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ORIGINAL ARTICLE

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Salivary thromboplastic activity in diabetics and healthy controls

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Abstract Coagulative function of saliva derives from the thromboplastin found in saliva. It may establish hemostasis in the mouth. Salivary disfunction and changes in salivary composition and are frequent complications of diabetes. This study investigated the influence of some local etiologic and systemic factors on salivary thromboplastic activity (STA) in diabetics. In this study, cytological smears and biochemical tests were used. STA was measured by Quick's one stage method, serum glucose by the glucose oxidase method, and salivary protein by the method of Lowry. STA was almost the same in the diabetic and control groups. The only statistically significant difference within the diabetic group was found to be due to antibiotic usage. STA, i.e. clotting time, was 30% longer (114 s) ($p<0.05$) and salivary protein (4.07 mg ml^{-1}) ($p<0.1$) was lower in diabetics not taking antibiotics than in those taking them. No such differences were observed in the healthy controls. Significant linear correlations ($p<0.05$) with respect to STA were with salivary protein in the control group (r=0.61) and in the diabetic group (r=0.51) and with antibiotic usage (r=0.29), with leukocyte cell count (r=0.27) in the diabetic group. It can be concluded that salivary cells, proteins and antibiotic usage are important for STA.

Keywords Diabetes · Saliva · Thromboplastic activity

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Introduction

Saliva plays an important role in the protection of oral cavity and alterations in either salivary flow rate or protein composition may have dramatic effects on oral health [8]. Diabetes induces multiple oral complications, including periodontitis, salivary disfunction, mucosal infections, and neurological problems of taste and smell [21]. Higher oral candidal incidence has been found in diabetic patients than in controls [10].

Thromboplastin, also known as tissue factor or factor III, is an important coagulation factor which initiates the extrinsic blood coagulation with FVII. It is not actively found in the blood but as a component of the cell membranes [4, 12, 15]. It has been shown that some body tissues and fluids have thromboplastic activities [2, 4, 13, 18, 19, 20]. As stated by several authors, coagulative function of the saliva derives from the thromboplastin found in saliva. Approximately 78% of salivary thromboplastic activity (STA) is attributable to the presence of cells in saliva and the remainder to particulate cell debris [20]. Physiopathology of human saliva thromboplastin has not yet been well demonstrated. It has been shown that oral cavity is affected from the disturbances of the hemostatic systems. Spontaneous bleedings of the dental tissues, pettechies of oral soft tissues and ecimosis are seen in routine examinations. Post-operative examinations show that minor oral surgeries can also cause bleeding [1]. Salivary thromboplastin may establish the hemostasis after oral traumas and treat bleeding peptic ulcer or diminish bleeding, as in tonsillectomies [5, 20]. There is no study related to diabetes and STA in the literature.

In this study, pH, total protein and thromboplastic activities of the saliva samples obtained from diabetic and healthy individuals were examined. Saliva imprint sam-

ples were evaluated cytologically. In addition, STA was compared with many local etiologic and systemic factors such as salivary flow rate, salivary pH, heredity, alcohol and smoking habits, antimicrobial therapy, wearing of dentures, burning sensation, dry mouth, taste alteration, and toothbrushing habit.

Material and methods

This study was performed on 59 diabetic patients and 55 non-diabetic control subjects having no systemic disease with the age range of 18–73 years. Diabetic patients were randomly selected from individuals who attended Turkish Diabetes Association, Harbiye, Istanbul, and control subjects from Marmara University, Faculty of Dentistry.

At 08:00–10:00 a.m., oral and dental examination were conducted by a dentist under natural lighting with the aid of a dental mirror and explorer. Subject history including the investigated parameters was recorded on forms prepared before the examination.

Unstimulated mixed saliva samples were collected, after overnight fasting and after the mouth had been rinsed with distilled water, by spitting into a funnel. Saliva volume and collection time were recorded to calculate salivary flow rate. Saliva samples were analysed for pH by using pH paper (Neutralit pH 5.5–9.0, Merck-pH Indikatepapier).

For cytological examinations, saliva samples were smeared over a glass microscope slide and fixed with air. Then they were stained with Giemsa stain [3]. All slides were examined microscopically (×100) for the presence of ephitelium, leucocyte, keratinization and bacterium.

Thromboplastic activities of saliva samples were evaluated according to Quick's one stage method using normal plasma [9, 20]. This was performed by mixing 0.1 ml saliva with 0.1 ml of 0.02 M $CaCl_2$, with the clotting reaction being started on addition of 0.1 ml of plasma. All reagents were brought to the reaction temperature (37°C) before admixture.

Serum glucose concentration was determined by glucose oxidase method (Randox, Cat No: GL 364) and total protein content of the saliva samples, by the method of Lowry [11].

Unistat 5.0 statistical computer programme was used to evaluate the results. Chi-square test, "t" test, one-way analysis of variance (ANOVA) test and Spearman correlation analysis were used.

Results and discussion

Ten percent of the diabetic patients were type I and 90% were type II diabetic. Twelve percent of the diabetic patients were under diet treatment, 49% under oral antidiabetic treatment (OAD) and 39% under insulin or insulin + OAD. Fifty percent of diabetic patients had a diabetic age <5 years, 20% between 5 and 10 years, 20% between 10 and 15 years, and 10% >15 years. In the diabetic group, females were 52 and males 48%, while in the control group, females were 62 and males 38%. In both groups, alcohol and smoking habits of men were higher while tooth brushing habit was less than in women.

The rates of diabetic patients wearing dentures, having diabetic heredity in the family, suffering from dry mouth, or having taste alteration were significantly higher than in the control group (Table 1).

Salivary flow rate and pH values of diabetic patients were significantly lower while serum glucose values were significantly higher than that of non-diabetic controls (Table 2). These findings were similar to those reported in

Table 1 Significant chi-squared test results of local etiologic factors in diabetic and non-diabetic control groups

		Diabetic group (n=59)	Nondiabetic group (n=55)	P
Heredity	Present	(n=36) 61%	(n=19) 34.5%	<0.01
	Not present	(n=23) 39%	(n=36) 65.5%	
Denture	Present	(n=24) 40.7%	(n=10) 18.2%	<0.01
	Not present	(n=35) 59.3%	(n=45) 45%	
Dry mouth	Present	(n=35) 59.3%	(n=1) 1.8%	<0.01
	Not present	(n=23) 40.7%	(n=54) 98.2%	
Taste alteration	Present	(n=5) 8.5%	(n=0) 0%	<0.05
	Not present	(n=54) 91.5%	(n=55) 100%	

Table 2 Mean levels of age, fasting serum glucose, salivary pH, saliva flow rate, saliva total protein and saliva thromboplastic activity for non-diabetic and diabetic group

	Diabetic group		Nondiabetic group		"t" test P
	Mean	SD	Mean	SD	
Age (years)	48.78 (n=59)	11.97	42.95 (n=55)	10.92	<0.01
pH	6.79 (n=59)	0.50	7.05 (n=51)	0.39	<0.01
Salivary flow rate (ml min^{-1})	0.82 (n=59)	0.56	1.03 (n=55)	0.65	0.05<p<0.1
Serum Glucose (mg dl^{-1})	170.31 (n=59)	63.41	87.16 (n=51)	6.77	<0.01
Saliva Total Protein (mg ml^{-1})	4.28 (n=57)	1.81	3.51 (n=53)	1.97	<0.05
Salivary Thromboplastic Activity (s)	107.95 (n=58)	52.42	103.22 (n=54)	44.02	>0.1

SD standard deviation

Table 3 Chi-squared test results of epithelium cells in saliva imprint samples between diabetics and non-diabetics

		Diabetic group		Nondiabetic group		*P*
Epithelium cell	1	(*n*=17)	29.3%	(*n*=9)	16.7%	>0.1
	2	(*n*=41)	70.7%	(*n*=45)	83.3%	
Nonepithelium cell	1	(*n*=44)	75.9%	(*n*=45)	83.3%	>0.1
	2	(*n*=14)	24.1%	(*n*=9)	16.9%	

1: cell count is normal, 2: more than normal

Table 4 Correlation analysis in non-diabetic and diabetic groups

	Variable I	Variable II	Correlation coeffiecent (r)
Nondiabetic group	STA	Saliva flow rate	−0.3469*
	STA	Saliva protein	+0.6070**
	Nonepithelium cells	Dry mouth	+0.3071*
	Nonepithelium cells	Denture usage	+0.2988*
Diabetic group	STA	Antibiotic usage	+0.2903*
	STA	Saliva protein	+0.5094**
	STA	Leucocyte cells	+0.2685*
	Saliva protein	Saliva flow rate	−0.4713**
	Saliva protein	Saliva pH	−0.4322**
	Saliva protein	Antibiotic usage	+0.2770*
	Epithelium cells	Saliva flow rate	−0.4050**
	Epithelium cells	Saliva protein	+0.3974**
	Nonepithelium cells	Alcohol usage	+0.3147*
	Nonepithelium cells	Toothbrushing	−0.3789*

* $p<0.05$, ** $p<0.01$
STA salivary thromboplastic activity

Table 5 Saliva thromboplastic activity and protein levels of individuals taking antibiotic therapy in control and diabetic group

	Individuals taking antibiotic		Individuals not taking antibiotics		*P*
	Mean	SD	Mean	SD	
Diabetic group					
Salivary protein (mg ml^{-1})	5.18 (*n*=11)	1.57	4.07 (*n*=46)	1.81	$0.05<p<0.1$
Salivary thromboplastic activity (s)	79.91 (*n*=11)	24.96	114.51 (*n*=47)	55.12	$p<0.05$
Nondiabetic group					
Salivary protein (mg ml^{-1})	3.50 (*n*=5)	1.95	3.51 (*n*=48)	1.99	$p>0.1$
Salivary thromboplastic activity (s)	102.2 (*n*=5)	24.95	103.33 (*n*=49)	45.69	$p>0.1$

SD Standard deviation

the literature [6, 7, 10, 14, 15, 17]. There was a significant increase in salivary total protein values and non-significant decrease in STA in diabetics compared with controls. No significant difference was found between epithelial cell counts and other cell counts in saliva imprint samples of both groups ($p>0.1$) (Table 3), nor did STA show significant difference; these findings were similar to those reported in the literature [20]. Approximately 78% of salivary thromboplastin is attributed to cells found in saliva. It has been reported that centrifuging the saliva samples caused a decrease in saliva thromboplastic activity and that it is necessary to centrifuge them at 500 g for 15 min in order to remove the debris [20]. Since we observed the decrease in STA in our preliminary experiments, we did not centrifuge the saliva samples. For easy sampling of saliva samples, we vortexed them at least 10 s before STA determinations.

In diabetes, the significant amount of yeast cells found in the oral imprint samples [10] and the non-significant decrease in STA in the present study may show that yeast cells do not contribute to STA. Positive correlation between leukocyte counts in imprint samples and STA may show that leukocytes contribute to STA (Table 4).

In the present study, no significant differences were found between Type I and Type II diabetic patients regarding STA activity and other parameters.

When we compared the parameters according to heredity, sex, diabetic treatment, diabetic age or the presence of local etiologic factors in diabetic and control groups, no significant differences were found in salivary total protein values and STAs. Only the STA of diabetics taking antibiotic therapy was significantly higher than diabetics not taking antibiotic therapy (Table 5) The

reason why antibiotic usage was not effective in the control group may be because of the significant increases of salivary total protein values in diabetics. Correlation analysis showed that there was a positive correlation between STA and salivary total protein amounts (Table 4).

Regarding correlation analysis, increase in antibiotic usage in the diabetic group caused an increase in STA while the decrease in salivary flow rate caused an increase of STA in the control group. The increase in protein amounts both in diabetic and control groups caused an increase in STA.

As a result of this study, it can be concluded that salivary cells, proteins and antibiotic usage were important for STA.

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References

1. Akyuz S, Atalay T (1991) Kanama ve pıhtılaşma zamanlarının uzamasına neden olan hastalıklar ve bunların dişhekimliği açısından önemi. I.U. Dishekimliği Fakültesi Dergisi 25:215–218
2. Ashkinazi IIa (1977) Functional deficiency of erythrocyte thromboplastin factor. Biull Eksp Biol Med 83:664–666
3. Atay Z, Topalidis T (1994) Cytodiagnostik der Serösen Höhlen. Atlas und Lehrbuch. Wolfgang Pabst Verlag, Lengerich, Berlin, Prag, Riga, Wien, Zagreb, pp 356
4. Bachli E (2000) History of thromboplastin. Br J Haematol 110:248–255
5. Belikov PP (1971) Blood coagulation factors in the saliva in injury of oral mucous membrane. Stomatol (Moscow) 50:72–74
6. Ben-Argen H, Serovya R, Kanter Y, Szargel R, Laufer D (1993) Oral health and salivary composition in diabetic patients. Diabetes Comp 7:57–62
7. Ciglar I, Sutalo J, Sjaljac-Stadt G, Bazikov J (1991) Saliva as a factor for caries in diabetic patients. Acta Stomatol Croat 25:143–149
8. Dawes C (1996) Factors influencing salivary flow rate and composition. In: Edgar WM, O'mualle DM (eds) Saliva and oral health. Thanet Press Limited, Margate, UK, pp 27–41
9. Ingram GIC, Hills M (1976) Reference method for the one stage prothrombin time test on human blood. Thromb Heamostas 36:237–238
10. Kadir T, Pisiriciler R, Akyuz S, Yarat A, Emekli N, Ipbuker A (2002) Mycological and cytological examination of oral candida, carriage in diabetic patients and non diabetic control subjects: thorough analysis of local aetiologic and systemic factors. J Oral Rehabil 29:452–457
11. Lowry OH, Rosebrough WI, Forr AL, Randal RJ (1951) Protein measurement with the folin phenol reagent. J Biol Chem 193:265–275
12. Lwaleed BA (2002) Tissue factor: biological function and clinical significance. Saudi Med J 23:135–143
13. Lwaleed BA, Francis JL, Chrisholm M (2000) Urinary tissue factor levels in neoplastic disease. Ann Saudi Med 20:197–201
14. Narhi Tao, Meurman JH, Odont D, Ainamo A, Tiluis R (1996) Oral health in the elderly with non-insulin dependent diabetes mellitus. Spec Care Dentist 16:116–122
15. Roberts HR, Monroe DM, Hoffman M (2001) Molecular biology and biochemistry of the coagulation factors and pathways of hemostasis. In: Beutler E, Lichtman MA, Coller BS, Kipps TJ, Seligsohn U (eds) Williams hematology. McGraw Hill, USA, pp 1409–1429
16. Sandberg GE, Sundberg HE, Fjellstrom CA, Wikblad KF (2000) Type 2 diabetes and oral health: a comparison between diabetic and non-diabetic subjects. Diabetes Res Clin Prac 50:27–34
17. Thurstenson H (1995) Periodontal disease in adult insulin dependent diabetes. Swed Dent J 107[Suppl]:1–68
18. Tutuarima JA, Hische EAH, Trotsenburg L, Helm HJ (1985) Thromboplastic activity of cerebrospinal fluid in neurological disease. Clin Chem 31:99–100
19. Utter GH, Owings JT, Jacoby RC, Gosselin RC, Paglieroni TG (2002) Injury induced increased monocyte expression of tissue factor: factors associated with head injury attenuate the injury-related monocyte expression of tissue factor. J Trauma 52:1071–1077
20. Zacharski LR, Rosenstein R (1979) Reduction of salivary tissue factor (thromboplastin) activity by warfarin therapy. Blood 53:366–374
21. Zielinski MB, Fedele D, Forman LJ, Pomerantz SC (2002) Oral health in the elderly with non-insulin-dependent diabetes mellitus. Spec Care Dentist 22:94–98