Original Article

The Impact of Smoking on Gingiva: a Histopathological Study

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ABSTRACT

Background and Objective: **Smoking can be associated with the decreasing gingival blood flow and epithelial changes. The aim of this study was to evaluate the histopathological changes of gingival epithelium and connective tissue in smokers.**

Methods: **The study was case-control. Sixty male patients (28 smokers and 32 nonsmokers) suffering chronic periodontitis were participated in the study. Periodontal parameters consisting the gingival (GI) and periodontal (PI) indexes were registered. Tissue samples were taken during flap surgery. The hemotoxylin and eosin stained slides were assessed for blood vessel density, inflammatory cells infiltration and epithelial changes. The histopathological findings were compared between smokers and nonsmokers. Data analyzed using t-test and chi-square tests.**

Results: **Smokers had lower GI (1.35 ± 0.48) and higher PI (2.87±0.68) than nonsmokers (2.72 ±0.31 and 1.87±0.25, respectively). The mean count of blood vessels with ≤ 0.5µ diameter was 18.78±10.06 and 5.90±2.93 in smokers, nonsmokers, respectively. The mean inflammatory cells infiltration in smokers, and nonsmokers were 0.89±1.03 and 70±0.46 that showed significant difference between two groups (P= 0.001, SD=1.21). The difference of epithelial hyperkeratosis, atrophy and acanthosis was not different in smokers and nonsmokers. Loss of normal epithelium pattern comprising of bulbous rete ridges, loss of polarity and increased parabasal cells was seen in 23(82.14%) of smokers and 2(6.25%) of nonsmokers.**

Conclusion: **Despite the normal appearance of gingiva in smokers, smoking increasing the epithelial changes resemble to early phases of dysplasia and decreasing inflammatory reaction.**

Key words: **Histopathological study, Nonsmoker, Smoker**

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Introduction

Smoking is a recognized risk factor for human health. It is related to many conditions such as respiratory problems, cardiovascular diseases and cancer (1).

Smoking can be associated with the incidence of gingivitis, periodontitis and epithelial malignancy in the oral cavity. Smoking increases the number and depth of periodontal pockets and attachment loss of periodontal ligaments. Loss of tissue strength which caused by harmful compounds in tobacco can increase gingival recession and changes in the oral mucosa (2). The prevalence of moderate and severe periodontitis is higher in smokers than nonsmokers is. Periodontitis are 2 to 20 times higher in smokers than nonsmokers (3, 4).

Smoking can effects on epithelial thickness (5). Moreover epithelial changes comprising basal layer hyperplasia and mild dysplasia has been demonstrated in pharynx, larynx and tongue mucosa of rats (6).

Generally, researches focused on the effect of smoking on treatment and outcome of destructive periodontal diseases and causal mechanism. In spit of the accepted fact about the effect of smoking on periodontal diseases, a histopathological study which assess both epithelial and connective tissue changes in smokers is not completed.

The aim of this study was to evaluate the histopathological changes of gingival epithelium and connective tissue in smokers.

Material and Methods

In this case-control study, sampling was based on target. Cases were collected from Periodontics Dept., Faculty of Dentistry, Shahed University, Tehran, Iran, from 2012-2013.

Sixty patients (28 smokers and 32 nonsmokers) 20 to 60 yr old were participated in the study. All patients had suffered from chronic periodontitis. Having at least 20 teeth, bleeding on probing, pocket depth of 5 mm and not receiving any periodontal treatment in the previous 6 months were inclusion criteria. Smoking in less than 3 years, having systemic diseases and taking medications were considered as exclusion criteria (7).

For eliminating the effect of hormonal changes on gingiva, all subjects were selected from male patients. All samples were obtained from patients who had need too periodontal surgery and did not impose any surgical treatment to them. An informed consent were taken of all participate subjects.

The gingival index (GI) and periodontal index (PI) were registered for all patients. For obtaining the GI, four gingival areas (facial, mesial, distal and lingual) were evaluated. The obtained GI was graded as follows:

0= normal, 1= mild inflammation (no bleeding on probing), 2= moderate inflammation (redness, edema, and bleeding on probing), 3= severe inflammation (edema, ulceration and tendency to spontaneous bleeding).

The total count of each tooth were summed and divided to four. A given number were assumed GI for each tooth. The teeth scored were added and divided in to the number of all teeth. The obtained score was GI. The PI was scored as follows: 0=negative, 1=mild gingivitis, inflammation in the free gingival 2 =gingivitis, Inflammation completely circumscribes the teeth, 3=gingivitis with pocket formation. The PI also calculated as GI for every tooth (2).

The quantum of smoking exposure was calculated by the number of packs × year (8).

Tissue samples were taken during flap surgery. Obtained samples were immediately fixed in 10% formalin and 3 µ sections of paraffinized blocked sections were prepared and stained by hemotoxylin and eosin .Stained slides were evaluated by optic microscope (ZEISS, Germany) at ×40 (objective) magnification for blood vessel density and inflammatory cells infiltration in 5 HPF.

The 5 fields with higher blood vessels density (hot

spots) were identified under 40 × magnifications (9). The mean number of blood vessels with ≤ 0.5µ diameter was determined.

Based on the obtained results from previous studies, the ≤ 0.5µ diameter was used as cut off point (7-10).

The inflammatory reaction were scored as follows: (11)

0 = no inflammatory cells (no reaction),

1 = less than 25 inflammatory cells (mild),

2 = 25 -125 inflammatory cells (moderate)

3= more than 125 inflammatory cells (severe)

The presence of epithelial changes comprising the keratinization, thickness (atrophy / acanthosis) and loss of normal pattern was recorded using optic microscope (ZEISS, Germany) at ×10 (objective) magnification.

The results were stated as mean±standard deviation for smokers and nonsmokers. Statistical analysis was performed using *t*-test and chi-square tests. Statistical Package for Social Sciences (SPSS) Version 20 (Chicago, IL, USA) was used. $P < 0.05$ was considered as significant.

Results

The mean age of smokers and nonsmokers were 43±8.06 and 39.28±9.86 years, respectively. The range of smoking duration was 3 to 20 years with the mean of 10.32 ±4.96 years. The minimum and maximum number of packs× years was 91 and 456, respectively. The mean of packs× years was 5.6±3.04.

In smokers and nonsmokers, the mean of GI was 1.35 ± 0.48 and 2.72 ±0.31, respectively. The GI of smokers was significantly lower than nonsmokers (P= 0.001, SD=0.39).

The mean of PI in smokers and nonsmokers was 2.87±0.68 and1.87±0.25, respectively. The PI of smokers was significantly higher than nonsmokers. (P= 0.001, SD=0.33)

Histopathological Findings

The mean count of blood vessels with ≤ 0.5µ diameter was 18.78±10.06 and 5.90±2.93 in smokers and nonsmokers, respectively. Although smokers had more blood vessels with≤ 0.5µ diameter than nonsmokers did, the difference was not significant (P =2.72, SD=2.93).

The mean inflammatory cells infiltration in smokers was 0.89±1.03. In nonsmokers, the mean of inflammatory cells infiltration was 2.70±0.46. The difference between smokers and nonsmokers was significant. (P = 0.001, SD=1.21). Table 1 shows the distribution of inflammatory reaction scores in smokers and nonsmokers.

Tissue necrosis was not present in any samples of smokers and nonsmokers cases.

The difference of epithelial hyperkeratosis, atrophy and acanthosis was not different in smokers and nonsmokers (P=0.85, SD=0.47/ P=0.2, SD=0.12 /P=0.84, SD=0.34).

Loss of normal epithelium pattern comprising of bulbous rete ridges, loss of polarity and increased parabasilar cells layer was seen in 23(82.14%) of smokers and 2(6.25%) of nonsmokers. The difference was significant (P = 0.001, SD= 0.5). Table2 shows the distribution of epithelial changes in smokers and nonsmokers.

Table 1: The distribution of inflammatory reaction scores in smokers and nonsmokers

Scores	0	1	2	3	Total
Smokers N (%)	13(46.5%)	8(28.6%)	4(14.2%)	3(10.7%)	28(100%)
Nonsmokers N(%)	0	0	9(28.2%)	23(71.8%)	32(100%)

Table 2: The distribution of epithelial changes in smokers and nonsmokers

Epithelial changes	Hyper keratosis	Atrophy	Acanthosis	* Loss of normal epithelium pattern
Smokers	10 (35.72%)	1(3.57%)	24(85.71%)	23(82.14%)
Mean±SD**	0.35±0.48	0.03±0.18	0.85±0.35	0.82±0.39
Nonsmokers	11 (34.37%)	0	28(87.5%)	2(6.25%)
Mean±SD	0.34±0.48		0.87±0.33	0.06±0.24

* Loss of normal epithelium pattern comprising of bulbous rete ridges, loss of polarity and increase parabasal cells

** Standard Deviation

Discussion

Smoking affects both gingival epithelium and connective tissue. Smokers have more blood vessel with≤ 0.5µ diameter and lesser inflammatory cells infiltration than nonsmokers do. The epithelial changes resemble to early phases of dysplasia is a common finding in smokers but not in nonsmokers.

Sreedevi et al. showed that the density of blood vessels and inflammatory cells infiltration are decreased in smokers (7). These findings are in harmony with the results of present study. The researchers believe that the effects of smoking on vascular status are caused by nicotine compounds. Nicotine due to stimulating the production of adrenaline and noradrenalin causes vasoconstriction and this leads to the decreasing of bleeding and exudates production (7).

Decrease in capillary diameter and density of blood vessels in the gingival tissues of smokers explains the reduction of gingival index in this group. Inflammatory responses will change in smokers. This causes the reduction of redness and bleeding. These signs are clearly mild in smokers. This finding is sometimes confused with gingival health status (12-14). Smoking cessation increases gingival bleeding in smokers as nonsmokers (15).

This study has shown that the rate of inflammation significantly is low in smokers than nonsmokers. Reduced infiltration of inflammatory cells in smokers compared with non-smokers is consistent with previous findings (6, 7-16).

The findings of present study showed that the gingival index is higher in nonsmokers and have significantly different with gingival index of smokers. Conversely, the periodontal index was higher in smokers and had significantly different with periodontal index of nonsmokers. This is consistent with previous reports (17-19).

Other studies have focused on increasing pocket depth and attachment loss in smokers (7,18-20). Bergeshtrom used the term "*chronic destructive periodontal disease*" to describe tooth loss because of tobacco use. Mild symptoms of inflammation are suppressed in the early stages, but at the same time bone loss and pocket formation have started and are in progress (21).

This effect can worsen the patient situation because at the time, which the periodontal disease is progressing, the symptoms of inflammation are mild.

The clinical findings of this study are in harmony with histopathological features of reduced diameter of blood vessels and decreased inflammatory cells infiltration. This finding is consistent with other studies that showed lesser clinical features of gingivitis and more severity of periodontal disease in smokers.

In this study, we assessed the patients who were surgical candidates for their periodontal status. Then, the patients put into two groups based on smokers or nonsmokers. All patients were men for removing the effect of hormonal changes.

These patients had no systemic diseases and were not taking medications. In this study, nonsmoker patients had no previous history of smoking. Accordingly, a more controlled condition was prepared to evaluate the effect of smoking.

The histopathological features of epithelium were different in smokers as compared with nonsmokers. These differences were included bulbous rete ridges, loss of polarity and increased in parabasal cells. These changes were not seen in lining epithelium of nonsmokers.

Based on our review this is the first study to report the effects of smoking on gingival epithelial lining in human respecting to report the pre dysplastic similar changes.

De oliveira semenzati et al. reported the effects of smoking on the tongue, pharynx and larynx mucosa in rats. This study represented the epithelial hyperplasia, basal cell hyperplasia and even mild to moderate dysplasia (6). This finding on animal model corresponded with the findings of this study on human. We did not find any study containing the effects of smoking on cellular polarity and related changes in human gingival epithelium.

The difference of epithelial hyperkeratosis, atrophy and acanthosis was not different in smokers and nonsmokers. This is not in consistent with Villar and Kumar that reported the higher thickness of the epithelium in smokers (5,10). We contributed this difference to sampling. Villar and Kumar evaluated the epithelial base thickness and the external / internal epithelial perimeters.

The oral cancer risk is higher in smokers. The risk is related to the amount and duration of smoking (22). Smoking can cause DNA instability. This is in consistent with increasing counts of mucosal micronucleus (8-23).

DNA instability can explain the genotoxic effect of smoking on the oral mucosa. Based on the findings of this study, smoking causes basal layer hyperplasia and loss of polarity. These changes may indicate the increased talent of epithelium for malignant transformation.

This study showed that the epithelial changes in smoker were similar to early stages of epithelial dysplasia. At the same time, smokers have lower GI than nonsmokers. This is consistent with this fact that despite the normal appearance of gingiva in smokers, smoking increasing the epithelial changes resemble to early phases of dysplasia and decreasing inflammatory reaction. By progressing the condition, smoking may lead to oral cancer.

Since these changes may be associated with cigarette constituents, further more investigation needed. In present study, the bacteriological compound and immunological changes in smokers compared to nonsmokers was not examined. Assessing the mechanisms by those smoking effects on oral mucosa need future studies containing clinical, immunological and histopathological parameters.

Conclusion

Despite the normal appearance of gingiva in smokers, smoking increasing the epithelial changes resemble to early phases of dysplasia and decreasing inflammatory reaction.

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Conflict of interest

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