

Original Article

The Impact of Smoking on Gingiva: a Histopathological Study

Noushin Jalayer Naderi ¹, Hassan Semyari², Zahra Elahinia³

1. Dept. of Oral and Maxillofacial Pathology, Shahed University, Tehran, Iran

2. Dept. of Periodontic, Shahed University, Tehran, Iran

3. Graduate from Faculty of Dentistry, Shahed University, Tehran, Iran

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 $\leq 0.5\mu$ 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

Received: 08 April 2014

Accepted: 26 September 2014

Address Communications to: Dr. Hassan Semyari, Department of Periodontic, Faculty of Dentistry, Shahed University, Tehran, Iran.

Email: h.semyari@gmail .com

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 \times 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 $\times 40$ (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 $40 \times$ magnifications (9). The mean number of blood vessels with $\leq 0.5\mu$ diameter was determined.

Based on the obtained results from previous studies, the $\leq 0.5\mu$ 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 $\times 10$ (objective) magnification.

The results were stated as mean \pm 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 \times years was 91 and 456, respectively. The mean of packs \times 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 and 1.87 ± 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 $\leq 0.5\mu$ diameter was 18.78 ± 10.06 and 5.90 ± 2.93 in smokers and nonsmokers, respectively. Although smokers had more blood vessels with $\leq 0.5\mu$ 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 $\leq 0.5\mu$ 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.

Acknowledgment

Authors thank Mr. Farhadi H. for statistical analyzing, Shahid Montazeri Dental Clinic and Imam Ali Dental Clinic from NAJA for their kindly assistance.

Conflict of interest

This study was completed under the financial support of the Deputy of Research, Shahed University by the grant of Drs. Noushin Jalayer Naderi. The authors declare that there is no conflict of interests.

References

1. Fauci AS, Braunwald E, Kasper DL, Hauser SL, Longo DL, Jameson JL, et al. Harrison Principles of internal medicine. 17th ed. New York :Mc Graw Hill;2008.p.2736-2739.
2. Newman MG, Takei HH, Klokkevold PR, Carranza FA. Carranza clinical periodontology. 10th ed. St.Louis: W.B.Saunders;2006.p.251-258.
3. 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(5):743-51.
4. Hyman JJ, Reid BC. Epidemiologic risk factors for periodontal attachment loss among adults in the United States. J Clin Periodontol 2003;30(3):230-7.
5. Villar CC, de Lima AF. Smoking influences on the thickness of marginal gingival epithelium. Pesqui Odontol Bras 17;2003):41-5.
6. de Oliveira Semenzati G, de Souza Salgado B, Rocha NS, Michelin Matheus SM, de Carvalho LR, Garcia Martins RH. Histological and immunohistochemical study of the expression of p53 and ki-67 proteins in the mucosa of the tongue, pharynx and larynx of rats exposed to cigarette smoke. Inhal Toxicol 2012; 24(11):723-31.
7. Sreedevi M, Ramesh A, Dwarakanath C. Periodontal status in smokers and nonsmokers: a clinical, microbiological, and histopathological study. Int J Dent 2012; 2012:571590.
8. Jalayer Naderi N, Farhadi S, Sarshar S. Micronucleus assay of buccal mucosa cells in smokers with the history of smoking less and more than 10 years. Indian J Pathol Microbiol. 2012; 55(4): 433-438.
9. Foote RL, Weidner N, Harris J, Hammond E, Lewis JE, Vuong T, et al. Evaluation of tumor angiogenesis measured with microvessel density (MVD) as a prognostic indicator in nasopharyngeal carcinoma: results of RTOG 9505. Int J Radiat Oncol Biol Phys 2005 1;61(3):745-753.
10. Kumar V, Faizuddin M. Effect of smoking on gingival microvasculature: A histological study. J Indian Soc Periodontol 2011; 15(4):344-8.
11. Gomes-filho J, Duarte P, Oliveria C, Watanabe S, Simonetti C. Tissue reaction to a triantibiotic paste used for endodontic self-generation of nonvital immature permanent teeth. J Endod 2012; 38(1):91-95.
12. Preber H, Bergstrom J. Occurrence of gingival bleeding in smoker and non-smoker patients. Acta Odontol Scand 1985; 43(5): 315-20.
13. Bergstrom J, Persson L, Preber H. Influence of cigarette smoking on the vascular reaction during experimental gingivitis. Scand J Dent Res 1988; 96(1): 34-9.
14. Lie MA, Timmermann MF, Van der Velden U, Van der Weijden GA. Evaluation of two methods to assess gingival bleeding in smokers and non-smokers in natural and experimental gingivitis. J Clin Periodontol 1998; 25(9): 695-700.
15. Morozumi T, Kubota T, Sato T, Okuda K, Yoshie H. Smoking cessation increases gingival blood flow and gingival crevicular fluid. J Clin Periodontol 2004; 31(4): 267-72.
16. Danielsen B, Manji F, Nagelkerke N, Fejerskov O, Baelum V. Effect of cigarette smoking on the transition dynamics in experimental gingivitis. J Clin Periodontol 1990; 17(3): 159-164.
17. Faddy MJ, Cullinan MP, Palmer JE, Westerman B, Seymour GJ. Ante-dependence modeling in a longitudinal study of periodontal disease: the effect of age, gender, and smoking status. J Periodontol 2000; 71(3):454-9.
18. Calsina G, Ramón JM, Echeverría JJ. Effects of smoking on periodontal tissues. J Clin Periodontol 2002; 29(8):771-6.
19. Rudziński R. Effect of tobacco smoking on the course and degree of advancement inflammation in periodontal tissue. Ann Acad Med Stetin 2010; 56(2):97-105.
20. Machuca G, Rosales I, Lacalle JR, Machuca C, Bullon P. Effect of cigarette smoking on periodontal status of healthy young adults. J Periodontol 2000 ;71(1):73-78.
21. Bergström J. Tobacco smoking and chronic

destructive periodontal disease. *Odontology* 2004 ; 92(1):1-8.

22. Neville BW , Damm DD , Allen CM , Bouquot JE . *Oral and Maxillofacial Pathology*. 3rd ed. Philadelphia : W.B.Saunders Company;2009.p. 410.

23. Nersesyan A, Muradyan R, Kundi M, Knasmueller S. Impact of smoking on the frequencies of micronuclei and other nuclear abnormalities in exfoliated oral cells: a comparative study with different cigarette types. *Mutagenesis* 2011; 26(2):295-301.