLTE³, MADHUR GUPTA⁴

ABSTRACT

Background: Chronic gingivitis and periodontitis are inflammatory diseases. An important function of host sialic acid is to regulate innate immunity. The aim of the study was to assess the concentration of Total sialic acid (TSA) in saliva and serum and also to find out their association if any, in periodontal health and disease.

Materials and Methods: A total of 90 subjects were clinically examined and distributed into three groups (n=30) according to the periodontal status namely healthy, chronic gingivitis and chronic periodontitis. Clinical measurements including probing depth, clinical attachment level, gingival index, oral hygiene index were recorded. TSA concentration was determined in saliva and serum of all subjects.

Results: In healthy group the mean salivary TSA level was 39.05mg/dl \pm 6.35 (p<0.0001), mean serum TSA level was 49.75 mg/dl \pm 4.87 (p<0.0001). In the chronic gingivitis group the mean salivary TSA level was 68.23 mg/dl \pm 2.71 (p<0.0001), mean serum TSA level was 65.65 mg/dl \pm 3.56 (p<0.0001). In the chronic periodontitis group the mean salivary TSA was 81.33 mg/dl \pm 3.94 (p<0.0001), mean serum TSA level was 75.98 mg/dl \pm 3.58 (p<0.0001).

Conclusion: The present data indicates that salivary & serum TSA levels can differentiate between chronic periodontitis patients and normal individuals. Thus it can be used as an adjunct to diagnose, monitor response to therapy, to determine the current periodontal disease status and to assess the treatment outcomes.

Keywords: Chronic periodontitis, N-Acetylneuraminic acid, Saliva, Serum

INTRODUCTION

Gingivitis and periodontitis are primarily bacterial infections caused by a diverse group of micro-organisms [1]. Acute phase inflammation have been suggested to associated with infectious diseases such as periodontal diseases [2]. Sialic acid (SA) is the generic term given to a family of acetylated derivatives of neuraminic acid [3]. SA is a 9 carbon monosaccharide. An important function of host SA is to regulate innate immunity. 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 [4]. 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 plaque formation and cause destruction of host tissue [5]. SA is present in several acute phase proteins which are known to be associated with periodontitis [6]. There exists a need for a biomarker, for early detection of disease evolution and more robust therapy efficacy measurements [7]. Till date, there is very little data regarding simultaneous estimation and comparison of total SA content in saliva and serum of chronic periodontitis patients. The present study was therefore undertaken with an aim, to not only assess the levels of TSA in saliva and serum and also to find out their association if any, in periodontal health, chronic gingivitis and chronic periodontitis.

OBJECTIVES

- 1) To understand the initiating aetiologic factor for elevated TSA level, that may help to design preventive and therapeutic strategies properly.
- 2) To evaluate a possible association between periodontal disease and TSA level.

MATERIALS AND METHODS

Study population- A total of 90 subjects visiting the out patient clinic of Department of Periodontics, VSPM Dental College & Research Centre, Nagpur, India, were recruited for this study. Written informed consent was obtained from those who agreed to participate voluntarily and ethical clearance was obtained from institution's ethical committee (VSPMDCRC/28/2012 dated on 07/09/2012).

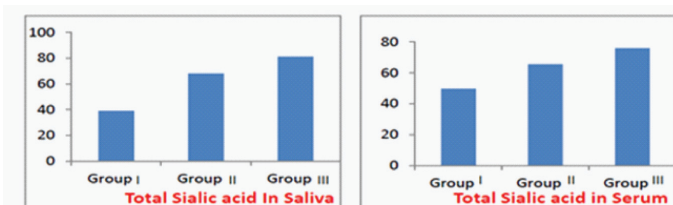
Inclusion criteria- All the subjects should be within the age group of 18 to 55 years, subject should have atleast 18 natural teeth excluding third molars.

Exclusion criteria- History of periodontal debridement or periodontal surgery in preceding six months or any antimicrobial therapy, anti-inflammatory drugs, corticosteroids, in previous three months, systemically compromised individuals, smokers, pregnant and lactating women. Medical history for absence of systemic disorders such as diabetes mellitus, cardiovascular diseases, pneumonia, tuberculosis, rheumatoid arthritis, which may trigger acute phase response, was recorded in detail.

Further clinical measurements such as probing depth (PD), clinical attachment loss (CAL), gingival index (Loe & Silness, 1963), oral hygiene index-simplified (OHI-S) (John C Greene & Jack Vermillon, 1964) were recorded for all subjects. All clinical measurements were performed by a single examiner using a William's graduated periodontal probe. Based on the clinical examination, the subjects were categorized into three different groups. Group I (healthy) consisted of 30 subjects with clinically healthy periodontium with no loss of clinical attachment. Group II (chronic gingivitis) consisted of 30 subjects who showed clinical signs of gingival inflammation (marked redness, oedema, ulceration, tendency of spontaneous

Parameters	Group 1		Group 2		Group 3		p-value
	Mean	S.D.	Mean	S.D.	Mean	S.D.	
Age (years)	27.37	9.69	27.73	7.51	31.23	6.74	<0.0001*
Probing Depth (mm)	1.66	0.36	1.83	0.26	3.58	0.45	<0.0001*
Clinical Attachment loss (mm)	0	0	0	0	3.72	0.52	<0.0001*
Gingival Index	0.0	0.0	1.15	0.26	1.26	0.31	<0.0001*
OHIS Index	1.35	0.42	2.22	0.41	2.37	0.49	<0.0001*
TSA in Saliva (mg/dl)	39.05	6.35	68.23	2.71	81.33	3.94	<0.0001*
TSA in Serum (mg/dl)	49.75	4.87	65.65	3.56	75.98	3.58	<0.0001*

[Table/Fig-1]: Clinical periodontal variables and sialic acid levels in saliva and serum among three groups (mean \pm SD), * statistically significant



[Table/Fig-2]: Distribution of mean TSA levels in (a) saliva and (b) serum amongst three groups

Pair-wise comparison among the groups	Mean Difference	Std. Error	p-value
Group I and Group II	-29.300*	1.193	<0.001
Group I and Group III	-42.333*	1.193	<0.001
Group II and Group III	-13.033*	1.193	<0.001

[Table/Fig-3]: Comparison of salivary sialic acid levels amongst the three groups by bonferroni multiple comparison test * statistically significant

bleeding without any CAL). Group III (chronic periodontitis) consisted of 30 subjects who had probing depth of ≥ 4 mm, CAL of ≥ 4 mm in 5 or more teeth. Sample size of 30 subjects in each group was determined using Power analysis ($\alpha=5\%$, power $(1-\beta)=90\%$).

Collection of samples- Saliva samples were collected from all participants between 9 and 11 AM. Spitting method was used for collection unstimulated whole saliva. Simultaneously, blood samples were collected from antecubital fossa by venipuncture. The samples were centrifuged immediately at 1000 g for 10 min, to separate the serum component. All the samples were stored at -20°C till analysis.

Biochemical analysis- SA content in saliva and serum was determined by a combined modification of the thiobarbituric acid method of Aminoff [8] and the method of Skoza and Mohos [9]. The samples (100 μl) were treated with 70 μl of periodate reagent and incubated at 37°C for 30 min. The reaction was terminated through the addition of 70 μl of sodium arsenite. 140 μl of thiobarbituric acid (0.1M) was then added and this solution was heated in a boiling water bath for 7.5min then cooled in ice water upto room temperature. Dimethyl sulphoxide (560 μl) was added and the solutions were measured on spectrophotometer at wavelength of 549 nm. Total SA content in saliva and serum was measured in mg/dl.

STATISTICAL ANALYSIS

Statistical analysis was performed using EPI info software, Analysis of variance (ANOVA) test for comparing between the three groups and comparison of salivary and serum SA levels amongst the three groups by Bonferroni multiple comparison test. The values of clinical and biochemical parameters were expressed as mean \pm standard deviation (SD) and p- values <0.0001 were considered statistically significant.

Pair-wise comparison among the groups	Mean Difference	Std. Error	p-value
Group I and Group II	-15.7*	1.036	<0.001
Group I and Group III	-26.1*	1.036	<0.001
Group II and Group III	-10.3*	1.036	<0.001

[Table/Fig-4]: Comparison of serum sialic acid levels amongst the three groups by Bonferroni multiple comparison test, * statistically significant

RESULTS

Clinical and biochemical results – Out of total 90 subjects , amongst the 30 healthy subjects the mean PD was 1.66 ± 0.36 mm, mean CAL was 0.0mm, mean GI was 0, mean OHI-S was 1.35 ± 0.42 , mean salivary TSA level was 39.05 ± 6.35 mg/dl and mean serum TSA level was 49.75 ± 4.87 mg/dl. Amongst the 30 chronic gingivitis subjects mean PD was 1.83 ± 0.26 mm , mean CAL was 0.0 mm, mean GI was 1.15 ± 0.26 , mean OHI-S was 2.22 ± 0.41 , mean salivary TSA level was 68.23 ± 2.71 mg/dl and mean serum TSA level was 65.65 ± 3.56 mg/dl. Amongst the 30 chronic periodontitis subjects the mean PD was 3.58 ± 0.45 mm and the mean CAL was 3.72 ± 0.52 mm, mean GI was 1.26 ± 0.31 , mean OHI-S was 2.37 ± 0.49 , mean salivary TSA level was 81.33 ± 3.94 mg/dl and mean TSA level was 75.98 ± 3.58 mg/dl , as indicated in [Table/Fig-1].

It is evident that with increase in severity of periodontal disease from group I to III, the mean TSA levels in saliva and serum also significantly increased from Group I to Group II and maximum in Group III. A significant correlation has been found between the periodontal findings and levels of TSA in saliva and serum, which signifies a relationship between periodontal diseases and salivary and serum TSA levels [Table/Fig-2].

Analysis in [Table/Fig-3], shows that differences salivary TSA levels in Group I and Group II , Group I and Group III and Group II and Group III . These differences were found to be statistically significant at ($p < 0.001$).

Analysis in [Table/Fig-4], shows that differences in serum TSA levels in Group I and Group II , Group I and Group III and Group II and Group III were statistically significant at $p < 0.001$.

Bonferroni multiple comparison test analysis showed that differences in salivary as well as serum TSA levels amongst the three groups, were statistically significant. With an increase in the severity of periodontitis, an increased in salivary as well as serum TSA levels was noted.

DISCUSSION

TSA is a marker of the acute - phase response [10]. Furthermore, it is a predictor of several systemic disorders, cardiovascular events, rheumatoid arthritis and diabetes [11,12]. Increased content of glucoconjugates such as TSA are detected in serum of head & neck cancer patients [13]. In our present study, we estimated TSA levels in saliva and serum. TSA is the combination of free SA and bound SA (LASA). The mean salivary and serum TSA levels of group III were significantly higher than group II & I. Even though TSA levels were detected in saliva & serum of healthy controls but they were significantly lesser than the levels in group II & III. Thus we found a positive correlation between periodontal statuses and salivary as well as serum TSA levels in patients and controls. The result of our present study is in accordance with result obtained, in various studies performed by Davis G et al., Shinohara and co-workers, Jawazaly J et al., Davis G et al., found a significant association between elevated SA levels in oral epithelial cells & gingivitis [14]. Shinohara and co-workers, studied the relationship between the salivary SA concentrations in rats having gingivitis. Their results suggested that the amount of SA elevated in saliva can be a useful index of

the severity of periodontal disease [15]. According to Jawazaly G, there exists a significant association between elevated levels of LASA (lipid associated SA) levels and periodontal diseases [16]. The elevation in TSA concentration could be attributed to the increase rise in the sialidase enzyme activity in periodontal diseases [17]. SA concentration increases rapidly following the inflammatory and injury process. In general, the acute phase proteins limits injury and aids healing [18]. Elevated levels of TSA in saliva and serum may have a protective role in periodontitis as the elevated levels of TSA might be considered as a defense molecule against the increased oxidative stress in inflammatory diseases including periodontitis. High mean concentration of SA might be attributed to a rapid synthesis of sialoproteins and to significant increase in release of globulins, resulting from tissue damage [19]. Sialic acid levels are markedly increased in those with periodontal diseases, confirming that this saccharide plays an important role in the immune system. Therefore, the present data indicates that salivary and serum TSA levels have a significant association with periodontal health and disease.

CONCLUSION

Based on the findings of the present study, increased TSA levels in both the saliva and serum may suggest its role in the pathogenesis of periodontal disease. Also, salivary and serum TSA levels can differentiate between periodontal disease and normal condition. Thus, it can be used as an adjunct to diagnose the current periodontal disease status and to assess the treatment outcomes. Estimation of is quite simple, reliable and reproducible and thus it could be an useful biomarker in periodontal diseases.

REFERENCES

- [1] Loe H, Teilade E, Jensen SB. Experimental gingivitis in man. *Journal Periodontol.* 1965 ;36:177-87.
- [2] Slade GD, Offenbacher S, Beck JD, Heiss G, Pankow JS. Acute phase inflammatory response to periodontal disease in the US population. *J Dent Res.* 2000;79:49-57.
- [3] Crook MA, Tutt P, Pickup JC. Elevated serum sialic acid concentration in NIDDM and its relationship to blood pressure and retinopathy. *Diabetes Care.* 1993;16:57-60.
- [4] Severi E, Hood DW, Thomas GH. Sialic acid utilization by bacterial pathogens. *Microbiology.* 2007;153(9):2817-22.
- [5] Critchley P, Wood JM, Saxton CA, Leach SA. The Polymerisation of Dietary Sugars by Dental Plaque. *Caries Res.* 1967;1:112-29.
- [6] Ide M, McPartin D, Coward PY, Crook M, Lumb P, Wilson RF. Effect of treatment of chronic periodontitis on levels of serum markers of acute phase inflammatory and vascular responses. *J Clin Periodontol.* 2003;30:334-40.
- [7] Colburn WA. Biomarkers in drug discovery and development: from target identification through drug marketing. *Journal of Clinical Pharmacology.* 2003;43(4):329-41.
- [8] Aminoff D. Methods for the quantitative estimation of N-acetylneuraminic acid and their application to hydrolysates of sialomucoids. *Biochem J.* 1961;81:384-92.
- [9] Skoza L, Mohos S. Stable thiobarbituric acid chromophore with dimethyl sulphoxide. *Biochem J.* 1976;159:457-62.
- [10] Lindberg G, Rastam L, Gullberg B, Lundblad A. Serum concentrations of total sialic acid and sialoglyco proteins in relation to coronary heart disease risk markers. *Atherosclerosis.* 1993;103:123-29.
- [11] Lindberg G, Rastam L, Gullberg B, Eklund GA. Serum sialic acid concentration predicts both coronary heart disease and stroke mortality. *Int J Epidemiol.* 1992;21:253-57.
- [12] Schmidt MI, Duncan BB, Sharrett AR, Lindberg G, Savage PJ, Offenbacher S, et al. Markers of inflammation and prediction of diabetes mellitus in adults (Atherosclerosis Risk in Communities Study): a cohort study. *Lancet.* 1999;353:1649-52.
- [13] Tewarson SL. Serum sialic acid an important cancer marker. *Indian J Cancer.* 1993;22:246-56.
- [14] Davis G, Gibbons RJ. Accessible sialic acid content of oral epithelial cells from healthy and gingivitis subjects. *J Periodontal Res.* 1990;25:250-53.
- [15] Shinohara M, Ohura K, Ogata K. The relationship between the sialic acid concentrations in the serum and whole saliva in rats with naturally occurring gingivitis. *Jpn J Pharmacol.* 1994;64:61-65.
- [16] Jawzaly JI, Hasan HG, Ahmed B M. Relationships of salivary sialic acid and its fraction in periodontitis with demographic properties and medical history. *International Journal of Dental Research and Development* 2013;3(2); 17-26.
- [17] Shetty PK, Pattabiraman TN. Salivary glycoproteins as indicators of oral diseases. *Indian J Clin Biochem.* 2004;19:97-101.
- [18] Manole C. Increased serum sialic acid levels in patients with rheumatoid arthritis. *Medical Connections.* 2012;2:26.
- [19] Tijima R, Takahashi H, Namme R, Ikegami S, Yamazaki M. Novel biological function of sialic acid as a hydrogen peroxide scavenger. *FEBS. Letters* 2004;561:163-66.

PARTICULARS OF CONTRIBUTORS:

1. Professor, Department of Periodontics, VSPM Dental College and Research Centre, Nagpur, India.
2. Post Graduate Student, Department of Periodontics, VSPM Dental College and Research Centre, Nagpur, India.
3. Professor and Head, Department of Periodontics, VSPM Dental College and Research Centre, Nagpur, India.
4. Professor and Head, Department of Biochemistry, NKPS Institute of Medical Sciences, Nagpur, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Dr. Farooque Khan,
Post Graduate Student, Department of Periodontics, VSPM Dental College and Research Centre,
Hingna Road, Nagpur, India.
Phone : +919960314612, E-mail : farooquekhan20@gmail.com

FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **Apr 11, 2014**
Date of Peer Review: **Jun 28, 2014**
Date of Acceptance: **Jul 05, 2014**
Date of Publishing: **Sep 20, 2014**