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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Received 5 April, 2015; Accepted 20 April, 2015

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≤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
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≤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
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=0.0002) 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.

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

### DISCUSSION

The objective of this study was to evaluate the influence of smoking on the levels of prostaglandin E₂ (GCF-PGE₂) in the gingival crevicular fluid of heavy, light and non-smokers according to G and CPD sites. This study revealed changes in the GCF-PGE₂ levels between G and PD sites when comparisons were done for HS, LS and NS groups.

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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 ≤ 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; Rudziński, 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 this fact, we decided to separate the periodontal disease sites in subgroups, gingivitis (G) and periodontitis (CPD) sites to investigate the GCF-PGE\textsubscript{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\textsubscript{2} in the GCF was measured to reveal differences among the three groups.

PGE\textsubscript{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\textsubscript{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\textsubscript{2} levels among G versus CPD sites disease (Preshaw and Heasman, 2002; Kurtiš et al., 2007). GCF-PGE\textsubscript{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; Kurtiš et al., 2007).

Indeed, our findings suggest that tobacco inhibit the PGE\textsubscript{2} release when G and CPD sites were compared (Figure 1). NS had higher levels of GCF-PGE\textsubscript{2} compared to HS and LS groups. Periodontitis sites (CPD) had higher PGE\textsubscript{2} levels than gingivitis sites (G). These results according to literature suggest the evidence that periodontal disease increase PGE\textsubscript{2} levels (Sánchez et al., 2013). Recent study with cell culture shows that tobacco has a detrimental effect on periodontal repair and PGE\textsubscript{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\textsubscript{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\textsubscript{2} compared to LS and HS. However, non-smokers had higher levels of GCF-PGE\textsubscript{2}. Indeed, this study confirmed that periodontal disease (CPD sites) exhibits higher GCF-PGE\textsubscript{2} levels compared to gingivitis (G sites), suggesting that periodontal disease can improved the GCF-PGE\textsubscript{2} levels.

**ACKNOWLEDGEMENTS**

This study was supported by Fundação de Amparo a Pesquisa Faperj, Rio de Janeiro, Brazil (E-26/100.491/2010). This manuscript was prepared with support of Ciências sem Fronteiras CsF, Brasília, Brazil (PDE - 248388/2013-4).

**Conflicts of interest**

The authors declare that they have no conflicts of interest.

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