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Histological changes of gingival epithelium in smokers and non-smokers

Research Article

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Abstract: Background: Smoking patients show a reduction of inflammatory clinical signs that might be associated with local vasoconstriction and an increased gingival epithelial thickness. The purpose of this work was to evaluate the S-thickness of the marginal gingival oral epithelium in smokers and non-smokers. Methods: Twelve biopsies were obtained from three different groups. Group I: non-smokers with gingivitis, group II smokers, and group III health persons without any periodontal disease. These biopsies were histologically processed, serially sectioned at 5 μm, and underwent evaluation of the major epithelial thickness, the epithelial base thickness, and the external and internal epithelial perimeters. Differences between the groups were analyzed using ANOVA test. The criteria for statistical significance were at the probablity level $p < 0.05$. Results: A greater epithelial thickness was observed in smokers. Conclusion: The increased epithelium thickness can contribute to the reduction of inflammatory clinical signs in the gingival tissue.

Keywords: *Gingiva* • *Epithelium* • *Tobacco*

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1. Introduction

Tobacco use has been directly associated with periodontal disease. Smokers have a higher number of diseased sites, 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 than current smokers [1-3]. The association between tobacco smoking and periodontal health has been studied in several clinical and epidemiological investigation [4]. Same early studies indicated that smoking patients showed more intense inflammatory gingival sings than non-smoking ones. 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].

There is an established biologic rationale for the negative effect of cigarette smoking on periodontal tissues. First and foremost, smoking has an immunosuppresive effect on the host, adversely affecting host-parasite interactions. Peripheral blood polymorphonuclear leukocyte motility, chemotaxis and phagocytosis are significantly impaired [6] thus, compromising this very important first line of defense against subgingival bacteria.

The net result is that periodontal organisms in current cigarette smokers escape specific and nonspecific

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immune clearance mechanisms allowing them to establish as subgingival inhabitants. Alteration in the physical subgingival environment, such as decreased oxygen tension, would allow the overgrowth of anaerobic flora [7] Cigarette smoking appears to trigger an anaerobic subgingival infection, leading to greater serverity of periodontal disease and impaired wound healing. Those studies suggest that by-products, originated from tobacco oxidation, modify the clinical characteristics and the progression of periodontal diseases and described smoking habit as a risk factor for periodontal desease [8]. Smokers displayed a less pronounced gingival inflammatory reaction as compared with non-smokers. The reduction of clinical inflammatory signs is confirmed by the decrease in gingival bleeding and suppuration on probing, tissue redness, edema and the amount of blood vessels in the marginal gingival tissue. The reduction of clinical inflammatory signs in smokers can be attributed to the cotinine, a nicotine metabolic by-product, which has a peripheral constrictive action on gingival vessels [9]. Although the literature indicates an increase in oral mucosa epithelium thickness in smokers, there is no morphometric study assessing the oral gingival epithelial thickness in those patients.

The objective of this work was to investigate the relation between the thickness of the marginal gingival oral epithelium in smokers and non–smokers, with clinically healthy gingivae or with gingivitis and to better understand the role of smoking in the relationship with periodontal disease.

2. Materials and methods

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

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 part of a routine periodontal treatment independent of this study. The 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 as control; group II (n-5): non–smokers with gingivitis; group III (n-5): smokers with gingivitis. The study protocol was approved by the Ethical committee of Medical Faculty in Nis, Serbia (No:01-2800-5). According to rule The Ethices committee in research, all patients gave a informed consent for all phases of the research.

The samplies 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) within 4 minutes and were observed in a light microscope at 10 X magnification. Hematoxylin stains are commonly employed for histological studies, often employed to color the nuclei of cells (and a few other objects, such as keratohyalin granules) blue. Eosin is a fluorescent red dye resulting from the action of bromine on fluorescein. It can be used to stain cytoplasm, collagen and muscle fibers for examination under the microscope [10].

Histologic assessment was carried at Institute for Pathology Medical Faculty in Nis.

The external epithelial (EE), internal epithelial (IE) 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 two cristae) were evaluated.

3. Statistical methods

The data showed homogeneity and the differences between the three groups were analyzed using the ANOVA test. The difference between groups II and III was analyzed using the Student`s t-test. The criteria for statistical significance was accepted at the probability level $p < 0.005$.

4. Results

Smokers with gingivitis (Group III) had consumed approximately 15 or more cigarettes per day during about 10 years (Table 1).

Of the 12 sections evaluated, 5 sections were from current smokers with gingivitis, 5 sections were from non-smokers with gingivitis and 2 from healthy persons. There was no statistical significant difference in MET between smokers and non-smokers, regardles of the

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	-	-

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

Clinical condition	Smokers	Non-smokers	Healthy	p value
MET* (μm)	422.1 ± 66.1	418.5 ± 33.1	430.5 ± 12	(p<0.05)
EBT** (μm)	260.1 ± 19.2	15.87 ± 16	216.9 ± 11	(p<0.05)

Footnotes: MET–distance between the external epithelial surface and the epithelial crista tip*
*EBT**–distance between the external epithelial surface and basal membrane located between two cristae*

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

Clinical condition	Smokers	Non-smokers	Healthy	p value
EE* (μm)	618.2 ± 3.1	601.5 ± 2.7	611.4 ± 2.1	(p<0.05)
IE** (μm)	2442.3	2290.6	2372.7	(p<0.05)

Footnotes: EE–external epithelium*
*IE**–internal epithelium*

Figure 1. Spinous stratum of gingival epithelium

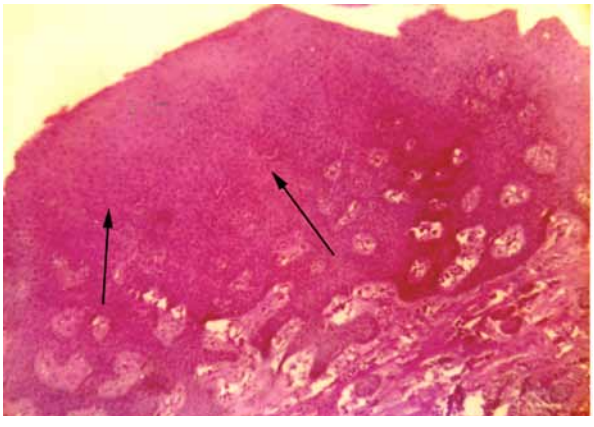


Figure 2. Stratum corneum of gingival epithelium

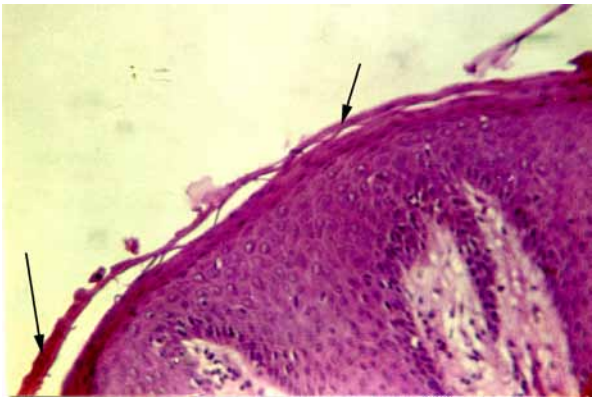
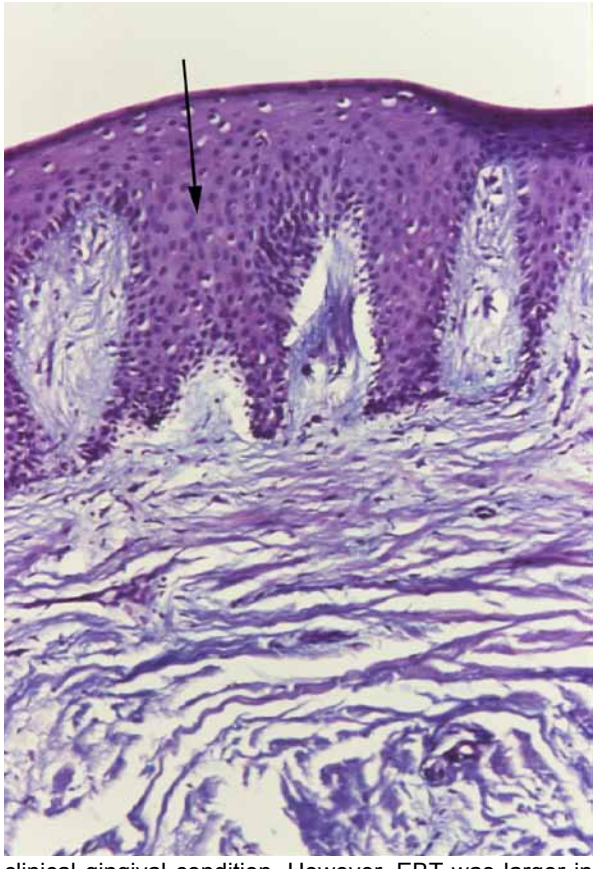


Figure 3. Normal gingival epithelium



clinical gingival condition. However, EBT was larger in smoking patients ($p < 0.05$) (Table 2).

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 (Figure 1). The stratum corneum was more exuberant in smoking patient samples (Figure 2). On the figure 3 is the epithelial thickness at health (Figure 3).

There was no significant statistical difference in both EE and IE between smoker and non-smokers, regardless of the clinical condition (Table 3)

5. 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 [11,12]. 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 [13]. Smoking has been associated with increased calculus deposition, deeper pockets and greater attachment loss, more

pronounced radiographic evidence of furcation involvement and increased alveolar bone loss. Variable levels of plaque and inflammation with evidence of decreased signs of clinical inflammation have also been 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 externed by smoking [14].

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 edema [15]. In the samples evaluated throughout this study, the spinous stratum occupied about 50% of total epithelium thickness and the keratinocytes were apart by small intercellular spaces. In the smokers samples, the stratum corneum was more pronounced. 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 [16].

Analysis showed an increase in the MET in clinically healthy gingival samples when compared to inflamed samples, in both smoking and non-smoking patients, but this difference did not achieve statistical significance ($p<0.05$). Gingival inflammation reduces the epithelial tickness and can potentially cause clinical ulceration [17].

In ours samples, we found, too, an increase in the gingival EBT in smoking with the spinous stratum wich occupied about 50% of total epithelium thickness and stratum corneum wich was more pronounced. Ours results were similar with the results the others studies [18]. Table 3. show that IE was larger in gingivitis cases smokers but there was no significant statistical difference. The epitheluim 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 [19]. The smoking patients showed increased epithelial base and stratum corneum thicknes. The increased epithelium S-thickness can contribute to the reduction of inflammatory clinical signs in the gingival tissue.

6. Conclusion

Our results suggest that among all the negative consequences of tobacco on the periodontium, the tobacco influence on epithilium S-tickness and so influence on 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 for chronic periodontal disease.

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