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Serum and Saliva Sialic Acid in Periodontitis Patients with and without Cardiovascular Disease

Sehkar Oktay[a] Isık Basar[c] Ebru Emekli-Alturfan[a] Ezgi Malali[b] Eser Elemek[b] Faruk Ayan[c] Lale Koldas[c] Ulku Noyan[b] Nesrin Emekli[a]

Departments of [a]Basic Sciences and [b]Periodontology, Faculty of Dentistry, Marmara University, and [c]Department of Cardiology, Faculty of Medicine, Istanbul University, Istanbul, Turkey

Key Words
Cardiovascular disease · Inflammation · Periodontitis · Saliva · Sialic acid

Abstract
Serum total sialic acid (sTSA) has recently been shown to be a cardiovascular risk factor. However, there is little information about the role of sTSA and TSA in saliva in periodontitis, a chronic and inflammatory disease known to be a risk factor for cardiovascular disease (CVD). We aimed to investigate the changes in sTSA and TSA levels in saliva in patients having both periodontitis and CVD versus periodontitis patients without diagnosed CVD. The study group consisted of 26 patients with proven periodontitis and 26 controls with no diagnosed systemic disease but periodontitis. sTSA and saliva TSA levels were determined by the thiobarbituric acid method, and C-reactive protein (CRP) was evaluated by the nephelometric method. The severity of periodontitis has been determined by the community periodontal index of treatment needs (CPITN). TSA in blood and saliva and CRP levels in blood were significantly increased in CVD patients compared with the control group. CPITN ranged from 2 to 4 in both groups. Significant and positive correlations were found between sTSA and saliva SA levels in patients and controls and between tooth loss and TSA both in blood and saliva. Therefore, TSA in saliva may be a useful marker similar to sTSA in CVD patients.

Introduction

Sialic acid (SA), a family of acetylated derivatives of neuraminic acid, is widely distributed in mammals. It usually occurs as a terminal component at the nonreducing end of carbohydrate chains of glycoproteins and glycolipids [1]. Normal human serum SA is largely bound to glycoproteins or glycolipids [2]. SA participates in multiple physiological functions, such as cell-to-cell interactions, cell migration and proliferation [3]. SA is an acute-phase reactant by itself and moieties are found also at terminal oligosaccharide chains of acute-phase proteins. Serum total SA (sTSA) has been proposed as a marker of an acute-phase response in different diseases [4, 5].

Studies have shown an association between sTSA and cardiovascular mortality. Crook [6] and Succarin et al. [7] found raised serum concentrations of SA in patients with

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Sehkar Oktay
Department of Basic Sciences
Faculty of Dentistry, Marmara University
TR–34365 Nisantasi, Istanbul (Turkey)
Tel. +90 212 231 9120 ext. 111, Fax +90 212 246 5247, E-Mail nsehkar@yahoo.com

acute myocardial infarction. It was shown that sTSA is a strong predictor of cardiovascular mortality and may also reflect the existence or activity of an atherosclerotic process [8].

In normal arteries, SA contributes to maintenance of endothelial cell integrity. Lindberg [8] recently suggested that SA plays a protective role in the endothelium. It has also been reported that the degree of desialylation of LDL cholesterol is associated with the formation of aggregates which are taken up by endothelial and smooth muscle cells leading to lipid accumulation in atherosclerotic patients. Rastam et al. [9] reported that subjects who presented with carotid thickening had significantly elevated sTSA. In contrast to these data, other studies have shown that sTSA concentration is not associated with the extent or severity of coronary artery disease [10].

Periodontitis is a chronic, tissue-destructive inflammatory disease, which degrades the attachment apparatus of the teeth, and progression of the disease may result in tooth loss. Periodontitis and atherosclerosis are common diseases; they start early in life, and progression is usually slow. Both diseases have some risk factors in common, e.g. inflammation, smoking and diabetes. In recent years, several studies have reported epidemiological associations between periodontitis and cardiovascular disease (CVD) [11–13]. Several mechanisms have been proposed to explain or support these theories. One of these is based on periodontitis having effects on the systemic dissemination of locally produced mediators such as C-reactive protein (CRP) [12]. We therefore investigated the relationship between sTSA and saliva TSA levels and CRP in chronic periodontitis patients with and without CVD.

Table 1. Characteristics of the CVD patients studied

Patient No.	CPITN score	Positive for
1	2	heparin, OW
2	4	heparin, DM, smoking, OW
3	2	
4	2	heparin, statin, DM
5	3	heparin, statin, DM
6	3	heparin, statin, smoking
7	2	heparin, statin, OW
8	2	heparin, statin, smoking, OW
9	2	DM, OW
10	3	heparin, statin, DM, OW
11	3	heparin, statin, OW
12	2	heparin, statin, smoking
13	2	heparin, statin
14	3	heparin, statin, smoking
15	3	heparin, statin, smoking, OW
16	2	OW
17	3	heparin, statin, DM, smoking, OW
18	4	heparin, statin, DM, smoking, OW
19	3	statin
20	3	DM, OW
21	4	OW
22	4	DM, smoking, OW
23	2	heparin, statin, DM, smoking
24	3	heparin, DM
25	4	heparin, statin, DM, smoking, OW
26	3	heparin, statin, OW

DM = Diabetes mellitus; OW = overweight.

Subjects and Methods

Patients and Control Group

The study group consisted of 26 patients (age: 40–85 years) with proven periodontitis (17 men and 9 women) presenting at the Department of Cardiology, Cerrahpasa Medical Faculty, Istanbul University, with electrocardiographically documented coronary artery disease and no clinical signs of ischemia within the previous month. The control group comprised 26 patients (age: 45–75 years) with periodontitis (14 women and 12 men) without diagnosed systemic disease. Characteristics of the patient group are given in table 1.

Blood and Saliva Collection

Saliva samples were collected in restful and quiet circumstances. All subjects gave informed consent to participate in the study. Unstimulated mixed saliva samples were collected before breakfast, after overnight fasting, between 08:00 and 10:00 a.m., and after the mouth had been rinsed with distilled water, by spitting into a funnel.

The blood samples of the patients and controls were centrifuged at 3,000 rpm for 10 min at room temperature and the serum was collected. Serum and saliva samples were stored at –80°C for SA determination.

SA Determination

Total serum and saliva SA levels were determined by the thiobarbituric acid method described by Warren [14].

CRP Determination

CRP levels were determined by the quantitative turbidimetric method. Reference values were up to 6 mg/l [15].

Severity of Periodontitis

The severity of periodontitis has been determined by the community periodontal index of treatment needs (CPITN). Both the patient and the control group consisted of subjects with CPITN score 2–4. Code 0: healthy periodontal tissues. Code 1: bleeding after probing. Code 2: supragingival and subgingival calculus. Code 3: 4- to 5-mm deep pathological pockets. Code 4: pathological pockets ≥6 mm.

Table 2. sTSA levels (mg/dl) in the CVD patients and the control group according to CPITN index

Group	All subjects	CPITN 2	CPITN 3	CPITN 4
Control	53.20 ± 7.89 (n = 26)	49.09 ± 9.89 (n = 10)	56.79 ± 4.82 (n = 11)	53.47 ± 5.72 (n = 5)
CVD	86.87 ± 15.61* (n = 26)	87.30 ± 7.79* (n = 10)	89.71 ± 21.54* (n = 11)	84.09 ± 14.89** (n = 5)

* $p \leq 0.001$, ** $p \leq 0.01$, vs. the control group.

Table 3. Saliva TSA levels (mg/dl) in the CVD and control groups according to CPITN

Group	All subjects	CPITN 2	CPITN 3	CPITN 4
Control	5.05 ± 1.49 (n = 26)	4.97 ± 1.31 (n = 10)	5.43 ± 1.80 (n = 11)	4.44 ± 1.51 (n = 5)
CVD	9.48 ± 4.55* (n = 26)	8.29 ± 4.34*** (n = 10)	11.19 ± 5.04* (n = 11)	7.82 ± 2.87 (n = 5)

* $p \leq 0.001$, *** $p \leq 0.05$, vs. the control group.

Statistical Analyses

The program SPSS 10.0 for Windows was used for statistical analysis. All results in the study are expressed as means ± SD. The Mann-Whitney test with repeated measures was used to determine any significant differences in the SA and CRP measurements. A value of $p < 0.05$ was considered to be statistically significant.

Results

sTSA and saliva TSA levels of the 26 CVD patients and 26 controls are shown in tables 2 and 3. Serum and saliva SA levels were significantly increased in CVD patients compared with the control group ($p < 0.001$ and $p < 0.001$, respectively). sTSA and saliva TSA levels were also increased in CVD patients with CPITN of 2 and 3 compared to controls with CPITN 2 and 3 ($p < 0.001$ and $p < 0.001$, and $p < 0.02$ and $p < 0.003$, respectively) and sTSA levels were increased in CVD patients with CPITN 4 compared to controls with CPITN 4 ($p < 0.01$). Although statistically nonsignificant, salivary TSA levels in CVD patients with CPITN 4 were found to be increased compared with controls with CPITN 4. This may be due to the limited number of patients and controls with CPITN 4. No significant differences were found in sTSA and saliva TSA levels between CVD patients with and without heparin and statin treatment (table 4). In addition, sTSA and saliva TSA levels were also not significantly different between CVD patients with and without diabetes mellitus (table 4). Serum CRP levels were significantly increased in CVD patients compared with the control group ($p \leq 0.001$) (table 5). Serum CRP levels were also increased in CVD patients with CPITN 2 and 3 compared to controls with CPITN 2 and 3 ($p \leq 0.003$ and $p \leq 0.008$, respectively). Similar to salivary TSA levels in patients with CPITN 4, although statistically nonsignificant, CRP levels in CVD patients with CPITN 4 were found to be increased compared with controls with CPITN 4. This may be due to the limited number of patients and controls with CPITN 4.

Table 4. sTSA and saliva TSA levels in the CVD patients with and without heparin treatment, with and without statin treatment and with and without diabetes mellitus (DM)

Group	sTSA, mg/dl	Saliva TSA, mg/dl
Heparin+ (n = 19)	85.18 ± 17.17	10.07 ± 4.99
Heparin– (n = 7)	91.46 ± 10.23	7.01 ± 1.61
Statin+ (n = 17)	85.05 ± 18.45	9.69 ± 4.87
Statin– (n = 9)	87.58 ± 9.19	8.62 ± 4.37
DM+ (n = 12)	84.92 ± 12.98	7.7 ± 4.07
DM– (n = 9)	89.12 ± 17.52	10.86 ± 4.55

Significant and positive correlations were found between sTSA and saliva TSA levels in periodontitis patients with and without CVD ($p < 0.01$, $r = 0.584$), between tooth loss and TSA both in sTSA and saliva ($p <$

Table 5. Serum CRP levels (mg/dl) in the CVD and control groups according to CPITN

Group	All subjects	CPITN 2	CPITN 3	CPITN 4
Control	0.90 ± 0.84 (n = 26)	0.33 ± 0.14 (n = 10)	0.70 ± 0.17 (n = 11)	1.75 ± 1.10 (n = 5)
CVD	6.47 ± 9.73* (n = 26)	5.87 ± 10.43** (n = 10)	6.94 ± 6.43** (n = 11)	7.75 ± 9.57 (n = 5)

* $p \leq 0.001$, ** $p \leq 0.01$, vs. the control group.

0.01, r = 0.319, r = 0.282), and between sTSA and saliva TSA and serum CRP ($p < 0.01$, r = 0.595, r = 0.589). In addition, significant and positive correlations were found between serum CRP levels according to the CPITN score in periodontitis patients without CVD ($p \leq 0.001$, r = 0.883).

Discussion

Succarin et al. [7] found raised serum concentrations of SA in patients with acute myocardial infarction. Beck and Offenbacher [13] reported a positive correlation between raised sTSA and the severity of CVD. In agreement with these studies, we noted increased TSA levels both in the serum and saliva of periodontitis patients with CVD. In order to obtain subjects in the same age range (40–85), patients without periodontal disease (CPITN 0) were not included in this study. Therefore, we cannot specify whether the increases in TSA levels were due to CVD or periodontitis.

Studies have confirmed that there is a strong positive association between markers of inflammation, especially CRP [8] and hypercoagulability (e.g. fibrinogen) [16] and sTSA. Reganon et al. [16] showed that the elevated plasma fibrinogen levels found in patients were usually accompanied by an increase in CRP and SA. Increases in fibrinogen and inflammatory markers such as SA and CRP in plasma may reflect the activity of the atherosclerotic process. However, the different correlations between fibrinogen, SA and CRP suggest that several biological mechanisms involved in the pathogenesis of atherosclerosis regulate the acute-phase response. Moreover, previous studies provided substantial evidence that high sTSA concentrations have been related to almost twofold increases in mortality from CVD and stroke [8].

The role of inflammation in the development of atherosclerosis has also been studied [17]. Previous studies indicated that atherosclerosis may be caused by several pathogens [18, 19]. The systemic implication of acute and chronic infections, such as chronic periodontitis, has received considerable attention over the past decade [20].

An association between periodontal disease severity and cardiovascular risk has been reported, and periodontitis has been ascribed an etiological or modulating role in CVD [13]. Different mechanisms have been proposed to explain or support such theories. One of these is based on the potential for the inflammatory phenomenon of periodontitis to exert effects via the systemic dissemination of locally produced mediators. Some studies reported that the association between periodontitis and CVD could be due to the release of bacterial products or proinflammatory cytokines from the chronic periodontal lesions into the blood stream [21]. This might lead to a systemic inflammatory response, which resembles a risk factor profile that is consistent with CVD. Although DNA from oral bacteria has been found in atherosclerotic plaques, a bacterial contribution to this plaque formation has yet to be demonstrated [22].

We therefore investigated the relationship between the concentration of TSA in serum and whole saliva in chronic periodontitis patients with and without CVD. We found a significant and positive correlation between serum and saliva SA levels in periodontitis patients with and without CVD. Although TSA and CRP levels were increased in CVD patients with CPITN 2 and 3 compared to controls with CPITN 2 and 3, there was no increase in CPITN 4. In our opinion, the number of patients having CPITN 4 is too low to disclose significant results in statistical analyses. The lack of patients with CPITN 0 or 1 may be explained by the age range of the patients included in the study; therefore, all study patients have periodontitis. Today it is known that SA levels increase with increasing diabetic complications. Even so, no significant differences were found in sTSA and saliva TSA levels between CVD patients with and without diabetes mellitus, which may be due to a slight diabetic condition.

Although a correlation between the severity of gingivitis and salivary SA concentration has been reported before [23], sTSA and saliva SA levels in periodontitis were firstly determined and correlated with CVD in our present study. Significant correlations were noted between tooth loss and TSA (both in serum and saliva), and between sTSA and saliva TSA and serum CRP. However, we found no significant correlation between CPITN score and TSA and CRP levels in the serum in the groups.

In agreement with previous studies [11, 12, 20], CRP levels were significantly higher in our patient group. Moreover, a positive correlation was observed between sTSA and serum CRP. Our study also showed a statistically significant correlation between serum CRP levels and sTSA and saliva TSA. We could not measure CRP levels in saliva because CRP levels in saliva were below the detection limit of the method. Although CRP is a traditional marker in blood, methods to assess CRP in saliva have to be improved.

In conclusion, in our study increased TSA levels – both in serum and in saliva – of periodontitis patients with CVD have shown that sTSA and saliva TSA may be used as effective markers for low-grade inflammation in periodontitis and patients with CVD. In addition, diagnosis of disease via TSA analysis may provide a cost-effective approach both for serum and saliva. Consequently, sTSA may be a more useful CVD risk marker than CRP. Moreover, a significant positive correlation was observed between sTSA and saliva TSA levels. However, in our study, in order to form age-matched groups, all subjects in the patient and control groups had periodontitis; therefore the use of SA as an inflammation marker for periodontitis cannot be assessed. To sum up, despite the limitation of including only a small number of participants, we suggest that saliva may also be used as an effective and non-invasive inflammation marker for CVD.

References

1 Ogasawara Y, Namai T, Yoshino F, Lee MC, Ishii K: Sialic acid is an essential moiety of mucin as a hydroxyl radical scavenger. FEBS Lett 2007;581:2473–2477.
2 Munday J, Floyd H, Crocker PR: Sialic acid binding receptor (siglecs) expressed by macrophages. J Leukoc Biol 1999;66:705–711.
3 Schauer R: Sialic acids and their role as biological masks. Trends Biochem Sci 1985;10:357–360.
4 Sillanaukee P, Ponnio M, Jaakelainen IP: Occurrence of sialic acids in healthy humans and different disorders. Eur J Clin Invest 1999;29:413–425.
5 Alturfan AA, Uslu E, Alturfan EE, Hatemi G, Fresko I, Kokoglu E: Increased serum sialic acid levels in primary osteoarthritis and inactive rheumatoid arthritis. Tohoku J Exp Med 2007;233:241–248.
6 Crook MA: Sialic acid and cardiovascular disease. Med Biochem 1999;1:123–130.
7 Succarin M, Foglietti MJ, Percheron F: Perchlorosoluble glycoproteins and myocardial infarct: modifications of the carbohydrate moiety. Pathol Biol (Paris) 1982;30:151–154.
8 Lindberg G: Resialylation of sialic acid deficit vascular endothelium, circulating cells and macromolecules may counteract the development of atherosclerosis: a hypothesis. Atherosclerosis 2007;192:243–245.
9 Rastam L, Lindberg G, Folsom AR, Burke GL, Nilsson-Ehle P, Lundblad A: Association between serum sialic acid concentration and carotid atherosclerosis measured by B-mode ultrasound. The ARIC Investigators. Atherosclerosis Risk in Communities Study. Int J Epidemiol 1996;25:953–958.
10 Knuiman MW, Watts GF, Divitini ML: Is sialic acid an independent risk factor for cardiovascular disease? A 17-year follow-up study in Busselton, Western Australia. Ann Epidemiol 2004;14:627–632.
11 Mattilla KJ, Pussinen PJ, Paju S: Dental infections and cardiovascular diseases: a review. J Periodontol 2005;76:2085–2088.
12 Persson GR, Pettersson T, Ohlsson O, Renvert S: High-sensitivity serum C-reactive protein levels in subjects with or without myocardial infarction or periodontitis. J Clin Periodontol 2005;32:219–224.
13 Beck JD, Offenbacher S: Systemic effects of periodontitis: epidemiology of periodontal disease and cardiovascular disease. J Periodontol 2005;76:2089–2100.
14 Warren L: The thiobarbituric acid assay of sialic acids. J Biol Chem 1959;234:1971–1975.
15 Malali E, Basar I, Emekli-Alturfan E, Elemek E, Oktay S, Ayan F, Emekli N, Noyan U: Levels of C-reactive protein and protein C in periodontitis patients with and without cardiovascular disease. Pathophysiol Haemost Thromb 2009–10;37:49–54.
16 Reganon E, Vila V, Martinez-Sales V, Vaya A, Mira Y, Ferrando F, Aznar J: Sialic acid is an inflammation marker associated with a history of deep vein thrombosis. Thromb Res 2007;119:73–78.
17 Ross R: Atherosclerosis – an inflammatory disease. N Engl J Med 1999;340:115–126.
18 Epstein SE, Zhou YF, Zhou J: Infection and atherosclerosis. Circulation 1999;100:20–28.
19 Viles-Gonzalez JF, Fuster V, Badimon JJ: Links between inflammation and thrombogenicity in atherosclerosis. Curr Mol Med 2006;6:489–499.
20 Noack B, Genco RJ, Treviisan M, Grossi S, Zambon JJ, Nardin E: Periodontal infections contribute to elevated systemic C-reactive protein level. J Periodontol 2001;72:1221–1227.
21 Dorn BR, Dunn WA, Progulske-Fox A: Invasion of human coronary artery cells by periodontal pathogens. Infect Immun 1999;67:5792–5798.
22 Haraszthy VI, Zambon JJ, Trevisan M, Zeid M, Genco RJ: Identification of periodontal pathogens in atheromatous plaques. J Periodontol 2000;71:1554–1560.
23 Ito F, Yamada S, Omori S, Yamada H, Nakashizuka T: The relationship between the sialic acid contents in saliva and the clinical findings (in Japanese). Nippon Shishubyo Gakkai Kaishi. 1975;17:110–114.