Original Article

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Dentistry Section

Effect of Smoking on Periodontal Health

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ABSTRACT

Aims and Objectives: The aim of the study was to assess and compare the effect of smoking on mRNA expression of MMP -8 and TIMP-1 in patients with untreated chronic periodontitis and in periodontally healthy subjects and to examine the correlation of MMP-8 and TIMP-1 levels with clinical parameters.

Materials and Methods: Out of 60 subjects, 40 were selected subjects for the study, and were divided into Group I (periodontitis subject) and Group II (healthy subjects). Each group was further subdivided into subgroups I-A (chronic periodontitis smokers CPS), and I-B (chronic periodontitis non-smokers CPN), subgroup II-A (healthy smokers HS) and II B (healthy non-smokers HS). Both the groups underwent periodontal examination and clinical parameters were recorded. Tissue samples from both groups were subjected to the isolation of RNA which was then followed by qRT-PCR and the expression of the mRNA levels of MMP-8 and TIMP-1 were analyzed.

Results: The mRNA expression of MMP-8 and TIMP-1 was further compared with the periodontal status of all the four groups. The mRNA expression of MMP-8 was compared between the groups and showed that Group I-A (CPS) had higher expression of MMP-8 compared to group I-B (CPN). Group I-B (CPN) vs Group I-A (CPS) showed statistically significant difference in MMP-8/TIMP-1 with higher values for Group I-A (CPS) than Group I-B (CPN). A positive correlation was found between MMP-8 expression and probing depth and clinical attachment level (CAL) among Group I-B (CPN) and Group I-A (CPS) subjects. A significant correlation was also found between MMP-8 and TIMP-1 expression with probing depth and CAL among Group II B(HN) group subjects. TIMP-1 also showed a positive correlation with gingival index (GI) among group II A (HS) subjects.

Conclusion: It is concluded that smoking has an impact on the periodontal status and mRNA expression of MMP-8 and TIMP-1 in chronic periodontitis patients. The earlier evaluation of MMP-8 and TIMP-1, can be used as a biomarker in predicting periodontal disease susceptibility.

Keywords: Chronic periodontitis, MMP's, Periodontal inflammation, Polymerase chain reaction, Smoking

INTRODUCTION

Matrix metalloproteinases (MMPs) are the key enzymes which have been associated with the severity of periodontal inflammation and disease and play an important role in the degradation of the host tissues. MMP-8, the neutrophil collagenase, is the major collagenolytic MMP in gingival tissue and oral fluids [1]. Among the various types, MMP-8, the type originating from neutrophils is more glycated than the one originating from fibroblasts. Therefore, MMP-8 expression is mainly regulated by protein degranulation of neutrophils. MMP-8 has been detected in healthy and inflamed periodontal tissues from untreated chronic periodontitis patients and is most prevalent in chronic periodontitis patients and smokers [2].

On the other hand, there are several proteinases that mediate MMP activation, including plasmin, furin and active MMPs that assemble in enzymatic amplifying loops. Tissue inhibitors of metalloproteinases (TIMPs) are the most important physiological inhibitors of MMPs that include TIMPs -1, -2, -3 and -4 [3]. TIMP-1, a 30 kDa glycoprotein, is the main inhibitor of MMPs synthesized by mast cells. It forms high-affinity complexes with the active forms of MMPs. TIMP-1 was selected in the study among all TIMPs because it inhibits MMP 1, -3, -9,-13 including MMP-8 as well. Smoking increases the susceptibility to periopathogenic bacteria and to periodontal tissue destruction. Limited information is available concerning the effect of smoking on the degradation of extracellular matrix and especially the activation of MMPs [4]. Hence, the present study was aimed to explore the possible effect of smoking on the Messenger RNA (mRNA) expression of MMP-8 and TIMP-1 in gingival tissues from subjects with untreated chronic periodontitis and to compare them with periodontally healthy subjects.

MATERIALS AND METHODS

Sixty subjects were recruited according to the computerized randomization from the outpatient pool of Department of Periodontology, Meenakshi Ammal Dental College and Hospital, out of which, 40 subjects were selected for the study. The "Meenakshi Institutional Review Board" approved this study and written informed consent was obtained from all participants of the study. The selected participants were divided into two groups GROUP one and GROUP II. Group I (Experimental group) comprised of 20 subjects and they were further divided into subgroup I A which consisted of 10 Smokers and subgroup II A which consisted of 10 non smokers. Group I included subjects between 18-60 years of age with generalized chronic periodontitis -having ≥ 20 teeth, BOP, presence of 7 teeth with ≥ 5 mm probing depth. Group I A included Subjects smoking ≥ 10 cigarettes/Day for the past five years. Group II (Control group) between 18-60 years of age comprised of 20 subjects and further divided into subgroup II A which consisted of 10 Smokers and subgroup II B which consisted of 10 nonsmokers. Inclusion criteria for Group II included subjects with healthy and intact periodontium, no BOP, no probing depth and no clinical attachment loss, Indication for crown lengthening, and the presence of third molar with indication for extraction. For Group

II A continuos smokers (Subjects smoking ≥ 10 cigarettes/Day for the past five years) were included. Subjects with any systemic disease or conditions that could affect periodontal tissues, history of periodontal treatment in the past six months, systemic medication or antibiotic treatment for the previous six months, indication for prophylactic antibiotics were excluded from the study. Both the groups underwent periodontal examination and clinical parameters such as, plaque index (PI), gingival index (GI), probing pocket depth (PPD), CAL, and bleeding on probing (BOP) were recorded [5].

SAMPLE COLLECTION

Collection of tissue samples

In the experimental group (group I), subgingival tissue samples were harvested from the deepest periodontitis sites with the help of Gracey's curettes and also during extraction of periodontally hopeless teeth. For the healthy controls (group II), the samples were collected during the surgical crown lengthening or third molar extraction. External bevel gingivectomy incision with BP blade no. 15 was used for sample collection. The tissue samples were stored at -70ºc until processing. The tissue samples from both the experimental group and the control group were subjected to the following process that consisted of first isolating the RNA which was then followed by RT-PCR.

PROCESSING

Gene-specific oligonucleotide primers were commercially obtained (Synergy) for MMP-8, TIMP-1 and glyceraldehyde 3-phosphate dehydrogenase GAPDH [Table/Fig-1].

Isolation of total RNA and RT-PCR

The RNA isolation was done in accordance with a study by Mouzakiti et al., briefly the tissue samples were harvested and transferred to a centrifuge tube followed by the preparation of the working stalk

Genes	Primers	Sequence 5'→3'	Fragment Size(bp)	PCR Protocol
GAPDH	Forward	TGGTATCGTGGAA GGACTCATGAC	188	94ºC (3 min); 32 cycles of 94ºC (30 s)
	Reverse	ATGCCAGTGAGCT TCCCGTTCAGC		56ºC (40 s), 72ºC (30 s); 72ºC (7 min)
MMP-8	Forward	GAT GCT ATC ACC ACA CTC CGT	283	94ºC (3 min); 38 cycles of 94ºC (30 s)
	Reverse	GCT GCG TCA ATT GCT TGG A		52ºC (30 s), 72ºC (40 s); 72ºC (7 min)
TIMP-1	Forward	AGTCAACCAGAC CACCTTATACCA	385	94ºC (3 min); 32 cycles of 94ºC (30 s),
	Reverse	TTTCAGAGCCTTG GAGGAGCTGGTC		54ºC (30 s), 72ºC (50 s); 72ºC (7 min)

[Table/Fig-1]: The primer sequences

Descriptive Analysis of Periodontitis Group CPS and CPN					
Parameters	Group I A (CPS)		Group I B (CPN)		p-value
	Mean	SD	Mean	SD	
PI	1.36	0.27	1.74	0.42	0.028*
GI	1.4	0.13	1.48	0.34	0.384
PD (mm)	4.75	0.8	4.85	0.78	0.82
CAL (mm)	4.98	0.75	4.99	0.7	0.976
Bleeding on probing (%)	49.7	24.37	74.6	18.17	0.017*
Existing Teeth	31.2	1.69	30.3	2.45	0.214
Teeth PD≥ 5mm	21.9	8.66	19.9	6.72	0.732
% Teeth PD≥ 5mm	70.31	27.57	65.5	20.27	0.622
% Sites PD 4 - 6mm	55.97	28.39	48.48	23.02	0.364
% Sites PD ≥ 7mm	0.39	0.52	2.78	2.82	0.047*

[Table/Fig-2]: Descriptive analysis among periodontitis group

Number and Percentage of Tissue Samples with MMP8 & TIMP1				
GENES	Group I-B (CPN) (%)	Group I-A (CPS) (%)	Group II-B (HN) (%)	Group II-A (HS) (%)
MMP8	10/10(100)	10/10(100)	8/10(80)	10/10(100)
TIMP-1	10/10(100)	10/10(100)	10/10(100)	5/10(50)

[Table/Fig-3]: Number and percentage of gingival tissue samples with MMP-8 and TIMP-1expression by study group

MMP 8 & TIMP 1 Expression & MMP8/TIMP 1 Ratios								
GENE	Group I-B (CPN)	Group I-A (CPS)	Group II-B (HN)	Group II-A (HS)	CPN vs HN	CPS vs HS	CPN vs CPS	HN vs HS
	Median (Q1-Q3)	Median (Q1-Q3)	Median (Q1-Q3)	Median (Q1-Q3)	p-Value	p-Value	p-Value	p-Value
MMP 8	-1.35 (-2.82 - -0.99)	-3.06 (-3.39 - -2.21)	-1.22 (-2.73 – 0)	0.50 (-8.66 – 5.10)	0.496	0.199	0.023*	0.677
TIMP-1	-1.12 (-1.87 - -0.37)	-0.96 (-2.00 - -0.60)	-2.44 (-3.81 – 1.41)	0 (-9.09 – 0)	0.496	0.446	0.597	0.703
MMP 8/ TIMP-1	1.43 (0.60 - 2.45)	3.651 (1.575-4.311)	0.65 (0.30-1.31)	0.81 (0.32 – 2.29)	0.173	0.1	0.049*	0.713

[Table/Fig-4]: MMP-8 and TIMP-1 expression and MMP 8/TIMP-1 ratios: analysis by study group and comparison among the study groups

Parameters	Genes	Group I-B (CPN)	Group I-A (CPS)	Group II-B (HN)	Group II-A (HS)
PI	MMP-8	-.128(.725)	-.139(.701)	.265(.460)	-.321(.365)
	TIMP-1	.213(.555)	.103(.777)	.350(.322)	-.162(.656)
GI	MMP-8	-.480(.160)	-.232(.519)	.402(.249)	-.432(.210)
	TIMP-1	-.140(.700)	.451(.191)	.426(.220)	-.737(.015)*
PD	MMP-8	-.721(.019)*	-.903(0)*	-.790(.007)*	.006(.987)
	TIMP-1	.067(.855)	-.309(.385)	-.661(.038)*	-.300(.399)
CAL	MMP-8	-.685(.029)*	-.709(.022)*	-.790(.007)*	.006(.987)
	TIMP-1	.042(.907)	.030(.934)	-.661(.038)*	-.300(.399)
BOP	MMP-8	-.006(.987)	.103(.777)	-	-
	TIMP-1	-.030(.934)	-.224(.533)	-	-

[Table/Fig-5]: Spearman correlation coefficients (p-values) of the expression of MMP-8 and TIMP-1 with the clinical measurements *p-value ≤ 0.05

and the RT-PCR was run. Total RNA was isolated, and the mRNA expression of MMP-8 and TIMP-1 was assessed by RT-PCR. Once the product was obtained densitometry analysis was carried out and the final graph was plotted [4].

STATISTICAL ANALYSIS:

To compare the two mean values, Mann – Whitney test was applied and level of significance was calculated. Mann-Whitney U-test was applied to compare the ratio of expression of MMP-8 and TIMP-1 among the groups. The possible existence of correlation between the MMP-8 and TIMP-1 expression and the clinical measurements (PI, GI, PD, clinical attachment level and bleeding on probing) was tested using the Spearman correlation coefficient. SPSS 16 statistical software was used and the level of significance ≤0.05.

RESULTS

[Table/Fig-2] shows descriptive analysis of the clinical parameters among both the periodontitis groups (Group I A and I B). Hence, it could be stated that the amount of plaque deposited (PI), bleeding on probing (BOP) and the percentage of sites with probing depth ≥7 mm was significantly higher in Group I-B (CPN) as compared to Group I-A (CPS). In Group I-B (CPN), and Group I-A (CPS), all the 10 tissue samples were found to have both MMP-8 and TIMP-1, showing 100% presence in the collected tissue samples.

In group II-B (HN), only eight out 10 tissue samples showed the presence of MMP-8. In the Group II-A (HS) only five out of 10 tissue samples showed the presence of TIMP-1, that is 50% presence of TIMP-1. MMP-8 was seen in all the 10 tissue samples showing 100% presence in the collected tissue samples [Table/Fig-3]. [Table/Fig-4] shows the mRNA expression of MMP-8 and TIMP-1. The mRNA expression of TIMP-1 expression was compared among all the groups and was found not to be statistically significant. The MMP 8/TIMP-1 ratios were compared between the groups, Group I-B (CPN) and II-B (HN), Group I-A (CPS) and II-A (HS), Group I-B (CPN) and I-A (CPS) and Group II-B (HN) and II-A (HS). Group I-A (CPS) showed a statistically significant higher value of MMP-8/TIMP-1 than Group I-B (CPN) (pvalue=0.049). Statistically significant positive correlation was found between MMP-8 expression and probing depth (p=0.019) and clinical attachment level (p=-.029) among Group I-B (CPN) subjects, MMP-8 expression and clinical attachment level (p=-.022) and probing depth (p=0) among Group I-A (CPS) subjects, MMP-8 expression and probing depth (p=0.007) and clinical attachment level (p=-.007) among Group II-B (HN) subjects, MMP-8 expression and gingival index (p=.015) among Group II-A (HS) subjects, whereas TIMP-1 also showed statistically significant correlations between the probing depth (p=.038) and clinical attachment levels (p=.038) among Group II-B (HN) [Table/Fig-5].

DISCUSSION

The present study shows that Group I-B (CPN) had a higher level of bleeding on probing (BOP) and plaque index (PI) when compared to Group I-A (CPS) [Table/Fig-2]. Preber & Bergström,[6] and Monteiro Da Silva et al., [7] in their previous studies suggested that the effect of tobacco smoking on the periodontium were independent of the plaque level. Calsina et al., [8] and Feldman et al., [9] also found significantly less plaque in smokers than in nonsmokers.

In our study, the clinical attachment level in smokers and non-smokers were relatively the same. However, Calsina et al., [8] found more amount of clinical attachment loss in smokers.

In our study the mean and percentage of teeth with probing pocket depth ≥5 mm was higher in group I-A (CPS) than group I-B (CPN). This was similar to the study done by Arno et al., [10] who also found the relationship between increased consumption of smoking and periodontal destruction. Haber & Kent [11] found that there were a higher percentage of current and former smokers with advanced periodontal destruction. Our study was inaccordance with the study done by Grossi et al., [12] who reported that the risk of having periodontitis was 2.05 times greater in moderate smokers and 4.75 times greater in heavy smokers than nonsmokers. However Mouzakiti et al., [4], he also found the PPD to be higher in chronic periodontitis nonsmokers in comparison to chronic periodontitis smokers which is in contrast with the present study.

In the present study the gingival index was found to be similar in both the groups whereas, Markkanen H [13] found a reduction in the clinical signs of gingivitis in smokers. It is suggested, although smoking is known to produce peripheral vasoconstriction, this is preceded by vasodilatation. In any particular instance, the effect produced is probably related to the degree of inhalation of the tobacco smoke and the rate of nicotine absorption [14]. Nicotine from cigarette stimulates the sympathetic ganglia to produce neurotransmitters including catecholamines [15]. These affect the alpha-receptors on blood vessels which in turn causes vasoconstriction. The vasoconstriction of peripheral blood vessels caused by smoking can also affect the periodontal tissue as smokers have less overt signs of gingivitis than nonsmokers and clinical signs of gingival inflammation such as redness, bleeding and exudation are not as evident in smokers [16]. The vasoconstrictive actions of nicotine may be responsible for the decreased gingival blood flow. This is in contrast with the study by Palmer et al., [17] who measured gingival blood flow, using a laser Doppler technique, and their data did not support the view that smoking compromised blood flow in the periodontal tissues, whereas our study supported the fact. Tobacco use has also been associated with reduced permeability of peripheral blood vessels. Overall, it is suggested that prolonged and heavy smoking can reduce gingival bleeding and therefore mask the clinical marker of bleeding on probing often used by dentists to monitor periodontal health.

In our study ,the mRNA expression of MMP-8 was compared between the four groups, Group I-B (CPN) and II-B (HN), Group I-A (CPS) and II-A (HS), Group I-B (CPN) and I-A (CPS) and Group II-B (HN) and II-A (HS) in our study [Table/Fig-4]. The mRNA expression was statistically significant only between the group I-B (CPN) and I-A (CPS), as MMP-8 expression was higher in among Group I-A (CPS). This could be due to the alterations in the neutrophil behavior as mentioned earlier. The same groups were compared for the expression of MMP-8 in a similar study by Mouzakiti et al., [4] and it was found that MMP-8 was statistically significant between Group I-B (CPN) and Group II-B (HN), with higher expression in Group I-B (CPN). This could be stated that, MMP-8 is mainly expressed by neutrophils, which are highly recruited to the area due to the inflammation process the greater MMP-8 expression found Group I-B (CPN) than Group II-B (HN) could be partly attributed to their association with inflammation [18].

Along with the MMP-8 expression, the mRNA expression of TIMP-1 was also compared in our study among all the groups Group I-B (CPN) and Group II-B (HN), Group I-A (CPS) and Group II-A (HS) Group I-B (CPN) and Group I-A (CPS) and Group II-B (HN) and Group II-A (HS) and it was found to be statistically nonsignificant. The nonsignificant indications of TIMP-1 expression might be partly due to smoking.But in the study by Mouzakiti et al., [4] TIMP-1 levels were found significant in the CPN vs CPS group as TIMP-1 showed greater expression in the CPS group. TIMP-1 expression found between nonsmokers and smokers might be attributed to smoking, because the groups were comparable in age, sex distribution and clinical measurements in their study. Zhou J et al., [19] stated in their study that there is an indicative data which suggests that smoking might modify although there is not sufficient information on the cell types and MMPs that smoking could influence.

MMP-8/TIMP-1 ratios also were compared between Group I-B (CPN) and Group II-B (HN), Group I-A (CPS) and Group II-A (HS) , Group I-B (CPN) and Group I-A (CPS) and Group II-B (HN) and Group II-A (HS). Group I-A (CPS) showed a statistically significant higher value of MMP-8/TIMP-1 than Group I-B (CPN). MMP-8 in Group I-A (CPS) showed a higher expression than TIMP-1 as smoking alters the expression of TIMP-1 and hence could not inhibit the expression of MMP-8. Whereas among Group I-B (CPN) MMP-8 and TIMP-1 were expressed at similar levels, that is TIMP-1 was able to inhibit the expression MMP-8, which gives us more evidence that smoking alters the expression of MMP's and TIMP's.

Correlations among MMP-8 and TIMP-1 expression with clinical parameters were detected in our study [Table/Fig-5]. Among the Group I-B (CPN), statistically significant positive correlation was seen between the MMP-8 expression and probing depth and also between clinical attachment level. In a study by Lee et al., [20] they reported a large increase of MMP-8 in patients with periodontitis causing progressive loss of connective tissue and bone. Similarly, in the study by Mouzakiti et al., [4] there was a statistically significant correlation between the expressions of MMPs and clinical measurements.

Among Group I-A (CPS) statistically significant positive correlation was seen between the expressions of MMP-8, probing depth along with Clinical attachment as well. In Group I-A (CPS), the MMP-8 levels

were higher to the levels expressed in Group I-B (CPN) which also had statistically positive correlation between MMP-8 and Probing depth and clinical attachment loss. Passoja A et al., [21] in their study found, MMP-8 concentrations to be significantly higher in smokers as compared to nonsmokers with relation to Probing pocket depth and clinical attachment. Similarly Gursoy UK, et al., [22] found in their study that smokers with periodontitis had a higher expression of MMP-8 with increase in the probing pocket depth as compared to Non-smokers with periodontitis. A significant correlation between increased MMP-8 levels and periodontal disease severity was suggested by Liu et al., [18] who reported increased local MMP-8 expression in the periodontal tissues of smokers compared to the nonsmokers. Our study was in contrast with the studies conducted by Söder et al., [23], where serum MMP-8 concentrations did not differ significantly between the smokers and nonsmokers.

In the present study the expression of MMP-8 and TIMP-1 was in correlation with the pocket depth and the clinical attachment level among Group II-B (HN). With probing pocket depth and clinical attachment level within the normal limits in healthy nonsmokers, the expression of MMP-8 was associated with the expression of TIMP-1 as well. It is well-accepted that the balance between MMPs and TIMPs plays a vital role in maintaining the integrity of healthy tissues, whereas disturbances in such balance is found in various pathological conditions, including rheumatoid arthritis, periodontitis, and cancer. The mRNA expression of MMP-8 was higher with gingival inflammation in Group II-A (HS) as compared to Group II-B (HN), this is in contrast with the study done by Mouzakiti et al., [4] who found a higher expression of MMP's among healthy nonsmokers. TIMP-1 showed a higher expression in the healthy smokers which is known to inhibit the expression of MMP's.

Some of the limitations in our study were the gingival tissue samplings, as in, for all healthy control subjects, the sampling site was clinically healthy, though the existence of slight, clinically undetectable inflammation cannot be excluded. Self-reports of nonsmoking people can sometimes be unreliable and could have hindered the results of our study. Further research is required to evaluate the immune expression of MMPs in the gingival tissues, since the mRNA expression does not always reflect protein expression, owing to possible post transcriptional modifications.

CONCLUSION

Overall, higher expression of MMP-8 and TIMP-1 were expressed among chronic periodontitis smokers which significantly related to the periodontal parameters as well. Future studies should be undertaken to investigate how the active components in cigarette smoke condensate influence the development of periodontal disease and its interaction with bacteria, such as *P. gingivalis*.

Further research in this field will definitely determine the relative importance of MMP's in contributing towards the increased periodontal destruction observed in smokers.

REFERENCES

[1] Mantyla P, Stenman M, Kinane D, Salo T, Suomalainen K, Tikanoja S,et al. Monitoring periodontal disease status in smokers and non-smokers using a gingival crevicular fluid matrix metalloproteinase-8-specific chair-side test. *J Periodontal Res.* 2006; 41 (6): 503-12.

[2] Kiili M, Cox SW, Chen HY, et al. 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: 224–32.

[3] Folgueras AR, Pendas AM, Sanchez LM, and Lopez-Otin C. Matrix metalloproteinases in cancer: from new functions to improved inhibition strategies. Int *J Dev Biol.* 2004; 48 (5-6): 411-24.

[4] Mouzakiti E, Pepelassi E, Fanourakis G, Markopoulou C, Tseleni-Balafouta S, Vrotsos I. The effect of smoking on the mRNA expression of MMPs and TIMP-1 in untreated chronic periodontitis patients: a cross-sectional study *J Periodontal Res.* 2011; 46(5): 576-83.

[5] Carranza FA. Textbook of Clinical Periodontology. Eighth Edition: *Elsiever publications, Illinois;* 61-81.

[6] Bergström J, Floderus-Myrhed B. Co-twin study of the relationship between smoking and some periodontal disease factors. *Community Dental Oral Epidemiology.* 1983; 11: 113–16.

[7] Monteiro Da Silva AM, Newman HN, Oackley DA, O'Leary R. Psychosocial factors, dental plaque levels and smoking in periodontitis patients. *J Clin Perio*. 1998; 25: 517–23.

[8] Calsina G, Ramon JM, Echeverria JJ: *Effects of smoking on periodontal tissues. J Clin Perio*. 2002; 29: 771–76.

[9] Feldman RS, Bravacos JS, Rose CL. Association between smoking different tobacco products and periodontal disease indexes. *Journal of Periodontology.* 1983; 54: 481–87.

[10] Arno A, Shei O, Lovdal A, Waerhaug J. Alveolar bone loss as a function of *tobacco consumption Acta Odontol Scand.* 1959; 17: 3–10.

[11] Haber J, Kent R. Cigarette smoking in a periodontal practice. *Journal of Periodontology.* 1992; 63: 100–06.

[12] Grossi SG, Zambon JJ, Machtei E, et al. Effects of smoking and smoking cessation on healing after mechanical periodontal therapy. *Journal of the American Dental Association*. 1997; 128: 599–607.

[13] Markkanen H, Paunio I, Tuominen R, Rajala M. Smoking and periodontal disease in the Finnish population aged 30 years and over. *Journal of Dental Research*. 1985; 64: 932–35.

[14] Lindeboom JA, Mathura KR, Harkisoen S, Van den Akker HP, Ince C. Effect of smoking on the gingival capillary density: assessment of gingival capillary density with orthogonal polarization spectral imaging. *J Clin Perio*. 2005; 32: 1208-12.

[15] Muller HP, Stadermann S, Heinecke A. Longitudinal association between plaque and gingival bleeding in smokers and non-smokers. *J Clin Perio*. 2002; 29: 287-94.

[16] Trauth. JA, Seidler FJ, Ali SF, Slotkin TA. Adolescent nicotine exposure produces immediate and long-term changes in CNS noradrenergic and dopaminergic function. *Brain Res.* 2001; 892:269-80.

[17] Palmer RM, Scott DA, Meekin TN, Odell EW, Wilson RF. Potential mechanisms of susceptibility to periodontitis in tobacco smokers. *J Perio Res.* 1999; 34: 363-9.

[18] Liu KZ, Hynes A, Man A, Alsagheer A, Singer DL, Scott DA. Increased local matrix metalloproteinase-8 expression in the periodontal connective tissues of *smokers with periodontal disease. Biochem Biophys Acta.* 2006; 1762: 775–80.

[19] Zhou J, Olson BL, Windsor LJ. Nicotine increases the collagen-degrading ability of human gingival fibroblasts. J *Periodontal Res.* 2007; 42: 228–35.

[20] Lee W, Aitken S, Sodek J, McCulloch CA. Evidence of a direct relationship between neutrophil collagenase activity and periodontal tissue destruction in vivo: role of active enzyme in human periodontitis. *J. Periodontal Res.* 1995; 30: 23–33.

[21] Passoja A, Ylipalosaari M, Tervonen T, Raunio T, Knuuttila M. Matrix metalloproteinase-8 concentration in shallow crevices associated with the extent of periodontal disease. *J Clin Periodontol.* 2008; 35(12): 1027-31.

[22] Gursoy UK, Könönen E, Pradhan-Palikhe P, Tervahartiala T, Pussinen PJ, Suominen-Taipale L,et al. Salivary MMP-8, TIMP-1, and ICTP as markers of advanced periodontitis. *J Clin Periodontol.* 2010; 37(6): 487-93.

[23] Söder 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 Perio.* 2002; 29(5): 384-91.

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