

Influence of Smoking on Clinical Parameters and Gingival Crevicular Fluid Volume in Patients with Chronic Periodontitis

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Abstract

Background: Tobacco smoking is regarded as one of the most significant risk factors for the development and progression of periodontal disease. In particular, studies have shown an alteration in Gingival Crevicular Fluid (GCF) volume and its components in smokers.

Objective: The purpose of this study was to compare the GCF volume in smoking and non-smoking Saudi subjects with chronic periodontitis.

Methods: In this study, 30 smoking patients and 30 non-smoking patients with chronic periodontitis were enrolled. Periodontal Probing Depth (PPD), Clinical Attachment Level (CAL), Plaque Index (PI), and Bleeding on Probing (BOP) were measured to assess the pattern of periodontal destruction for each patient at six sites in selected teeth. Gingival inflammation was registered at six sites, where Gingival Crevicular Fluid (GCF) was also collected. The GCF volume was measured with a Periotron 8000®. Comparisons were made between smoking and non-smoking groups with periodontitis.

Results: Smokers demonstrated significantly deeper periodontal pockets (4.64 ± 0.30 mm) than non-smokers (4.24 ± 0.38 mm). Smoking subjects also presented significantly greater attachment loss (3.08 ± 0.28 mm) than non-smoking subjects (2.74 ± 0.42 mm), whereas the GCF volume was found to be significantly lower in smokers (0.25 ± 0.04 μ l) than in non-smokers (0.31 ± 0.05 μ l) ($P < 0.01$). Among smoking subjects, lingual sites showed reduced GCF levels compared to facial sites (0.22 ± 0.03 μ l vs. 0.25 ± 0.03 μ l).

Conclusion: Smoking appears to have considerable adverse effects on the inflammatory process, thereby promoting the progression of periodontal disease in smokers.

Clinical Significance: The adverse effect of smoking on the initiation and progression of periodontal disease is highlighted in this study. In particular, estimation of the GCF volume may serve as an indicator to assess the severity as well as the prognosis of periodontitis in smokers.

Key words: Smoking, GCF, GCF volume, Attachment loss, Gingivitis, Periodontitis

Introduction

Tobacco smoking is an important risk factor responsible for the loss of attachment and destruction of alveolar bone in patients with periodontitis [1]. Epidemiological studies on the relationship between tobacco use and periodontal diseases have consistently shown that cigarette smokers are two to six times more likely to develop severe periodontitis than non-smokers [2-4]. In addition, smoking was found to be a significant predictor of further attachment loss and bone loss in smoking patients with periodontitis when compared to non-smokers [5-8].

It is also known that smoking alters the host response, including changes in vascular function, neutrophil/monocyte activities, adhesion molecule expression, antibody production, and release of cytokine and inflammatory mediators [9-16]. Furthermore, smoking can mask the early signs of periodontal disease by suppressing the inflammatory response, which can result in diagnostic problems, especially in young patients with early periodontitis [17,18].

The precise mechanism by which tobacco smoking influences the periodontal tissues remains unclear. However, plausible biological mechanisms of the effects of smoking on the periodontium have been proposed with supportive evidence. For example, tobacco components can stimulate the production of proinflammatory cytokines, such as interleukin (IL)-1, IL-6, IL-8, tumor necrosis factor- α , as well

as transforming growth factor- β , thereby promoting increased bone resorption and tissue destruction [16,19,20].

Gingival Crevicular Fluid (GCF) is found in the sulcus or periodontal pocket between the surface of the tooth and the gingival epithelium. In healthy gingiva, small amounts of this fluid represent the transudate of gingival tissues. However, during the course of periodontal disease, this fluid is transformed into an inflammatory exudate [21]. GCF contains a complex mixture of substances derived from serum, leukocytes, periodontal cells and oral bacteria. The host-derived substances present in GCF include antibodies, cytokines, enzymes and tissue degradation products [22].

The volume of GCF has been shown to be associated with the status of periodontal disease and is an indicator of gingival inflammation [23,24]. In particular, the flow rate of GCF can increase up to 30-fold at sites of periodontitis compared to the healthy sulcus [24]. Holmes [25] studied crevicular fluid flow in smokers in areas of the oral cavity exposed to smoke (maxillary palatal) as well as areas less exposed to smoke (maxillary buccal). The results indicated that smokers had significantly less crevicular fluid flow than non-smokers. Interestingly, the exposed lingual areas of smokers showed no significant difference from the less exposed buccal areas, which suggests that the effect of nicotine may not be local; or, if this effect is local, it may be modified and distributed by the saliva [26,27].

Nicotine can cause vasoconstriction in peripheral blood

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vessels and thus may reduce the clinical signs of gingivitis [28]. Evidence for this reduction in clinical disease expression comes from various sources, including the study of Bergstrom [18], which compared the compliance of smokers to that of non-smokers in an oral hygiene intervention program. However, traditional clinical parameters, such as probing pocket depth, bleeding on probing, and clinical attachment loss, which are commonly used for periodontal diagnosis, are often of only limited usefulness because they are indicators of previous periodontal disease rather than current disease activity. Thus, a more accurate assessment of disease activity may assist with early intervention in patients with this disease. In the present study, the estimation of GCF volume and evaluation of clinical periodontal disease parameters were performed in smokers and non-smokers with periodontitis. The GCF volumes at facial and lingual surfaces of smokers with periodontitis were also compared.

Materials and Methods

Study population

A total of 30 smoking (Group-1) and 30 non-smoking (Group-2) male subjects with periodontitis (age 20-35 years) were enrolled in this study. These subjects were selected from patients attending the outpatient clinics of the College of Dentistry. Approval from the ethics committee was obtained from the College of Dentistry Research Center (CDRC), King Saud University, Riyadh, Saudi Arabia.

Only patients with a periodontal probing depth ≥ 4 mm and clinical attachment loss ≥ 2 mm in at least 30% of teeth were included in the present study [10]. Periodontal diagnosis was assessed according to the classification of the American Academy of Periodontology [29]. Smoking status was determined based on daily consumption, with smoking patients identified as those who smoked a minimum of 20 cigarettes per day for at least two years [30]. The following criteria were also used to exclude subjects from the study: (1) the presence of any chronic medical condition, including diabetes and viral, fungal or bacterial infections; (2) aggressive periodontitis, periodontal abscess, or necrotizing ulcerative gingivitis or periodontitis; and (3) periodontal treatment or use of antibiotics within the preceding three months.

Clinical periodontal examination

The medical history of each patient was recorded using a written questionnaire and an interview lasting 20 to 30 min. For each patient, complete examination and recording of the intraoral clinical parameters was performed. One clinical examiner performed all the clinical measurements. Calibration exercises for probing measurements were performed in five patients before the actual study. Intra-examiner agreement was good, with a k value of 0.82. Periodontal Probing Depth (PPD), Clinical Attachment Level (CAL), Plaque Index (PI), and Bleeding on Probing (BOP) were measured using the Williams periodontal probe at the mesio-buccal, mid-buccal, disto-buccal, mesio-lingual, mid-lingual and disto-lingual surfaces of each tooth. Smoking history was assessed according to a standardized interview and self-reported questionnaire.

The plaque score percentage was calculated after applying a disclosing agent (Displaque[®], Pace-maker Corporation,

Oregon, USA) by the method of O'Leary et al. [31]. After rinsing the oral cavity the mesial, distal, buccal and lingual surface were examined for the staining. The presence of plaque was marked and the surfaces which do not have soft accumulations at the dentogingival junction were not recorded. After all teeth were examined and scored, the index was calculated by dividing the number of plaque containing surfaces by the total number of available surfaces.

The bleeding on probing was assessed using the Gingival Bleeding Index (GBI) performed through gentle probing of the orifice of the gingival crevice [32]. If bleeding occurs within 10 seconds, a positive finding was established and the number of positive sites was recorded and then expressed as the percentage of the number of sites examined.

Gingival Crevicular Fluid (GCF)

After careful removal of all supragingival plaque, teeth were washed with water spray, isolated with cotton rolls and gently dried for 30 seconds. Paper strips (Periopaper, Ora Flow Inc., Amityville, NY, USA) were gently inserted until slight resistance was felt, and the strip was kept in the selected site for 30 seconds [33]. GCF volume was measured using a calibrated Periotron 8000 (OraFlow, PlainView, New York, USA) according to the manufacturer's instructions. Paper strips with traces of blood were discarded. Eight teeth (#16, 11, 24, 26, 36, 32, 44 and 46) were examined at 6 sites [34]. The filter paper strip was immediately placed between the recording sensors, such that the entire moistened area of the filter strip was in contact with the sensors. With the switch on the no-hold mode, the highest numerical readings were recorded. Raw Periotron scores were converted to GCF volume measurements in microliters [33].

Data analysis

Statistical analysis of the data was performed using GraphPad InStat[®] software (GraphPad Software, San Diego California USA, www.graphpad.com). Mean and standard deviation scores were calculated for age, plaque index (PI), Bleeding on Probing (BOP), Periodontal Probing Depth (PPD), Clinical Attachment Level (CAL), and GCF volume. Comparisons were made between smoking and non-smoking groups using the unpaired ' t ' test. The mean differences in GCF volume between lingual and facial sites among smokers were analyzed using paired ' t ' test.

Results

This study was conducted to estimate the Gingival Crevicular Fluid (GCF) volume among smoking and non-smoking subjects with chronic periodontitis. The mean age of the smoker group and non-smoker group was 27.90 ± 3.22 years and 27.33 ± 3.84 years, respectively. Clinical parameters such as the plaque index, bleeding on probing, periodontal probing depth and clinical attachment level were assessed for both smokers and non-smokers, and the results are presented in *Table 1* (PI-Plaque Index, BOP-Bleeding on Probing, PPD-Probing Pocket Depth and CAL-Clinical Attachment Loss).

Smokers demonstrated significantly higher plaque index levels compared to non-smokers ($74.90 \pm 9.89\%$ and $67.63 \pm 15.48\%$, respectively) ($p < 0.05$) (*Table 1*). Bleeding on

Table 1. Characteristics of the study population, including age, Plaque Index (PI), Bleeding on Probing (BOP), Probing Pocket Depth (PPD) and Clinical Attachment Loss (CAL), in smokers and non-smokers.

Groups (n=30)	Age (years)	PI (%)	BOP (%)	PPD (mm)	CAL (mm)
	Mean \pm SD (min-max)	Mean \pm SD (min-max)	Mean \pm SD (min-max)	Mean \pm SD (min-max)	Mean \pm SD (min-max)
Smokers with Periodontitis	27.90 \pm 3.22	74.90 \pm 9.89*	60.20 \pm 17.14**	4.64 \pm 0.30***	3.08 \pm 0.28***
	(20-35)	(47-94)	(31-91)	(4.13-5.50)	(2.63-3.85)
Non-smokers with Periodontitis	27.33 \pm 3.84	67.63 \pm 15.48	72.43 \pm 15.49	4.24 \pm 0.38	2.74 \pm 0.42
	(20-37)	(38-92)	(43-97)	(3.41-5.16)	(1.91-3.66)

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, SD- Standard Deviation, min-Minimum, max- Maximum

probing was lower in smokers (60.20 \pm 17.14%) than non-smoker patients (72.43 \pm 15.49%), and this difference was statistically significant ($p < 0.01$).

The periodontal probing depth and clinical attachment levels were measured at six sites, and the mean values were calculated for each subject in the smoker and non-smoker groups (Table 1). The mean periodontal probing depth for smokers was 4.64 \pm 0.30 mm, whereas this value was significantly lower for non-smokers (4.24 \pm 0.38 mm; $p < 0.001$). In addition, the clinical attachment loss was significantly higher ($p < 0.001$) among smokers (3.08 \pm 0.28 mm) compared to non-smokers (2.74 \pm 0.42 mm).

The mean GCF volume was 0.25 \pm 0.04 μ l in smokers and 0.31 \pm 0.05 μ l in non-smokers (Figure 1), and this difference was statistically significant ($p < 0.001$). Among smoking subjects, lingual sites demonstrated a relatively lower GCF volume (0.22 \pm 0.03 μ l) compared to facial sites (0.25 \pm 0.03 μ l), although this difference was not statistically significant.

Discussion

Smoking is one of the major risk factors for periodontal disease [35]. Earlier studies have shown the adverse effects of smoking on the periodontium [15,26,36]. Out of the various components of tobacco smoke, nicotine act on the periodontal tissues causing destruction of the supporting tissues [5,37]. Smokers have decreased blood flow to the tissues of periodontium, which may manifest clinically as reduced bleeding on probing [38].

The present study was performed in smokers and non-smokers with periodontitis to assess differences in periodontal destruction and the gingival crevicular fluid volume. Smokers had significantly low GCF volume compared to non-smokers. The periodontal probing depth and attachment loss were higher in smokers compared to non-smokers, which is in agreement with earlier studies [2,4,5,26,35,37,39]. Machuca et al. [40] evaluated the relationship between periodontal disease and smoking and found a positive correlation with greater probing depth and attachment loss in smokers. Clinical studies have also reported that cigarette smokers with periodontitis have relatively increased periodontal destruction in the maxilla, especially at the palatal region [36,41]. Preber [3] suggested that physical exposure to cigarette smoke at the palatal maxillary surfaces leads to a significant difference in periodontal destruction. Van der Weijden et al. [39] reported a possible local effect of smoking on the palatal surfaces of the maxillary teeth. Haffajee [37] have also reported the

differences in pocket depth and attachment level in the palatal area of smokers.

Gingival crevicular fluid nicotine concentrations can be up to nearly 300 times than that of plasma concentrations in smokers [42]. Nicotine binds to root surface in smokers and *in vitro* studies show that it can be stored and released from periodontal fibroblasts. Nicotine may inhibit fibroblast attachment and integrin expression, fibronectin and collagen production and increase fibroblast collagenase activity [43].

The volume of GCF was also lower in smokers, suggesting a suppression of the normal inflammatory response to plaque, which is in agreement with previous studies [3,27]. Bergstrom [3] reported a diminished GCF flow in chronic smokers, while McLaughlin and colleagues [44] reported that cigarette smoking may cause an immediate increase in GCF flow. These apparently contradictory findings may be related to the acute and chronic effects of smoking on blood flow and the inflammatory response [27]. Holmes [25] demonstrated a reduction in GCF flow in smokers with clinically healthy gingival tissues compared to matched non-smokers, and this study also compared GCF flow in smokers in the areas physically exposed to smoke (maxillary palatal) as well as areas not physically exposed to smoke (maxillary buccal). The results indicated that smokers had significantly lower GCF flow than non-smokers. However, the exposed lingual areas of smokers showed no significant difference compared to the less exposed buccal areas. In the present study, we also did not detect any significant changes in GCF volume between the facial and lingual sites, which is in agreement with previous observations (Figure 2) [25].

The results of the present study also suggest a possible local effect of cigarette smoking in addition to the known systemic effect. The decreased levels of GCF flow observed in smokers may consequently affect the levels of antibodies and other defense molecules present in the serum, which could influence the host defense system [45]. In addition, smoking may affect the vasculature, the humoral immune system, the cellular and soluble inflammatory system, and the cytokine and adhesion molecule network [10,15,16]. However, the relative importance of these smoking-related alterations and their precise mode of action in increasing the risk of periodontal disease remain to be elucidated.

Conclusion

The findings of the present study indicate that smoking appears to have considerable adverse effects on the inflammatory

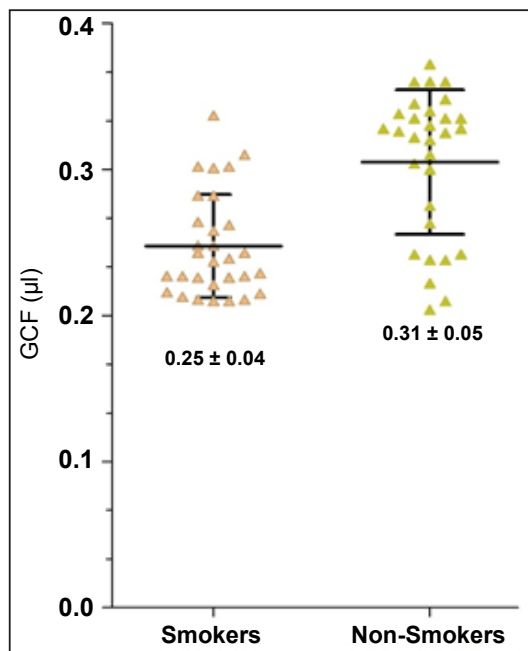


Figure 1. The GCF volume in smokers and non-smokers with periodontitis.

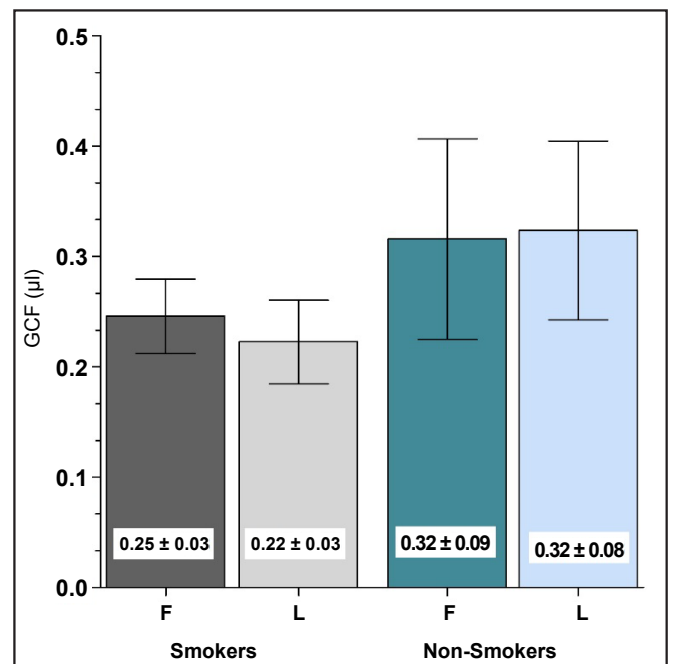


Figure 2. The GCF volume in facial and lingual sites in smokers and non-smokers with periodontitis (F- facial, L- lingual).

process, thereby promoting the progression of periodontal disease. Further studies are needed to evaluate whether GCF components may serve as indicators to monitor the effects of smoking on the periodontium.

References

- Palmer RM, Wilson RF, Hasan AS, Scott DA. Mechanisms of action of environmental factors--tobacco smoking. *Journal of Clinical Periodontology* 2005; **32**: 180-195.
- Anand PS, Kamath KP, Shekar BR, Anil S. Relationship of smoking and smokeless tobacco use to tooth loss in a central Indian population. *Oral Health & Preventive Dentistry*. 2012; **10**: 243-252.
- Bergstrom J, Preber H. The influence of cigarette smoking on the development of experimental gingivitis. *Journal of Periodontal Research*. 1986; **21**: 668-676.
- Razali M, Palmer RM, Coward P, Wilson RF. A retrospective study of periodontal disease severity in smokers and non-smokers. *British Dental Journal*. 2005; **198**: 495-498; discussion 485.
- Anand PS, Kamath KP, Bansal A, Dwivedi S, Anil S. Comparison of periodontal destruction patterns among patients with and without the habit of smokeless tobacco use--a retrospective study. *Journal of Periodontal Research*. 2013; **48**: 623-631.
- Graetz C, Ehrenthal JC, Senf D, Semar K, Herzog W, Dorfer CE. Influence of psychological attachment patterns on periodontal disease - a pilot study with 310 compliant patients. *Journal of Clinical Periodontology*. 2013; **40**: 1087-1094.
- Grossi SG, Zambon JJ, Ho AW, Koch G, Dunford RG, Machtei EE, Norderyd OM, Genco RJ. Assessment of risk for periodontal disease. I. Risk indicators for attachment loss. *Journal of Periodontology*. 1994; **65**: 260-267.
- Machtei EE, Dunford R, Hausmann E, Grossi SG, Powell J, Cummins D, Zambon JJ, Genco RJ. Longitudinal study of prognostic factors in established periodontitis patients. *Journal of Clinical Periodontology*. 1997; **24**: 102-109.
- Raulin LA, McPherson JC, 3rd, McQuade MJ, Hanson BS. The effect of nicotine on the attachment of human fibroblasts to glass

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and human root surfaces in vitro. *Journal of Periodontology*. 1988; **59**: 318-325.

10. Al-Ghamdi HS, Anil S. Serum antibody levels in smoker and non-smoker saudi subjects with chronic periodontitis. *Journal of Periodontology*. 2007; **78**: 1043-1050.

11. Andersen P, Pedersen OF, Bach B, Bonde GJ. Serum antibodies and immunoglobulins in smokers and nonsmokers. *Clinical and Experimental Immunology*. 1982; **47**: 467-473.

12. Ryder MI, Fujitaki R, Johnson G, Hyun W. Alterations of neutrophil oxidative burst by in vitro smoke exposure: implications for oral and systemic diseases. *Annals of Periodontology*. 1998; **3**: 76-87.

13. Sopori M. Effects of cigarette smoke on the immune system. *Nature Reviews Immunology*. 2002; **2**: 372-377.

14. Stampfli MR, Anderson GP. How cigarette smoke skews immune responses to promote infection, lung disease and cancer. *Nature Reviews Immunology*. 2009; **9**: 377-384.

15. Barbour SE, Nakashima K, Zhang JB, Tangada S, Hahn CL, Schenkein HA, Tew JG. Tobacco and smoking: environmental factors that modify the host response (immune system) and have an impact on periodontal health. *Critical Reviews in Oral Biology and Medicine*. 1997; **8**: 437-460.

16. Anil S, Preethanath RS, Alasqah M, Mokeem SA, Anand PS. Increased levels of serum and gingival crevicular fluid monocyte chemoattractant protein-1 in smokers with periodontitis. *Journal of Periodontology*. 2013; **84**: e23-28.

17. Bergstrom J, Floderus-Myrhed B. Co-twin control study of the relationship between smoking and some periodontal disease factors. *Community Dentistry and Oral Epidemiology*. 1983; **11**: 113-116.

18. Bergstrom J. Oral hygiene compliance and gingivitis

expression in cigarette smokers. *Scandinavian Journal of Dental Research*. 1990; **98**: 497-503.

19. Stein SH, Green BE, Scarbecz M. Augmented transforming growth factor-beta1 in gingival crevicular fluid of smokers with chronic periodontitis. *Journal of Periodontology*. 2004; **75**: 1619-1626.

20. Bostrom L, Linder LE, Bergstrom J. Smoking and cervicular fluid levels of IL-6 and TNF-alpha in periodontal disease. *Journal of Clinical Periodontology*. 1999; **26**: 352-357.

21. Griffiths GS. Formation, collection and significance of gingival crevice fluid. *Periodontology 2000*. 2003; **31**: 32-42.

22. Uitto VJ. Gingival crevice fluid--an introduction. *Periodontology 2000*. 2003; **31**: 9-11.

23. Poulsen S, Holm-Pedersen P, Kelstrup J. Comparison of different measurements of development of plaque and gingivitis in man. *Scandinavian Journal of Dental Research*. 1979; **87**: 178-183.

24. Goodson JM. Gingival crevice fluid flow. *Periodontology 2000*. 2003; **31**: 43-54.

25. Holmes LG. Effects of smoking and/or vitamin C on crevicular fluid flow in clinically healthy gingiva. *Quintessence International*. 1990; **21**: 191-195.

26. Kinane DF, Chestnutt IG. Smoking and periodontal disease. *Critical Reviews in Oral Biology and Medicine*. 2000; **11**: 356-365.

27. Morozumi T, Kubota T, Sato T, Okuda K, Yoshie H. Smoking cessation increases gingival blood flow and gingival crevicular fluid. *Journal of Clinical Periodontology*. 2004; **31**: 267-272.

28. Clarke NG, Shephard BC, Hirsch RS. The effects of intra-arterial epinephrine and nicotine on gingival circulation. *Oral Surgery, Oral Medicine, Oral Pathology*. 1981; **52**: 577-582.

29. Armitage GC. Development of a classification system for periodontal diseases and conditions. *Annals of Periodontology*. 1999; **4**: 1-6.

30. Bostrom L, Bergstrom J, Dahlen G, Linder LE. Smoking and subgingival microflora in periodontal disease. *Journal of Clinical Periodontology*. 2001; **28**: 212-219.

31. O'Leary TJ, Drake RB, Naylor JE. The plaque control record. *Journal of Periodontology*. 1972; **43**: 38.

32. Ainamo J, Bay I. Problems and proposals for recording gingivitis and plaque. *International Dental Journal*. 1975; **25**: 229-235.

33. Bickel M, Cimasoni G. Reliability of volume measurements with the new Periotron 6000. *Journal of Periodontal Research*. 1984; **19**: 313-316.

34. Ramfjord SP, Knowles JW, Nissle RR, Burgett FG, Shick RA. Results following three modalities of periodontal therapy. *Journal of Periodontology*. 1975; **46**: 522-526.

35. Bergstrom J, Preber H. Tobacco use as a risk factor. *Journal of Periodontology*. 1994; **65**: 545-550.

36. Kamma JJ, Nakou M, Baehni PC. Clinical and microbiological characteristics of smokers with early onset periodontitis. *Journal of Periodontal Research*. 1999; **34**: 25-33.

37. Haffajee AD, Socransky SS. Relationship of cigarette smoking to attachment level profiles. *Journal of Clinical Periodontology*. 2001; **28**: 283-295.

38. Kazor C, Taylor GW, Loesche WJ. The prevalence of BANA-hydrolyzing periodontopathic bacteria in smokers. *Journal of Clinical Periodontology*. 1999; **26**: 814-821.

39. van der Weijden GA, de Slegte C, Timmerman MF, van der Velden U. Periodontitis in smokers and non-smokers: intra-oral distribution of pockets. *Journal of Clinical Periodontology*. 2001; **28**: 955-960.

40. Machuca G, Rosales I, Lacalle JR, Machuca C, Bullon P. Effect of cigarette smoking on periodontal status of healthy young adults. *Journal of Periodontology*. 2000; **71**: 73-78.

41. Anil S. Study of the patterns of periodontal destruction in smokers with chronic periodontitis. *Indian Journal of Dental Research*. 2008; **19**: 124-128.

42. Chen X, Wolff L, Aepli D, Guo Z, Luan W, Baelum V, Fejeskov O. Cigarette smoking, salivary/gingival crevicular fluid cotinine and periodontal status. A 10-year longitudinal study. *Journal of Clinical Periodontology*. 2001; **28**: 331-339.

43. Alpar B, Leyhausen G, Sapotnick A, Gunay H, Geurtsen W. Nicotine-induced alterations in human primary periodontal ligament and gingiva fibroblast cultures. *Clinical Oral Investigations*. 1998; **2**: 40-46.

44. McLaughlin WS, Lovat FM, Macgregor ID, Kelly PJ. The immediate effects of smoking on gingival fluid flow. *Journal of Clinical Periodontology*. 1993; **20**: 448-451.

45. Kinane DF, Radvar M. The effect of smoking on mechanical and antimicrobial periodontal therapy. *Journal of Periodontology*. 1997; **68**: 467-472.