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Proinflammatory Factors in Saliva as Possible Markers for Periodontal Disease

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A B S T R A C T

Studies have indicated that host inflammatory proteins, enzymes and indicators of bone metabolism present in saliva differ in different types of periodontal disease. However, the number of markers analyzed was limited and the effect of edentulousness was not examined. We measured the concentration of host inflammatory proteins: C-reactive protein (CRP), C3 and C4 complement components, alpha-2-macroglobulin (α-2M) and tumor-necrosis factor (TNF) in unstimulated saliva of 14 periodontally healthy (PH), 9 edentulous persons (EP), 10 patients with chronic periodontitis (CP) and 18 with aggressive periodontitis (AgP). TNF was below the level of detection in all samples except one. Edentulous persons and patients with CP had significantly reduced concentrations of CRP, C3 and α-2M. Edentulous persons and AgP patients had lower C4 concentrations. We can conclude that edentulous persons and CP patients have reduced salivary concentrations of host inflammatory proteins. These findings suggest that a reduction in host responsiveness might play a role in the pathogenesis of CP.

Key words: *saliva, markers, proinflammatory factors, periodontitis*

Introduction

The diagnostics of periodontal disease relies primarily on clinical and radiographic parameters. These measures are useful in detecting evidence for past disease, or verifying periodontal health, but provide limited information about patients at risk for future periodontal breakdown[1,2].

The gingival crevicular fluid (GCF) is derived from gingival capillary beds (serum components) and resident and migrating inflammatory cells. Factors derived from GCF and the subgingival plaque is found in whole saliva, rather than gland-specific saliva[3].

Saliva can be easily collected, contains locally derived and systemically-derived markers of periodontal disease, and hence may offer the basis for a patient specific diagnostic test for periodontitis[4]. Furthermore, the analysis of saliva may offer a cost-effective approach to assess periodontal disease incidence in large populations[1,5].

The use of saliva in periodontal diagnostics has been the subject of considerable research activity, and proposed markers for disease include proteins of host origin, phenotypic markers, host cells, hormones, bacteria and bacterial products, volatile compounds and ions[6–8].

Studies have examined enzyme activity in saliva in relation to periodontal status and in response to periodontal treatment[1,4,5,8]. Their results indicate that saliva might contain markers useful for diagnosis of periodontal disease. However, their exact value or the optimal markers combination has not been defined. Also, the influence of other factors, such as edentulousness, on saliva constituents has not been analyzed.

We therefore measured following markers of inflammation: C-reactive protein (CRP), C3 and C4 components of complement (C3, C4), α-2 macroglobulin (α-2M) and tumor necrosis factor (TNF) in whole saliva of patients with chronic periodontitis (CP), aggressive periodontitis (AgP), periodontal healthy (PH) and edentulous persons (EP) to identify potential markers useful for diagnosis of periodontal disease.

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Material and Methods

Subjects

The study included 51 persons, 25 males and 26 females. Two study groups consisted of 18 untreated AgP patients, 9 males and 9 females and 10 untreated CP patients, 5 males and 5 females, refered to our department at the Dental School University of Zagreb, for treatment of periodontal disease. The mean age in the AgP group was 33 years, ranging from 19–52 years, and the mean age in the CP group was 38 years, ranging from 29–49 years. Control group consisted of 14 PH persons, 7 males and 7 females with mean age 24 years, ranging from 23–25 years, and 9 EP persons, 4 males and 4 females with mean age of 73 years, ranging from 65–81 years.

Criteria for AgP were: familial history of periodontal disease, generalized interproximal attachment loss affecting at least 4 permanent teeth other than first molars and incisors, and radiographically-proven vertical bone resorption[9]. Criteria for CP were: gingival inflammation, pocketing with clinical attachment loss of at least 10 sites with more than 5 mm of clinical attachment loss spread over several teeth and radiographical evidence of alveolar bone loss of more than 1/3 of the root length on at least 1 tooth per quadrant[10]. Periodontal screening was part of initial periodontal examination, using following clinical measurements: plaque index (PI)[11], sulcus bleeding index (SBI)[12], probing depth and clinical attachment loss (CAL)[13]. Periodontal tissue destruction was determined by measuring the attachment level loss calculated from the periodontal probing depth and gingival retraction. Probing depth was measured on mesial, distal, oral and vestibular sides of all teeth while gingival retraction was measured on oral and vestibular sides of all teeth using a calibrated manual probe. Diseased groups did not differ regarding the number of teeth.

PH persons were without clinical signs of destruction of the periodontal ligament, had low plaque and gingival inflammation levels, and showed no signs of pocketing.

According to anamnestic data reasons for teeth loss in the EP group were caries and its complications or periodontal disease.

A dietary and medical history was obtained from all subjects and all participants received a complete dental check-up. Individuals were encouraged to follow their normal daily nutritional regimen; no specific instructions regarding food or fluid intake were given.

Exclusion criteria were: individuals who had serious systemic diseases, such as diabetes or cancer, smoking (including persons who stopped smoking less than 2 yrs. ago) extensive carious lesions, medication in the 6 months preceding the study (e.g. immunosuppressive drugs, antibiotics, antiphlogistics) or pregnancy.

After the purpose and scope of the study was explained to them, all subjects gave an written informed consent for participation in this study.

Approval for this investigation was given by the Ethical Committee.

Collection of saliva

All saliva samples were collected in the morning, at least 2 hours after any food intake. Unstimulated saliva was collected after oral cavity irrigation with water. After the irrigation, the subjects took 5 ml of water and held it for 5 minutes in oral cavity, after which the sample was taken. Unstimulated saliva was obtained by passive drooling during 10 min with the subjects sitting in a relaxed position. About 4–5 ml of saliva was placed in an ice chilled test tube and transported to the Department of Laboratory Diagnostics, University Hospital Center Zagreb. All samples were homogenized, stored at –70°C and tested simultaneously.

To determine the temporal variability of tested salivary substances in 5 patients with AgP and 5 with CP, samples were obtained 3 times during a period of 1 h. Furthermore, the concentration of tested substances was determined in the same subjects on a standard diet for 3 consecutive days.

Laboratory methods

CRP concentrations were determined by simple radial immunodiffusion, using specific anti-human CRP antibodies forming insoluble aggregates after binding (intra assay CV%=1.02, inter-assay CV%=1.29)[14]. C3 and C4 were determined photometrically after radial immunodiffusion on LC-Partingen plates (Behring, Frankfurt, Germany), based on formation of immune complexes with specific antibodies (intra-assay CV% = 1.9, 1.8; inter-assay CV%=5.9, 2.9 for C3 and C4 respectively)[15]. α-2M concentrations were determined using the same method (intra-assay CV%=1.6, inter-assay CV%=3.0)[15]. TNF was determined employing linked immunosorbent assay ELISA with specific monoclonal antibodies (Endogen, Cambridge, Ma, USA) (inter-assay CV%=1.6, intra-assay CV%=3.3)[14].

Statistical analyses

Differences between groups for tested variables were assessed using variance analysis, and for clinical attachment loss using t-test. Normality of distribution was tested with Kolmogorov-Smirnov test. The correlation of variables was tested with Pearson correlation coefficient.

Results

Sex did not influence the finding. The mean CAL level in CP patients was 3.4 mm, range 1.8–4.7 mm, and in AgP patients 4.2 mm, range 3.0–6.4 mm. This difference was not significant (p=0.07). Maximal attachment loss (CALMAX) is different in CP and AgP groups. In the CP group CALMAX was 5.4 mm, range 2.8–9.0 mm. In the AgP group CALMAX was 6.7 mm, range 4.8–9.5 mm (Table 1).

The mean PI for the CP group was 0.8, and 1.9 for the AgP group. Mean SBI scores were 1.26 in CP patients and 3.02 in AgP patients. In the PH group mean PI was 0.6, mean SBI score was 0.5 and mean CAL 0.3 mm, range 0–0.8 mm.

TNF was below the level of detection in all samples except one from a patient with AgP.

Table 1 shows mean values and standard deviations (SD) of variables: CRP, C3, C4 and α-2M. The groups differ in proinflammatory marker concentrations observed in unstimulated saliva. CRP values in saliva were highest in the AgP group (102.11±79.02), then in the PH group (90.20±79.67), while CP and EP groups show significantly lower values (27.45±29.59) and (20.72±29.51). Observing the paired-differences significant difference exists in CRP concentrations between EP and AgP, and CP and AgP groups.

Tested groups show similar relationship when C3 concentrations are analysed. Similarly high values are observed in PH and AgP groups (10.74±8.03 and 10.39±5.08), while EP and CP groups show almost three times lower C3 concentrations in saliva (3.79±2.25 and 3.62±2.94). The differences between pairwise PH and EP, PH and CP, AgP and EP, and AgP and CP groups are statistically significant.

The highest C4 concentration was observed in the PH group (35.36±11.74), somewhat lower in CP group (24.10±10.67). For AgP group C4 concentration was 20.55±7.82, and EP group showed lowest concentrations C4 in saliva (17.50±12.84). Statistically significant differences in C4 concentrations were found between following pairs: PH and EP, PH and CP, EP and AgP, and CP and AgP.

When differences in α-2M concentrations in the saliva are analysed between the groups, they show relation similar to differences of CRP and C3 values between the groups. Mean concentration of α-2M in saliva of PH group is 401.14±484.58, and 384.57±383.48 in the AgP group. In the EP and CP group α-2M concentrations were approximately seven times lower (57.00±

TABLE 1
VARIABLES FOR MARKERS OF PERIODONTAL DISEASE IN SALIVA AND ATTACHMENT LOSS FOR CP AND AGP GROUPS

Variables	Groups	N	X	SD	Standard error	Test statistics	df	p
CRP	PH	14	90.20	79.67	21.29			
	EP	18	20.72	29.51	9.84			
	CP	10	27.45	29.59	9.36	4.95[a]	3, 47	0.005
	AgP	9	102.11	79.02	18.62			
	Total	51	69.84	73.18	10.25			
C3	PH	14	10.74	8.03	2.15			
	EP	18	3.79	2.95	0.98			
	CP	10	3.62	2.94	0.93	6.15[a]	3, 47	0.001
	AgP	9	10.39	5.08	1.20			
	Total	51	8.00	6.30	0.88			
C4	PH	14	35.36	11.74	3.14			
	EP	18	17.50	12.84	4.28			
	CP	10	24.10	10.67	3.37	7.18[a]	3, 47	<0.001
	AgP	9	20.55	7.82	1.84			
	Total	51	24.77	12.29	1.72			
α-2M	PH	14	401.14	484.58	129.51			
	EP	18	57.00	42.25	14.08			
	CP	10	58.70	42.97	13.59	3.74[a]	3, 47	0.017
	AgP	9	384.57	383.48	90.39			
	Total	51	267.42	371.89	52.07			
CAL	CP	10	3.40	0.89	0.28			
	AgP	18	4.18	1.12	0.26	–1.89[b]	26	0.070
	Total	28	3.91	1.09	0.21			
CALMAX	CP	10	5.40	1.80	0.57			
	AgP	18	6.69	1.49	0.35	–2.03[b]	26	0.050
	Total	28	6.23	1.69	0.32			

[a]F-test, [b]t-test, CP – chronic periodontitis, AgP – aggressive periodontitis, PH – periodontally healthy, EP – edentulous persons, CAL – mean clinical attachment loss,CALMAX – maximal clinical attachment loss, CRP – C-reactive protein, α-2M – α-2 macroglobulin, C3 – C3 complement component, C4 – C4 complement component

TABLE 2
CORRELATION OF VARIABLES FOR CP GROUP (N=10)

Variables	CRP	C3	C4	α-2M	CAL	CALMAX
CRP	–	0.903*	–0.031	0.326	–0.327	–0.229
C3	0.903*	–	0.118	0.092	–0.205	–0.224
C4	–0.031	0.118	–	0.021	0.010	–0.333
α-2M	0.326	0.092	0.021	–	0.082	–0.010
CAL	–0.327	–0.205	0.010	0.082	–	0.890*
CALMAX	–0.229	–0.224	–0.333	–0.010	0.890*	–

*correlation is significant at the 0.01 level, CP – chronic periodontitis, CAL – mean clinical attachment loss, CALMAX – maximal clinical attachment loss, CRP – C-reactive protein, α-2M – α-2 macroglobulin, C3 – C3 complement component, C4 – C4 complement component

TABLE 3
CORRELATION OF VARIABLES FOR AGP GROUP (N=18)

Variables	CRP	C3	C4	α-2M	CAL	CALMAX
CRP	–	0.387	–0.132	0.384	0.123	0.245
C3	0.387	–	–0.090	0.000	–0.257	–0.297
C4	–0.132	–0.090	–	0.222	–0.035	0.063
α-2M	0.384	0.000	0.222	–	0.023	0.183
CAL	0.123	–0.257	–0.035	0.023	–	0.917*
CALMAX	0.245	–0.297	0.063	0.183	0.917*	–

*correlation is significant at the 0.01 level, AgP – aggressive periodontitis, CAL – mean clinical attachment loss, CALMAX – maximal clinical attachment loss, CRP – C-reactive protein, α-2M – α-2 macroglobulin, C3 – C3 complement component, C4 – C4 complement component

42.25, and 58.70±42.97). Statistically significant differences in the α-2M concentrations were found pairwise between PH and EP, CP and AgP, and EP and CP.

Correlation between the tested variables (CRP, C3, C4 and α-2M) was tested using Pearson correlation coefficient. In the PH group significant positive correlation was observed between α-2M and CRP (r=0.703, p=0.005). Significant positive correlation was also observed between α-2M and CRP (r=0.922, p<0.001).

Between immunological and clinical variables, there was no significant correlation in the CP group (Table 2). Similarly, no correlation was observed in the AgP group between immunological and clinical variables (Table 3).

Discussion

Our data show that patients with different types of periodontitis differ between themselves and from healthy persons in their salivary content of inflammatory or bone destruction markers. This supports the hypothesis that biochemical analyses of saliva might be an important non-invasive way of studying disease processes in periodontitis[16]. However, since teeth loss exerts a very strong influence on these variables, future studies must take this factor into account. TNF concentration in saliva is below the level of detection of commercially available assays and investigators who wish to measure it should use alternative methods.

CRP, C3 and α-2M are humoral inflammatory markers and acute phase reactants. Reduced levels of these proteins in saliva of patients with CP, indicates that in this chronic disorder host inflammatory response is reduced[17–20]. C4 is also a humoral inflammatory marker and acute phase reactant. In contrast to C4, C3 and α-2M is higher in AgP in comparison to CP patients or PH group. Reduced concentrations of these proteins and C4 in EP group can be explained by the lack of gingival fluid component in their saliva.

In summary, our data show that the measurement of host inflammatory markers in the saliva might be a valuable path to follow in studying periodontal health and disease, taking into account that teeth loss profoundly affects these findings. CP is characterized by a reduction in concentrations of different inflammatory markers, indicating that a reduced host response might be important in the pathogenesis of this disorder[21].

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FAKTORI UPALE U SLINI KAO MOGUĆI BILJEZI PARODONTNE BOLESTI

SAŽETAK

Slina čini pogodan medij za otkrivanje proupalnih biljega u usnoj šupljini. Brojni serumski i tkivni sastojci koji prolaze kroz stijenku spojnog epitela gingivnog sulkusa i ulaze u slinu, mogu pomoći u određivanju aktivnosti parodontitisa. Stoga smo istraživali međuzavisnost medijatora lokalne imunosti u slini domaćina i kliničke parametre destrukcije u oboljelih od kroničnog (CP) i agresivnog (AgP) parodontitisa. Koncentraciju upalnih proteina domaćina određivali smo enzim-imunokemijskim testovima i u slini bezubih (E), što do sada nije istraživano, te u parodont zdravih osoba (PH). Stupanj destrukcije parodonta mjeren je gubitkom vezivnog pričvrstka (CAL). Istraživanje je obuhvatilo 51 osobu. Struktura navedenih uzoraka i njihova međuzavisnost testirana je neparametrijskim metodama. Dobiveni rezultati pokazuju kako se razina C-reaktivnog proteina (CRP), C3-komponente komplementa (C3), α-2 makroglobulina značajno razlikuju u nativnoj slini oboljelih od CP i AgP. Bezube osobe, slično CP skupini, pokazivale su značajnu redukciju koncentracije CRP, C3 i α-2M. C4 komponenta komplementa ne mjeri razliku među bolesnima. Zaključiti se može kako se razina medijatora obrane domaćina razlikuje za pojedine vrste parodontitisa u nativnoj slini.