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Original Article

Estimation of volume density of interdental papilla components in patients with chronic periodontitis and interleukin-6 (-174G/C) gene polymorphisms

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ABSTRACT

Background: The association between interleukin-6 (IL-6) (-174G/C) gene polymorphisms and level of tissue breakdown and periodontal disease progression is unknown. The present study investigated quantitative parameters of interdental papilla in chronic periodontitis (CP) patients with IL-6 (-174G/C) gene polymorphisms.

Materials and Methods: Sixty gingival samples were studied. After determination of IL-6 (-174G/C) gene polymorphisms using a tetra-primer amplification refractory mutation system-polymerase chain reaction (T-ARMS-PCR) technique, 45 gingival tissue samples of CP patients (GG and GC+CC genotypes) were considered as case groups. Fifteen control samples were also collected from healthy individuals. After tissue processing, interdental gingival tissues were exhaustively sectioned into 4 μm-thick sections. Ten to thirteen sections were sampled by systematic uniform random sampling and stained with Masson trichrome, and the volume density (Vv) of the gingival components was estimated using Cavalier's point counting method. Statistical analysis was performed by Student *t*-test to compare differences between groups. The significance level was set at $P < 0.05$.

Results: There were statistically significant differences in the Vv of epithelium, connective tissue, collagenous and non-collagenous matrix, and blood vessels between the control and CP groups ($P < 0.0001$). There were no statistically significant differences in the Vv of epithelium, connective tissue of gingiva, collagenous and non-collagenous matrix, and blood vessels among GG, GC, and CC genotypes in CP patients ($P > 0.05$).

Conclusion: Results of the current study showed that there was no association between IL-6 (-174G/C) gene polymorphisms and quantitative parameters of interdental papilla in CP patients.

Key Words: Chronic periodontitis, genes, gingiva, interleukin-6, polymorphism

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INTRODUCTION

Chronic periodontitis (CP) as an inflammatory disease may cause progressive demolition of periodontal ligament and in the long-term destruction of alveolar bone and tooth loss. Many microorganisms are involved

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in CP.[1-4] It seems that the basic mechanism of this destructive process is by the direct effects of bacterial products of dental plaque and bacterial induction of the host immune and inflammatory reactions.[5-7]

Histological features of this condition include decreased extracellular matrix ingredients that have an association with a penetration of numerous inflammatory cells into the gingival epithelium and connective tissue. As chronic inflammation progresses, several autocrine and paracrine loops of cytokines affect the cells inside the lesion.[1,2,8]

Cytokines, inflammatory mediators, have regulatory role in immune and inflammatory responses. Pro-inflammatory cytokines have an important role in microbe-induced devastating inflammation. Fibroblasts of the periodontal ligament and gingiva secrete many cytokines and signal molecules during the progression of periodontitis. Many bacterial-derived infective factors increase the production of several pro-inflammatory cytokines hereby culminate in the demolition of soft tissue and bones.[1,3,9,10]

Some cytokines are encoded by polymorphic genes showing genotypes associated with inflammatory diseases that may confer vulnerability to periodontal diseases. In CP, increasing gene expression was reported in affected tissue, which was compared to normal tissue. Hence, it is proposed that cytokines are diagnostic markers of periodontitis.[1,3,9,10]

Interleukins (ILs), members of cytokines, have an increasing role in studies on periodontal diseases and have attracted a good deal of attention.[2]

IL-6 is a cytokine that has numerous roles including distinction or activation of macrophages and T lymphocyte. It can also develop and differentiate lymphocyte B. IL-6 stimulate hematopoiesis and also collagen and glycosaminoglycan synthesis. It induces the production of fibroblasts and proliferation of epithelial cells. IL-6 is a stimulator for osteoclast differentiation and bone absorptive process.[9,11-14]

Serum IL-6 levels increase in the inflammatory conditions such as rheumatoid arthritis, psoriasis, and in patients with Sjögren's syndrome.[2,13,14]

Most studies have shown that IL-6 expression at the sites of periodontal inflammation and in gingival crevicular fluid collected from these sites is high.[2]

It seems that the variations in carriage alleles can change the production of inflammatory mediators in periodontal disease.[3,9,13]

The IL-6 gene has been localized to chromosome 7p21. Some studies demonstrated that polymorphisms of this gene can affect the concentration of IL-6 in serum.[9,12]

IL-6 (-174G/C) promoter gene polymorphism can affect the gene expression.[9,11-13]

The -174 R-allele carrier individuals have decreased the plasma levels of IL-6 and present lower IL-6 gene transcriptional activity when compared with N/N individuals. Therefore, a genetically determined low IL-6 response (the -174 R-allele carriers) may hamper individual's defense against periodontal pathogens.[11]

Our previous study showed that there was no relationship between IL-6 -174G/C gene polymorphism and CP. Distribution frequency of genotypes and alleles showed no difference between CP patients and healthy controls.[3]

In the present study, we used tissue samples of the same persons who had surgical gingival operations and aimed to examine the association between IL-6 -174G/C gene polymorphisms and level of tissue destruction using stereological methods. To date, there are no data concerning the quantitative parameters of gingival tissues of individuals with IL-6 polymorphisms. In this study, quantitative analysis of interdental gingiva in patients with CP and IL-6 (-174G/C) gene polymorphisms are investigated.

MATERIALS AND METHODS

Sample selection

The patients were determined on the basis of the criteria defined in the International Workshop for Classification of Periodontal Diseases and Conditions.[4] The project was approved by the Local Ethics Committee of Zahedan University of Medical Sciences (No. 89–2880), and written informed consent was obtained from all the participants.

Characteristics of patients and controls and selection criteria are listed in our previous study. Then, 2 mL of peripheral venous blood were collected in Na-EDTA tubes from each participator to detect IL-6 (-174G/C) polymorphisms. Tissue samples of persons who had gingival surgical operations during treatment processes were obtained as described previously.[1,15]

Preparation of tissues and stereology

After detection of IL-6 (-174G/C) gene polymorphisms by tetra-primer amplification refractory mutation system-polymerase chain reaction T-ARMS-PCR in the

samples of our previous study, 45 interdental gingival tissues from CP patients with known genotypes were considered as three case groups (GG, GC, and CC).[3]

Tissue preparation was done, according to the protocol described previously.[1]

Because of the low frequency of samples with CC genotype and scant number of tissue samples of this group, the CC and GC genotypes were considered as a group (n = 19) and were compared with GG genotype group (n = 26).

In the control group, gingival samples (n = 15) were acquired from healthy volunteers that came for tooth extraction due to orthodontic or prosthodontic treatments.

The gingival tissues were submerged in Lillie fixative for 1 week at room temperature and then were processed and embedded vertically in paraffin wax. Then, each interdental gingival sample was exhaustively sectioned into 4 μm-thick sections.

From each gingival sample, 10–13 sections were chosen by systematic uniform random sampling (SURS) method as described previously.[1,16,17]

Cavalieri's principle was used to estimate the volume of interdental papilla using the formula:

$$V = \frac{\sum_{i=1}^{m} P \times a(p) \times t}{M^2}$$

Where V is the estimated volume of the interdental papilla, $\sum_{i=1}^{m} P$ is the sum of the number of points landing within the interdental papilla profiles, a (p) is the area associated with each point, t is the distance between sections, and M is the magnification.[1,16,17]

Six to eight fields were selected via SURS on each section, by movement of the microscope's stage in X and Y directions by dint of the vernier scale of a projection microscope.

Then, a probe of points was projected randomly on these fields. The points that hit to the desired components were counted. An estimate of the volume density (Vv) of the components in the reference space was obtained using the formula:

$$(Vv) = P_{part}/P_{total}$$

P_{part} is the number of test points falling in all structure profiles of desired part of the tissue (for example collagenous matrix) and P_{total} is the number of points that hit to all gingival tissue.[1,17]

All stereological analyses were done by two expert histologists (ZH and HM-S) on slides that were mask-coded.

Statistical analyses

Data were presented as means ± standard division for each parameter investigated. Student's t-test was used to compare differences between two groups. The significant level was $P < 0.05$.

All statistical analyses were performed employing SPSS version 16.0 Chicago, SPSS Inc for windows software system.

RESULTS

The demographic data showed that the mean ages for patients with CP and healthy subjects did not differ between the two groups (respectively, 28.33 ± 5.765 and 29.22 ± 3.597 years, $P > 0.05$). There were no significant differences between subjects with CP and controls regarding the ethnicity and gender ($P > 0.05$). The CP group exhibited a significantly greater mean of PD (5.58 ± 0.63 mm vs. 1.50 ± 0.86 mm), CAL (5.44 ± 0.58 mm) and a higher percentage of sites with BOP (85.86 ± 3.68%) than the control group ($P < 0.05$).

Quantitative analysis of gingival samples indicated that there were statistically significant differences in the Vv of epithelium, connective tissue, collagenous and non-collagenous matrix, and blood vessels between control and all CP groups ($P < 0.0001$). The results of this comparison are shown in Figure 1.

Comparing the volume fraction of epithelium, connective tissue, extracellular matrix components and blood vessels did not show any statistically significant differences between GG, and GC+CC groups ($P > 0.05$) [Table 1 and Figure 2].

DISCUSSION

Our results showed that the basic stereological parameters of interdental papilla differ statistically between healthy and periodontitis groups. It was according to our previous studies. There was a statistically significant difference in the Vv of gingival epithelium and connective tissue between the CP patients and the healthy controls, increase in Vv of epithelium might be the result of hyperplasia and infiltration of inflammatory cells in epithelium.[1,18,19]

Table 1: Quantitative parameters of interdental gingiva in chronic periodontitis patients with IL-6 (-174G/C) gene polymorphisms (GG, GC+CC genotypes)

Groups volume density (%)	GG genotype (*n*=26)	95% (CI) GG genotype	GC+CC genotype (*n*=19)	95% (CI) GC+CC genotype	*t*-test
Epithelium	37.6±4.07	35.97-39.30	35.1±4.08	33.37-37.68	NS
Connective tissue	62.4±4.07	60.74-64.02	64.9±4.08	62.32-66.63	NS
Collagenous matrix	35.3±7.02	32.45-38.16	35.7±7.56	31.84-39.00	NS
Noncollagenous compartment	27.1±5.61	24.81-29.34	28.7±6.97	25.37-31.90	NS
Blood vessel	16.2±4.68	14.30-18.08	16.6±4.53	14.19-18.55	NS
Noncollagenous matrix	10.9±4.67	9.00-12.77	12.6±6.39	9.77-15.81	NS

Values are mean±SD. CI for mean of volume densities. NS: Not significant; CI: Confidence interval; SD: Standard deviation

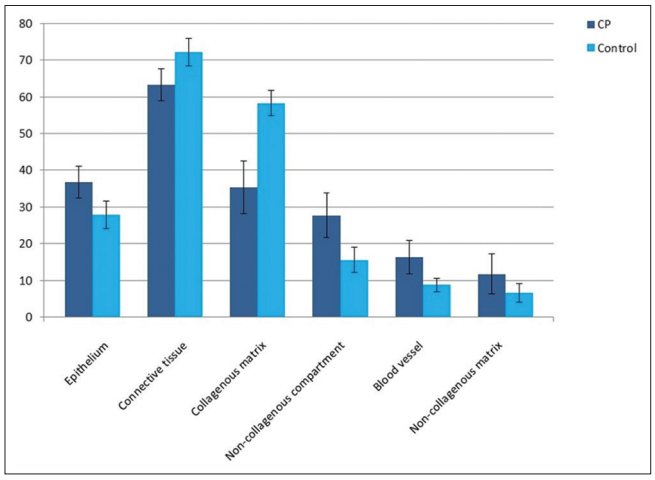

Figure 1: Quantitative parameters of interdental gingiva in patients with chronic periodontitis and healthy controls.

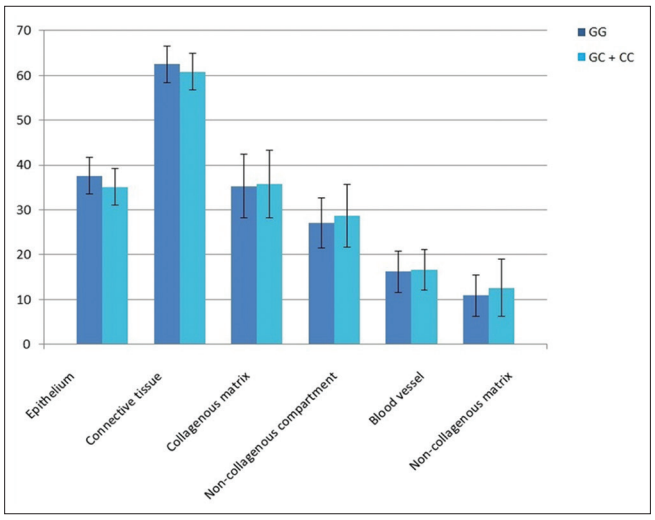

Figure 2: Quantitative parameters of interdental gingiva in patients with chronic periodontitis and interleukin-6 (-174G/C) gene polymorphisms (GG, GC+CC genotypes).

According to many studies on periodontal diseases, there are local infiltration of inflammatory cells in association with a degeneration of extracellular matrix macromolecules. Prominent macromolecules of this matrix are collagen fibers which constitute the major component of the gingival connective tissue quantitatively. Collagenous matrix has a primordial role in gingival architecture and loss of it may reflect the severity of periodontal disease.[1,18,19]

Zoellner *et al*.[20] showed that there was a statistically significant increase in the number of vessels in the deepest part and in the active front of the periodontal pocket wall in CP and also reported that the diameter of vessels was greater in periodontitis and gingivitis than in minimally inflamed tissues. Increased vasculature occurred throughout the entire thickness of gingival tissues.[20]

Pinchback *et al*. demonstrate the much higher vascular levels of type I collagenase and urokinase-type plasminogen activator in periodontitis specimens compared with minimally inflamed tissues.[21]

To date, there are no data concerning the quantitative parameters of gingival tissues of individuals with IL-6 polymorphisms.

Results of the present study showed that there was no statistically significant difference in the Vv of epithelium, connective tissue, collagenous matrix and noncollagenous compartment of gingival connective tissue, and blood vessels between GG, and GC+CC genotypes of IL-6 (-174G/C) gene polymorphisms in CP patients.

Not having difference in quantitative parameters of histological structure between two genotype groups may conclude that CP is not associated with IL-6 (-174G/C) polymorphism. The same as our previous study on total blood samples of CP and controls indicated that there was no association between IL-6 (-174G/C) polymorphism and CP in this population.[3]

In our similar studies on total blood samples of CP and controls, it was showed that there was no association between tumor necrosis factor-alpha

(-308 G/A) polymorphisms and CP. Histological quantitative studies on gingival tissues of patients with different gene polymorphism also showed that there was no finding for significant differences in quantitative parameters of interdental papilla.[1,22]

In our previous study we investigated the association of transforming growth factor-beta1 (29C/T) with quantitative parameters of interdental gingiva tissue and found that tissue breakdown rate differ between CP patients with different genotypes.[15]

It was concluded that if there was no association between CP and a gene polymorphism, histological parameters of gingival tissues of patients with different genotypes or polymorphisms would not be statistically different from each other. Thus, quantitative histological results are in the line of PCR-related association results. More studies are needed in this concern.[8]

Disease prevalence pattern often differs in geography and ethnic origin, and allele frequencies can vary widely worldwide. Genetic risk factors for disease susceptibility are different from one population to another population.[1,8]

Genes obviously play a crucial role in predisposition to CP and progression and severity of CP.[8]

However, more researches with larger sample sizes on genotype and allele diversity of IL-6 gene polymorphism in CP patients and molecular mechanisms by which IL-6 is involved in susceptibility to CP are needed to clarify the issues discussed.

CONCLUSIONS

Results of the current study showed that there was no association between IL-6 (-174G/C) gene polymorphisms and quantitative parameters of interdental papilla in CP patients. Undoubtedly, further investigations about gene expression and serum level of IL-6 and immunohistochemical changes in these different polymorphisms are necessary for precise interpretation of histological changes.

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Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or non-financial in this article.

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