ACTA FAC MED NAISS

UDC 616.311.2:613.84

Original article

ACTA FAC MED NAISS 2006; 23 (3): 151-154

Vukadin Bajagic[1], Ana Pejcic[1],
Vesna Zivkovic[2], Aleksandar Petrovic[3]

[1] Department of Periodontology and Oral Medicine, Clinic od Dentistry Nis
[2] Faculty of Medicine, Institute of Pathology
[3] Faculty of Medicine, Institute of Histology

HISTOCHEMICAL STUDY OF GINGIVAL EPITHELIUM IN SMOKERS AND NON-SMOKERS

SUMMARY

Smoking patients show reduction in inflammatory clinical signs that might be associated with local vasoconstriction and increased gingival epithelial thickness. The aim of the paper was to evaluate the thickness of the marginal gingival oral epithelium in smokers and non-smokers. Twelve biopsies were obtained from three different groups. Group I: non-smokers with gingivitis, group II: smokers and group III - healthy persons without any periodontal disease. The biopsies were histologically processed, serially sectioned at 5 µm, followed by the evaluation of the major epithelial thickness, the epithelial base thickness and the external and internal epithelial parameters. The difference between the groups was analyzed using ANOVA test. The criteria for statistical significance were at the probablity level p< 0.05. A greater epithelial thickness was observed in smokers.

Key words: **gingiva, epithelium, tobacco**

INTRODUCTION

Tobacco use is directly associated with periodontal disease. A greater number of diseased sites can be registered in smokers as well as a greater loss of alveolar bone and increased tooth loss. The severity of the disease increases with both the extent and duration of the smoking exposure. Former smokers are at lower risk compared to current smokers (1-3). The association between tobacco smoking and periodontal health has been studied in several clinical and epidemiological investigations (4). Same early studies have indicated that smokers show more intense inflammatory gingival signs than non-smokers. Conversely, high tobacco consumption seemed to reduce gingival bleeding. The gingivitis experimental model in smoking and non-smoking patients showed that the plaque formation rate was similar in both groups (5).

Those studies suggest that by-products originating from tobacco oxidation modify the clinical characteristics and progression of periodontal diseases and described smoking habit as a risk faktor for periodontal desease (6). Smokers displayed less marked gingival inflammatory reaction when compared to non-smokers. The reduction of clinical inflammatory sings is confirmed by decrease in gingival bleeding and suppuration on probing, tissue redness, oedema and the amount of blood vessels in the marginal gingival tissue. The reduction of clinical inflammatory sings in smokers can be attributed to the cotinine, a nicotine metabolic by-product which has a peripheral constrictive action on gingival vessels (7). Although the literature indicates an increase in oral mucosa epithelium thickness in smokers, there has not been any morphometric study assessing the oral gingival epithelial thickness in those patients.

The aim of the paper was to investigate the relation between the thickness of marginal gingival oral epithelium in smokers and non –smokers with clinically healthy gingivae or with gingivitis and to

Corresponding author. Tel. 069/ 662-218

better understand the role of smoking in the relationship with periodontal disease.

MATERIAL AND METHODS

Study population – Twelve patients (27 to 55 years old) were selected with clinical signs of gingival health (n–2) or gingivitis/parodontopathia (n-10) with clinical indication for periodontal surgery at one intraoral site per patient. The periodontal surgeries were carried out at the Department of Periodontology and Oral Medicine, Dentistry Clinic in Nis. Of twelve patients, there were 10 patients who smoked on average 15 or more cigarettes per day for at least 10 years and they were considered smokers. Pregnant women, former smokers, individuals with systemic and immunologic abnormalitiers or those who had used any drug 4 weeks prior to the experiment were excluded from the investigation.

Tissue preparation – All gingival biopsies (0.4 cm to 0.2 cm) from different parts of the oral gingival tissue were obtained during periodontal surgery as a part of a routine periodontal treatment independent of this study. Gingival biopsies were divided into three groups, according to the donor`s gingival health and smoking habit. Group I (n-2): patients with clinically healthy gingivae represented the control group; group II (n-5): non –smokers with gingivitis; group III (n-5): smokers with gingivitis. According to the rule of The Ethic Committee, all patients gave a written informed consent for all phases of the research.

The samples were immediately fixed in 10% phosphate-buffered formalin, pH-7.4, and later embedded in paraffin and serially sectioned at 5µm. The samples were cut at right angles to the oral vestibular epithelium, resulting in a section exhibiting both sulcular and oral epithelium. The slides were stained with hematoxylin and eosin (HE) and were observed under a light microscope at magnification10 X. The histologic assessment was carried out at the Institute of Pathology of the Faculty of Medicine in Nis.

The external (EE), internal (IE) epithelial perimeters, the major epithelial thickness (MET–distance between the external epithelial surface and the epithelial crista tip) and the epithelial base thickness (EBT–distance between the external epithelial surface and basal membrane located between the two cristae) were evaluated.

Statistical analysis – The data showed homogeneity and the differences between these three groups were analyzed using the ANOVA test. The difference between groups II and III was analyzed using the Student`s t-test. The criterion for statistical significance was accepted at the probability level $p < 0,005$.

RESULTS

Smokers with gingivitis (Group III) had consumed approximately 15 or more cigarettes per day for approximately 10 years. Their smoking habits' characteristics are presented in *Table 1*.

Table 1. Smoking habits' characteristics

	Smokers	Non-smokers	Healthy
Number of patients	5	5	2
Daily consumption	15 ± 1.1	-	-
Habit duration -years	10 ± 2.1	-	-

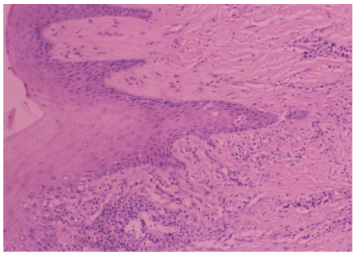
Of the 12 sectiones evaluated, 5 sections were from the current smokers with gingivitis, 5 section were from non-smokers with gingivitis and 2 from healthy persons. There was no statistically significant difference in MET between smokers and non-smokers, regardless of the clinical gingival condition. However, EBT was larger in smoking patients ($p< 0.05$), as the *Table 2* presents.

Table 2. Major epithelial thickness and epithelial base thickness expressed in µm

Clinical condition	Smokers	Non-smokers	Healthy
MET	422.1 ± 66.1	418.5 ± 33.1	430.5 ± 12 (p<0,05)
EBT	260.1 ± 19.2	15.87 ± 16	216.9.01±11 (p<0,05)

The marginal gingival epithelium was classified as keratinized, stratified, squamous epithelium with small intercellular spaces. The spinous stratum occupied about 50% of the total epithelial thickness.The stratum corneum was more exuberant in smoking patients' samples. Chronic inflammatory infiltrate in smokers' gingivae is presented in *Figure 1*.

Figure 1. Spinous stratum of gingival epithelium shows acanthotic change (hematoxylin-eosin, overall magnification x 200)



Normal gingival epithelium is presented in *Figure 2*.

Figure 2. Normal gingival epithelium with the presence of stratum corneum (hematoxylin – eosin, overall magnification x 400)

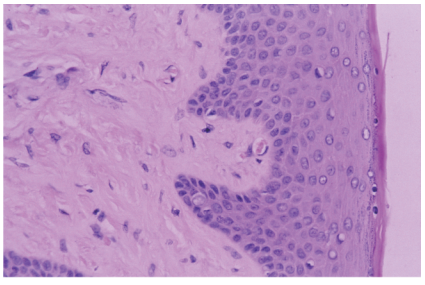
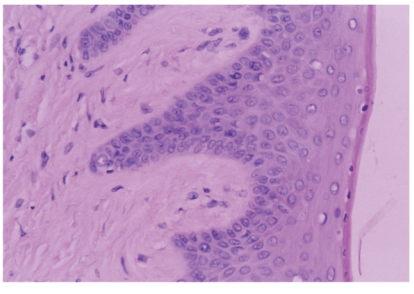


Figure 3 presents the epithelial thickness of the healthy gingiva.

Figure 3. Normal gingival epithelium (hematoxylin-eosin, overall magnification x 400)



There was no statistically significant difference in both EE and IE between smokers and non-smokers, regardless of the clinical condition, which is presented in *Table 3*.

Table 3. External and internal epithelial parameter in smokers and non-smokers (μm)

Clinical condition	Smokers	Non-smokers	Healthy
EE	618.2 ± 3.1	601.5 ± 2.7	611.4 ± 2.1 p <0,05
IE	2442.3	2290.6	2372.7 p<0,05

DISCUSSION

Tobacco use has been directly associated with a variety of medical conditions, including various types of cancer, pulmonary and cardiovascular diseases, and low birth weight (8,9). Although gingivitis and periodontitis are elicited by bacteria, cigarette smoking has been strongly implicated as a risk factor for the initiation and progression of periodontal disease(10). Smoking has been associated with increased calculus deposition, deeper pockets and greater attachment loss, more marked radiographic evidence of furcation involvement and increased alveolar bone loss. Variable levels of plaque and inflammation with evidence of decreased signs of clinical inflammation were also noted in smokers. It is possible that the reduced intensity of the gingival response is due to the vascular changes and the thicknes of marginal gingival epithelium damaged by smoking (11).

The inflammatory response induced by dental plaque accumulation can be modified by tobacco by-products, such as cotinine, a by-product of nicotine that has a peripheral vasoconstriction action that reduces gingival clinical signs of bleeding, redness and oedema (12). In the samples evaluated throughout this study, the spinous stratum occupied about 50% of the total epithelium thickness and the keratinocytes were set apart by minor intercellular spaces. In the samples of smokers, the stratum corneum was more marked. These evants were similar to the ones already described in literature where the increase in local temperatures and by-product from tobacco oxidation induce an increase in oral mucosa and in the oral gingival epithelium thickness (13).

Analysis pointed to an increase in the MET in clinically healthy gingival samples when compared to inflamed samples in both smoking and non-smoking patients. However, this difference did not amount to statistical significance ($p<0.05$). Gingival inflammmation reduces the epithelial thickness and can potentially cause clinical ulceration (14).

In our samples, we also found an increase in the gingival EBT in smokers, with the spinous stratum which occupied about 50% of total epithelium thickness and stratum corneum which was more marked. Our results were similar to the results of some other studies (15). *Table 3*. shows that IE was larger in gingivitis present in smokers, but there was no statistically significant difference. The epithelium is a non-vascular tissue that depends on the subjacent connective tissue. The inflammation causes connective disorganization, modifying the blood availability and impending the elimination of metabolites from the epithelium. Epithelium projection are more frequent and protuberant during gingival inflammation (16).

Our resuts suggest that among all the negative consequences of tobacco on the periodontium, tobacco influences epithilium thickness, influencing thus the signs and symptoms of gingival inflammation induced by plaque accumulation. Although the exact mechanism of its influence is still unclear, smoking must be considered as a high risk factor of chronic periodontal disease.

CONCLUSION

The smoking patients showed increased epithelial base and stratum corneum thickness. The increased epithelium thickness can contribute to the reduction of inflammatory clinical signs in the gingival tissue.

REFERENCES

1. Axelsson P, Paulander J, Lindhe J. Relationships between smoking and dental status in 35-,50-,65- and 75- year old individuals. J Clin Periodontol 1998;25:297-305.

2. Bergstrom J, Eliasson S, Dock J. Exposure to tobacco smoking and periodontal health. J Clin Periodontol 2000;27:61-68.

3. Haber RL, Walttles J, CroweyM, MandellR, Joshipura K, Kent RL. Evidence for cigarette smoking as a major risk factor for periodontitis. J Periodontol 1993;64:16-23.

4. Bergstrom J. Oral hygiene complance and gingivitis expression in cigarette smokers. Scand J Dent Res 1990;98:497-503.

5. Bergstrom J, Preber H. The influence of cigarette smoking on the development of experimental gingivitis. J Periodontal Res 1986;21:668-676.

6. Bergstrom J, Preber H. Tobacco use as a risk factor. J Periodontal 1994;65:545-550.

7. Danielsen B, Manji F, Nageilkerke N, Fejerskov O, Baelum V.Effect of cigarette smoking on the transition dynamics in experimental gingivitis. J Clin Periodontol 1990;17:159-164.

8. Palmer RM, Wilson RF, Hasan AS, Scott DA. Mechanisms of action of environmental factors-tobacco smoking. J Clin Periodontal 2005;32:180-195.

9. Dietrich T, Bernimoulin JP, Glynn R. The effect of cigarette smoking on gingival bleeding. J Periodontology 2004;75:16-22.

10. Salvi GE, Ramseier CA, Kandylaki M, Sigrist L, Awedowa E, Lang NP. Experimental gingivitis in cigarette smokers.A clinical and microbiological stydy. J Clin Periodontol 2005;32:441-447.

11. Biddle AJ, Palmer RM, Wilson RF, Watts TL. Comparison of the validity of periodontal probing measurements in smokers and non-smokers. J Clin Perioidontol 2001;28:806-812.

12. Giannopoulou C, Roehrich N, Mombelli A. Effect of nicotine-treated epithelial cells on the proliferation and collagen production of gingival fibroblasts. J Clin Periodontol 2001;28:769-775.

13. Alonge OH, Ashrafi SH, Colvard MD. Mitochondrial volumen densities in the smokless tobacco-treated hamster cheek pouch epithelium. Oral Diseases 2003;9:128-143.

14. Polson Am, Greenstein G, Caton J. Relationshion between epithelium and connective tissue in inflamed gingiva J Periodontol1981;52:743-774.

15. Villar CC, Lima AF. Smoking influences on the thickness of marginal gingival epithelium. Pesqui Odontol Bras 2003 ;17:41-45.

16. Mirbod MS, Ahing IS, Pruthi KV. Immunohistochemical study of vestibular gingival blood vessel density and internal circumference in smokers and non-smokers. J Periodontol 2001;72:1318-1323.

HISTOHEMIJSKO ISPITIVANJE GINGIVALNOG EPITELA KOD PUŠAČA I NEPUŠAČA

Vukadin Bajagić[1], Ana Pejčić[1], Vesna Živković[2], Aleksandar Petrović[3]

[1]Odeljenje za parodontologiju i oralnu medicinu, Klinika za stomatologiju, Niš
[2]Medicinski fakultet, Institut za patologiju
[3]Medicinski fakultet, Institut za histologiju

SAŽETAK

Kod pušača je prisutno smanjenje kliničkih znakova inflamacije koji mogu biti udruženi sa lokalnom vazokonstrikcijom i povećanjem debljine gingivalnog epitela. Cilj ovog rada bio je ispitivanje debljine marginalnog oralnog gingivalnog epitela kod pušača i nepušača. Uzeto je 12 biopsija iz tri različite grupe. Grupa I - nepušači sa gingivitisom; grupa II - pušači i grupa III - zdrave osobe bez parodontopatije. Biopsije su histološki obrađene, serijski sečene na 5 μm, ispitivana je najveća debljina epitela, debljina baze epitela i spoljašnji i unutrašnji epitelijalni parametri. Razlika između grupa je analizirana pomoću ANOVA testa. Kriterijumi za statističku signifikantnost su na nivou verovatnoće od $p<0.05$. Velika debljina epitela bila je kod pušača.

Ključne reči: **gingiva, epitel, duvan**