Hacettepe Dişhekimliği Fakültesi Dergisi
Cilt: 30, Sayı: 3, Sayfa: 25-32, 2006

The Enzyme Activity of Alkaline Phosphatase in Gingival Crevicular Fluid of Smokers and Non-Smokers with Chronic Periodontitis

Kronik Periodontitisli Sigara İçen ve İçmeyen Bireylerin Dişeti Oluğu Sıvısı Alkalen Fosfataz Enzim Aktivitesi

***Ebru Olgun ERDEMİR DDS, PHD, **İsmet DURAN DDS, PhD, ***Seyfullah HALİLOĞLU DVM, PhD**

*Kırıkkale University, Faculty of Dentistry, Department of Periodontology
**Selcuk University, Faculty of Dentistry, Department of Periodontology
***Selcuk University, Faculty of Veterinary Medicine, Department of Biochemistry

ABSTRACT

Objective: The aim of this study was to evaluate the effects of smoking on clinical parameters and the gingival crevicular fluid (GCF) levels of alkaline phosphatase (ALP) in patients with chronic periodontitis after initial periodontal therapy.

Material and Methods: The study base consisted of 41 volunteer patients including 22 current smokers and 19 non-smokers. The clinical parameters including plaque index (PI), gingival index (GI), bleeding on probing (BOP), probing depth (PD), clinical attachment loss (CAL) were recorded and GCF samples were collected for analysis of ALP levels. At the 3rd and the 6th months all of these procedures were repeated.

Results: In smokers, only CAL was significantly higher at the 3rd month compared to non-smokers ($P<0.05$). GI and BOP were higher in non-smokers than smokers in both periods ($P<0.05$). PI showed increases from initial to the 6th month in smokers ($P<0.05$). Total activity of ALP was significantly higher in non-smokers at the 6th month ($P<0.05$) and decreased from baseline to the 6th month in smokers ($P<0.017$).

Conclusion: The present study demonstrated that cigarette smoking decreases ALP levels of GCF in smokers.

KEYWORDS
Smoking, ALP, Chronic periodontitis

ÖZET

Amaç: Bu çalışmanın amacı sigara kullanımının kronik periodontitisli hastalarda klinik parametreler ile DOS ALP enzim aktivitesi üzerine etkisinin değerlendirilmesidir.

Gereç ve Yöntem: Araştırma, sigara içen 22 hasta, hiç sigara içmemiş 19 hasta olmak üzere toplam 41 gönüllü hasta üzerinde yürütüldü. Bütün hastalardan gingival indeks (Gİ), plak indeksi (Pİ), sondalama cep derinliği (SCD), klinik ataşman kaybı (KAK) ve sondalamada kanama (SK) dahil klinik parametreler ve alkalen fosfataz (ALP) enzim aktivitesi değerlendirmeleri için dişeti oluğu sıvısı (DOS) örneklemeleri yapıldı. Bütün ölçümler 3. ve 6. aylarda tekrarlandı.

Bulgular: 3. ve 6. aylardaki ölçümlerde sigara içmeyen grupta, Gİ ve SK ortalama değerleri istatistiksel olarak anlamlı bir şekilde daha yüksek bulunmuştur ($P<0.05$). DOS ALP total aktivitesinin sigara içmeyenlerde 6. ayda istatistiksel olarak anlamlı bir şekilde daha yüksek olduğu ve sigara içen grupta ise DOS ALP total aktivitesinin 6. aya doğru azaldığı görülmüştür.

Sonuç: Bu araştırmada sigara kullanımının DOS ALP total seviyesini azalttığı sonucuna varılmıştır.

ANAHTAR KELİMELER
Sigara kullanımı, ALP, Kronik periodontitis

INTRODUCTION

There is well-documented evidence that bacteria and their products found in dental plaque comprise the primary etiologic agents responsible for periodontal disease[1,2]. In the periodontal tissue destruction that develops due to periodontal disease, the products of tissue destruction are seen in gingival crevicular fluid (GCF). It's shown that the levels of enzymes, in GCF such as β-glukronidase (BG),[3] elastase,[4] collagenase,[5] aspartate amiotransferase (AST)[6] and alkaline phosphatase (ALP)[7] have a possible correlation with periodontal destruction.

ALP is a membrane-bound glycoprotein found on most cell membranes in the body.[8] It is produced by many cells within the periodontal environment, the principal source being polymorphonuclear leukocytes (PMN),[9] bacteria within the supra and subgingival plaque[10] and through fibroblast and osteoblast activity.[11,12] ALP is one of the potentially powerful markers of periodontal disease activity. This was first recognized by Ishikawa and Cimasoni,[13] who demonstrated levels of enzyme in GCF three times those of serum and showed a significant correlation between ALP concentration in GCF and pocket depth. Binder et. al.[14] demonstrated that ALP concentration in GCF showed a positive relationship with attachment loss.

There are many risk factors in the onset and progression of periodontal diseases[15,16] and in a number of studies, cigarette smoking has also been found to increase the risk for periodontitis[17-24]. In general, smoking could lead to increased periodontal destruction by altering the host response through 2 mechanisms: 1) impairment of the normal host response in neutralizing infection and 2) alterations that result in destruction of the surrounding healthy periodontal tissues[25]. It has also been demonstrated that there is an interaction between cigarette smoking and the products of tissue destruction[26, 27].

In literature, there isn't a study that searches whether smoking has an effect on the levels of ALP in GCF or not. The aim of this study was to evaluate the effects of smoking on clinical parameters and enzyme activity of ALP in GCF in patients with chronic periodontitis after initial periodontal treatment at the 3rd and the 6th months.

MATERIAL and METHODS

Subject and site selection

The present investigation included the following subjects: 41 patients, 21 men and 20 women, in the age range of 32-59 years (45.59±7.13). The patients had moderate to severe periodontal disease as evidenced by multiple sites with a probing depth of 5 mm or more and bone loss by radiographs. All participants were in principal periodontally untreated and had not previously received surgical therapy and were drawn from the patients with chronic periodontitis at the Department of Periodontology. Patients with a history of systemic diseases or having received antibiotic therapies during the previous 6 months were excluded.

Patients were classified as either current smokers [S(+)], i.e., regular daily smoke 20 cigarettes (22 patients), or non-smokers [S(-)], i.e., who had never smoked tobacco (19 patients). All smokers were cigarette smokers. The mean age of current smokers and non-smokers was 44.41±7.88 and 46.94±6.07 years, respectively. The age differences between groups were found no statistically significant using t-test ($P>0.05$).

All participants received primary phase of non-surgical treatment including oral hygiene instruction, scaling and root planing. First month after non-surgical periodontal therapy was accepted as the baseline of the study. At the 3rd and the 6th months clinical recordings and sampling procedures were repeated.

Clinical recordings

Prior to crevicular fluid collection, supragingival plaque was scored using Plaque index (PI)[28]. Gingival inflammation was scored follow-

ing crevicular fluid collection using gingival index (GI)[29]. Bleeding on probing (BOP) was measured dichotomously[30]. Probing depth (PD) and clinical attachment loss (CAL) measures were obtained from sample sites (mesial or distal midpoints) of teeth using a conventional periodontal probe (Hu-Friedy, Chicago, IL, USA). The probe was directed parallel to the long axis of the tooth. CAL measurements were made from the cemento-enamel junction to the bottom of the sulcus. All clinical data were recorded by one examiner (EOE).

Crevicular fluid sampling

After supragingival plaque was removed from each tooth, the individual tooth site was gently air-dried and isolated with cotton rolls. Each GCF sample was collected with paper strips (Periopaper, Amityville, NY, USA) from randomly selected 4 sites of each patient with 5mm or more PD. The paper strips were consecutively inserted into the crevice at the mesial or distal midpoints until mild resistance was felt. The strips were left in situ 30 seconds and then transferred, for volume determination, to the chair-side located Periotron 8000 (Oraflow Inc., Plainview., NY, USA) which was calibrated using known volumes of phosphate-buffered saline (PBS). Four strips of each patient were immediately placed in a labeled tube containing 500 µl PBS and transported to the laboratory. Following 10 s vortexing and 20 min shaking, the strips were removed and the eluates centrifuged for 5 min at 5800 g to remove plaque and cellular elements. The samples were stored at -80°C until enzyme analysis.

Enzyme assay

ALP levels in samples were determined by using an appropriate commercial ALP kit (Bio-Clinica, Biobak, Istanbul, Turkey), 100 µl of eluted sample was assayed according to the kit's instructions. When samples were added to ALP kit, following reaction occurred.

P-nitrofenil fosfat+H_2O D fosfat+p-nitrofeneol

The absorbance of mixture was read 1 min. after and subsequent readings were repeated at 1st, 2nd and 3rd minutes. The absorbance changes in each well were determined using a computer-interfaced visible light spectrophotometer designed to read color reactions in 96-well microtiter plates. Optical density readings of each well were read at 405 nm and recorded on floppy disks. The concentration and total activity of ALP calculated following formula:

(1st reading) - (Initial absorption)=x

(2nd reading) - (Initial absorption)/2=y

(3rd reading) - (Initial absorption)/3=z

(x+y+z)/3=t = rALP

ALP=(3,298x500xrALP)/GCF amount=(U/L)/1000=U/ml

Total activity = (U/ml x GCF amount)/4=(U/ml)/4 sites

Concentrations of ALP were corrected for GCF volume and were defined as U/ml. Total activity of ALP was expressed as U/4 sites.

Statistical analysis

Data were expressed as means and standard deviations. The statistical significance of differences between groups was tested according to Univariate analysis of variance. Baseline measurements of parameters and age were taken as covariate factors to eliminate the effects of individual differences between subjects. Therefore, analysis of co-variance was used to analyze the differences in the levels of ALP in GCF and clinical parameters between S(+) and S(-) groups at the 3rd and the 6th months. The intra-group measurements over time were analyzed by using Friedman two-way ANOVA, including Bonferroni adjusted Wilcoxon signed ranks test (P=0.017) post hoc multiple comparison procedure when significant associations were observed. Simple pairwise correlations were calculated according to the rank correlation of Spearman (r_s). The null-hypothesis was rejected at $P<0.05$.

RESULTS

Clinical characteristics

The clinical characteristics of this study at initial, 3rd and 6th months are shown in Table I. Although we applied non-surgical periodontal therapy to match the periodontal conditions of the patients, there were differences among initial measurements. The confounding factors were taken as covariate for homogenizing these measurements.

When the clinical parameters were compared between groups, in S(+) group, only CAL was significantly higher compared to S(-) at 3rd month (P<0.05). GI and BOP were significantly higher in S(-) group compared to S(+) in both evaluation periods (P<0.05).

Once comparisons of clinical parameters were evaluated in each group, in S(+) group, PI showed increases from initial to 6th month (P<0.05). In S(-) group, GI and BOP showed an increase from 3rd month to 6th month (P<0.05). Therefore a significance was obtained between initial and 6th months (P<0.05). In each group, from initial to 3rd month significant decrease or increase wasn't seen.

GCF sample levels of ALP

The concentration and total activity measurements of the levels of ALP in GCF are shown in Table 2. Although in 3rd month, there was no significant difference between groups in the concentration and total activity of ALP (P>0.05), the concentration and total activity of ALP were significantly higher in non-smokers at 6th month (p<0.05).

TABLE I

The mean values of clinical parameters at initial, 3rd and 6th months in sampling sites (mean ± SD)

Parameters	Initial values		3rd month values		6th month values	
	S(+) (n=22)	S(-) (n=19)	S(+) (n=22)	S(-) (n=19)	S(+) (n=22)	S(-) (n=19)
PI	1.56±0.37	1.33±0.31	1.68±0.40	1.68±0.18	1.90±0.30[a]	1.84±0.24
GI	1.48±0.40	1.59±0.34	1.29±0.26	1.59±0.22*	1.40±0.28	1.89±0.17*[,a,b]
BOP	0.48±0.40	0.59±0.34	0.29±0.26	0.59±0.22*	0.40±0.28	0.88±0.15*[,a,b]
PD	5.28±0.24	5.21±0.29	5.35±0.19	5.27±0.18	5.33±0.28	5.17±0.19
CAL	5.31±0.78	4.54±0.49	5.10±0.85*	4.65±0.52	5.17±0.72	4.52±0.58

*P<0.05 according to S(+) or S(-) (initial values were used as covariates).
[a] P<0.05 according to initial value (Friedman and Bonferroni adjusted Wilcoxon signed ranks test).
[b] P<0.05 according to 3rd month value (Friedman and Bonferroni adjusted Wilcoxon signed ranks test).

TABLE II

The mean values of GCF ALP at initial, 3rd and 6th months in sampling sites (mean ± SD)

Parameter	Initial values		3rd month values		6th month values	
	S(+) (n=22)	S(-) (n=19)	S(+) (n=22)	S(-) (n=19)	S(+) (n=22)	S(-) (n=19)
Concentration (u/ml)	24.17±9.66	22.43±10.64	26.72±14.68	27.39±14.24	16.28±6.67[a,b]	22.19±8.87*
Total activity (u/4 sites)	8.22±1.23	8.04±2.09	8.03±1.86	7.63±1.76	7.19±0.84[a]	7.89±1.28*

*P<0.05 according to S(+) or S(-) (initial values were used as covariates).
a P<0.05 according to initial value (Friedman and Bonferroni adjusted Wilcoxon signed ranks test).
b P<0.05 according to 3rd month value (Friedman and Bonferroni adjusted Wilcoxon signed ranks test).

In S(+) group, the concentration of ALP decreased from initial and 3rd month to 6th month (P<0.05). The total activity of ALP in GCF decreased from initial to 6th month (P<0.05).

Correlations

Correlations between mean total activity of ALP levels in GCF and clinical parameters are shown in Table 3. There was a significant moderate correlation between the mean total activity of ALP in GCF and initial PD values in S(+) group (P<0.05). In S(-) group, it was found that there was a moderate negative correlation between the mean total activity of ALP in GCF and initial PI values (P<0.05). We didn't observe any correlations in the 3rd and the 6th months.

DISCUSSION

It has been reported that smoking affects the levels of some enzymes.[27,31,32] In this study, the effect of smoking on the levels of ALP in GCF, which is a potentially powerful marker of periodontal disease activity, was evaluated, however, it was observed that the level of ALP in GCF was higher in non-smokers than smokers during six-month study period.

In this study, non-surgical periodontal therapy was performed to match the amount of dental plaque and degree of gingival inflammation of patients with chronic periodontitis who regularly smoke and never smoke. Because of that the first month after periodontal therapy was accepted as the baseline of the study.

It has been interpreted that the effect of cigarette smoking on the periodontium to be indirect and due to inadequate levels of oral hygiene and increased plaque accumulation among smokers relative to non-smokers[33-35]. Adversely, the decreased plaque levels in smokers due to smoking can act as an anti-plaque agent and change the contents of saliva[17,36,37]. It has been also suggested that there was no difference between smokers and non-smokers in plaque levels after surgical periodontal therapy.[38] In this study, PI levels of both groups were the same after non-surgical periodontal therapy in all evaluation periods. This result supports the concept that cigarette smoking does not have a direct effect on dental plaque. However, when intra-group comparisons were evaluated, increase of PI levels from baseline to 6th month in S(+) group may be dependent on altered environmental factors in the long term by smoking.

Non-smokers were found to have higher GI and BOP values than smokers in some cross-sectional studies searching the effect of smoking on the clinical parameters[17,20,39]. However, there are studies that show no significant difference between smokers and non-smokers[40] and smokers have higher values than non-smokers[41]. It was reported that GI and BOP values were similar in smokers and non-smokers 9 months after periodontal therapy[42]. In our study, increased mean values of GI and BOP from baseline to the 6th month may depend on poor oral hygiene in both groups. In addition, GI and BOP values were

TABLE III

Correlations between total activity (U/4 sites) of ALP and clinical parameters in S(+) and S(-) (r values)

	PI		GI		BOP		PD		CAL	
Month	S(+) (n=22)	S(-) (n=19)	S(+) (n=22)	S(-) (n=19)	S(+) (n=22)	S(-) (n=19)	S(+) (n=22)	S(-) (n=19)	S(+) (n=22)	S(-) (n=19)
0	-0.002	-0.495*	-0.022	-0.096	-0.022	-0.096	0.433*	-0.163	0.041	0.192
3	0.121	-0.235	0.171	0.258	0.171	0.258	-0.121	0.258	-0.182	0.034
6	0.035	0.048	0.022	0.071	0.022	0.037	0.326	0.364	0.414	-0.325

*Significant correlation at alfa=0.05 (Spearman rank correlation coefficients)

found higher in non-smokers than smokers in both evaluation periods. The suppressive effect of smoking on bleeding, one of the markers of inflammation, can mask gingivitis signs in smokers. Besides, it has been found that the amount of gingival vessels in smokers was half of that in the non-smokers[43]. The higher GI scores of non-smokers can depend on nicotine, which causes vasoconstriction of blood vessels such as in forearm, skin, and hands. However, the effects of nicotine have been disputed, some claim that the blood flow is reduced[44] and others claim it is significantly increased[45] or unchanged[46].

It has been demonstrated that PD values were higher in smokers than non-smokers[17,19,34-37,40,47] and a significant positive correlation between smoking and CAL[24,35,37,39,40,48] has also been demonstrated. The increased PD and CAL levels in smokers may be dependent on accumulation of dental plaque and poor oral hygiene[33,35,49]. However, it was shown that deleterious effects of smoking on periodontium resulted not only from plaque amount and poor oral hygiene, but also from the effect of direct tissue destruction of smoking in homogen groups[18,19]. Some researchers found that smokers had higher mean values of PD than non-smokers after non-surgical periodontal therapy[50,51]. However, Pucher et al.[42] could not find any difference in PD and CAL scores between smokers and non-smokers 9 months after initial periodontal therapy. Similarly, in the present study, no difference was detected in the mean values of PD for 6-month period. Surprisingly, in this study mean values of CAL were higher in S (+) group at the 3rd month and we couldn't find any significant difference between groups at the 6th month.

GCF is now widely used as the primary collection source for components of host and bacterial cell metabolism that may prove to be reliable indicators of disease activity or potential activity. However it is not without its problems. The principal problem associated with GCF, is that the extremely small volumes of fluid collectable from a single site, necessitate highly sensitive analytical techniques to allow accurate quantification of fluid components. GCF volume is influenced by many factors such as flow rate[52], gingival trauma[53] and repeat sampling.[3] Because of that, the total activities of some enzymes in gingival fluid will correlate better with disease than enzyme concentrations. One of the potential problem is saliva contamination and this could clearly influence results expressed as enzyme concentration. The total activity of enzymes is more associated with disease activity than concentration.[54] Therefore, the comparisons of enzyme between groups, the changes of enzyme intra-groups and the correlations between ALP and clinical parameters were evaluated and discussed according to total activity in our study.

Some of the ways of collecting GCF are paper strips, capillary tubes, paper cones and gingival washing. Standardization in sampling is required to allow comparison between samples. This is achieved by sampling all sites for the same length of time with a reproducible collection procedure. We preferred to use paper strips as some of the authors[55-61]. Presently, GCF collection is usually made by paper strips and 30 s time of sampling is generally preferred[55,58,60,61].

ALP is enriched in the membranes of mineralizing tissue cells (e.g., osteoblasts) and is also present in PMN granules, plaque bacteria, fibroblasts and osteoclasts.[8] Chapple et. al.[62] suggested that host-derived ALP contributed to >80% of the enzyme in GCF in levamisole inhibition and studies on suspensions of washed plaque. Additionally, it's demonstrated that the major source of ALP within GCF was host derived and in early inflammatory disease was likely to be of PMN origin. In literature, although there are several studies about association between periodontal disease and ALP level of GCF[13,14,54,62-64,65], there isn't a study about the effect of smoking on the level of ALP in GCF. In the present study, after initial treatment, we expect an increase in tissue destruction products in GCF because of smoking as being a risk factor. In contrast, there was not a difference between groups at 3rd month; ALP enzyme activity was higher in non-smokers than smokers at 6th month. The lower level of ALP enzyme activity of smokers and decrease of enzyme activity at 6th month may be due to the effect of smoking on the functions and products of PMN, which are main sources of ALP. In addi-

tion, it is demonstrated that there was a correlation between concentration and total activity of ALP in GCF and both GI and PD scores[54]. In this study, there was a positive correlation between only initial measurements and PD in smokers.

Finally, the degree of gingival inflammation is not affected by cigarette smoking, and the amount of dental plaque increases over time in smokers. Moreover, this study shows that cigarette smoking has an effect on the levels of ALP in GCF in smokers.

Acknowledgements

This study was supported by a grant from the Research Fund of Selcuk University, Turkey. The authors thank the following individuals for their input and expert assistance on this clinical study: Said Bodur and Behic Serpek.

REFERENCES

1. Ebersole JL, Taubman MA. The protective nature of host responses in periodontal diseases. Periodontology 2000 1980; 5: 112-141.
2. Haffajae AD, Socransky SS. Microbial etiological agents of destructive periodontal diseases. Periodontology 2000 1994; 5: 78-111.
3. Lamster IB, Harper DS, Goldstein S, Celenti RS, Oshrain RL The effect of sequential sampling on crevicular fluid volume and enzyme activity. J Clin Periodontol 1989; 16: 252-258.
4. Jin L, Yu C, Corbet EF. Granulocyte elastase activity in static and flow gingival crevicular fluid. J Periodont Res 2003; 38: 303-310.
5. Kiili M, Cox SW, Chen HW, Wahlgren J, Maisi P, Eley BM, Salo T, Sorsa T. Collagenase-2 (MMP-8) and collagenase-3 (MMP-13) in adult periodontitis: molecular forms and levels in gingival crevicular fluid and immunolocalisation in gingival tissue. J Clin Periodontol 2002; 29: 224-232.
6. Tsalikis L, Malaka E, Pavlitou E, Konstantinidis A. Aspartate aminotransferase levels in gingival crevicular fluid before and after initial periodontal treatment. J Int Acad Periodontol 2001; 3: 68-74.
7. Chapple IL, Garner I, Saxby MS, Moscrop H, Matthews JB. Prediction and diagnosis of attachment loss by enhanced chemiluminescent assay of crevicular fluid alkaline phosphatase levels. J Clin Periodontol 1999; 26: 190-198.
8. Fedde KN, Cole DE, Whyte MP. Pseudohypophosphatasia: Aberrant localisation and substrat specifity of alkaline phosphatase in cultured skin fibroblasts, Am J Human Gene, 1990; 47: 776-783.
9. Cohn ZA, Hirsch JG. The isolation and properties of the specific cytoplasmic granules of rabbit PMNL. J Exp Med 1960; 112: 983-1004.
10. Bowen WH. Phosphatase in microorganisms cultured from carious dentin and calculus. J Dent Res 1961; 40: 571-577.
11. Cabrini RL, Carranza FA. Histochemical study on alkaline phosphatase in normal gingiva, varying the pH and the substrate. J Dent Res 1951; 30: 28-32.
12. Harris H. The human alkaline phosphatases: what we know and what we don't know. Clinica Chimica Acta 1989; 186: 133-150.
13. Ishikawa, Cimasoni G. Alkaline phosphatase in human gingival fluid and its relation to periodontitis, Arch Oral Biol 1970;15: 1401-1404.
14. Binder TA, Goodson JM, Socransky SS. Gingival fluid levels of acid and alkaline phosphatase, Arch Oral Biol 1987; 22, 14-19.
15. Genco RJ. Host responses in periodontal diseases current concepts. J Periodontol 1992; 63: 338-355.
16. Brown LJ, Brunelle JA, Kingman A. Periodontal status in the United States. J Dent Res 1996; 75, 672-683.
17. Feldman RS, Bravacos JS, Rose CL. Associations between smoking different tobacco products and periodontal disease indexes. J Periodontol 1983; 54: 481-487.
18. Bergström J, Eliasson S. Cigarette smoking and alveolar bone height in subjects with high standard of oral hygiene. J Periodontol 1987a; 14: 466-469.
19. Bergström J, Eliasson S. Noxious effect of cigarette smoking on periodontal health. J Periodont Res 1987b; 22: 513-517.
20. Bergström J. Cigarette smoking as risk factor in chronic periodontal disease. Comm Dent Oral Epidemiol 1989; 17: 245-247.
21. Genco RJ, Löe H. The role of systemic conditions and disorders in periodontal disease. Periodontology 2000 1993; 2: 98-116.
22. Bergström J, Preber H. Tobacco use as a risk factor. J Periodontol 1994; 65: 545-550.
23. Grossi SG., Zambon JJ, Ho AW et al. Assessment of risk for periodontal disease.I. Risk indicators for attachment loss. J Periodontol 1994; 65: 260-267.
24. Grossi SG, Genco RJ, Machtei EE et al. Assessment of risk for periodontal disease.II. Risk indicators for alveoler bone loss. J Periodontol 1995; 66: 23-29.
25. The American Academy of Periodontology. Tobacco use and the periodontal patient, (position paper) J Periodontol 1999; 70: 1419-1427.
26. Persson L, Bergstrom J, Ito H, Gustafsson A. Tobacco smoking and neutrophil activity in patients with periodontal disease. J Periodontol 2001; 72: 90-95.
27. Soder B, Jin LJ, Wickholm S. Granulocyte elastase, matrix metalloproteinase-8 and prostaglandin E2 in gingival crevicular fluid in matched clinical sites in smokers and non-smokers with persistent periodontitis. J Clin Periodontol 2002; 29: 384-391.
28. Silness J, Löe H. Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal condition. Acta Odontol Scand 1964; 22: 121-135.
29. Löe H, Silness J. Periodontal disease in pregnancy. I. Prevalence and severity. Acta Odontol Scand 1963; 21: 533-551.
30. Ainomo J, Bay I. Problems and proposals for recording gingivitis and plaque. Int Dent J 1975; 25: 229-235.
31. Soder B. Neutrophil elastase activity, levels of prostaglandin E2, and matrix metalloproteinase-8 in refractory periodontitis sites in smokers and non-smokers. Acta Odontol Scand 1999; 57: 77-82.
32. Pauletto NC., Liede K, Nieminen A, Larjava H, Uitto VJ. Effect of cigarette smoking on oral elastase activity in adult periodontitis patients. J Periodontol 2000; 71:58-62.

33. Preber H, Kant T, Bergström J. Cigarette smoking oral hygiene and periodontal health in Swedish army conscripts. J Clin Periodontol 1980; 7: 106-113.
34. Linden GJ, Mullally BH. Cigarette smoking and periodontal destruction in young adults. J Periodontol 1994; 65: 718-723.
35. Zambon JJ, Grossi SG, Machtei EE, Ho AW, Dunford R, Genco RJ. Cigarette smoking increases the risk for subgingival infection with periodontal pathogens. J Periodontol 1996; 67: 1050-1054.
36. Bergström J, Eliasson S, Dock J. A 10-year prospective study of tobacco smoking and periodontal health. J Periodontol 2000; 71: 1338-1347.
37. Machuca G, Rosales I, Lacalle JR, Machuca C, Bullon P. Effect of cigarette smoking on periodontal status of healthy young adults. J Periodontol 2000; 71: 73-78.
38. Boström L, Linder LE, Bergström J. Influence of smoking on the outcome of periodontal surgery. A 5-year follow-up. J Clin Periodontol 1998, 25: 194-201.
39. Axelsson P, Paulander J, Lindhe J. Relationship between smoking and dental status in 35- 50- 65- and 75- year old individuals. J Clin Periodontol 1998; 25: 297-305.
40. Van der Weijden GA, De Slegte C, Timmerman MF, van der Velden U. Periodontitis in smokers and non-smokers: intra-oral distribution of pockets. J Clin Periodontol 2001; 28: 955-960.
41. Haber J, Wattles J, Crowley M, Mandell R. Evidence for cigarette smoking as a major risk factor for periodontitis. J Periodontol 1993; 64: 16-23.
42. Pucher JJ., Shibley O, Dentino AR, Ciancio SG. Results of limited non-surgical periodontal therapy in smokers and non-smokers. J Periodontol 1997; 68: 851-856.
43. Danielsen B, Manji F, Nagelkerke N, Fejerskov O, Baelum V. Effect of cigarette smoking on the transition dynamics in experimental gingivitis. J Clin Periodontol 1990; 17: 159-164.
44. Clarke NG, Carey SE. Etiology of chronic periodontal disease an alternative perspective. JADA 1985; 110: 689-691.
45. Baab DA, Öberg PA. The effect of cigarette smoking on gingival blood flow in humans. J Clin Periodontol 1987; 14: 418-424.
46. Meekin TN, Wilson RF, Scott DA, Ide , Palmer RM. Laser doppler flowmeter measurement of relative gingival and forehead skin blood flow in light and heavy smokers during and after smoking. J Clin Periodontol 2000; 27: 236-242.
47. Stoltenberg JL, Osborn JB, Philström BL. Association between cigarette smoking, bacterial pathogens, and periodontal status. J Periodontol 1993; 64: 1225-1230.
48. Albandar JM, Streckfus CF, Adesanya MR, Winn D. Cigar pipe and cigarette smoking as risk factors for periodontal disease and tooth loss. J Periodontol 2000; 71: 1874-1881.
49. Ismail A, Burt B, Eklund S. Epidemiologic patterns of smoking and periodontal disease in United States. JADA 1983; 106: 617-621.
50. Preber H, Bergström J. The effect of non-surgical treatment on periodontal pockets in smokers and non-smokers. J Clin Periodontol 1985; 13: 319-323.
51. Kaldahl WB, Johnson GK, Patil KD, Kalkwarf KL. Levels of cigarette consumption and response to periodontal therapy. J Periodontol 1996; 67: 675-681.
52. Challacombe SJ. Passage of serum immunoglobulins into the oral cavity. In Borderland between caries and periodontal disease, eds. Lehner T. & Cimasoni G. 1980; pp. 51-67, London: England.
53. Curtis MA, Griffiths GS, Price SJ, Coulthurst SA, Johnson NW. The total protein concentration of GCF variation with sampling time and gingival inflammation. J Clin Periodontol 1988; 15: 628-632.
54. Nakashima K, Demeurisse C, Cimasoni G. The recovery efficiency of various materials for sampling enzymes and polymorphonuclear leukocytes from gingival crevices. J Clin Periodontol 1994; 21: 479-483.
55. Rossomando EF, Kennedy JE, Hadjimichael J. Tumor necrosis factor alpha in gingival crevicular fluid as a possible indicator of periodontal disease in humans. Arch Oral Biol 1990; 35: 431-434.
56. Geivelis M, Turner DW, Pederson ED, Lamberts BL. Measurements of interleukin-6 in GCF from adults with destructive periodontal diseases. J Periodontol 1993; 64: 980-983.
57. Heasman PA, Collins JG, Offenbacher S. Changes in crevicular fluid levels of interleukin-1β leukotriene B4 prostaglandin E2 thromboxane B2 and tumour necrosis factor α in experimental gingivitis in humans. J Periodont Res 1993; 28: 241-247.
58. Reinhardt RA, Masada MP, Kaldahl WB, DuBois IM, Kornman KS, Choi JI, Kalkwarf KL, Allison AC. Gingival fluid IL-1, and IL-6 levels in refractory periodontitis. J Clin Periodontol 1993; 20: 225-231.
59. Lee HJ, Kang IK, Chung CP, Choi SM. The subgingival microflora and gingival crevicular fluid cytokines in refractory periodontitis. J Clin Periodontol 1995; 22: 885-890.
60. Atilla G, Kütükçüler N. Crevicular fluid interleukin-1β tumor necrosis factor-α and interleukin-6 levels in renal transplant patients receiving cyclosporine A. J Periodontol 1998; 69: 784-790.
61. Ataoğlu H, Alptekin NÖ, Haliloğlu S, Gürsel M., Ataoğlu T, Serpek B, Durmuş E. Interleukin-1β tumor necrosis factor-α levels and neutrophil elastase activity in peri-implant crevicular fluid. Clin Oral Imp Res 2002; 13: 470-476.
62. Chapple ILC, Sokransky SS, Dibart S, Glenwright HD, Matthews JB. Chemiluminescent assay of alkaline phosphatase in human gingival crevicular fluid: investigations with an experimental gingivitis model and studies on the source of the enzyme within crevicular fluid, J Clin Periodontol 1996; 23: 587-594.
63. Chapple ILC, Matthews JB, Thorpe GH, Glenwright HD, Smith JM, Saxby MS. A new ultrasensitive chemiluminescent assay for the site-spesific quantification of alkaline phosphatase in gingival crevicular fluid, J Periodont Res 1993; 28: 266-273.
64. Chapple ILC, Glenwright HD, Matthews JB, Thorpe GH, Lumley PJ. Site-specific alkaline phosphatase levels in gingival crevicular fluid in health and gingivitis: cross-sectional studies, J Clin Periodontol 1994; 21: 409-414.
65. Nakashima K, Roehrich N, Cimasoni G. Osteocalcin, prostaglandin E2, and alkaline phosphatase in gingival crevicular fluid: their relations to periodontal status, J Clin Periodontol 1996; 21: 327-333.

CORRESPONDING ADDRESS

Ebru Olgun ERDEMİR DDS, PhD

Kırıkkale University, Faculty of Dentistry, Department of Periodontology 71200, Kırıkkale

Tel: 0318 224 49 27 Fax: 0318 224 36 18 e-mail: ebruerdemir@hotmail.com