Original Article

Influence of Smoking on Gingival Crevicular Fluid Interleukin 1β and Interleukin-8 in Patients with Severe Chronic Periodontitis among a Rural Population in India

Ritu Prabha Patel, Ramesh Amirisetty, Butchibabu Kalakonda[1], Narendra Varma Penumatsa[2], Pradeep Koppolu[3]

Department of Periodontics, Chhattisgarh Dental College and Research Institute, Rajnandgaon, Chhattisgarh, India, [1]Department of Preventive Dental Sciences, Al Farabi Colleges, Riyadh, [2]Department of Preventive Dental Sciences, College of Dentistry, Prince Sattam Bin Abdul Aziz University, Alkharj, [3]Department of Preventive Dental Sciences, College of Dentistry, Dar Al Uloom University, Riyadh, Saudi Arabia

Abstract

Background and Objective: Smoking is widely prevalent among rural Indian populations. Smoking is considered a significant risk factor for periodontal diseases. The aim of the present study was to evaluate the influence of smoking on the inflammatory cytokines interleukin (IL) IL-1β and IL-8 in gingival crevicular fluid (GCF) and compare these between smokers and nonsmokers with periodontitis in diseased and healthy sites. **Materials and Methods:** A total of thirty patients with severe chronic periodontitis (15 smokers and 15 nonsmokers) participated in this study. Clinical parameters assessed were gingival index, bleeding on probing, probing depth (PD), and clinical attachment level. One diseased and one healthy site from each of the periodontitis patients were selected for GCF collection and assigned to the following four groups: healthy sites in smoker (SH), diseased sites in smoker (SD), healthy sites in nonsmoker (NH), and diseased sites in nonsmoker (ND) and were analyzed by enzyme-linked immunosorbent assay test (Quantikine®). **Results:** Intragroup comparisons revealed statistically significant levels of IL-1β in ND sites compared to NH sites ($P < 0.005$) and SD sites showed statistically significantly higher levels of IL-1β compared to SH sites ($P < 0.0001$). However, there was no significant difference in IL-8 levels between NH and ND sites as well as between SH and SD sites. Intergroup comparisons revealed that SH sites exhibited significantly lower amounts of IL-8 ($P < 0.01$) compared to NH sites and SD sites showed significantly lower levels of IL-8 compared to ND sites ($P < 0.001$). However, the comparison of NH and SH sites as well as ND and SD sites showed no significant difference in the levels of IL-1β. **Conclusions:** Cytokine levels were significantly elevated in periodontitis patients. Smokers exhibited a decrease in IL-8 and increase in IL-1β levels. Hence, this reflects the influence of smoking on immunosuppression and its role in the pathogenesis of periodontal disease.

Keywords: Chronic periodontitis, cytokines, enzyme-linked immunosorbent assay, gingival crevicular fluid, interleukins 1β and 8, smoking

Introduction

Periodontitis is a chronic, multifactorial, infectious disease of the supporting tissues of the teeth. The onset and progression of periodontitis is due to an imbalance of the interaction between bacterial pathogens and host immunity.[1,2]

Host immunity is greatly influenced by both genetic susceptibility and environmental risk factors. Cigarette smoking is considered to be one of the most important environmental risk factors for periodontitis.[3,4] More clinical attachment loss and bone loss have been observed among smokers than among nonsmokers.[5]

Cigarette smoking alters the host response through a plethora of changes that include antibody production, vascular function, activities of neutrophil/monocyte, and finally influencing cytokine and inflammatory mediator release, and thus exhibits a negative impact on the periodontium.[6] Cytokines are low-molecular-weight proteins involved in the initiation

Access this article online

Quick Response Code:

Website: www.nigeriamedj.com

DOI: 10.4103/nmj.NMJ_142_17

Address for correspondence: Dr. Butchibabu Kalakonda, Department of Preventive Dental Sciences, Al Farabi Colleges, Riyadh, Saudi Arabia.
E-mail: docbutchi@gmail.com

How to cite this article: Patel RP, Amirisetty R, Kalakonda B, Penumatsa NV, Koppolu P. Influence of smoking on gingival crevicular fluid interleukin 1β and interleukin-8 in patients with severe chronic periodontitis among a Rural Population in India. Niger Med J 2018;59:33-8.

and effector stages of immunity and inflammation. The interrelationships of various cytokines and their correlation with periodontitis have been explored through the biochemical analysis of gingival crevicular fluid (GCF). Sampling of GCF provides ease of access, is relatively atraumatic, and can be repeatedly collected. GCF exhibits the lowest bias, the highest reproducibility, and the strongest validity.[7]

Cytokines such as interleukin (IL) IL-1, IL-6, IL-8, and TNF-α are involved in the host response of periodontal disease as mediators of tissue destruction. IL-lβ has pro-inflammatory effects and acts on endothelial cells to promote adhesion and migration of leukocytes into inflamed tissue sites.[8,9] In this context, IL-8 may also play a role in the pathogenesis of inflammation because it promotes neutrophil chemotaxis and activation.[10] Considering the above facts, it seems logical to hypothesize the role of IL-1β and IL-8 in the pathogenesis of periodontal disease.

Smoking alters many aspects of the host's immune response, and these alterations may be a major contribution to the pathogenesis of the disease. Smokers have elevated GCF levels of IL-8.[11,12] In other studies, smokers showed decreased levels of certain cytokines in GCF, such as IL-1β and IL-8.[13,14] In a recent study by Fredriksson *et al*., smoking was found to reduce the sensitivity of peripheral neutrophil to stimulation by IL-8 when comparing smoking to nonsmoking patients.[15] This suggests that smoking may interfere with the inflammatory process by affecting the release of pro-inflammatory cytokines. Smoking's effect on IL-1β and IL-8 in GCF has not been well characterized. Therefore, this study evaluated the impact of smoking on these two cytokines.

The present study was designed to determine the cytokine levels of IL-1β and IL-8 in GCF from smokers and nonsmokers with periodontitis, with an objective to determine the differences in these levels from disease and healthy sites of smokers and nonsmokers with periodontal disease.

Materials and Methods

The present study comprised a cross-sectional study design. A total of thirty male patients aged between 40 and 70 years (15 smokers and 15 nonsmokers) with severe chronic periodontitis were enrolled in this study during January 2014 to April 2014. The patients were randomly selected from the Outpatient Department of Periodontology, Chhattisgarh Dental College and Research Institute, Rajnandagaon, India. Ethical clearance for the study was obtained from the college's ethical committee. All the patients had the procedure explained to them, and a written informed consent was obtained from those willing to be part of this study.

Sample size

The sample size for the present study was based on the preliminary findings related to the variations in IL-β levels expressed in picograms per microliter reported by Tymkiw *et al*.[16] Based on the standard deviation with an error of 0.05 and power at 80%, the calculated sample size was 15 for each representative group.

Selection criteria for the patients

Inclusion criteria

Presence of good general health and clinical diagnosis of periodontal disease (periodontally diseased patients had a diagnosis of generalized severe chronic periodontitis [30% of sites with CAL and PD ≥5 mm]).

Exclusion criteria

Pregnancy or lactation, gingival overgrowth, chronic systemic disease such as diabetes, administration of systemic antibiotics in the last 6 months, and regular use of anti-inflammatory medications in the last 6 months.

Patient selection

Classification of smokers and nonsmokers was adopted from the study by Tymkiw *et al*.[16] Smokers were classified and enrolled if they regularly smoked ≥20 cigarettes per day, and nonsmokers were classified as not having smoked one hundred or more cigarettes in their lifetime.

Site selection

One diseased and one healthy site were selected from each of the smoking and nonsmoking groups. The diseased sites in the smoking patients were labeled as diseased sites in smoker (SD) and healthy sites as healthy sites in smoker (SH). In the nonsmoking group, the diseased sites were labeled as diseased sites in nonsmoker (ND) and the healthy sites were labeled as healthy sites in nonsmoker (NH).

The clinical parameters recorded were bleeding on probing (BOP), probing depth (PD), clinical attachment level (CAL), and gingival recession (REC). PD, CAL, and recession measurements were recorded by a calibrated examiner and rounded off to the nearest millimeter with a periodontal probe (UNC-15, Hu-Friedy, Chicago, IL, USA).

Gingival crevicular fluid sample collection

The patients were informed about the protocol prior to the collection of GCF. Due to the contamination of the GCF sample with saliva and the difficulties involved in the isolation process, GCF sample collection was predominantly done from the maxillary teeth. The test sites for GCF sample collection in the periodontitis patients comprised of five different nonadjacent sites that exhibited the deepest PD on clinical examination. In the healthy group, to facilitate sufficient volume for the assay, GCF was collected from numerous sites without any inflammation and was pooled up. The samples were collected a day after the clinical parameters were recorded to avoid sample contamination.

Prior to the sampling, each of these selected test sites was air dried gently with a blast of air and isolated with cotton rolls. Supragingival plaque was removed with Gracey curettes (Hu-Friedy), avoiding any contact with the marginal gingiva to pave way for the micropipette in the sample collection. The GCF sample was collected by placing the 1–5 μl calibrated

volumetric microcapillary pipettes (Sigma-Aldrich, Chemical Company, St. Louis, Missouri,USA) at the entrance of the gingival sulcus [Figure 1]. The GCF sample was collected from all the five sites with a collection time of no more than 5 min from each site, and the collected sample was pooled to calculate the volume. Pooled GCF sample of 3 µl was collected from all these sites. Care was taken to discard the micropipettes contaminated with blood and saliva. GCF collected was immediately transferred to vials and stored at −70°C in a deep freezer till the time of the assay.

Interleukin-1β and interleukin-8 assays

The pooled GCF samples were assayed to measure the IL-1β and IL-8 levels using a quantitative sandwich enzyme-linked immunosorbent assay (ELISA) (Quantikine R and D systems, MN, USA) that was specific to human IL-1β and IL-8 assays. The test was run by an expert, blinded to the clinical results as per the manufacturer's instructions. The calorimetric reaction in the wells was recognized by an ELISA reader with an optical density value set at 450 nm. The concentrations of IL-1β and IL-8 were reported as picograms per microliter (pg/µl) of the sample.

Statistical analysis

Statistical analysis was performed using IBM Statistical Program for Social Sciences Version 20.0 (SPSS Inc., Chicago, IL, USA). Intergroup comparisons were done using unpaired *t*-test. Smokers and nonsmokers were compared in relation to healthy and diseased sites. Intragroup comparisons were done using the Wilcoxon matched-pairs signed-rank test between healthy and diseased sites in smokers and nonsmokers. In each case, the level of significance was set at $P \leq 0.05$.

Results

A total of thirty patients were examined for this study, with 15 participants in each group. The mean age of the smokers group was 50.2 ± 7.8 and that of the nonsmokers group was 60.2 ± 8.7, with the difference in age being not statistically significant.

Clinical parameters

Table 1 represents the distribution of the values of the clinical parameters among the study groups. PD was significantly high for the SD group (5.9 [0.5], $P = 0.03$) compared to the ND group (5.5 [0.9]). PD was high in the SH group (2.8 [0.4]) compared to the NH group (2.6 [0.6]) and was statistically significant ($P = 0.06$). CAL was also significantly high in the SD group (6.9 [1.3], $P = 0.04$) compared to the ND group (6.2 [1.3]).

Table 1: Distribution of clinical parameters of the study population

Clinical parameters	NH	SH	ND	SD
PD (mm)	2.6±0.6	2.8±0.4	5.5±0.9	5.9±0.5
Recession (mm)	0.3±0.7	0.3±0.4	0.7±0.9	1.0±1.1
CAL (mm)	2.9±0.9	3.0±0.3	6.2±1.3	6.9±1.3
BOP (percentage positive)	0.0±0.0	0.0±0.0	100±0.0	100±0.0

NH – Healthy sites in nonsmoker; SH – Healthy sites in smoker; ND – Diseased sites in nonsmoker; SD – Diseased sites in smoker; CAL – Clinical attachment level; BOP – Bleeding on probing; PD – Probing depth

Cytokine levels

Table 2 represents the intragroup comparison of IL-1β and IL-8 levels between healthy and diseased sites in smokers and nonsmokers.

There was a statistically significant elevation in the mean levels of IL-1β in ND sites (131.5 [62.2] pg/µl) compared to NH sites (37.2 [17.1] pg/µl) ($P < 0.005$). SD sites showed significantly higher levels of IL-1β (192.2 [26.4] pg/µl) compared with SH sites (42.6 [4.1] pg/µl) ($P < 0.0001$). However, there was no significant difference in IL-8 levels between NH and ND sites (1342.4 [134.5] and 2409.1 [298.1] pg/µl, respectively) as well as between SH and SD sites (743.6 [76.7] and 809.1 [61.5] pg/µl, respectively).

Tables 3 and 4 represents the intergroup comparison of IL-1β and IL-8 levels between healthy and diseased sites in smokers and nonsmokers.

There was a statistically significant decrease in the mean levels of IL-8 in SH sites (743.6 [76.7] pg/µl) compared to NH sites (1342.4 [134.5] pg/µl) ($P < 0.01$), and SD sites (809.1 [61.5] pg/µl) showed significantly lower levels of IL-8 compared to ND sites (2409.1 [298.1] pg/µl) ($P < 0.001$). However, the comparison of NH and SH sites (37.2 [17.1] and 42.6 [4.1] pg/µl, respectively) as well as ND and SD sites (131.5 [62.2] and 192.2 [26.4] pg/µl, respectively) showed no significant difference in the mean levels of IL-1β.

Discussion

The objective of the present study was to investigate the influence of smoking on the etiopathogenesis of periodontal diseases through the assessment of potential mediators of inflammation. Literature is replete with abundant evidence[17-19] on the positive influence smoking has on periodontal disease, with most of these studies centered on the urban population.[20,21]

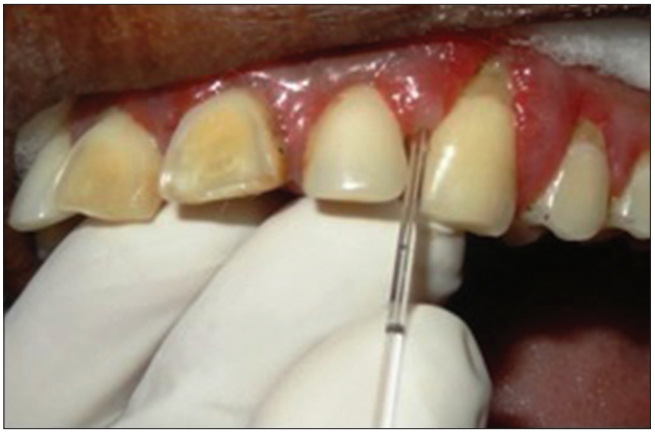

Figure 1: Gingival crevicular fluid sample collection from a diseased periodontal site

There is a dearth of articles that have assessed this association in a rural setting. Hence, our study was designed to explore the role of smoking in the pathogenesis of periodontal disease in a rural population through the assessment of levels of mediators of inflammation.

The participants in our study comprised only males, probably because smoking among females is considered a taboo in rural India. Females are, therefore, more addicted to smokeless tobacco and other related products.[22] This point was taken into consideration in our previous study that had included females in correlating the role of gutka chewing on periodontitis and was published elsewhere. Further, GCF volume is influenced by variables such as ovulation, menstruation, and use of hormonal contraceptives; hence, females were excluded to avoid this bias in our study.

Our study evaluated GCF biomarkers using ELISA. A major limitation was that this assay has the capability to evaluate only a limited number of mediators in each sample. A multiplex assay would have made it possible to evaluate a wide range of cytokines, but the fact that the study was self-funded and had no financial help from external sources limited our possibility only to the rural population attending a private dental college.

Comparison of periodontal parameters in our study revealed that patients in the SD and ND groups had higher mean percentage of BOP compared to the NH and SH groups. Our findings attain support from a study by Ramseier *et al*.[23] who reported that mean BOP significantly increased with periodontal disease severity, irrespective of the smoking status. However, there was an absence of BOP in healthy smoker group.

This could be explained by the fact that smoking leads to decrease in vascular density and reduced lumen area of gingival vessels and causes vasoconstriction in peripheral blood vessels and also an increase in the thickness of gingival epithelium.[24-26]

A significant finding in our study was that diseased sites in nonsmokers and smokers had significantly elevated IL-1β profiles as compared with healthy sites. This relative increase in IL-1β in diseased smokers is consistent with a previous research by Kamma *et al*.[14] and Zhong *et al*.[27] who reported greater total amounts of IL-1β in smokers. This can be explained by the fact that nicotine, an active content of smoke, impairs the gingival blood flow with its vasoconstrictive properties and creates an anaerobic environment conducive for the growth of periodontal bacteria.[28,29] An increase in these anaerobes enhances greater stimulation of the gingival monocytes and macrophages, resulting in an increased production of IL-1β.[29] Further, the oral keratinocytes are quite sensitive to cigarette smoke and react with an increased production of inflammatory mediators such as PGE_2 and IL-1, contributing to periodontal destruction.[30]

Table 2: Intragroup comparisons: Healthy and diseased sites in smokers and nonsmokers

Cytokine	Mean±SD		*P*	Mean±SD		*P*
	NH	ND		SH	SD	
IL-1β	37.2±17.1	131.5±62.2	<0.005*	42.6±4.1	192.2±26.4	<0.0001*
IL-8	1342.4±134.5	2409.1±298.1	NS	743.6±76.7	809.1±61.5	NS

*Statistically significant. IL-1β and IL-8 levels of NH versus ND and SH versus SD compared using Wilcoxon matched-pairs signed-rank test.
NH – Healthy sites in nonsmoker; SH – Healthy sites in smoker; ND – Diseased sites in nonsmoker; SD – Diseased sites in smoker; SD – Standard deviation; IL – Interleukin; NS – Not significant

Table 3: Intergroup comparisons: Healthy and diseased sites in smokers and nonsmokers

	NH	SH	*P*	ND	SD	*P*
PD (mm)	2.6±0.6	2.8±0.4	0.06	5.5±0.9	5.9±0.5	0.03*
Recession (mm)	0.3±0.7	0.3±0.4	0.06	0.7±0.9	1.0±1.1	0.03*
CAL (mm)	2.9±0.9	3.0±0.3	0.08	6.2±1.3	6.9±1.3	0.04*
BOP (percentage positive)	0.0±0.0	0.0±0.0	-	100±0.0	100±0.0	-
IL-1β	37.2±17.1	42.6±4.1	NS	131.5±62.2	192.2±26.4	NS
IL-8	1342.4±134.5	743.6±77.8	<0.01	2409.1±298.1	809.1±61.5	<0.001*

*Statistically significant. NH – Healthy sites in nonsmoker; SH – Healthy sites in smoker; ND – Diseased sites in nonsmoker; SD – Diseased sites in smoker; IL – Interleukin; NS – Not significant; CAL – Clinical attachment level; BOP – Bleeding on probing; PD – Probing depth

Table 4: Comparison of cytokine levels and clinical parameters among all the groups

	Mean±SD		*P*	Mean±SD		*P*
	NH	SH		ND	SD	
IL-1β	37.2±17.1	45.6±4.1	NS	131.5±62.2	192.2±26.4	NS
IL-8	1342.4±134.5	743.6±77.8	<0.01*	2409.1±298.1	809.1±61.5	<0.001*

*Statistically significant. NH – Healthy sites in nonsmoker; SH – Healthy sites in smoker; ND – Diseased sites in nonsmoker; SD – Diseased sites in smoker; IL – Interleukin; NS – Not significant; SD – Standard deviation

In contrast, Rawlinson *et al*.[13] reported lower GCF levels of IL-1β among smokers. The differences in IL-1β levels among smokers could be attributed to a number of factors such as nature of the host response, variables associated with smoking, techniques employed in the collection of GCF, and lab technicalities in the analysis of a GCF assay.[16]

There was also a reported increase in IL-1β in diseased nonsmokers compared to the healthy controls. This finding corroborates with other studies which reported increased GCF amounts of pro-inflammatory cytokines, especially IL-1β in periodontitis patients.[31,32]

The present study also analyzed GCF levels of IL-8. Our results exhibit a reduction of IL-8 among smokers. The findings in our study corroborate with those of Tymkiv *et al*.[16] who reported similar reductions of IL-8 in GCF of smokers. Our study did not find any significant difference in the chemokine levels of smokers with periodontitis when compared with the healthy smokers. However, a significant difference in chemokine levels has been found when nonsmokers with periodontitis were compared with smokers, both healthy and those with periodontitis. This reduction in IL-8 levels in smokers leads to impaired neutrophil chemotaxis and migration in the periodontium, paving way for the progression of periodontal disease.[33] Our results are at variance with those of Lütfioğlu *et al*.[34] who reported increases in GCF IL-8 levels in smokers.

Our study revealed that the periodontal parameters such as PD, REC, and CAL were higher and statistically significant in the SD and SH groups when compared with the ND and NH groups. Our findings agree with the studies by Rosa *et al*.[35] and Calsina *et al*.[36] who also reported a direct relation between an increased consumption of smoking and periodontal destruction. Literature is replete with evidence that supports that smokers are at a higher risk of having periodontitis with higher attachment loss as compared with nonsmokers.[37,38]

Our study shows a correlation between clinical parameters and cytokine levels in the study groups. There was a positive association in the SD and ND groups reflected by an increased PD and CAL associated with elevated IL-1β levels. Our findings were similar to those of Engebretson *et al*.[39] who reported a statistically significant correlation between IL-1β GCF levels and clinical parameters with severe disease sites, showing a higher IL-1β GCF level when compared to the shallow sites.

Conclusions

Within the limitations of the present study, it can be concluded that smoking appears to have an inhibitory effect on chemokine production reflected by a reduced amount of IL-8 levels in GCF. Hence, smoking induces an immunosuppressed state reducing the individual's capacity to fight periodontal diseases. Smokers with periodontitis exhibited higher levels of IL-1β compared with nonsmokers, which could explain the increased periodontal destruction in smokers.

However, a small sample size restrains us in giving a robust conclusion. A larger sample employing novel assay methods would help us in correlating the influence of smoking on the cytokine profile and periodontal destruction.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Page RC, Kornman KS. The pathogenesis of human periodontitis: An introduction. Periodontol 2000 1997;14:9-11.
2. Pihlstrom BL, Michalowicz BS, Johnson NW. Periodontal diseases. Lancet 2005;366:1809-20.
3. Bergström J, Preber H. Tobacco use as a risk factor. J Periodontol 1994;65:545-50.
4. Grossi SG, Zambon JJ, Ho AW, Koch G, Dunford RG, Machtei EE, *et al.* Assessment of risk for periodontal disease. I. Risk indicators for attachment loss. J Periodontol 1994;65:260-7.
5. Albandar JM, Streckfus CF, Adesanya MR, Winn DM. Cigar, pipe, and cigarette smoking as risk factors for periodontal disease and tooth loss. J Periodontol 2000;71:1874-81.
6. Ryder MI. The influence of smoking on host responses in periodontal infections. Periodontol 2000 2007;43:267-77.
7. Wilton JM, Bampton JL, Hurst TJ, Caves J, Powell JR. Interleukin-1 beta and IgG subclass concentrations in gingival crevicular fluid from patients with adult periodontitis. Arch Oral Biol 1993;38:55-60.
8. Bevilacqua MP, Pober JS, Wheeler ME, Cotran RS, Gimbrone MA Jr. Interleukin 1 acts on cultured human vascular endothelium to increase the adhesion of polymorphonuclear leukocytes, monocytes, and related leukocyte cell lines. J Clin Invest 1985;76:2003-11.
9. Koppolu P, Durvasula S, Palaparthy R, Rao M, Sagar V, Reddy SK, *et al.* Estimate of CRP and TNF-alpha level before and after periodontal therapy in cardiovascular disease patients. Pan Afr Med J 2013;15:92.
10. Lagdive SS, Marawar PP, Byakod G, Lagdive SB. Evaluation and comparison of interleukin-8 (IL-8) level in gingival crevicular fluid in health and severity of periodontal disease: A clinico-biochemical study. Indian J Dent Res 2013;24:188-92.
11. Giannopoulou C, Cappuyns I, Mombelli A. Effect of smoking on gingival crevicular fluid cytokine profile during experimental gingivitis. J Clin Periodontol 2003a; 30:996-1002.
12. Giannopoulou C, Kamma JJ, Mombelli A. Effect of inflammation, smoking and stress on gingival crevicular fluid cytokine level. J Clin Periodontol 2003b; 30:145-53.
13. Rawlinson A, Grummitt JM, Walsh TF, Ian Douglas CW. Interleukin 1 and receptor antagonist levels in gingival crevicular fluid in heavy smokers versus non-smokers. J Clin Periodontol 2003;30:42-8.
14. Kamma JJ, Giannopoulou C, Vasdekis VG, Mombelli A. Cytokine profile in gingival crevicular fluid of aggressive periodontitis: Influence of smoking and stress. J Clin Periodontol 2004;31:894-902.
15. Fredriksson M, Bergström K, Asman B. IL-8 and TNF-alpha from peripheral neutrophils and acute-phase proteins in periodontitis. J Clin Periodontol 2002;29:123-8.
16. Tymkiw KD, Thunell DH, Johnson GK, Joly S, Burnell KK, Cavanaugh JE, *et al.* Influence of smoking on gingival crevicular fluid cytokines in severe chronic periodontitis. J Clin Periodontol 2011;38:219-28.
17. Albandar JM. Global risk factors and risk indicators for periodontal diseases. Periodontol 2000 2002;29:177-206.
18. Bergström J, Eliasson S, Dock J. A 10-year prospective study of tobacco smoking and periodontal health. J Periodontol 2000;71:1338-47.
19. Tomar SL, Asma S. Smoking-attributable periodontitis in the United States: Findings from NHANES III. National health and nutrition examination survey. J Periodontol 2000;71:743-51.
20. Norderyd O, Hugoson A. Risk of severe periodontal disease in a

Swedish adult population. A cross-sectional study. J Clin Periodontol 1998;25:1022-8.
21. Susin C, Oppermann RV, Haugejorden O, Albandar JM. Periodontal attachment loss attributable to cigarette smoking in an urban Brazilian population. J Clin Periodontol 2004;31:951-8.
22. Joshi U, Modi B, Yadav S. A study on prevalence of chewing form of tobacco and existing quitting patterns in urban population of Jamnagar, Gujarat. Indian J Community Med 2010;35:105-8.
23. Ramseier CA, Mirra D, Schütz C, Sculean A, Lang NP, Walter C, *et al.* Bleeding on probing as it relates to smoking status in patients enrolled in supportive periodontal therapy for at least 5 years. J Clin Periodontol 2015;42:150-9.
24. Scardina GA, Messina P. Morphological changes in microcirculation induced by chronic smoking habit: A video capillary study on gingival mucosa. J Periodontol 2001;72:1318-23.
25. Persson L, Bergström J. Smoking and vascular density of healthy marginal gingiva. Eur J Oral Sci 1998;106:953-7.
26. Anil S. Study of the patterns of periodontal destruction in smokers with chronic periodontitis. Indian J Dent Res 2008;19:124-8.
27. Zhong Y, Slade GD, Beck JD, Offenbacher S. Gingival crevicular fluid interleukin-1beta, prostaglandin E2 and periodontal status in a community population. J Clin Periodontol 2007;34:285-93.
28. Zambon JJ, Grossi SG, Machtei EE, Ho AW, Dunford R, Genco RJ. Cigarette smoking increases the risk for subgingival infection with periodontal pathogens. J Periodontol 1996;67:1050-4.
29. Yoshinari N, Kawase H, Mitani A, Ito M, Sugiishi S, Matsuoka M, *et al.* Effects of scaling and root planing on the amounts of interleukin-1 and interleukin-1 receptor antagonist and the mRNA expression of interleukin-1beta in gingival crevicular fluid and gingival tissues. J Periodontal Res 2004;39:158-67.
30. Tambwekar KR, Kakariya RB, Garg S. A validated high performance liquid chromatographic method for analysis of nicotine in pure form and from formulations. J Pharm Biomed Anal 2003;32:441-50.
31. Teles R, Sakellari D, Teles F, Konstantinidis A, Kent R, Socransky S, *et al.* Relationships among gingival crevicular fluid biomarkers, clinical parameters of periodontal disease, and the subgingival microbiota. J Periodontol 2010;81:89-98.
32. Tsai CC, Ho YP, Chen CC. Levels of interleukin-1 beta and interleukin-8 in gingival crevicular fluids in adult periodontitis. J Periodontol 1995;66:852-9.
33. Palmer RM, Wilson RF, Hasan AS, Scott DA. Mechanisms of action of environmental factors – tobacco smoking. J Clin Periodontol 2005;32 Suppl 6:180-95.
34. Lütfioğlu M, Aydoğdu A, Sakallioğlu EE, Alaçam H, Pamuk F. Gingival crevicular fluid interleukin-8 and lipoxin A4 levels of smokers and nonsmokers with different periodontal status: A cross-sectional study. J Periodontal Res 2016;51:471-80.
35. Rosa GM, Lucas GQ, Lucas ON. Cigarette smoking and alveolar bone in young adults: A study using digitized radiographs. J Periodontol 2008;79:232-44.
36. Calsina G, Ramón JM, Echeverría JJ. Effects of smoking on periodontal tissues. J Clin Periodontol 2002;29:771-6.
37. Grossi SG, Zambon J, Machtei EE, Schifferle R, Andreana S, Genco RJ, *et al.* Effects of smoking and smoking cessation on healing after mechanical periodontal therapy. J Am Dent Assoc 1997;128:599-607.
38. Haffajee AD, Socransky SS. Relationship of cigarette smoking to attachment level profiles. J Clin Periodontol 2001;28:283-95.
39. Engebretson SP, Grbic JT, Singer R, Lamster IB. GCF IL-1beta profiles in periodontal disease. J Clin Periodontol 2002;29:48-53.