Original Article

Is Sialic Acid a Promising Marker for Periodontal Diseases?

S Oktay, Ö Özöner Bal[1], L Kuru[2], A Yarat, Ü Noyan[3]

Department of Basic Medical Science, Biochemistry, Faculty of Dentistry, Marmara University, [1]Center of Oral and Dental Department, Atasehir Hospital, [2]Department of Periodontology, Faculty of Dentistry, Marmara University, [3]Oral and Dental Department, Acıbadem Healthcare Group, Istanbul, Turkey

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ABSTRACT

Objective: Periodontal diseases are inflammatory chronic infections. Sialic acid (SA) is an acute phase reactant by itself. The aim of this study is to investigate the relationship between salivary and serum SA levels and clinical parameters in different forms of periodontal diseases. **Subject and Methods:** Systemically healthy subjects were included in the study; patients with chronic gingivitis (CG) (n = 10), chronic periodontitis (CP) (n = 10), and aggressive periodontitis (AgP) (n = 10), and ten volunteers with healthy periodontium as the control group. Total SA levels were determined by Warren's thiobarbituric acid method in whole saliva, parotis saliva, and serum samples of subjects before and 3 months after nonsurgical periodontal treatment. Full mouth clinical parameters including plaque index, gingival index, probing depth, and bleeding on probing were also recorded. **Results:** Before treatment, in both periodontitis groups salivary and serum SA levels were higher than those of controls (P = 0.001). Both salivary and serum SA levels decreased significantly in the patient groups after treatment (P < 0.001). Multiple comparisons of baseline clinical parameters in all groups revealed significant differences (P = 0.001) and these parameters decreased significantly on the 90th day (P < 0.01). There were positive correlations between SA levels and periodontal indices of the CG, CP, and AgP groups (P < 0.05). **Conclusion:** Our results suggest that SA level in both saliva and serum may be a potentially useful marker to determine inflammatory changes and investigate different forms of periodontal diseases.

Keywords: *Gingivitis, periodontitis, saliva, serum, sialic acid*

Introduction

Sialic acid (SA) is a general term for acetylated derivative of neuraminic acid and forms a part of various glycoproteins and glycolipids by being one of the terminal residues. SA has several biological actions such as increasing the viscosity of a glycoprotein, binding, and transporting molecules and viruses, stabilizing the conformation of proteins including enzymes.[1] SA has a protective effect on molecules and cells from attack by proteases or glycosidases, extending their lifetime and function.[2] Also, it is an important structural component of serum and saliva.[3] Further, SA has been proposed as a marker of acute phase response.[4,5] As in all biological membranes, acute phase reactants also contain SA in their structure. Therefore, SA levels increase in blood during inflammatory processes as a result of elevated levels of richly sialylated acute phase glycoproteins.[6]

It has been demonstrated that SA is associated with a number of inflammatory diseases including periodontal disease.[7] In previous studies, it was reported that salivary SA levels are affected by oral disease[8,9] and patients with periodontal disease may have elevated circulating levels of inflammatory markers.[10,11] SA is a good source of carbon for bacterial pathogens and has a key role in the colonization of periodontal pathogens. It enhances bacterial aggregation and

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Address for correspondence: Dr. S Oktay, Marmara University, Faculty of Dentistry, Department of Basic Medical Science, Maltepe - 34854, Istanbul, Turkey.
E-mail: nsehkar@yahoo.com

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participates in the formation of pellicle and dental plaque.[12,13]

Periodontal disease, a chronic inflammatory disease caused by specific microorganisms, is one of the most common bacterial infections affecting a quarter of the adult population.[11,14] It occurs as a result of an imbalance between host immune response and pathogenic bacteria.[15]

Previous studies suggested that both salivary and serum SA levels may be useful parameters to determine the severity of periodontal diseases.[6,16-18] Since SA is defined as a mediator for bacterial adhesion,[19] it may be utilized as a diagnostic tool as well as a novel method to assess the outcome of periodontal treatment. The use of saliva has been studied commonly as a potential diagnostic tool due to its noninvasive and easy accessibility.[20]

Therefore, the aim of this study was to evaluate and compare the SA levels in whole saliva, parotis saliva and serum of patients with chronic gingivitis (CG), chronic periodontitis (CP), and aggressive periodontitis (AgP) along with clinical parameters before and after nonsurgical periodontal treatment.

Subject and Methods

Subject population

The study was approved by the Ethical Committee of Marmara University with the number of MAR-YC-2009-0282.

The subject population consisted of 30 patients (male/female (M/F) 22/18; mean age 35,16 ± 6,81 years) who attended to the Periodontology Clinics of Dental Faculty, Marmara University Istanbul, Turkey and diagnosed with CG (n = 10), CP (n = 10), and AgP (n = 10). In addition, ten healthy volunteers (M/F 5/5; mean age 22.20 ± 1.55 years) without any systemic and periodontal disease served as the control group. Exclusion criteria included followings: Having past systemic illness and undergoing any periodontal treatment, taking drugs at least 6 months which may affect periodontium, smoking, and for female individuals being pregnant or lactating.

According to the power analysis performed by using the values (12.99 ng/ml mean serum extracellular glutathione peroxidase (eGPx) level, 7.16 ng/ml standard deviation with 95% power, and significance set at $P < 0.05$ level) obtained from a clinical study,[21] comparing serum eGPx level between the groups. It was determined that a minimum of 8 individuals should be included into each group. So, ten patients were decided to be included into each group considering the possible drop outs.

Sample collection

Fasting whole and parotis saliva samples were collected in the morning and approximately at the same time on days 0 and 90. Individuals were not allowed to brush their teeth, chew gums, or eat before sampling of saliva. After rinsing their mouths with distilled water, approximately 5 ml of nonstimulated whole saliva was collected in a 15 ml test tube under resting conditions. Parotis saliva samples were collected immediately after collection of whole saliva samples. The patients' buccal mucosa was dried by sterile tampons to remove any remnants of whole saliva. The salivary flow was stimulated by citric acid and 60 µl of parotis saliva was taken by microcapillary tubes that placed to region of the Wharton channel, which is the channel of parotis gland, opening into the oral cavity. Saliva samples were frozen immediately and stored at -20°C until required for experiment.

Blood samples were centrifuged at 4000 rpm for 10 min, then serum was carefully removed from tubes into Eppendorf tubes, aliquoted, and stored at -20°C prior to experiment.

Determination of total sialic acid

Total SA levels in whole saliva, parotis saliva, and serum samples were assayed by the thiobarbituric acid (TBA) method described by Warren.[22] Briefly, the samples were incubated with 0.1 N sulfuric acid at 80°C for 1 h to obtain the hydrolysate which was used for detection. SA was oxidized with sodium periodate in concentrated phosphoric acid. The periodate oxidation product was coupled with TBA, and the resulting chromophore was extracted into cyclohexanone. The absorbances of the samples were measured at 549 nm spectrophotometrically and the SA levels were expressed as mg/dl.

Clinical measurements

Clinical periodontal parameters included plaque index (PI), gingival index (GI), probing depth (PD), and bleeding on probing (BoP).

PI developed by Silness and Löe[23] scores the amount of dental plaque according to 4 grades as 0: No plaque, 1: A film of plaque adhering to the free gingival margin and adjacent area of the tooth, which cannot be seen with the naked eye, 2: moderate accumulation of plaque within the gingival pocket, on the gingival margin and/or adjacent tooth surface, which can be seen with the naked eye, and 3: abundance of soft matter within the gingival pocket and/or on the tooth and gingival margin.

GI developed by Löe and Silness[24] scores clinical signs of gingival inflammation according to 4 grades, as 0: No inflammation, 1: Mild inflammation, slight change in color, slight edema, no bleeding on probing, 2: moderate

inflammation, moderate glazing, redness, bleeding on probing, 3: severe inflammation, marked redness and hypertrophy, ulceration, tendency to spontaneous bleeding.

PD, the distance between the gingival margin and the bottom of the sulcus/pocket, was measured on mesiobuccal, midbuccal, distobuccal, mesiolingual, midlingual, and distolingual of all teeth except third molars using a North Carolina probe.

The level of inflammation was evaluated by assessing BoP immediately after probing. If bleeding occurs within 30 s, a positive score is given. All clinical measurements were recorded at baseline in all groups and 90 days after nonsurgical periodontal therapy in the CG, CP, and AgP groups following collection of saliva samples.

Periodontal therapy

Patients with periodontal diseases received nonsurgical periodontal treatment including oral hygiene instruction, scaling and root planning (SRP) on days 0, 7, and 14. Following therapy, all patients were checked once a month for 3 months follow-up period and if necessary, oral hygiene instructions were repeated and professional dental cleaning was performed.

Statistical analyses

Data of the study were presented as the mean ± standard deviation. The statistical analyses were performed using the Graph Pad 5.0 Prism program. The distribution of all data was assessed by the Kolmogorov–Smirnov test for normality and parametric tests were used since the data demonstrated normal distribution. One-way analysis of variance (Anova) test was performed for multiple comparisons and the *Post Hoc* Tukey test was used as a binary comparison for determining the group that caused the difference. The paired *t* test was used for intra-group comparison. Correlation analysis of clinical and laboratory data was performed by Spearman test. The significance level was set at 0.05.

Results

Laboratory results

SA levels at baseline

Baseline SA levels in whole saliva, parotis saliva, and serum of all groups are shown in Table 1. Salivary and serum SA values were significantly different among groups ($p_{Anova} < 0.001$). Whole saliva, parotis saliva, and serum SA levels in the CP and AgP groups were significantly higher than those of control group ($P < 0.05$, $P < 0.001$). Whole saliva SA in the CG group was significantly lower than those in the CP and AgP groups ($P < 0.001$). Parotis saliva in the CG and CP groups were significantly lower than those in the AgP group ($P < 0.001$). Serum saliva in the CG group was lower than those in the CP and AgP groups ($P < 0.01$, $P < 0.001$) and also in the CP group than that in the AgP group ($P < 0.05$).

SA levels after treatment

Comparisons of whole saliva, parotis saliva, and serum SA levels before and after periodontal treatment in the

Table 1: Whole saliva, parotis saliva and serum SA levels (mg/dl) of all groups at baseline

	Control (*n*=10)	CG group (*n*=10)	CP group (*n*=10)	AgP group (*n*=10)	P_{Anova}
Whole saliva SA	3.54±0.93	4.72±1.08$^{**,\Delta\Delta}$	8.87±1.57$^{\varepsilon\varepsilon}$	10.01±1.92$^{\varepsilon\varepsilon}$	0.001
Parotis saliva SA	3.01±0.57	3.72±0.84$^{\Delta\Delta}$	4.16±1.10e$^{-\Delta\Delta}$	5.83±0.74$^{\varepsilon\varepsilon}$	0.001
Serum SA	60.03±10.62	80.69±9.09$^{*,\Delta\Delta}$	108.65±17.71$^{\varepsilon\varepsilon,\Delta}$	134.28±27.84$^{\varepsilon\varepsilon}$	0.001

Values are given as mean±standard deviation. SA; Sialic acid, CG: Chronic gingivitis, CP: Chronic periodontitis, AgP: Aggressive periodontitis. Paired *t* test, $^{\varepsilon}P<0.05$, $^{\varepsilon\varepsilon}P<0.001$ significantly different from control. $^{*}P<0.01$, $^{**}P<0.001$ significantly different from CP. $^{\Delta}P<0.05$, $^{\Delta\Delta}P<0.001$ significantly different from AgP

Table 2: Comparisons of whole saliva, parotis saliva and serum SA levels (mg/dl) before and after periodontal treatment in the CG, CP, and AgP groups

Parameters	CG group (*n*=10)	CP group (*n*=10)	AgP group (*n*=10)
Baseline whole saliva SA	4.72±1.08	8.87±1.57	10.01±1.92
After treatment whole saliva SA	2.18±0.37$^{\ddagger,*,\Delta\Delta}$	3.22±0.70$^{\ddagger,\Delta}$	5.23±1.62‡
Change whole saliva SA	2.54±0.95$^{**,\Delta\Delta}$	5.64±1.39	4.77±2.05
Baseline whole saliva SA	3.72±0.84	4.16±1.10	5.83±0.74
After treatment whole saliva SA	2.11±0.56‡	2.05±0.63‡	2.29±0.73‡
Change whole saliva SA	1.61±1.02$^{\Delta\Delta}$	2.11±1.07$^{\Delta}$	3.53±0.91
Baseline whole saliva SA	80.69±9.09	108.65±17.71	134.28±27.84
After treatment whole saliva SA	51.16±9.70$^{\ddagger,*,\Delta\Delta}$	64.19±9.63$^{\ddagger,\Delta}$	80.81±19.91‡
Change whole saliva SA	29.53±12.07	44.46±25.06	53.47±27.10

Values are given as mean±standart deviation. SA; Sialic acid, AT; CG: Chronic gingivitis, CP: Chronic periodontitis, AgP: Aggressive periodontitis. $^{\ddagger}P<0.001$ significantly different from baseline. $^{*}P<0,01$; $^{**}P<0,001$ significantly different from CP. $^{\Delta}P<0,05$; $^{\Delta\Delta}P<0,001$ significantly different from AgP

Table 3: Comparison of clinical parameters (PI, GI, BoP, PD) before and after periodontal treatment in the CG, CP, and AgP groups

Clinical Parameters	CG (*n*=10)	CP (*n*=10)	AgP (*n*=10)
Baseline PI	1.63±0.20	2.51±0.25	2.64±0.28
After treatment PI	0.30±0.23‡	0.52±0.34‡	0.78±0.44‡
Change PI	1.32±0.31*, Δ	1.99±0.38	1.91±0.56
Baseline GI	1.28±0.26	2.06±0.33	2.26±0.37
After treatment GI	0.08±0.13‡	0.22±0.24‡	0.45±0.39‡
Change GI	1.20±0.32*,Δ	1.84±0.41	1.81±0.40
Baseline BoP (%)	29.75±6.48	58.51±12.69	70.73±20.11
After treatment BoP (%)	1.95±1.73‡	4.06±3.61‡	9.62±7.20‡
Change BoP (%)	27.80±6.42**,ΔΔ	54.45±12.50	61.11±16.86
Baseline PD (mm)	2.23±0.23	3.62±0.26	4.40±1.30
After treatment PD (mm)	1.44±0.30‡	1.90±0.36‡	3.15±1.07‡
Change PD (mm)	0.78±0.23*	1.71±0.53	1.25±0.23

Values are given as mean±standart deviation. PI: Plaque index; GI: Gingival index; BoP; Bleeding on probing; PD: Probing depth; CG: Chronic Gingivitis, CP: Chronic periodontitis, AgP: Aggressive periodontitis. ‡*P*<0.001 significantly different from baseline. **P*<0,01; ***P*<0,001 significantly different from CP. Δ*P*<0,05; ΔΔ*P*<0,001 significantly different from AgP

Table 4: Correlations between clinical parameters and SA levels in the CG, CP, AgP groups

Parameters	*r* (Spearman correlation coefficient)		
	CG	CP	AgP
PI- whole saliva SA	0.811+	0.730+	0.811+
PI- parotis saliva SA	0.562#	0.669+	0.562+
PI- serum SA	0.578+	0.762+	0.578#
GI- whole saliva SA	0.771+	0.738+	0.771+
GI- parotis saliva SA	0.51#	0.707+	0.511+
GI- serum SA	0.639+	0.756+	0.639+
BoP- whole saliva SA	0.749+	0.758+	0.749+
BoP- parotis saliva SA	0.666+	0.765+	0.666+
BoP- serum SA	0.802+	0.710+	0.802+
PD- whole saliva SA	0.700#	0.775+	0.700#
PD- parotis saliva SA	0.474	0.678+	0.264
PD- serum SA	0.466	0.830+	0.089

PI: Plaque index; GI: Gingival index; BoP; Bleeding on probing; PD: Probing depth; SA: Sialic acid, CG: Chronic gingivitis; CP: Chronic periodontitis; AgP: Aggressive periodontitis. **P*<0.05; #*P*<0.01; +*P*<0.001

CG, CP, and AgP groups are shown in Table 2. After treatment, whole saliva SA levels decreased significantly in all groups compared to their respective baseline values ($P < 0.001$). Whole saliva SA level in the CG group was significantly lower than the CP and AgP groups ($P < 0.01$, $P < 0.001$) and in the CP group than the AgP group ($P < 0.05$). The change in the whole saliva SA level of the CG group was significantly lower than those of the CP and AgP groups ($P < 0.001$), without any difference in the changes of whole saliva SA levels between the CP and AgP groups.

After treatment, parotis saliva SA levels decreased significantly in the CG, CP, and AgP groups compared to their respective baseline values ($P < 0.001$). Although no differences were observed in the parotis saliva SA levels among the CG, CP, and AgP groups after treatment, the changes in the parotis saliva SA level between baseline and post-treatment values of the CG and CP groups were significantly lower than that of the AgP group ($P < 0.001$, $P < 0.05$), without any difference between the CG and CP groups ($P > 0.05$).

In concordance with the salivary SA findings, serum SA levels decreased significantly in the CG, CP, and AgP groups after treatment ($P < 0.001$). Serum SA level of the CG group was significantly lower than those of the CP and AgP groups ($P < 0.01$, $P < 0.001$) and of the CP group than the AgP group ($P < 0.05$). However, the changes in serum SA levels of the CG, CP, and AgP groups were similar ($P > 0.05$).

Clinical results

Values of PI, GI, BoP, and PD in all groups decreased significantly after treatment ($P < 0.001$), as expected [Table 3]. The changes in the PI, GI, BoP, and PD of the CG group were significantly lower than the CP and AgP groups ($P < 0.05$), although no significant difference was detected in clinical parameters between the CP and AgP groups ($P > 0.05$) [Table 3].

There was a positive correlation between whole saliva, parotis saliva, and serum SA levels and PI, GI, and BoP of the CG, CP, and AgP groups ($P < 0.05$) [Table 4]. Additionally, a positive correlation was detected between whole saliva SA level and PD of the CG, CP, and AgP groups; whole saliva, parotis saliva, and serum SA levels and PD of the CP group ($P < 0.05$).

Discussion

Patients suffering from periodontal disease are suggested to be more susceptible to inflammation,

and the circulating levels of inflammatory mediators elevated in patients with periodontitis lead to increased risk of developing several systemic diseases, such as atherosclerosis, diabetes, coronary heart diseases, and rheumatic diseases.[11,25,26] Recent studies established a strong association between inflammation and SA.[17,27] Inflammatory conditions, irritants, or damaged cells cause elevation of serum SA level which was reported to serve as an inflammation marker in osteoarthritis, rheumatoid arthritis, cardiovascular disease, and atherosclerosis.[5,27-29]

In the present study, we investigated SA levels in whole saliva, parotis saliva, and serum in patients with gingivitis and periodontitis. At baseline, high SA levels detected in saliva and serum of the patients indicated that the periodontal pathogens activated the immune system. Inflammation is the primary reaction of the immune response to eliminate stimuli and pathogen microorganisms in order to restore the damaged host cells to normal state.[30] The inhibitory effect of pathogens may lead to an increase in tendency of gingival bleeding on gentle probing. The overproduction of reactive oxygen species by inflammatory cells causes tissue damage at active inflammatory response.[31] As a marker of inflammation, SA may have a regulatory role in immunological processes and may play a role in the prevention of oxidative stress and removal of reactive oxygen species.[32] In periodontitis, an increase in the secretion of proinflammatory cytokines occurs as a result of pathogenic microorganisms, leading to the formation of oxidative stress. This, in turn, increases radical formation and leads to loss of terminal SA residues from glycoproteins.[19,33] In addition, released proinflammatory mediators stimulate liver and extra hepatic tissues causing elevation of acute phase reactants in the circulatory system.[34]

Following inflammation and injury processes, SA concentration rises up rapidly as a result of increased levels of richly sialylated acute phase glycoproteins, so that SA acts as an acute phase protein to limit injury and encourage healing.[35] Markedly increased SA levels in inflammatory diseases including periodontitis confirm the evidence that saccharides play a crucial role in the immune system.[17] Elevated levels of SA were attributed to the synthesis of sialoproteins and cleavage of globulins from damaged tissue.[36]

In the present study, nonsurgical treatment of periodontal disease provided significant decreases in salivary and serum SA levels in all groups parallel to significant reductions in the clinical parameters. The 90th day after treatment is the timing which has been described in relevant studies[37,38] and shows the potential to enable patient adherence to oral health measures, and to benefit in reducing subgingival treatment needs. Removal of supra and subgingival plaque and calculus deposits by applying SRP gave rise to healing the soft tissue wall of periodontal pockets and disappearance of gingival edema by decreasing inflammation.

Saliva is the first biological fluid to come in contact with bacteria. The reason for the increase in salivary SA levels in gingivitis and periodontitis patients may be attributed to the presence of SA molecules cleaved from the cell membranes of oral tissues by the effects of periodontopathogens. The oral bacteria, passing into the circulation system through lymph vessels,[39] may also induce a response in the immune system and increase blood SA level in patients with periodontal disease.

Clinical parameters, such as PI, GI, PD, and BoP provide information on the severity of periodontal disease; however, they are not able to measure the disease activity.[40] Our results and previous studies[6,17,18] showed that SA levels in saliva and serum may be potential markers for determining different forms of periodontal diseases. Saliva has known to contain a number of acute phase response molecules released against pathogen microorganisms. Additionally, saliva is more suitable to be sampled than serum because of its ready availability, noninvasive, and easy collection. Among the samples used in this study, whole saliva is the best for predicting periodontal disease status because SA levels in whole saliva demonstrated significant differences between the pre- and posttreatment values. CP and AgP can be identified easily from healthy controls in terms of SA determined in whole saliva, parotis saliva, and serum; however, this method cannot distinguish between gingivitis and healthy periodontium. CP may be only differentiated from AgP regarding SA levels in parotis saliva and serum. Additionally, due to the positive correlation detected between SA levels and PI, GI, and BoP parameters of the periodontitis groups. SA can be concluded as a promising marker in patients with periodontitis. Determination of biomarkers for screening and evaluating the periodontal disease activity and the efficacy of therapy is considered valuable for diagnostics.[41] It would be desirable to develop reliable and simple diagnostic methods for early detection of disease status and for monitoring the response to periodontal therapy.[42] SA levels can be used in combination with different diagnostic methods measuring oxidative stress markers, inflammatory cytokines, and host-derived enzymes. In future, a novel chair side kit may be developed for early diagnosis of periodontitis, so that it may enable determination and/or prevention periodontal tissue destruction process.

Further studies with larger sample size are needed to support the findings of this pilot study.

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Conflicts of interest

There are no conflicts of interest.

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