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Original Article

Effect of Cigarette Smoking on Eosinophil Count in Periodontal Inflammation: A Histopathologic Study

Noushin Jalayer Naderi[1*], Mohaddeseh Mehrparvar[2]

Abstract

Background: It has been shown that cigarette smoking is associated with decreased number of eosinophil cells in blood and lung. Cigarette smoking is one of the major causes of gingival problems and periodontitis. The effect of cigarette smoking on eosinophils in gingiva has not been elucidated. The aim of this study was to determine the effect of cigarette smoking on eosinophil count in periodontal inflammation.

Methods: The study was a case-control study. Forty paraffin embedded block of periodontitis obtained from 20 cigarette smokers and 20 nonsmokers were evaluated histochemically for eosinophil count. Using hematoxylin-eosin stained sections, the number of eosinophils was determined per high power field at ×400 magnification. One-way analysis of variance (ANOVA), *t* test, Duncan and Pearson correlation coefficient tests were employed for data analyses at the level of $P \leq 0.01$.

Results: The mean number of eosinophils in nonsmokers was significantly higher than that in smokers ($P < 0.001$). The intensity of gingivitis and periodontitis in none of nonsmokers (GI: r = 0.2, $P = 0.37$; PI: r = 0.01, $P = 0.95$) and smokers (gingival index [GI]: r = 0.04, $P = 0.83$; periodontal index [PI]: r = 0.23, $P = 0.31$) were correlated to eosinophil count. The eosinophil count was higher in heavy smokers ($P = 0.03$).

Conclusions: The eosinophil count plays no effective or critical role in smoking-induced periodontal inflammations. Increasing time of exposure to cigarette smoke affects eosinophil count in adult gingivitis/periodontitis. The dual effect of eosinophils in progressing the periodontal inflammation needs more investigation.

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***Correspondence to**
Noushin Jalayer Naderi, DMD; Department of Oral and Maxillofacial Pathology, Faculty of Dentistry, Shahed University, Tehran, Iran/
Tel: +982188959210
Fax: +982188967618
Email: jalayer@shahed.ac.ir

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Background

Eosinophils are multiple functional granulocytes which originate from the bone marrow. Their regulatory/initiating role in the pathogenesis of allergic reactions, parasitic infections and neoplastic disorders is known. Eosinophils contain cytotoxic materials such as cytokines, chemokine and lipid mediators, and are therefore able to sustain inflammatory process (1,2). Blood and tissue eosinophilia is formed due to the loss of the balance between the production and apoptosis of eosinophils (3).

Existing reports on eosinophil count in gingiva is very scant and controversial. Examination of Appelgren et al on human gingivitis revealed that no eosinophils were situated in gingiva (4). However, Bertão et al) reported that eosinophil counts were progressively increased in the inflammatory disease in rat gingiva (5). It has been shown that cigarette smoking affects inflammation and oxidative stress. Acute cigarette smoking was associated with decreased number of blood eosinophils. The suppressive effect of smoking on eosinophils is attributed to the anti-inflammatory effect of carbon monoxide (6-8).

Highlights

- Number of eosinophil cells was higher in nonsmokers than smokers
- The severity of gingivitis and periodontitis were not related to eosinophils count.
- The cigarette smoke reduces the number of eosinophils, but, this did not affect the progression of the periodontal disease.

Smoking is one of the major causes of periodontal destruction. Severity and prevalence of periodontitis is higher in smokers compared to nonsmokers and increases with the number of smoked cigarettes (9).

Eosinophils play a complex and mysterious role in inflammatory responses and modulation of immune system (10). Our information about the role of eosinophil in inflammatory related conditions of gingiva is very low. Yet, no reports on the effect of cigarette smoking on extravascular gingival eosinophil are available.

[1]Department of Oral and Maxillofacial Pathology, Faculty of Dentistry, Shahed University, Tehran, Iran. [2]Faculty of Dentistry, Shahed University, Tehran, Iran.

Investigating the role of eosinophil and its effect on the progression of periodontitis may help to control the gingivitis/periodontitis in the smokers. The aim of this study was to determine the effect of cigarette smoking on eosinophil count in periodontal inflammation. This is the first study on determining the effect of cigarette smoking on eosinophil counts in gingiva.

Methods

The study was a case-control study. The samples were retrieved from our archive. The study was carried out in Department of Pathology, Faculty of Dentistry, Shahed University, Tehran, Iran, during February -May 2017.

Twenty formalin-fixed, paraffin embedded samples of chronic periodontitis were selected from our archive. The demographic and medical data were registered from samples' medical records. The samples from males (25 to 50 years) who did not have systemic diseases and did not take medication were included in the study. Subjects suffering from diabetes and immune system diseases, those under radiotherapy, and pregnant women were excluded from the study. The subjects who were not alcoholic and were not drug users entered the study. The samples belonged to the patients that did not take periodontal surgery in the previous 6 months and were cases of gingival flap surgery. The samples were obtained from free gingiva of labial aspects of attached gingiva.

The information about gingival index (GI), periodontal index (PI), and exposure time to cigarette smoke were obtained from registered records of each sample. The GI and PI were graded according to Loe & Sillness and CPITN scaling system as follows (9):

0 = normal, 1 = mild inflammation (GI: no bleeding on probing, PI: inflammation in the free gingiva), 2 = moderate inflammation (GI: bleeding on probing, PI: Inflammation completely circumscribes the teeth), 3 = severe inflammation (GI: ulceration and tendency to spontaneous bleeding, PI: pocket formation, probing pocket depth ≥5 mm)

The exposure time to cigarette smoke was determined by the number of pack × years (11). Based on pack × years, the samples were divided into 4 groups:

1 to 100 pack × years: Group 1
101 to 200 pack × years: Group 2
201 to 300 pack × years: Group 3
301 to 400 and more pack × years: Group 4

The 5 µm thick, hematoxylin-eosin stained sections were studied for evaluating the eosinophil count. The nucleated cells with red, intense cytoplasmic granules were calculated (12,13) (Figure 1).

The hot spot areas with the highest eosinophil density were selected. The eosinophil number was determined per high power field (12).

Blind counting was completed using optical microscopy (Zeiss, Japan) at ×400 magnification.

One-way analysis of variance (ANOVA), *t* test, Duncan and Pearson correlation coefficient tests were employed for data analyses at the level of $P \leq 0.01$. IBM SPSS statistical software (Version 19; Chicago, IL, USA) was employed for statistical analyses.

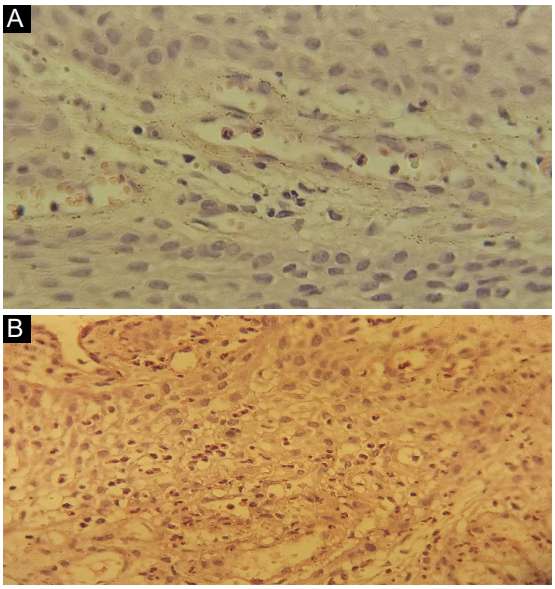

Figure 1. Eosinophils Within Connective Tissue Section Obtained From (A) Cigarette Smoker and (B) Nonsmoker: Images Contain Nucleated Cells With Red, Intense Cytoplasmic Granules (Hematoxilin & eosin ×400).

Results

The mean age of nonsmokers and smokers was 39 and 43.75, respectively. The mean time of smoking was 269.3 ± 103.59 packs × years. The mean number of eosinophil count in nonsmokers and smokers was 5.75 ± 3.82 and 1.25 ± 1.40, respectively. The mean of GI in nonsmokers and smokers was 2.71 ± 0.42 and 1.46 ± 0.52, respectively. The mean of PI in nonsmokers and smokers was 1.80 ± 0.26 and 2.67 ± 0.61, respectively.

The mean number of eosinophil in nonsmokers was significantly higher than that in smokers ($P < 0.001$). The mean of GI and PI in nonsmokers was significantly higher ($P=0$) and lower than that in smokers ($P=0$), respectively (Table 1).

One-way ANOVA showed a significant difference among 4 groups regarding exposure time to cigarette smoke (pack× years) in terms of eosinophil count ($P=0.03$). The Duncan multiple range test indicated higher significant eosinophil count among smokers with 301 to 400 and more pack × years ($P=0.03$).

The intensity of gingivitis and periodontitis in none of nonsmokers (GI: r = 0.2, $P=0.37$; PI: r = 0.01, $P=0.95$) and smokers (GI: r = 0.04, $P=0.83$; PI: r = 0.23, $P=0.31$) was correlated to eosinophil count.

Discussion

The study showed a significantly higher eosinophil count in periodontal inflammation of non-smokers. Since

Table 1. Eosinophil Counts in Nonsmokers (n = 20) and Smokers (n = 20)

Variables	Nonsmokers		Smokers		Sig (two-tailed)
	Number	Eosinophil Count Mean ± SD	Number	Eosinophil Count Mean ± SD	*t* test
Eosinophil count					
0-5	9	2.8 ± 1.6	20	1.25 ± 1.3	
6-10	9	6.4 ± 0.8	0	0	
11-15	1	12	0	0	
16-20	1	17	0	0	$P<0.001$[b]
Gingival index					
Grade 0	0	0	0	0	
Grade 1	1	8	12	0.5 ± 0.7	
Grade 2	2	4 ± 2.8	7	2.5 ± 1.3	
Grade 3	17	5.2 ± 4	1	0	
Periodontal index					
Grade 0	0	0	0	0	
Grade 1	7	5.4 ± 3.9	1	1	
Grade 2	13	8.4 ± 8.2	2	1.5 ± 1.3	
Grade 3	0	0	17	1.2 ± 1.3	
Pack × years[a]					
0-100	0	0	2	2.75 ± 0.3	$P=0.03$[b]
101-200	0	0	5	2.4 ± 1.1	
201-300	0	0	6	2.4 ± 0.8	
301-400	0	0	6	3.2 ± 0.2	
401-500	0	0	1	3	

[a] Number of smoking per year.
[b] Significant.

the GI and PI in none of smokers and non-smokers were related to eosinophil count, the findings indicated that eosinophil cells did not play any effective role in periodontal inflammation.

The only published research about extravascular eosinophil in gingivitis dates back to 1977. Appelgren et al reported no eosinophilia in the 60 studied samples of gingivitis (4). This is inconsistent with the present study. The different results may be due to different study methods. Appelgren et al had used different tissue fixative and stains in their study. This may have affected the results.

Bertão et al, who studied the experimentally induced periodontitis in rats, showed increased number of eosinophil during progression of the inflammation in the connective tissue of gingiva (5). The results of our study were in agreement with the results of Bertão et al.

Eosinophil cells play a key role in immune system functions. Antigen presentation, promoting T cell proliferation and cytokine secretion are among the different functions of eosinophils in immune system (14-16). It has been shown that different cytokines were involved in eosinophils trafficking at inflammatory sites (17).

Different functions of eosinophil cells make them unique cells in inflammatory-immune responses. Contrary to neutrophils (18-21), few researchers have studied the eosinophil role in chronic adult periodontitis (22,23).

The reported findings about the effect of smoking on blood eosinophils are controversial. van der Vaart et al showed a decrease in the number of eosinophils (24), while Higuchi et al reported an elevated count of blood eosinophils in cigarette smokers (25).

The present study showed that eosinophil count in periodontal inflammation of nonsmokers was significantly higher than that of cigarette smokers. Eosinophil count was not previously investigated in the periodontal inflammation in cigarette smokers. The subject needs further investigation.

In the present study, the PI of smokers was significantly higher than that of nonsmokers. This is in consistence with previous studies which showed the higher bone loss, periodontal attachment loss and gingival recession in smokers (9,26,27). The eosinophil count was higher in nonsmokers than smokers, while the GI and PI in none of nonsmokers and smokers were correlated to eosinophil count. It seems that the cigarette smoke reduces the

number of eosinophils, but, this cellular decrease, does not affect the progression of the disease. Toxic substances of cigarette smoke such as carbon monoxide may cause the eosinophils apoptosis in periodontal inflammation. Heme oxygenase-1/carbon monoxide metabolism is a new approach in biological sciences for considering the pathogenesis of diseases (28). As a possibility, this route may be involved in the initiation of periodontitis in cigarette smokers. The issue needs further investigations.

In the present study, the samples were limited to male smokers. Due to increasing interest of women on cigarette smoking, comparative studies on the effect of cigarette smoking between males and females are recommended. Eosinophil count in periodontal inflammation may be affected by female hormones. The present study was the first investigation about the effect of cigarette smoking on eosinophils in periodontal inflammation. Finding the involved mechanisms needs supplementary researches.

Conclusions

The eosinophil count in periodontal inflammation in cigarette smokers was significantly lower than that in nonsmokers. The eosinophil count played no effective or critical role in smoking-induced periodontal inflammation. Increasing time of exposure to cigarette smoke affects eosinophil count in adult gingivitis/periodontitis. The dual effect of eosinophil cells in progressing the periodontal inflammation needs more investigation.

Authors' Contribution

NJN contributed to the development of draft, design the work, analysis and interpretation of data. MM contributed to the acquisition and collection of data. All authors contributed to the final draft.

Ethical Statement

Used samples were obtained from "Faraji M. Effect of smoking on epithelial appoptosis and cell proliferation in chronic periodontitis: A histological study using ki-67 and p53markers" presented for the DDS degree, Shahed University, 2017 under IR.Shahed.REC.1396.6 ethics code.

Conflict of Interest Disclosures

The authors declare that they have no conflict of interests.

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