

THE EFFECT OF SMOKING ON GINGIVAL CREVICULAR FLUID VOLUME DURING THE TREATMENT OF GINGIVITIS

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ABSTRACT

Smoking is detrimental to periodontal tissues, and periodontal destruction is greater among smokers. Paradoxically, smokers seem to have less gingival bleeding than never-smokers with comparable supragingival plaque. There is scarce information about the impact of smoking on gingival crevicular fluid (GCF) volume. This single-arm study clinical trial assessed the effect of smoking on GCF volume during the treatment of gingivitis. The sample included 24 never-smokers (47.3 ± 6.7 years old, 41.7% males) and 21 smokers (45.8 ± 5.1 years old; 55% males; 19.6 ± 11.8 cigarettes/day; 24.1 ± 8.7 years of smoking) with gingivitis and chronic periodontitis. After baseline supragingival scaling, patients received oral hygiene instructions weekly for 180 days. Participants were examined at baseline, 30, 90 and 180 days, and gingival bleeding index (GBI), bleeding on prob-

ing (BOP), periodontal probing depth (PPD) and GCF volume were recorded. Statistical analysis was performed using linear models (Wald test, $p < 0.05\%$). Smokers had significantly smaller GCF volumes than never-smokers. This finding was not attributed to GBI, BOP or PPD. Higher volumes of GCF were significantly associated with deeper pockets. GCF was significantly reduced throughout the study for both smokers and never-smokers, and the largest reductions were seen at 30 days. Smoking affected the GCF crevicular fluid volume independently of the presence of gingival bleeding, BOP and PPD. Smoking status and PPD should be taken into account when GCF volume and components are under investigation.

Key words: smoking, oral hygiene, gingivitis, periodontitis, gingival crevicular fluid.

EFEITO DO TABAGISMO SOBRE O VOLUME DE FLUIDO CREVICULAR GINGIVAL DURANTE O TRATAMENTO DA GENGIVITE

RESUMO

O tabagismo é capaz de alterar a resposta periodontal determinando maior expressão de destruição periodontal em pacientes fumantes. Paradoxalmente, estes pacientes apresentam menos sangramento gengival frente a uma quantidade semelhante de biofilme dental, quando comparados a pacientes que nunca fumaram. Por outro lado, existe pouca informação sobre o impacto do tabagismo sobre o volume de fluido crevicular gengival (FCG). O presente ensaio clínico de braço único teve como objetivo avaliar o efeito do tabagismo sobre o volume de FCG durante o tratamento da gengivite. A amostra foi composta por 24 pacientes que nunca fumaram (47.3 ± 6.7 anos, 41.7% homens) e 21 fumantes (45.8 ± 5.1 anos; 55% homens; 19.6 ± 11.8 cigarros por dia; 24.1 ± 8.7 anos de exposição ao tabaco), com diagnóstico de gengivite e periodontite crônica. Os exames periodontais: Índice de Placa Visível (IPV), Índice de Sangramento Gengival (ISG), Sangramento à Sondagem (SS) e Profundidade

de Sondagem (PS) e a coleta de FCG foram realizados nos dias 0, 30, 90 e 180. Após a raspagem supragengival realizada no dia zero (dia 0), os pacientes receberam instrução de higiene bucal semanalmente, até o dia 180. A análise estatística utilizou modelos lineares (Teste de Wald, $p < 0.05\%$). Os fumantes apresentaram um volume significativamente menor de FCG. Este resultado não esteve associado ao ISG, SS ou PS. Sítios com maiores valores de PS apresentaram maior volume de FCG. Durante o tratamento, uma redução significativa do volume de FCG foi observada em fumantes e pacientes que nunca fumaram. Conclui-se que o tabagismo influenciou o volume de FCG independente da presença de sinais inflamatórios clínicos e que o hábito de tabagismo e a PS devem ser observados quando o volume de FCG e seus componentes estiverem sob investigação.

Palavras-chave: tabaco, higiene bucal, gengivite, periodontite, fluido do sulco gengival.

INTRODUCTION

Smoking is a well-established risk factor for destructive periodontal disease. Epidemiological and clinical studies showed that smokers have deeper periodontal probing depth (PPD) and greater clinical attachment

loss (CAL) and gingival recession than never- or former-smokers¹⁻⁵. Paradoxically, several studies demonstrated that current-smokers have less gingival bleeding than never- and former-smokers⁶, but there were no differences in bleeding on probing (BOP)⁷.

Gingival crevicular fluid (GCF) is an inflammatory exudate that results from the interaction between the host and dental biofilms⁸. GCF volume, the net result of gingival and periodontal inflammatory exudates, has been used to evaluate the inflammatory status of the gingival and periodontal tissues in health and disease⁹⁻¹². Recent studies demonstrated that GCF volume increases with dental plaque accumulation^{12,13} and decreases after microbial challenge is reduced or removed¹⁴.

The effect of smoking on GCF volume has not been consistently studied in the periodontal literature. Some studies raised questions about whether differences observed in GCF between smokers and never-smokers may be attributed to smoking or to confounding factors, such as gingival bleeding and microbiota¹⁵⁻²². This study assessed the effect of smoking on GCF volume in patients undergoing treatment for gingivitis.

MATERIALS AND METHODS

Study design and sample

The present study was a single-arm, 6-month follow-up clinical trial; supragingival plaque control was the intervention, and smoking, the effect modifier²¹. A consecutive sample of 45 individuals was selected according to smoking status from the pool of patients of the Department of Periodontology, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil. Twenty-four never-smokers (47.3 ± 6.7 years old, 41.7% males) and 21 smokers (45.8 ± 5.1 years old; 55% males; 19.6 ± 11.8 cigarettes/day; 24.1 ± 8.7 years of smoking) fulfilled the following selection criteria and were included in the study:

1. No known systemic disease or previous periodontal treatment;
2. No anti-inflammatory or antibiotic therapy in the previous 3 and 6 months. No pregnancy, hormone therapy or chemical plaque control;
3. At least 12 or more teeth, excluding third molars;
4. At least 4 teeth with one site with periodontal pocket depth (PPD) of 3 to 5 mm and 4 additional teeth with one site with PPD 6 to 10 mm;
5. Availability to return weekly and to be enrolled in a plaque control program.

The study was approved by the Committee on Ethical Affairs of the Universidade Estadual de Sao Paulo, Araraquara, Brazil.

Experimental procedures

All participants underwent strict supragingival plaque control for 6 months. Initially, full mouth supragingival debridement, extraction of hopeless teeth, endodontic treatment, and placement of temporary fillings and crowns were performed. Participants received oral hygiene instructions according to their needs, and supragingival plaque control was evaluated weekly throughout the study. All procedures were performed by three periodontists.

Clinical examination

A full-mouth, excluding third molars, periodontal examination was conducted at baseline, 30, 90 and 180 days. Gingival bleeding index (GBI), bleeding on probing (BOP) and PPD were recorded. The nearest upper millimeter was used to record PPD. Six sites in each tooth were examined: mesiobuccal, midbuccal, distobuccal, mesiolingual, midlingual, and distolingual. All clinical examinations were performed by one calibrated and blinded periodontist (FP). The unweighted and weighted kappa (±1 mm) values for each site PPD were 0.65 and 0.95.

Gingival Crevicular Fluid sampling

Gingival crevicular fluid (GCF) was randomly collected from 8 sites (1 site per tooth) with visible plaque, gingivitis and chronic periodontitis. Sites were selected according to initial PPD: 4 sites with PPD <6 mm and 4 with PPD >6 mm. 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 Strips, OraFlow, PlainView, New York, USA) were gently inserted until slight resistance was met, and the strip was kept in the selected site for 30 seconds. GCF volume was measured using a calibrated Periotrom 8000 (OraFlow, PlainView, New York, USA) according to manufacturer's instructions. Paper strips with traces of blood were discarded. One examiner (SCG) performed all GCF sampling.

Statistical analysis

Raw Periotrom scores were converted to volume of GCF in microliters (µl). Data were normalized using logarithmic transformation. Results were reported as back-transformed values. Linear models were used to estimate GCF volume according to smoking status and study time point. Measurements

for each tooth were used, and estimates were adjusted for the clustering of teeth for participants using a robust variance estimator. Wald tests were used for comparisons, and the p-value was adjusted for multiple comparisons (STATA software). The level of significance was set at 5%, and 95% confidence intervals (CI) were calculated. Additionally, generalizing estimating equations were used to model the association between GCF and various factors. An exchangeable correlation structure was used to model correlations within participants.

Results

Never-smokers had significantly greater GCF volumes than smokers, and this difference was constant over 6 months (Table 1). The volume of GCF significantly decreased in both groups along the study. The greatest decrease was found at 30 days. Additional significant, but not substantial, decrease in GCF was observed between 30 and 180 days. The amount of GCF was associated with PPD, and deep pockets had significantly greater amounts of

GCF than shallow pockets. After the study time, no differences in mean GCF volume were observed in shallow and moderate pockets (PPD <6 mm) according to smoking status, but never-smokers had a significantly greater GCF volume than smokers in sites with PPD >6 mm (Fig. 1).

At 30 days, reductions in the GCF volume were associated with a decrease in gingival bleeding and BOP (Fig. 2). Further decrease in GCF was mostly associated with a reduction in BOP only, because gingival bleeding was practically non-existent. Never-smokers had greater GCF volumes than smokers, regardless of presence of gingival bleeding or BOP (Table 2).

The multivariable analysis showed that smoking, gingival bleeding, BOP and PPD were significant independent factors of GCF volume (Table 3). After adjusting for other factors, PPD had the greatest effect on GCF volume, followed by smoking. The magnitude of the effect of gingival bleeding and BOP on GCF volume was similar after adjusting for cofactors.

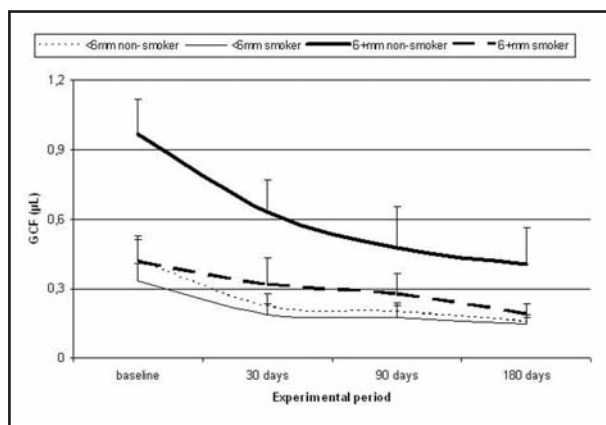


Fig. 1: Mean gingival crevicular fluid volume in never-smokers (non-smoker) and smokers according to periodontal probing depth at all study time points (baseline, 30, 90 and 180 days). Bars represent 95% confidence interval.

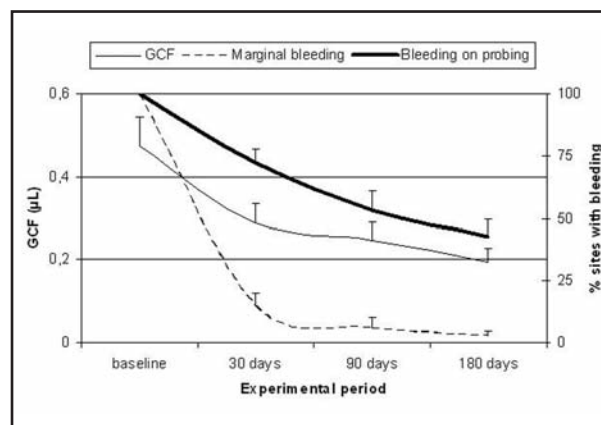


Fig. 2: Mean gingival crevicular fluid volume and percentage of sites with gingival bleeding and bleeding on probing at all study time points (baseline, 30, 90 and 180 days). Bars represent 95% confidence interval.

Table 1: Mean (CI) gingival crevicular fluid volume in microliters according to study time points and smoking habits.

Time point	Never-smokers	Smokers	Mean (95% CI)
Baseline	0.59 (0.52; 0.69)Aa	0.36 (0.29; 0.45)Ba	0.47 (0.41; 0.55)a
30 days	0.33 (0.28; 0.41)Ab	0.23 (0.19; 0.29)Bb	0.29 (0.25; 0.34)b
90 days	0.28 (0.23; 0.34)Ac	0.21 (0.17; 0.27)Ac	0.25 (0.21; 0.29)c
180 days	0.23 (0.18; 0.29)Ad	0.16 (0.14; 0.19)Bd	0.20 (0.17; 0.23)d

Uppercase letters indicate comparisons between never-smokers and smokers: means followed by the same uppercase letters are not statistically different ($p < 0.05$). Lowercase letters indicate comparisons between different time points: means followed by the same lowercase letters are not statistically different ($p < 0.05$).

Table 2: Mean values of gingival crevicular fluid volume according to gingival bleeding index, bleeding on probing (BOP) and periodontal probing depth (PPD) for never-smokers and smokers.

Gingival Bleeding	BOP	Never-smokers		Smokers		p
		Mean	95%CI	Mean	95%CI	
PPD <6 mm						
Negative	Negative	0.36	0.22; 0.58	0.17	0.13; 0.22	0.008
Negative	Positive	0.49	0.38; 0.62	0.26	0.21; 0.33	0.0005
Positive	Positive	0.53	0.46; 0.63	0.40	0.32; 0.50	0.04
PPD >6 mm						
Negative*	Negative*	-	-	-	-	-
Negative	Positive	0.74	0.62; 0.89	0.30	0.23; 0.40	<0.0001
Positive	Positive	0.96	0.82; 1.09	0.42	0.33; 0.53	<0.0001

* Statistical analysis was not performed due to small number of observations

Table 3: Multivariable longitudinal analysis of the effect of smoking, gingival bleeding, bleeding on probing (BOP) and periodontal probing depth (PPD) on the volume of gingival crevicular fluid volume.

Variable	Categories	Coefficient*	95% CI	p
Smokers	Never-smokers	Reference		
	Smokers	-0.45	-0.63; -0.26	<0.001
Gingival bleeding	Absent	Reference		
	Present	0.24	0.11; 0.39	<0.001
BOP	Absent	Reference		
	Present	0.27	0.15; 0.39	<0.001
PPD	Continuous (mm)	0.24	0.19; 0.28	<0.001

*estimates adjusted for clustering of observation within participants and study time points.

Discussion

This study showed that smoking affected the volume of GCF negatively regardless of the presence gingival bleeding or subgingival bleeding (BOP). Smokers and never-smokers had significant reductions in GCF volume during the treatment of gingivitis although smokers had lower GCF values before and at the end of the intervention. The smaller GCF volumes observed among smokers could not be attributed to other factors, such as gingival bleeding, BOP and PPD.

To our knowledge this is the first study to show that smoking affects the volume of GCF regardless of the inflammatory conditions associated with gingival and subgingival bleeding. Other studies showed that smokers had less gingival bleeding than never- or former-smokers²³. However, no significant differences have been reported for BOP according to smoking⁷. These findings may suggest that smoking has a major effect on gingival tissues but not on periodontal

inflammation. According to this study, smoking has a detrimental effect on both gingival and periodontal inflammatory parameters in terms of GCF volume. Most of the reduction in GCF volume was found after one month of supragingival plaque control, which may be associated with the decrease in the percentage of sites with gingival bleeding. As gingival bleeding was virtually absent for the remaining time of the study, additional reductions were probably associated with the decrease in BOP and PPD.

Our results showed that the presence of gingival inflammation is associated with increased GCF volume. Consequently, supragingival biofilm control may help to differentiate between GCF volumes found in gingivitis and those found in periodontitis. Gingivitis and periodontitis are currently classified as two different diseases²⁴. The fact that fluid volumes are different in these diseases may be relevant for the analysis of immune-inflammatory markers and indicators. These observations suggest that the rec-

ommendation to clearly distinguish between former- and never-smokers when studying the relationship between periodontal diseases and systemic health²⁵ should be expanded to the study of GCF formation and composition. In this regard, GCF volume should be accounted for when investigating the composition of fluid and its impact on etiology, diagnosis and prognosis of periodontal diseases, as suggested by Giannopoulou et al., 2003²⁶. The effect of smoking can be better appreciated by the observation that the initial GCF volume at PPD>6 mm sites in smokers was similar to the volume in PPD<6 mm sites in never-smokers. Throughout the study, never-smokers showed a faster volume reduction, although no differences were found at 180 days. The reasons for these findings are unclear, but may be associated with the fact that smokers respond more slowly to periodontal therapy than never- or former-smokers^{26,27}.

ACKNOWLEDGMENTS

The authors acknowledge Élcia Silveira and Graziela Benemann for their clinical support and CNPq (National Research Council, Brasília, DF, Brazil), CAPES (National Coordination of Post-Graduate Education, Brasília, DF, Brazil) and FAPESP (São Paulo State Research Foundation, São Paulo, SP, Brazil) for financial support.

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GCF volume decreases after subgingival scaling and root planing^{28,29}. Our results showed that the treatment of gingivitis, based on mechanical supragingival plaque control, yielded significant reductions in GCF volume. This is in accordance with evidence that supragingival control may affect the subgingival environment and lead to a decrease in inflammation and subgingival microbiota²¹. Smoking affected gingival and periodontal clinical parameters of inflammation negatively. Smokers had significantly lower GCF values than never-smokers at baseline and throughout the study, regardless of gingival bleeding, BOP or PPD. Future studies should take in consideration that GCF volume is associated with smoking status, gingival bleeding, BOP and PPD, and that these variables should be taken into account when GCF volume and components are under investigation.

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