Original Paper

Pathophysiology of Haemostasis and Thrombosis

Pathophysiol Haemost Thromb
DOI: 10.1159/000323418

Received: September 17, 2010
Accepted after revision: December 8, 2010
Published online: February 4, 2011

Plasma Tissue Factor Levels and Salivary Tissue Factor Activities of Periodontitis Patients with and without Cardiovascular Disease

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Key Words
Tissue factor · Saliva · Periodontitis · Cardiovascular disease

Abstract
The association between periodontal and cardiovascular disease has received considerable attention. Studies have demonstrated a higher incidence of atherosclerotic complications in patients with periodontal disease. Tissue factor (TF) has been known as a key initiator of the coagulation cascade, and the TF pathway is the primary physiological mechanism of initiation of blood coagulation. Recently, it has been shown that the circulating pool of TF in blood is associated with increased blood thrombogenicity in patients with coronary artery disease (CAD). Various tissues and saliva have been known to have TF activity. Consequently, the aim of this study was to investigate plasma TF levels and TF activity of saliva in periodontitis patients with and without diagnosed CAD. Twenty-six patients with a diagnosis of CAD and 26 systemically healthy patients were examined in the dental clinic, and the Community Periodontal Index Treatment Needs (CPITN) scores were recorded. Plasma TF levels were determined using commercially available ELISA kit. Salivary TF activities were determined according to Quick's one-stage method. Plasma TF levels were significantly increased in patients with CAD when compared with the control group. There was no difference in salivary TF activities between the 2 groups, but there was a strong and negative correlation between salivary TF activities and CPITN indexes in both groups. In order to determine the possible role of TF activity as a salivary marker in CAD and periodontitis and to fully understand the negative correlation between salivary TF activities and CPITN, TF activity of gingival crevicular fluid that may also affect saliva can be evaluated.

Introduction

Periodontitis is a chronic inflammatory disease, initiated by colonization of dental plaque bacteria in the gingival sulcus, which causes destruction of the surrounding and supporting tissues of the teeth and can finally lead to tooth loss. The global prevalence of periodontal diseases is high, and the severe forms of chronic periodontitis affect approximately 10–15% of individuals in most popu-

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lations, whereas 35% exhibit moderate or mild signs of the disease [1–3].

Atherosclerosis is also a very common disease and starts early in life; however, since disease progression is usually slow, clinical symptoms or hospitalization are rare before the age of 40 years [4]. The association between periodontal disease and cardiovascular disease (CVD) can be due to bacteremia by periodontal pathogens or due to cell-mediated inflammatory response by the local effect of the bacteria. Periodontal pathogens may enter the circulation during chewing, tooth brushing, flossing and gentle mastication. A casual link between periodontitis and CVD has been postulated on the basis of some periodontal pathogens, such as *Porphyromonas gingivalis,* to invade arterial endothelial and smooth muscle cells, and thereby, to promote platelet aggregation and thrombus formation. This local invasion can trigger a chronic inflammatory response that favors the onset and progression of atherosclerosis [5]. Due to the release of bacterial lipopolysaccharides and endotoxins, inflammatory (polymononuclear cells, macrophages and lymphocytes) and endothelial cells get activated and promote the proinflammatory cytokine secretion, leading to systemic inflammation [6]. Accordingly, studies have demonstrated a higher incidence of atherosclerotic complications in patients with periodontal disease [7–10].

Tissue factor (TF; thromboplastin, factor III) has long been known as a key initiator of the coagulation cascade. Consequently, the TF pathway is the primary physiological mechanism of initiation of blood coagulation [11, 12]. Binding of native coagulation factor VII (FVII) to TF converts FVII to the activated form (FVIIa). The resulting TF-FVIIa complex then activates factors IX and X to factors IXa and Xa, respectively, leading to the formation of the prothrombinase complex and to thrombin generation. TF, which is present in the adventitia of normal blood vessels and is highly expressed in atherosclerotic plaques, has been recognized to initiate coagulation and thrombus formation when the vessel wall is injured or plaques are fissured [13]. More recently, it has been shown that there is a circulating pool of TF in blood that is associated with increased blood thrombogenicity in patients with CVD [14], sickle cell disease [15], antiphospholipid antibody syndrome [16], hyperlipidemia [17] and disseminated intravascular coagulation [18]. In addition, bacterial lipopolysaccharides have been shown to upregulate the TF activity in monocytes [19].

Besides blood, various tissues and body fluids (saliva, amniotic fluid, bile, semen, sweat or tears) have been known to have TF activity [20–25]. Most of them are not easily obtained; thus, saliva is especially suitable for study because of its ready availability. Consequently, the aim of this study was to investigate plasma TF levels and TF activity of saliva in periodontitis patients with and without diagnosed coronary artery disease (CAD).

Materials and Methods

Patients and Control Group

The study group consisted of 26 patients (17 men and 9 women) with stable CAD, who were staying at the Istanbul University Cerrahpasa Medical Faculty, Department of Cardiology, Istanbul University, Istanbul, Turkey, aged 40–85 years, with proven periodontitis. Patients with electrocardiographically documented CAD with no clinical signs of ischemia within the previous month were considered to have stable CAD. The control group consisted of 26 patients (14 women and 12 men) aged 40–75 years without diagnosed systemic disease but with periodontitis. The severity of periodontitis has been evaluated by the Community Periodontal Index Treatment Needs (CPITN) score using a World Health Organization periodontal probe. The CPITN index ranged between 0 and 4. Code 0: healthy periodontal tissues; code 1: bleeding after probing; code 2: supragingival and subgingival calculus; code 3: 4- to 6-mm-deep pathological pockets; code 4: 6-mm and deeper pathological pockets. The patient and the control groups were matched in order to have CPITN score 2–4. Informed consent was signed by all patients before the study. The study was approved by the local hospital ethical committee.

Blood and Saliva Collection

One tube of sodium citrate blood was drawn by venipuncture for plasma TF assay, and samples were separated by centrifugation. 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, after overnight fasting, before breakfast, between 08.00 and 10.00 a.m., and after the mouth had been rinsed with distilled water, by spitting into a funnel.

TF Enzyme-Linked Immunosorbent Assay

Plasma TF levels were determined using a commercially available enzyme-linked immunosorbent assay (ELISA) kit, Assaypro (St. Charles, Mo., USA; catalog No. ET 1002-1), according to the manufacturer's protocol. Briefly, the 96-well plate was precoated with capture antibody by the manufacturer. The plasma samples were then diluted 1:2 in enzyme immunoassay diluent. Standards and samples were added to the wells, incubated at room temperature for 2 h. After washing, biotinylated TF antibody was added to the wells and left for 1 h at room temperature. Subsequently, streptavidin-peroxidase conjugate was added to the wells and incubated for 30 min. After washing 5 times with wash buffer, chromogen substrate was added and incubated for 10 min until the optimal blue color density developed. This enzymatic reaction was stopped by adding 0.5 N HCl. Absorbance on the plate was then read at 450 nm using an ELISA plate reader (Medispec ESR 200, Germantown, Md., USA) immediately.

TF Activity Assay

TF activities of saliva samples were determined according to Quick's one-stage method using pooled plasma obtained from healthy subjects [26]. This was performed by mixing 0.1 ml saliva with 0.1 ml of 0.02 M $CaCl_2$ (Merck, Germany), with the clotting reaction being started in addition of 0.1 ml of plasma. All reagents were brought to the reaction temperature (37°C) before admixture. Since the clotting time is inversely proportional to the TF activity, the lengthening of the clotting time is a manifestation of decreased TF activity.

Statistical Analyses

Data are presented as the mean ± standard deviation. The differences between the values of the groups were tested by the Mann-Whitney test, and Spearman's correlation test was used to determine the correlations. Differences with p values ≤ 0.05 were considered significant. All statistical analyses were performed with the SPSS software for Windows, version 11.

Results

Plasma TF levels significantly increased in patients with CAD when compared with the control group ($p < 0.001$; table 1). No significant change was observed in the salivary TF activities of the CAD patient group compared with the control group ($p > 0.05$; table 1).

In the CAD patient group, salivary TF activity was found to be decreased in patients with CPITN index 4 when compared with patients with CPITN indexes 2 and 3 ($p < 0.01$, $p < 0.05$). Moreover, a significant decrease was observed in the salivary TF activity of patients with CPITN index 3 when compared with patients with CPITN index 2 ($p < 0.01$; table 2). Similarly, in periodontitis patients without CAD (control group), TF activity was decreased in patients with CPITN indexes 3 and 4 when compared with patients with CPITN index 2 ($p < 0.001$ and $p < 0.005$, respectively; table 3).

There was a strong and negative correlation between salivary TF activities and CPITN indexes in periodontitis patients with and without CAD ($r = -0.793$, $p < 0.01$; $r = -0.762$, $p < 0.01$).

Discussion

Although a number of studies on the possible association between CVD and periodontitis have been performed recently, this association is not yet thoroughly understood [27, 28]. Consequently, in the present study, hypothesizing that TF may play a role in this association, we aimed to evaluate TF levels in plasma as well as TF activity of saliva in periodontitis patients with and without CAD.

Table 1. Plasma TF levels and salivary TF activities of the control and CAD patient groups

	Control group	Patient group
TF, pg/ml	47.32 ± 14.64	67.16 ± 19.00[a]
TF activity, s	66.77 ± 9.06	63.69 ± 7.10

Lengthening in seconds is a manifestation of decreased TF activity. [a] $p < 0.001$, significantly different when compared with the control group.

Table 2. Plasma TF levels and salivary TF activities of CAD patients with CPITN indexes 2, 3 and 4

	CPITN 2	CPITN 3	CPITN 4
TF, pg/ml	60.44 ± 20.05	75.59 ± 16.96	62.04 ± 17.06
TF activity, s	57.9 ± 3.87[a, b]	64.55 ± 4.89[a]	73.4 ± 4.28

Lengthening in seconds is a manifestation of decreased TF activity. [a] $p < 0.05$, significantly different from the CPITN 4 group; [b] $p < 0.05$, significantly different from the CPITN 3 group.

Table 3. Plasma TF levels and salivary TF activities of the control group with CPITN indexes 2, 3 and 4

	CPITN 2	CPITN 3	CPITN 4
TF, pg/ml	46.48 ± 12.30	46.35 ± 15.42	51.14 ± 19.55
TF activity, s	58.0 ± 4.27[a, b]	69.91 ± 6.40	77.4 ± 3.13

Lengthening in seconds is a manifestation of decreased TF activity. [a] $p < 0.001$, significantly different from the CPTIN 3 group; [b] $p < 0.005$, significantly different from the CPTIN 4 group.

As the principal biological initiator of blood coagulation, TF is believed to play a critical role in thrombosis and thrombogenesis [29], and concentrations in several biological fluids correlate with different pathological conditions [30]. Inappropriate expression of TF may result in thrombosis contributing to acute clinical consequences of CAD. Many studies demonstrated elevated TF plasma levels in patients with myocardial infarction [31], stable angina [32], unstable angina and an increased risk of unfavorable outcomes in patients with unstable angina and raised TF levels. Accordingly, in the present study, TF levels in plasma were significantly increased in periodontitis patients with CAD. The presence of an in-

flammatory focus in the oral cavity may potentiate the atherosclerotic process by stimulation of humoral and cell-mediated inflammatory pathways. Also, the presence of periodontal infection may lead to brief episodes of bacteremia with inoculation of atherosclerotic plaques by periodontal pathogens such as *P. gingivalis, Actinobacillus actinomycetemcomitans* and *Treponema denticola*. The degree of inflammation in periodontal disease is clearly sufficient to cause a systemic inflammatory response, as evidenced by increases in C-reactive protein [5, 6, 33].

In the present study, increased plasma TF levels in periodontitis patients with CAD compared with those without CAD are consistent with other results given in the literature, since a circulating pool of TF in blood has been shown to be associated with increased blood thrombogenicity in patients with CVD [14]. However, no changes were observed in plasma TF levels in periodontitis patients with or without CAD when patients were categorized according to their CPITN indexes. Consequently, in our results, the degree of inflammation in the oral cavity does not seem to affect TF levels in plasma.

Saliva has long been recognized as having thromboplastic properties [34, 35]. However, our study is the only one in the literature which evaluated salivary TF activity in periodontitis patients with and without CAD. Salivary TF activities did not change significantly in periodontitis patients with and without CAD. On the other hand, significant decreases were observed in the salivary TF activities in both groups as the CPITN index increased. Accordingly, a strong and negative correlation was found between the CPITN indexes and the salivary TF activities both in periodontitis patients with and without CAD. Based on this finding, we may suggest that TF activity of saliva decreases as the severity of periodontitis increases.

The coagulant of normal human saliva has been identified as TF which is related to cells and cell fragments in saliva [25]. Rai [36] has found a significantly higher amount of tumor necrosis factor (TNF)-α in saliva samples obtained from patients with periodontitis in comparison with the healthy controls. Kambas et al. [37] have shown that TNF signaling upregulated TF-dependent procoagulant properties of bronchoalveaolar lavage fluid in patients with acute respiratory distress syndrome and suggested a primary role for inflammatory mediators in the upregulation of TF. However, within the limits of this study, as the CPITN indexes increased, salivary TF activities decreased. We believe that investigation of inflammatory mediators such as TNF-α in saliva may illuminate this debate.

As a diagnostic fluid, saliva is underused. The physiopathology of salivary TF has not yet been well demonstrated. The coagulative function of saliva derives from the TF found in saliva, and TF has been reported to establish hemostasis in the mouth [20, 22]. Disturbances of the hemostatic system may affect the oral cavity, which in turn may cause spontaneous bleedings of the dental tissues, petechies of oral soft tissues and echimosis as seen in routine examinations. Postoperative examinations show that minor oral surgeries can cause bleeding as well [20]. The role of a permanent or prolonged decrease in salivary TF activity in the progression of periodontitis needs to be investigated.

To our knowledge, TF levels in saliva have not been measured before. The ELISA kit used in the present study has been designed for detection of human TF in plasma, serum, tissue and cell culture lysate. The minimum detectable dose of TF has been reported to be 10 pg/ml for this assay. In our present study, salivary TF activities were determined; therefore, further studies are necessary to evaluate TF levels in saliva.

As the bleeding in periodontitis takes place in the ulcerated tissue of the gingival sulcus, we believe that the content of gingival crevicular fluid may also play a crucial role in understanding the relationship between TF activity and disease severity. The present study makes the observation that while the plasma TF level is an important marker of CAD, the salivary TF activity may be an indicator for periodontal disease severity. Our findings add to the understanding of the role of TF in pathways linked to inflammation and coagulation and further help to explain the link between periodontal infections and atherosclerosis-related vascular disease.

Acknowledgement

This study was supported by project number 25-DPT-070401 from the Commission of Scientific Investigations Projects of Yildiz University.

References

1 Papapanos P: Periodontal diseases: epidemiology. Ann Periodontol 1996;1:1–36.
2 Hugoson A, Norderyd O, Slotte C, Thorstensson H: Distribution of periodontal disease in a Swedish adult population 1973, 1983 and 1993. J Clin Periodontol 1998;25:542–548.
3 Lalla E, Lamster IB, Hofmann MA, Bucciarelli L, Jerud AP, Tucker S, Lu Y, Papapanou PN, Schmidt AM: Oral infection with a periodontal pathogen accelerates early atherosclerosis in apolipoprotein E-null mice. Arterioscler Thromb Vasc Biol 2003;23:1405–1411.
4 Joshipura K, Ritchie C, Douglass C: Strength of evidence linking oral conditions and systemic disease. Compend Contin Educ Dent 2000;21(suppl):12–23.
5 Elkaïm R, Dahan M, Kocgozlu L, Werner S, Kanter D, Kretz JG, Tenenbaum H: Prevalence of periodontal pathogens in subgingival lesions, atherosclerotic plaques and healthy blood vessels: a preliminary study. J Periodontal Res 2008;43:224–231.
6 Roth GA, Moser B, Roth-Walter F, Giacona MB, Harja E, Papapanou PN, Schmidt AM, Lalla E: Infection with a periodontal pathogen increases mononuclear cell adhesion to human aortic endothelial cells. Atherosclerosis 2007;190:271–281.
7 Syrjanen J, Peltola J, Valtonen V, Iivanainen M, Kaste M, Huttunen JK: Dental infection associated with cerebral infarction in young and middle aged men. J Intern Med 1989; 225:179–184.
8 Buhlin K, Gustafsson A, Håkansson J, Klinge B: Oral health and cardiovascular disease in Sweden. J Clin Periodontol 2002;29:254–259.
9 Grau AJ, Buggle F, Ziegler C, Schwarz W, Meuser J, Tasman AJ, Bühler A, Benesch C, Becher H, Hacke W: Association between acute cerebrovascular ischemia and chronic recurrent infection. Stroke 1997;28:1724–1729.
10 Arbes SJ, Slade GD, Beck JD: Association between extent of periodontal attachment loss and self-reported history of heart attack: an analysis of NHANES III data. J Dent Res 1999;78:1777–1782.
11 Rauch U, Nemerson Y: Tissue factor, the blood, and the arterial wall. Trends Cardiovasc Med 2000;10:139–143.
12 Mackman N: Role of tissue factor in hemostasis, thrombosis, and vascular development. Arterioscler Thromb Vasc Biol 2004; 24:1015–1022.
13 Wilcox JN, Smith KM, Schwartz SM, Gordon D: Localization of tissue factor in the normal vessel wall and in the atherosclerotic plaque. Proc Natl Acad Sci USA 1989;86: 2839–2843.
14 Tutar E, Ozcan M, Kilickap M, Gülec S, Aras O, Pamir G, Oral D, Dandelet L, Key NS: Elevated whole-blood tissue factor procoagulant activity as a marker of restenosis after percutaneous transluminal coronary angioplasty and stent implantation. Circulation 2003;108:1581–1584.
15 Key NS, Slungaard A, Dandelet L, Nelson SC, Moertel C, Styles LA, Kuypers FA, Bach RR: Whole blood tissue factor procoagulant activity is elevated in patients with sickle cell disease. Blood 1998;91:4216–4223.
16 Amengual O, Atsumi T, Khamashta MA, Gughes GR: The role of the tissue factor pathway in the hypercoagulable state in patients with the antiphospholipid syndrome. Thromb Haemost 1998;79:276–281.
17 Sambola A, Osende J, Hathcock J, Degen M, Nemerson Y, Fuster V, Crandall J, Badimon JJ: Role of risk factors in the modulation of tissue factor activity and blood thrombogenicity. Circulation 2003;107:973–977.
18 Asakura H, Kamikubo Y, Goto A, et al: Role of tissue factor in disseminated intravascular coagulation. Thromb Res 1995;80:217–224.
19 Meszaros K, Aberle S, Dedrick R, Machovich R, Horwitz A, Birr C, Theofan G, Parent JB: Monocyte tissue factor induction by lipopolysaccharide (LPS): dependence on LPS-binding protein and CD14, and inhibition by a recombinant fragment of bactericidal/permeability-increasing protein. Blood 1994;83: 2516–2525.
20 Yarat A, Tunali T, Pisiriciler R, Akyuz S, Ipbuker A, Emekli N: Salivary thromboplastic activity in diabetics and healthy controls. Clin Oral Investig 2004;8:36–39.
21 Emekli-Alturfan E, Kasikci E, Yarat A: Peanuts improve blood glutathione, HDL-cholesterol level and change tissue factor activity in rats fed a high-cholesterol diet. Eur J Nutr 2007;46:476–482.
22 Emekli-Alturfan E, Kasikci E, Yarat A: Tissue factor activities of streptozotocin induced diabetic rat tissues and the effect of peanut consumption. Diabetes Metab Res Rev 2007;23:653–658.
23 Emekli-Alturfan E, Demir G, Kasikci E, Tunali-Akbay, Pisiriciler R, Calikan E, Yarat A: Altered biochemical parameters in the saliva of patients with breast cancer. Tohoku J Exp Med 2008;214:89–96.
24 Alturfan AA, Alturfan EE, Dariyerli N, Zengin E, Aytac E, Yigit G, Kokoglu E: Investigation of tissue factor and other hemostatic profiles in experimental hypothyroidism. Endocrine 2006;30:63–67.
25 Zacharski LR, Rosenstein R: Reduction of salivary tissue factor (thromboplastin) activity by warfarin therapy. Blood 1979;53:366–374.
26 Ingram GIC, Hills M: Reference method for the one stage prothrombin time test on human blood. Thromb Haemostas 1976;36: 237–238.
27 Emingil G, Buduneli E, Aliyev M, Akilli A, Atilla G: Association between periodontal disease and acute myocardial infarction. J Periodontol 2000;71:1882–1886.
28 Genco R, Offenbacher S, Beck J: Periodontal disease and cardiovascular disease: epidemiology and possible mechanisms. J Am Dent Assoc 2002;133:14–22.
29 Nemerson Y: Tissue factor: then and now. Thromb Haemost 1995;74:180–184.
30 Fareed J, Callas DD, Hoppensteadt D, Bermes EW Jr: Tissue factor antigen levels in various biological fluids. Blood Coagul Fibrinolysis 1995;6:32–36.
31 Saigo M, Abe S, Ogawa M, Yamashita T, Biro S, Minagoe S, Maruyama I, Tei C: Imbalance of plasminogen activator inhibitor-I/tissue plasminogen activator and tissue factor/tissue factor pathway inhibitor in young Japanese men with myocardial infarction. Thromb Haemost 2001;86:1197–1203.
32 Kim H, Song K, Park Y, et al: Changes of plasma tissue factor and tissue factor pathway inhibitor antigen levels and induction of tissue factor expression on the monocytes in coronary artery disease. Cardiology 2000;93:31–36.
33 Haynes WG, Stanford C: Periodontal disease and atherosclerosis: from dental to arterial plaque. Arterioscler Thromb Vasc Biol 2003; 23:1309–1311.
34 Bellis C, Scott FH: Saliva and blood coagulation. Proc Soc Exp Biol Med 1933;30:1373.
35 Ersner MS, Myers D, Ersner W: A clinical and experimental study of the action of saliva on blood coagulation and wound healing in surgery of the oral cavity and throat. Ann Otol Rhinol Laryngol 1934;43:114.
36 Rai B: Salivary levels of tumor necrosis factor-alpha in periodontitis. Adv Med Dent Sci 2008;2:40–41.
37 Kambas K, Markiewski MM, Pneumatikos IA, et al: C5a and TNF-alpha up-regulate the expression of tissue factor in intra-alveolar neutrophils of patients with the acute respiratory distress syndrome. J Immunol 2008; 180:7368–7375.