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Effect of Smoking on Interleukin-10 and Interferon-Gamma Levels in Gingival Crevicular Fluid of Patients with Periodontitis

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Abstract

Background: Periodontitis is an inflammatory disease of tooth-supporting tissues; several factors are involved in the development and severity of periodontitis among which smoking can be mentioned; however, the exact mechanism of the effect of smoking on progression of periodontitis is not still well known. In this regard, the present study was conducted to evaluate Interleukin-10 and gamma interferon levels in gingival crevicular fluid (GCF) of patients with chronic periodontitis.

Materials and Methods: This case-control study was carried out on 60 men referred to the Department of Periodontology of Babol Faculty of Dentistry; 30 smokers and 30 age-matched non-smokers, both with chronic periodontitis, entered the case and the control group respectively. Those with systemic disease were excluded from the study. Assessment of periodontal health was performed by using the dental plaque, Barnett gingival bleeding and probing pocket depth (PPD) indices. Cytokines level in the GCF evaluated by Enzyme linked Immunosorbent Assay (ELISA). Data were analyzed by SPSS18 statistical software by using Mann-Whitney and Spearman rho tests.

Results: The mean dental plaque index showed no significant difference between the two groups (P=0.1). Although gingival bleeding index was higher in control compared to the case group, the difference was not significant (P=0.08). The mean PPD was lower in the case that the control group (P=0.02). The mean Interleukin-10 and gamma interferon levels was respectively 1.25 (±0.04) pg/ml and 0.82 (±0.44) pg/ml in smoking and 1.22 (±0.44) pg/ml and 0.75 (±0.32) pg/ml in non-smoking group (P>0.05). In addition, a reverse correlation has been found between gamma interferon and Interleukin-10 in both groups which was not significant (P>0.05).

Conclusion: IL-10 level in GCF was higher in the case than the control group; however, the difference was not significant. Further investigations are, therefore, required to confirm this observation.

Keywords: Chronic Periodontitis; Smoking; Interleukin-10; IFN-γ; GCF.

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Introduction

Periodontitis is a chronic inflammatory disease of tooth supporting tissues which leads to clinical attachment loss, alveolar bone loss and in the end tooth loss (1-2). Among the factors influenced the disease, genetic, lifestyle, dental plaque and the bacteria living in it can be listed (1, 3). Oral cavity and mucosa are the first surfaces of the body influenced by the direct contact of particles and gases produced by the combustion of tobacco smoke passing through the filters. Based on various studies,

it has been identified that no influencing factor has been recognized so far as known as smoking on periodontal tissue health. Smoking-inducedde struction of teeth supporting tissues occurs in many ways. On one side, it suppresses signs of inflammation through the interaction with immune and vascular responses and, on the other hand, it eliminates the function of supportive tissues by destroying them, thereby leading to tooth loss (4). Smoking is also influential on the process of healing and duration of periodontitis treatment (3-4). In Kubota et al. study on subgingival microbial flora of smokers with chronic periodontitis, it has been revealed that subgingival microflora changes under the effects of smoking (5); they concluded that there is a positive relation between gingival bleeding by Campylobacter rectus and Prevote ll a intermedia and smoking; the prevalence of Aggregatibacter actino mycetemcomitans was also lower in dental plaque of smokers compared with non-smokers (5).

Although, no significant difference has been found between the diversity of bacteria in dental plaque of smokers and non-smokers in different studies (6-9), the relation between periodontitis and smoking is dose dependent (10). However, the mechanism by which smoking can contribute to periodontitis exacerbation has not been still well clarified. Smoking can result in the inhibition of gingival bleeding, and gingival bleeding which is as an important indicator in assessing the health of the gums may not exist while periodontal examination. Anti-inflammatory and anti-hemorrhagic effects of smoking can cause inflammation signs, including redness, hemorrhage and edema, to appear later in most smokers (11-12). When smoking stops, gingival blood flow, gingival bleeding, volume and flow of GCF returns to normal as it is in healthy subjects (11, 13-14). Cytokines play an important role in periodntitis since it is an inflammatory disease (15-16). Cytokines such as IL-1β, TNF-α and IL-6 play a tissue-destructive role in immune response to pathogens in dental plaque (17). Cytokines, in fact, can regulate and develop the host response (16, 18). Environmental factors such as smoking may affect the production of proinflammatory, inflammatory and anti-inflammatory cytokines in the periodontium, as well as the host responses to stimulating factors (19-20). IL-10 and gamma interferon are considered as anti-inflammatory and intermediate cytokines respectively (21). Since smoking have an anti-inflammatory impact on the periodontium (20, 22), it may delay signs of inflammation in the periodontium and inhibit host responses to pathogens and, thus, the development and exacerbation of periodontal disease by interfering with the production of anti-inflammatory cytokines. Therefore the aim of present study to investigate the relationship between levels of cytokines IL-10 and gamma interferon in GCF with periodontal health indices in smokers and non smokers with chronic periodontitis referred to the periodontology department of Babol dentistry faculty.

Materials and Methods

The study protocol was approved by the ethics committee of the Babol University of medical sciences. This case-control study was carried out on male patients referred to the periodontology department of dentistry faculty. People with the following conditions entered the study;

1. Lack of any systemic disease affecting the periodontal tissues,
2. No antibiotic treatment during the last month and/or any medication affecting the periodontal tissues,
3. No intraoral inflammatory and non-inflammatory lesions,
4. No alcohol consumption and 5. No history of scaling and root planning during the last six months.

Those who enrolled were oriented about the study process and obtained a written informed consent; the participants were then divided into two groups as follows;

1. Men with generalized chronic periodontitis (clinical attachment loss [CAL] ≥ 4mm in 30% of the probing sites), consumed at least 10 cigarettes a day (case group)
2. Men with chronic generalized periodontitis (clinical attachment loss (CAL ≥ 4mm in 30% of the probing sites), nonsmokers and not even passive smokers (control group)

To eliminate the confounding effect of age on the results, both the case and control groups were matched in terms of age. In each quadrant of the jaw, teeth probing pocket depth greater than or equal to 4 mm was randomly selected, and CGF was collected by paper points # 30. Before collecting the CGF, supragingival plaque was gently removed using a cotton roll and washed with saline. In the end, isolation was accomplished by cotton roll. Paper point was gently inserted into the sulcus to a one-millimeter depth and kept in place for 30 seconds. Paper points contaminated with blood or saliva were not applied. Four paper points were collected in each subject; The samples were placed in 1.5 mm capped microtubes and transferred to the laboratory and stored in the -80 °C freezer until the day of the experiments. One the day of experiment, 200 µl of PBS was added to each microtube which was centrifuged (×1800 rpm) after being mixed, and the supernatant was collected. Cytokines measurements were performed by commercial ELISA kits (Bender Med Systems, Austria) according to the manufacturer's instructions. Periodontal clinical assessment using

dental plaque index (Silness and Loe) (23), Barnett bleeding index (24) and PPD were performed on all teeth except the third molars. To determine the pocket depth using the William's probe, each tooth underwent probing in mesiobuccal, buccal, distobuccal, and lingual sites. Probe was in serted in to the pocket in parallel with the long axis of the tooth, and periodontal pocket depth was calculated from the gingival margin to the total probing depth. All measurements were performed by a single calibrated examiner.

Statistical analysis

Collected data were analyzed by $SPSS_{18}$ statistical software. The Mann-Whitney test for the evaluation of the mean age, gender, dental plaque, gingival bleeding, periodontal pocket depth index, and IFN-γ and IL-10 levels in both smokers and nonsmokers was used. Quantitative data are presented as mean (± Standard Deviation). Statistical significance was assumed on the basis of a P-value <0.05.

Results

Sixty male patients (30 in case and 30 in control group) with the mean age of 41.16 (±9.85) years, ranging from 26 to 62 years, entered the study. The mean age of participants was 42.83 (±8.32) years in the case and 39.50 (±11.06) years in the control group (P=0.19). The mean plaque index, Barnett bleeding index, and PPD was 1.81 (±0.72), 1.75 (±0.77), and 5.18 (±0.64) mm respectively. There was no significant difference between the two groups in terms of clinical parameters and only the mean PPD was significantly higher in smoking group (P<0.05).

Table 1. The mean (SD) clinical parameters evaluated in smokers and non-smokers patients.

Clinical Parameters	Non-smokers		Smokers		P-Value
	Mean	Standard Deviation	Mean	Standard Deviation	
Plaque index	1.66	0.71	1.96	1.93	0.1
Barnett bleeding index	1.93	0.78	1.56	0.72	0.08
Probingpocket depth(mm)	4.99	0.62	5.37	0.61	*0.02

The mean IL-10 and gamma interferon levels were found to be higher in smokers; however, the difference was not statistically significant. A reverse correlation was observed between IL-10 and gamma interferon levels in GCF in both groups, though the correlation was not statistically significant (r= -0.096, P=0.615 and r=0.113, P=0.553 in smoking and non-smoking group respectively).

Table 2. The mean (±SD) level of gingival crevicular fluid cytokines.

Cytokines	Non-smokers		Smokers		P-Value
	Mean	Standard Deviation	Mean	Standard Deviation	
IL-10 (pg/ml) ‡	1.22	0.44	1.25	0.4	0.976
IFN Gamma (pg/ml) †	0.75	0.32	0.82	0.44	0.662

‡ Interleukin-10
† Gamma interferon

Discussion

In this study, the effect of smoking has been evaluated on IL-10 and gamma interferon levels in GCF of female patients with chronic periodontitis and its correlation with periodontal health indicators. Dental plaque index was not statistically different between the two groups. Gingival bleeding was higher in non-smokers, though the difference was statistically insignificant (P=0.08); whereas, the measured pocket depth was significantly higher in smokers. However, in Rai et al. Study, bleeding on Probing (BOP) and PPD were found to be significantly higher in smoking group. It should be mentioned that 12 participants with periodontitis were smoker and 10 were non-smokers in above study (25).

In an investigation by Tabibzadeh et al., the mean PPD was higher in smokers, but the difference was not significant (26). In Lafzi et al. study, PPD was

significantly higher in heavy smokers as compared with non-smokers; however, the difference was not significant in light smokers. Moreover, the BOP was found to be significantly lower in heavy smokers in comparison with light and non-smokers (27). PPD was reported to be significantly higher in smokers compared with non-smokers and those who quitted smoking in Haffajee et al. study (28). In addition, in Bergstrom research, pocket depth was higher in former smokers than non-smokers (29). Significant reduction in BOP index in smokers compared with non-smokers has been demonstrated in Haffajee (28), Calsina (30) and Bergstrom (14) studies. In an investigation by Abolfazli et al., BOP index was lower in heavy smokers than nonsmokers and probing pocket depth was significantly higher in nonsmokers (31). The difference in the results of different studies may, therefore, be due to difference in the number of cigarettes consumed and the duration of cigarette consumption (27, 31-32). Higher gingival bleeding index in non-smokers might possibly reflect the suppressive impact of smoking on blood vessels. Gingival bleeding is indicative of the severity of periodontium inflammation and smoking has been strongly able to decrease the severity of inflammation in periodontium through vasoconstriction effect by nicotine or reduction in blood vessel density and luminal surface (33); while, the PPD was higher in smokers. Of course, the impact of smoking on dental plaque bacteria, which cause increase in certain species affecting the periodontitis pathogenesis, may be the reason behind the difference in bleeding index between smoking and non-smoking groups.

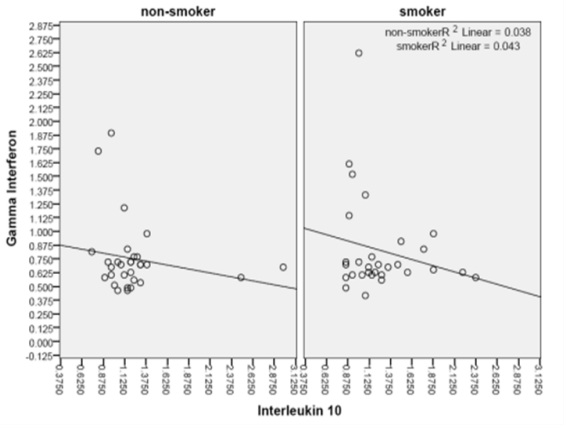

Figure 1. The correlation between interleukin-10 and gamma interferon levels in GCF of smokers and non-smokers with chronic periodontitis.

The mean IL-10 level in GCF was higher in our case than the control group; however, the difference did not reach a statistically significant level. Furthermore, GCF gamma interferon level was higher, but not statistically significant, in the case than the control group. As shown in figure 1, increase in IL-10 level is associated with decrease in gamma interferon concentration and such a reduction occurs with greater slope in smoking group. This finding partly supports a hypothesis indicating the inhibition of host inflammatory responses by smoking (20, 22). GCF sampling as well as the sites of sampling can be influential on the concentration of mediators in crevicular fluid (34-35). If the sites with bleeding on probing are selected, the disease would be concluded to be more active and the inflammation to be more severe in comparison with the sites with no BOP during examination; therefore, this point should be taken into consideration in the selection of GCF sampling sites, as a deep pocket may have no BOP (inactive disease and reduced inflammatory symptoms), but a pocket with less or equal depth may show BOP and higher concentration of inflammatory cytokines, and the two cannot, thus, be compared with each other; hence, those sites which are identical in terms of the presence or absence of BOP should be compared with each other.

IL-10 is one of the cytokines produced by regulatory T cells that can inhibit the host immune responses, especially those produced by activated macrophages. Cytokines such as gamma interferon, IL-2, and IL-3 are produced by Th1 cells, and IL-13, IL-10, IL-4 and IL-5 by Th2 cells. The presence of gamma interferon and IL-10 is indicative of cell immune response and humoral immune response respectively. Torres et al. showed that the balance between helper T cells is disturbed and moves toward Th2 in smokers (36). Byron (37), Cozen (38) and Hagiwara (39) also revealed that gamma interferon production has been decreased byTh1in smokers compared with non- smokers. Thus, smoking can alter the balance of cytokines produced by T helper cells. Higher level of IL-10 has been observed in smoking group in the present study which represents the predominance of Th2 cell activity in periodontal tissues of smokers with periodontitis. Imbalance in Th1 and Th2 cytokines production in infectious diseases may be associated with disease progression and deterioration (40). Studies have shown that in early stable lesions, Th1 are the dominant cytokines, while in advanced progressive lesions, Th2 are the predominant category (41). In addition to periodontitis in other inflammatory diseases, smoking may worsen the situation and deviate the immune response toward the Th2 cells (37-39). Thus, it appears that smoking can intervene with the development and exacerbation of periodontitis in smokers through affecting the Th cells. However, this claim needs histological examination on periodontal tissues. Moreover, through the culture of immune cells existing in periodontal tissue of these patients,

activity and cytokine production of the tissue can be measured to avoid the influence of other factors on cytokine production that are not construable in human body.

Conclusions

The findings of the present study showed that IL-10 and IFN-γ levels in the GCF of smokers were higher comparison with non-smokers with chronic periodontitis, however the difference was not significant. Further investigations including evaluation of the other inflammatory mediators are required.

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Conflict of interest

The authors declare that no conflict of interest.

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