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Levels of prostaglandin E_2 (PGE_2) in gingival crevicular fluid from smokers and non-smokers with gingivitis and chronic periodontal disease

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The aim of this study was to evaluate the levels of prostaglandin E_2 (PGE_2) on the gingival crevicular fluid (GCF) of smokers (light and heavy) and non-smokers with gingivitis (G) and chronic periodontal disease (CPD). Forty-five patients were selected: 15 heavy smokers whose daily tobacco consumption was more than 10 cigarettes/day (HS), 15 light smokers whose daily tobacco consumption was fewer than 10 cigarettes/day (LS), and 15 non-smokers who had never smoked tobacco (NS). Clinical periodontal parameters (plaque index (PI), bleeding on probing (BOP), probing depth (PD), gingival recession (GR), and clinical attachment level (CAL)) were recorded for all groups. Each group was separated in both sites: G and CPD, and GCF samples were collected, and analyzed for PGE_2 content by enzyme-linked immunosorbent assay. The results indicated that the non-smoking group had higher PI (88.53±17.08%) and BOP (82.80±17.14%) scores than the two smoking groups. PD, GR and CAL scores did not differ significantly among the three groups. Statistically significance differences in GCF-PGE_2 were found among G versus CPD sites ($P \leq 0.05$) for the three groups. This study confirms that heavy and light smokers have less BOP and GCF-PGE_2 levels than non-smokers and that the GCF-PGE_2 was higher to CPD sites when compared with G sites.

Key words: Periodontal disease, gingival crevicular fluid, smoker, prostaglandin E_2.

INTRODUCTION

Periodontal disease is a local inflammation in the tissues that support the teeth, which leads to progressive loss of periodontal ligament tissue and bone. Periodontal destruction is directly related to smoking (Gera, 1999). Several reports have shown that the prevalence and severity of periodontitis is significantly higher in smokers than in non-smokers (Bernzweig et al., 1998). This high risk of periodontal disease is due to systemic and local

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effects of nicotine, a major component of cigarette smoke. There is evidence that nicotine may distort the clinical signs and symptoms of periodontal inflammation (e.g. periodontal bleeding, erythema and edema), indicating a suppressive influence of smoking on inflammatory responses (Bernzweig et al., 1998; Boström et al., 1998; Bergström et al., 2000). Other factors, such as the type of tobacco product, amount consumed and duration of exposure to tobacco, can exacerbate the periodontal destructive effects of tobacco (Schuller and Holst, 2001).

The relationship between tobacco and the pathogenesis of periodontal disease is less clear. Cigarette smoking is known to affect systemic and local immune responses. Prostaglandin E_2 (PGE_2), a pro-inflammatory mediator synthesized from cell membrane phospholipids by the action of cyclooxygenase enzyme, is considered a key inflammatory mediator in periodontal disease and is associated with periodontal disease progression and alveolar bone resorption (Bernzweig et al., 1998). The levels of PGE_2 in the gingival crevicular fluid (GCF) of individuals with periodontitis are elevated when compared with normal subjects, a situation believed to arise from the stimulation of PGE_2 secretion from peripheral mononuclear cells (monocytes and lymphocytes) by nicotine (Bernzweig et al., 1998). However, few studies have quantitatively analyzed the effects of cigarette smoking on PGE_2 levels in GCF or whether the daily dose of tobacco in smokers is correlated with PGE_2 secretion.

Thus, this study hypothesized that cigarette smokers have high levels of prostaglandin E_2 (GCF-PGE_2) expressed in the GCF in gingivitis and periodontitis sites. Based on this, the objective of this study was to evaluate the levels of prostaglandin E_2 (GCF-PGE_2) in the GCF of each group heavy, light and non-smokers according to gingivitis and periodontitis sites.

MATERIALS AND METHODS

Forty-five patients were recruited for this study and were distributed into three groups: 15 heavy smokers, with consumption of more than 10 cigarettes/day (HS); 15 light smokers, with consumption of less than 10 cigarettes/day (LS); and 15 non-smokers, who had never smoked (NS) (Coady et al., 2012). All subjects were recruited from the Department of Periodontology, School of Dentistry, Fluminense Federal University, Nova Friburgo, Rio de Janeiro, over a period of 6 months between 2010 and 2011. The study protocol was approved (protocol number, CAAE - 0070.0.258.000-10) by the ethics committee of the Fluminense Federal University School of Medicine. Prior to participation, the purpose and procedures were fully explained to all patients, who consequently gave written informed consent in accordance with the Helsinki Declaration. Medical and dental histories were taken and patients received clinical evaluation at prescreening visits. Inclusion criteria were: presence of periodontal disease and bleeding on probing in sites where probing depth was ≥5 mm; and radiographic bone loss ranging from 30 to 50%, diagnosis of chronic periodontal disease; however, patients had sites with gingivitis and periodontitis. Exclusion criteria were: patients with systemic diseases, diabetes, osteoporosis; pregnant lactating females; use of immune suppressive medication, phenytoin, cyclosporine, calcium channel blockers or any use of antibiotics or nonsteroidal anti-inflammatory drugs in the past 3 months; and any medical conditions requiring immunotherapy or diagnosed as HIV+ or with AIDS that could interfere with the periodontium.

The selected patients reported the age, mean of daily tobacco consumption and the time-span over which they had been smoking (years). An experienced periodontist determined the number of sites presenting with periodontal disease and evaluated the clinical parameters using a PCP15 (PCP-UNC15, Hu-Friedy, Chicago, IL) periodontal probe at six sites per tooth for all teeth, excluding third molars. Additionally, the following parameters were recorded: plaque index (PI), bleeding on probing (BOP), probing depth (PD), gingival recession (GR), and clinical attachment level (CAL).

After one week, the collections of the samples were performed. The supragingival biofilm was removed with sterile gauze and the sites dried gently with an air syringe and isolated with cotton rolls. GCF samples were taken from two different sites from the same patient from different groups: G = gingivitis sites, the deepest PD were ≤3 mm, bleeding on probe and chronic periodontal disease (CPD) = periodontitis sites, the deepest PD were ≥5 mm, each patient had both conditions. All patients were allocated in groups: NS, LS and HS. GCF samples were obtained by placing calibrated, volumetric microcapillary pipette of internal diameter of 1.1 mm with a capacity of 5 µl. Sites which did not express appropriate volume of fluid and micropipettes which were contaminated with blood and saliva were not included in the study (Koregol et al., 2011). The GCF was immediately placed into separate tubes containing 250 µl phosphate-buffered saline. The samples were stored at -20°C for subsequent assays. The samples were analyzed by a single-blinded examiner using a commercial PGE_2-specific enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN, USA).

Statistical analysis

The required sample size was determined by G*Power (G*Power, Franz Faul, Kiel University, Germany, Version 3.1.2, 2009) and was calculated to detect a 0.05 difference between PI (NS group) and PI (HS group) with power level of 89%. The power calculation analysis revealed that the required sample size was a minimum of 15 subjects for each study group. The primary efficacy variables were whole-mouth mean PI (NS group) and PI (HS group).

Statistical analysis was performed on data obtained from all patients who completed the trial. The decision about whether to use parametric or nonparametric tests was made based on the results of Shapiro-Wilk Normality Test for normal distribution. Statistical tests were performed using the Statistix software (Analytical Software, Tallahassee, FL, USA, Version 8.0, 2003). A two-sample T-test was performed to compare clinical parameters (PI, BOP, PD, GR and CAL) among NS, LS and HS groups. Comparison between groups was considered (NS × LS, NS × HS and LS × NS) to test variables age, total sites, number of sites with PD, daily cigarette consumption, duration of consumption, number of missing teeth, PI and BOP were considered to full mouth. PD, GR and CAL were analyzed according to G and CPD sites. All variables were normally distributed, except GCF-PGE_2. The Mann Whitney test was used to analyze differences in GCF-PGE_2 levels among G versus PD and NS, LS and HS groups. Statistical significance for all variables was defined as $p \leq 0.05$.

RESULTS

Descriptive statistics of each variable measured (mean ± standard deviation, with statistical significance assessed by two-sample T-test) are shown in Table 1. Statistically significant differences in the number of sites with

Table 1. Clinical parameters of members of the heavy smoker (HS), light smoker (LS) and non-smoker (NS) groups with gingivitis (G) and chronic periodontal disease (CPD).

Parameter	NS (n=15)	LS (n=15)	HS (n=15)
Age	48.27 ± 9.27	33.93 ± 10.53	38.53 ± 12.68
Total sites	134.0 ± 20.74	137.2 ± 27.01	133.2 ± 22.92
Number of sites with PD	40.93 ± 32.62	12.53 ± 14.6*	15.33 ± 8.34†‡
Mean daily cigarette consumption	N/A	7.93 ± 2.46	19.66 ± 7.02‡
Duration of consumption (years)	N/A	14.53 ± 10.94	20.46 ± 13.09
Mean number of missing teeth	9.66 ± 3.43	9.13 ± 4.15	9.8 ± 3.82
PI (%)	88.53 ± 17.08*	68.66 ± 33.33	91.73 ± 17.44†
BOP (%)	82.80 ± 17.14*	44.33 ± 30.37	42.2 ± 28.33‡
PD (mm)			
G	1.93 ± 0.78	2.2 ± 0.56	2.73 ± 0.46
CPD	5.13 ± 0.35	5.0 ± 0.0	5.0 ± 0.0
GR (mm)			
G	0	0	0
CPD	0.733 ± 0.96	0.86 ± 0.99	1.33 ± 1.04
CAL (mm)			
G	1.93 ± 0.78	2.2 ± 0.56	2.73 ± 0.46
CPD	6.2 ± 1.26	5.87 ± 0.99	6.47 ± 1.12

Data are means ± standard deviation. Statistical testing was by two-sample T-test. *†‡Statistically significant differences ($p \leq 0.05$) between the NS and LS groups, NS and HS groups, and LS and HS groups, respectively. PI: Plaque index; BOP: bleeding on probing; PD: probing depth; CAL: clinical attachment level; N/A: not applicable.

periodontal disease were observed in comparisons between the NS and LS groups (p=0.0024), the NS and HS groups (p<0.0001) and the LS and HS groups (p<0.0221). For the mean daily cigarette consumption, a statistically significant difference was observed between the LS and HS groups (p=0.0002). PI was significantly different between the NS and LS (p=0.0088) and NS and HS (p=0.0106) groups, with the highest mean PI being in the HS group (PI=91.73%), followed by the NS (88.53%) and LS (68.66%) groups, respectively. BOP was significantly different between the NS and LS (p=0.0202) and NS and HS (p=0.0202) groups, with the rank order of mean BOP values being NS (BOP=82.80%) > LS (44.33%) ≥ HS (42.2%). No significant differences among the groups were found for the PD, GR and CAL. However, significantly statistical difference was found between the G and CPD sites ($p \leq 0.0001$) to PD and CAL to NS, LS and HS sites.

GCF-PGE_2 production in each subject in the three groups is shown in Figure 1. Differences among groups and sites G versus CPD were assessed by Mann-Whitney test. Statistically significant difference in GCF-PGE_2 levels was detected when comparing NS versus LS (p=0.0576), NS was higher than LS and NS into G groups, and NS versus LS (*p*=0.0576), also NS was higher than HS group into CPD sites. Comparisons among sites were HS/G versus HS/CPD (p=0.0002), LS/G versus LS/CPD (p=0.0158) and NS/G versus NS/CPD (p=0.0382). Periodontal disease sites showed higher levels of GCF-PGE_2 when they were compared to G sites.

DISCUSSION

The objective of this study was to evaluate the influence of smoking on the levels of prostaglandin E_2 (GCF-PGE_2) in the gingival crevicular fluid of heavy, light and non-smokers according to G and CPD sites. This study revealed changes in the GCF-PGE_2 levels between G and PD sites when comparisons were done for HS, LS and NS groups.

The samples were characterized by daily cigarette and number of consumption by years. High daily consumption of tobacco and long history of consumption have been shown to increase periodontal destruction compared with non-smokers or patients that has sporadic tobacco consumption (Bergström et al., 2000). In this study, HS group exhibited the high number of sites with probing depth higher than 5 mm in full mouth periodontal evaluations. Daily and duration consumption of cigarette were higher to HS followed by LS group to confirm the

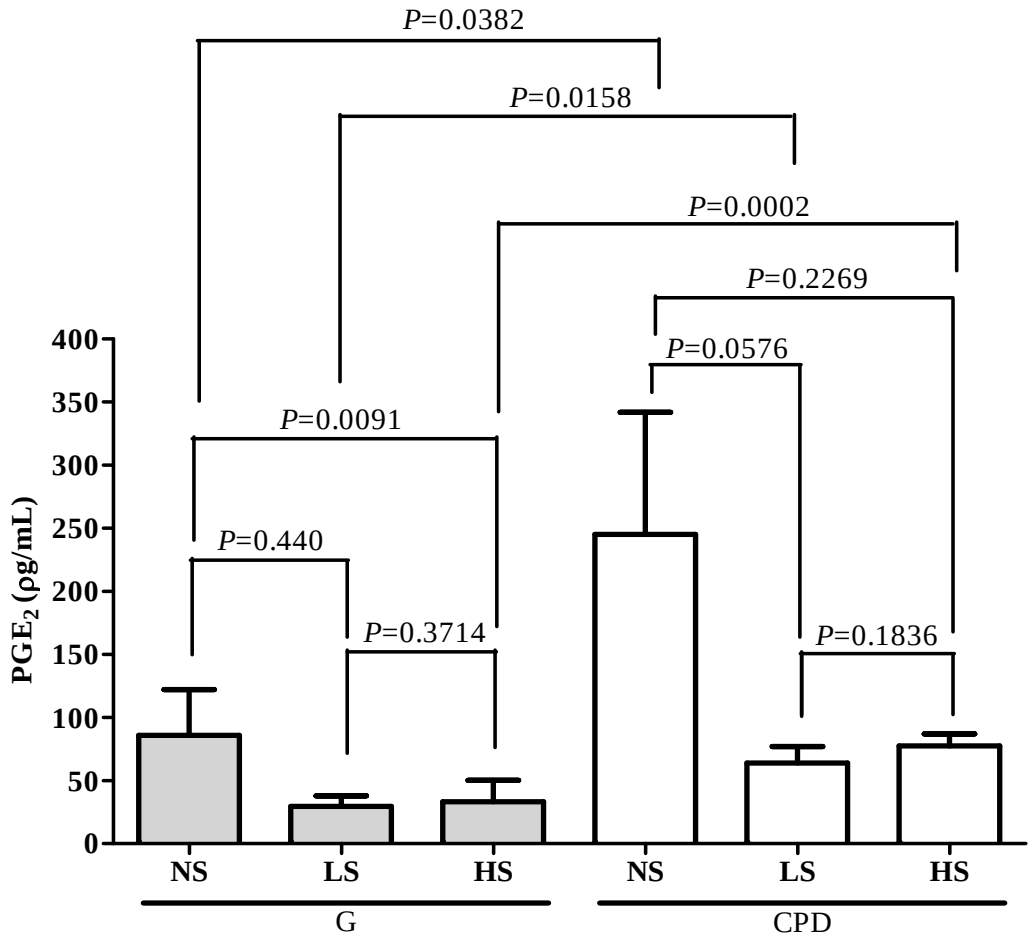

Figure 1. Levels of PGE_2 (pg/ml) at GCF, considering different groups: non-smokers (NS), light smokers (LS) and heavy smokers (HS) with gingivitis (G) and chronic periodontal disease (CPD). Statistically significant differences in PGE_2 levels were detected among any of these groups ($P \leq 0.05$, Mann-Whitney Test).

profile of the groups. However, no differences between age, PD, GR and CAL were found for the three groups (Table 1).

The smoking habit should increase teeth loss in smokers compared to non-smokers (Haffajee and Socransky, 2001; Chen et al., 2001). Previous studies have shown a high means of numbers of missing teeth to smokers (5.1) than non-smokers (2.8), respectively (Krall et al., 1999; Albandar et al., 2000). However, this study found higher means to missing teeth than previous study, but no statistically significant differences were found between the three groups (Table 1).

The comparisons for plaque index are controversial in the literature to smokers and non-smokers (Haffajee and Socransky, 2001; Chen et al., 2001). Studies have shown that cigarette smokers have more calculus and more plaque than non-smokers (Feldman et al., 1983; Luzzi et al., 2007), others reported similar plaque index between smokers and non-smokers (Gomes et al., 2007). However, this study shows that PI was different between groups; HS had higher means than NS followed by LS groups (Table 1).

Bleeding on probe has been reported to be higher in non-smokers than in smokers (Ah et al., 1994; Bergström and Preber, 1986). Previous report showed that various symptoms of periodontal inflammation (e.g. gingival bleeding, erythema and edema) can be suppressed by smoking owing to its inhibitory action on the inflammatory response. Cytotoxic and vasoactive substances, including nicotine, are responsible for this local effect but can also cause systemic effects including the inhibition of peripheral blood and oral neutrophils and reduced antibody production (Van der Weijden et al., 2001; Matthews et al., 2011). This study according to these results, BOP was higher to NS group than HS and LS groups.

Clinical periodontal parameters were investigated in the three groups (Table 1). No difference among groups was noted for periodontal parameters PD, GR and CAL. Various studies have reported that attachment loss is

higher in smokers than non-smokers (Feldman et al., 1983; Bergström et al., 1991; Haffajee and Socransky, 2001; Kerdvongbundit and Wikesjö, 2002; Jansson and Hagström, 2002; Gonzalez et al., 2009; Guarnelli et al., 2010; Rudzińiski, 2010), because smoking suppressed the system of host defense against the bacterial products of the biofilm and increased the risk of suffering extensive and severe alveolar bone loss. However, in this study, no differences were found to PD and CAL for the three groups, due to the fact that all patients had previous diagnosis of chronic periodontal disease.

Despite of this fact, we decided to separate the periodontal disease sites in subgroups, gingivitis (G) and periodontitis (CPD) sites to investigate the GCF-PGE_2 levels per sites. Statistically significance difference confirmed the differences between PD and CAL to gingivitis (G) and periodontitis (CPD) sites (Table 1). Subgroups were characterized by sites with probing depth ≤ 3 mm, gingivitis sites or sites with periodontitis, probing depth ≥ 5 mm, all sites bleeding on probe. The level of PGE_2 in the GCF was measured to reveal differences among the three groups.

PGE_2 was selected because it is one of the most important biochemical mediators of periodontal inflammation and plays a significant role in the pathogenesis of periodontal disease. PGE_2 stimulates bone resorption and it is expected to increase in GCF samples from periodontal sites compared with healthy and gingivitis sites (Offenbacher et al., 1986; Preshaw and Heasman, 2002). This study is in agreement with previous reports and finds differences of GCF-PGE_2 levels among G versus CPD sites disease (Preshaw and Heasman, 2002; Kurtiş et al., 2007). GCF-PGE_2 levels of CPD sites were higher than G group. Differences were found among NS and HS for G group and NS and LS in CPD group. No differences were found among LS and HS groups. These results are similar to previous studies that found no differences in GCF-PGE levels between smokers and non-smokers in adults with periodontal disease (Preshaw and Heasman, 2002; Kurtiş et al., 2007).

Indeed, our findings suggest that tobacco inhibit the PGE_2 release when G and CPD sites were compared (Figure 1). NS had higher levels of GCF-PGE_2 compared to HS and LS groups. Periodontitis sites (CPD) had higher PGE_2 levels than gingivitis sites (G). These results according to literature suggest the evidence that periodontal disease increase PGE_2 levels (Sánchez et al., 2013). Recent study with cell culture shows that tobacco has a detrimental effect on periodontal repair and PGE_2 levels are diminished in cells stimulated by cigarette smoke condensate (CSC) (Romero et al., 2014). However, further evidence of the effects of smoking on the PGE_2 release is necessary to demonstrate the effects of nicotine on the periodontal tissues.

Conclusion

Based on these findings, HS did not exhibit high levels of GCF-PGE_2 compared to LS and HS. However, non-smokers had higher levels of GCF-PGE_2. Indeed, this study confirmed that periodontal disease (CPD sites) exhibits higher GCF-PGE_2 levels compared to gingivitis (G sites), suggesting that periodontal disease can improved the GCF-PGE_2 levels.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

REFERENCES

Ah MK, Johnson GK, Kaldahl WB, Patil KD, Kalkwarf KL (1994). The effect of smoking on the response to periodontal therapy. J. Clin. Periodontol. 21:91-97.

Albandar JM, Streckfus CF, Adesanya MR, Winn DM (2000). Cigar, pipe, and cigarette smoking as risk factors for periodontal disease and tooth loss. J. Periodontol. 71(12):1874-1881.

Bergström J, Eliasson S, Dock J (2000). Exposure to tobacco smoking and periodontal health. J. Clin. Periodontol. 27:61-68.

Bergström J, Eliasson S, Preber H (1991). Cigarette smoking and periodontal bone loss. J. Periodontol. 62:242-246.

Bergström J, Preber H (1986). The influence of cigarette smoking on the development of experimental gingivitis. J. Periodontal Res. 21:668-676.

Bernzweig E, Payne JB, Reinhardt RA, Dyer JK, Patil KD (1998). Nicotine and smokeless tobacco effects on gingival and peripheral blood mononuclear cells. J. Clin. Periodontol. 25:246-252.

Boström L, Linder LE, Bergstrom J (1998). Influence of smoking on the outcome of periodontal surgery. A 5-year follows up. J. Clin. Periodontol. 25(10):767-773.

Coady MH, Jasek J, Davis K, Kerker B, Kilgore EA, Perl SB (2012). Changes in smoking prevalence and number of cigarettes smoked per day following the implementation of a comprehensive tobacco control plan in New York City. J. Urban Health 89(5):802-808.

Chen X, Wolff L, Aeppli D, Guo Z, Luan W-M, Baelum V, Fejeskov O (2001). Cigarette smoking, salivary/gingival crevicular fluid cotinine and periodontal status. A 10-year longitudinal study. J. Clin. Periodontol. 28:331–339.

Feldman RS, Bravacos JS, Rose CL (1983). Association between smoking different tobacco products and periodontal disease indexes. J. Periodontol. 54:481-487.

Gera I (1999). The effect of smoking on the spread and frequency of periodontal disease. Fogorv Sz. 92(4):99-110.

Gomes SC, Piccinin FB, Susin C, Oppermann RV, Marcantonio RA (2007). Effect of supragingival plaque control in smokers and never-smokers: 6-month evaluation of patients with periodontitis. J. Periodontol. 78(8):1515-1521.

Gonzalez R, Arancibia R, Cáceres M, Martínez J, Smith PC (2009). Cigarette smoke condensate stimulates urokinase production through the generation of reactive oxygen species and activation of the mitogen activated protein kinase pathways in human gingival fibroblasts. J. Periodont. Res. 44:386-394.

Guarnelli ME, Farina R, Cucchi A, Trombelli L (2010). Clinical and

microbiological effects of mechanical instrumentation and local antimicrobials during periodontal supportive therapy in aggressive periodontitis patients: smoker versus non-smoker patients. J. Clin. Periodontol. 37:998-1004.
Haffajee AD, Socransky SS (2001). Relationship of cigarette smoking to attachment level profiles. J. Clin. Periodontol. 28:283–295.
Jansson LE, Hagström KE (2002). Relationship between compliance and periodontal treatment outcome in smokers. J. Periodontol. 73:602-607.
Kerdvongbundit V, Wikesjö UM (2002). Prevalence and severity of periodontal disease at mandibular molar teeth in smokers with regular oral hygiene habits. J. Periodontol. 73:735-740.
Koregol AC, More SP, Nainegali S, Kalburgi N, Verma S (2011). Analysis of inorganic ions in gingival crevicular fluid as indicators of periodontal disease activity: A clinico-biochemical study. Contemp. Clin. Dent. 2(4):278-282.
Krall EA, Garvey AJ, Garcia RI (1999). Alveolar bone loss and tooth loss in male cigar and pipe smokers. J. Am. Dent. Assoc. 130:57-64.
Kurtiş B, Tüter G, Serdar M, Pinar S, Demirel I, Toyman U (2007). Gingival crevicular fluid prostaglandin E(2) and thiobarbituric acid reactive substance levels in smokers and non-smokers with chronic periodontitis following phase I periodontal therapy and adjunctive use of flurbiprofen. J. Periodontol. 78:104-111.
Luzzi LI, Greghi SL, Passanezi E, Sant'ana AC, Lauris JR, Cestari TM (2007). Evaluation of clinical periodontal conditions in smokers and non-smokers. J. Appl. Oral Sci. 15(6):512-517.
Matthews JB, Chen FM, Milward MR, Wright HJ, Carter K, McDonagh A, Chapple ILC (2011). Effect of nicotine, cotinine and cigarette smoke extract on the neutrophil respiratory burst. J. Clin. Periodontol. 38:208–218.
Offenbacher S, Odle BM, van Dyke TE (1986). The use of crevicular fluid prostaglandin E2 levels as a predictor of periodontal attachment loss. J. Periodontal Res. 21:101-112.
Preshaw PM, Heasman PA (2002). Prostaglandin E2 concentrations in gingival crevicular fluid: Observations in untreated chronic periodontitis. J. Clin. Periodontol. 29:15-20.
Romero A, Cáceres M, Arancibia R, Silva D, Couve E, Martínez C, Martínez J, Smith PC (2014). Cigarette smoke condensate inhibits collagen gel contraction and prostaglandin E(2) production in human gingival fibroblasts. J. Periodontal Res. DOI: 10.1111/jre.12216 [Epub ahead of print]
Rudziński R (2010). Effect of tobacco smoking on the course and degree of advancement inflammation in periodontal tissue. Ann. Acad. Med. Stetin. 56(2):97-105.
Sánchez GA, Miozza VA, Delgado A, Busch L (2013). Salivary IL-1β and PGE2 as biomarkers of periodontal status, before and after periodontal treatment. J. Clin. Periodontol. 40(12):1112-1117.
Schuller AA, Holst D (2001). An "S-shaped" relationship between smoking duration and alveolar bone loss: generating a hypothesis. J. Periodontol. 72:1164-1171.
Van der Weijden GA, de Slegte C, Timmerman MF, van der Velden U (2001). Periodontitis in smoker and non-smokers: intra oral distribution of pockets. A retrospective study. J. Clin. Periodontol. 28:955-960.