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IL29 expression in gingival tissues of chronic periodontitis and aggressive periodontitis patients: An immunohistochemical analysis

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ABSTRACT

Background: Interleukin-29 (IL-29) is one of the cytokines which has immunomodulatory properties and might play a role in the pathogenesis of periodontal diseases. The aim of this study was an immunohistochemical analysis of IL-29 in gingival tissues of chronic and aggressive periodontitis.
Materials and Methods: In this cross-sectional study based on clinical evaluation and inclusion and exclusion criteria, 20 patients with generalized chronic periodontitis, 13 patients with generalized aggressive periodontitis, and 20 periodontally healthy individuals were enrolled. Gingival tissue samples were obtained during periodontal flap and crown lengthening surgery in periodontal patients and healthy individuals, respectively. Tissue samples were examined to determine the level of IL-29 expression by immunohistochemistry. The data were analyzed using SPSS and paired *t*-test, ANOVA test, and Tukey's test ($P < 0.05$).
Results: A total of 53 participants (34 females and 19 males) were enrolled in this study. IL-29 expression in the connective tissue of the patient groups was more than the healthy one ($P < 0.001$). In the aggressive periodontitis group, there was a significant increase of IL-29 expression compared to the other two groups, but there was no significant difference between the chronic periodontitis and healthy groups.
Conclusion: According to the results of this study, IL-29 expression was increased in the gingival tissue of aggressive and chronic periodontitis. IL-29 local expression in aggressive periodontitis is higher than the chronic periodontitis and healthy groups, which could suggest the role of IL-29 in the etiopathogenesis of aggressive periodontitis.

Key Words: Aggressive periodontitis, chronic periodontitis, cytokines, interleukin-29, Immunohistochemistry

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INTRODUCTION

Periodontitis is a multifactorial disease that is affected from both bacterial pathogens and host inflammatory responses. This means that, with increased microbial activity, the host response's balance is broken up and inflammation occurs. In case of stimulus continuity, the periodontal tissue is destroyed.[1,2] Periodontitis is an inflammatory process in which the immune system plays an important role in its pathogenesis. Moreover, in fact, it is the periodontal inflammatory response to the bacterial pathogens of the dental plaque and

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their compounds. Not only the inflammatory response causes the resistance of tissue to bacterial invasion and preventing its spread to other tissues but also provides mechanisms led to tissue degradation and will be harmful to the host.[3] According to the classification presented by the American Academy of Periodontology in 1999, based on the scientific and clinical findings, the different forms of periodontitis are divided into three types of chronic periodontitis, aggressive periodontitis, and periodontitis, as a manifestation of systemic diseases.[4]

Actinobacillus actinomycetemcomitans, *Porphyromonas gingivalis*, and *Bacteroides forsythus* are considered as major pathogenic species in periodontal disease.[5] In addition to periodontal pathogenic bacteria, a number of herpesviruses including Epstein–Barr virus type 1, human cytomegalovirus (HCMV), and herpes simplex virus have been associated with periodontal disease as well.[5,6] In tissue involved, biochemical biomarkers were also found such as type interferons, interleukin-28 (IL-28), and IL-29 were induced by viral infection and showed antiviral activity.[7,8] They have been produced in three biologic phases such as inflammation, connective tissue degeneration, and alveolar bone destruction and cause clinical symptoms.[9] They are in the gingival tissue of patients with periodontitis and indicate the role of these factors in the pathogenesis of periodontal disease.[10,11]

One of the cytokines recently studied in relation to periodontal diseases is IL-29. IL-29 production is induced by viral infection and has antiviral and antitumor activities. IL-29 is often produced by dendritic cells in response to viral proteins or toll-like receptor agonists.[8,12,13] IL-29 also increases the production of IL-8, IL-6, and IL-10 through active macrophages and active monocytes.[14] IL-29 levels positively correlated well with clinical parameters such as probing pocket depth (PPD), clinical attachment level (CAL), and gingival index. More the pocket depth and attachment loss, more the IL-29 level was found, which suggests that the increased prevalence of herpesviruses in deeper pockets might induce the increased production of IL-29.[15]

Researchers have conducted several studies on various cytokines that are effective in periodontitis.[5,15-18] Among them, Tabari *et al.* in 2017 examined the expression of tissue visfatin between chronic and aggressive periodontitis and concluded that visfatin may play a role in the etiopathogenesis of the periodontitis.[19] In 2015, Shivaprasad and Pradeep reported the increasing level of IL-29 in the gingival fluid and plasma of patients with periodontitis.[15] Further studies are needed to detect the therapeutic effect of IL-29 in periodontitis. The aim of this study was to evaluate the level of IL-29 expression in the gingival tissue of patients with chronic and aggressive periodontitis using immunohistochemistry.

MATERIALS AND METHODS

This cross-sectional study was performed at the Periodontics Department of Qazvin University of Medical Sciences, Qazvin, Iran, from 2014 to 2015. In this study, 13 patients with generalized aggressive periodontitis (because of rarity), 20 patients with generalized chronic periodontitis, and 20 individuals with healthy periodontium, who visited in the Periodontics Department at Qazvin University of Medical Sciences, were enrolled.

The protocol was approved by the Ethical Committee of Qazvin University of Medical Sciences (# 28/20/9838).

Clinical evaluation

Measurements of the clinical parameters including plaque index (PI),[19] bleeding index (BI),[20] PPD,[5] and CAL[5] were performed at six points around all teeth using UNC probe (Hu-Friedy, USA) by a periodontist. Diseased groups had the diagnosis of generalized chronic periodontitis and generalized aggressive periodontitis established based on the classification of the American Academy of Periodontology and the following criteria:[4]

- Generalized chronic periodontitis: Aged over 35 years, more than 30% of sites with CAL ≥3 mm, and PPD ≥5 mm with bleeding on probing (BOP).
- Generalized aggressive periodontitis: Aged between 18 and 35 years, at least 6 permanent incisors and first molars with at least one site of CAL ≥5 mm, and PPD ≥5 mm with BOP. At least three other permanent teeth had PPD ≥5 mm and CAL ≥5 mm in at least one site.

Periodontally healthy individuals as a control group had BI ≤1, with no evidence of radiographic bone loss, attachment loss, and PPD >3 mm.

The inclusion criteria of the study were good general health, a minimum of 18 teeth, aged over 18 years,

good patient cooperation, no periodontal treatment during the past two years, and no use of orthodontic appliances. Exclusion criteria included history of alcoholism and smoking, infectious diseases, inflammatory bowel disease, rheumatoid arthritis, granulomatous diseases, hypertension, diabetes, atherosclerosis, organ transplantation, or cancer therapy. In addition, patients with the use of glucocorticoids, antibiotics, or immunosuppressant medication during the past 3 months; pregnancy or lactation; and need for antibiotics for infective endocarditis prophylaxis during dental procedures were excluded from the study.

Tissue collection

Before entering the study, a simple informational letter was given to the patients about the description of the tests. Then, informed consent was obtained from them. For each person, a checklist was used to collect demographic and clinical data and to record the biomarker tissue concentration, and written consent was obtained. Then, Phase I periodontal treatment (nonsurgical periodontal therapy) was performed for patients and healthy participants (if needed), and in the Phase I treatment, antibiotics were not given to patients. One month after completion of nonsurgical therapy, clinical indices were measured again at six sites on each tooth where the tissue sample was to be prepared. Afterward, tissue samples were prepared during periodontal flap surgery and crown lengthening surgery in periodontal patients and healthy individuals (which is needed), with an approximate thickness of 1.5 mm and a height of 2–3 mm.[19]

Samples were collected from sites with a probing depth of 5 mm or more and the presence of BOP. In all groups, periodontal tissues were excised by internal bevel and sulcular incisions using a 15c blade (Sterile Scalpel Blade, Stainless Steel, Hu-Friedy). The tissue samples were sent to the pathology laboratory in 10% formalin containers.

Histological evaluations and immunohistochemistry

First, the tissue samples were fixed in 10% formalin, dehydrated in graded alcohol, and washed in xylene. Then, the samples were embedded in paraffin and cut into 3 μm sections. In the standard envision immunohistochemical staining, sections were mounted on poly-L-lysine-coated slides. After deparaffinization and rehydration, the sections were incubated in 0.01M citrate buffer in a microwave oven for antigen retrieval. The slides were then washed in phosphate-buffered saline (PBS) and incubated in 0.5% H_2O_2 in methanol to block endogenous peroxidase activity. IL-29 primary antibody, anti-IL-29 ab38569 (Abcam, Cambridge, UK), was applied for 1 h at room temperature. After rinsing in PBS for 5 min, the sections were incubated with secondary antibody to enhance the sensitivity of the procedure: Goat Anti-Rabbit IgG H and L (HRP) ab6721 (Abcam, Cambridge, UK) for 30 min at room temperature.

After rinsing with PBS, the immunoreactivity was visualized by a diaminobenzidine tetrahydrochloride, (K5007, Dako REAL™ Substrate Buffer and Dako REAL™ DAB + Chromogen, Glostrup, Denmark) as the chromogen for 5 min. Sections were finally counterstained with hematoxylin, cleared, and mounted with PV mount [Figure 1]. Negative controls consisted of PBS instead of primary antibody, and samples of positive controls for IL-29 were human hepatocarcinoma tissue.

All specimens, in ten randomly selected high-power fields (×400), were evaluated using light microscope (Olympus, BX41TF, Tokyo, Japan) by a pathologist in a masked manner (who had no prior knowledge of patient's clinical status). The percentage of colored cells, demonstrating cytoplasmic positivity for IL-29, was estimated and classified: 0 – absence of colored cells, +1 – 25% or fewer positive cells, +2 – 26%–50% positive cells, +3 – 51%–75% positive cells, and +4 – 76% or more positive cells.[20]

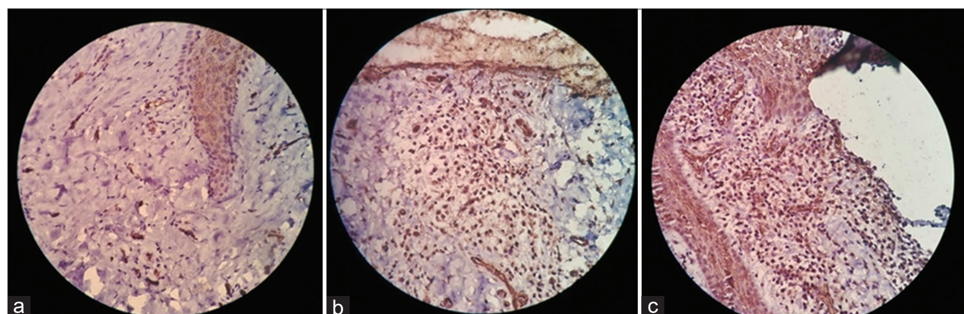

Figure 1: Interleukin-29 expression in the healthy group (a). Chronic periodontitis (b). Aggressive periodontitis (c).

Furthermore, the intensity of staining with these two markers was examined based on the following criteria: 0 – cells with no staining, +1 – the possibility of stained cell recognition with ×40 magnification, +2 – the possibility of stained cell recognition with ×100 magnification, and +3 – the possibility of stained cell recognition with ×400 magnification.[20]

Finally, the staining intensity distribution (SID) score of the stained cell proportion for each field was multiplied by the score of the staining intensity in that field to provide a SID score.[21]

Statistical analysis

Statistical analysis was performed using statistical software, SPSS version 20 (IBM, Armonk, NY, USA). Moreover, the data were analyzed using a paired *t*-test, ANOVA test, and Tukey's test. $P < 0.05$ was considered statistically significant.

RESULTS

A total of 53 participants (34 females and 19 males) were enrolled in this study. They were 20 healthy individuals (12 females and 8 males/mean age: 33.5) and 20 patients with chronic periodontitis (14 females and 6 males/mean age: 42.58) and 13 aggressive periodontitis patients (8 females and 5 males/mean age: 29.3).

Using *t*-test, there was a significant difference between the PPD, BI, and CAL indices in the three groups (in the three PPD, BI, and CAL indices, $P < 0.001$). According to the average score obtained in the healthy and patient groups, these indicators in the patient groups are higher than in the healthy group.

ANOVA test have been shown significant differences in connective tissue IL-29 expression between three groups($P < 0.001$). This amount in the patient groups was more than the healthy one [Table 1].

With Tukey's test, IL-29 expression was significantly different between the healthy and aggressive periodontitis groups ($P < 0.001$) and the aggressive and chronic periodontitis groups ($P < 0.001$). In the aggressive periodontitis group, there was a significant increase of IL-29 expression compared to the other two groups, but there was no significant difference between the chronic periodontitis and healthy groups ($P = 0.638$).

Table 1: Comparison of mean expression of interleukin-29 in connective tissue in three groups with Tukey's test

Group	Mean expression of IL-29	SD	*P* with Tukey's test
Healthy group	3	1.09	<0.001
Chronic periodontitis	3.41	1.32	
Aggressive periodontitis	4.91	1.5	

IL-29: Interleukin-29; SD: Standard deviation

DISCUSSION

Periodontal diseases are initiated by host response to gram-negative microbial biofilms which result osseous and soft-tissue destruction.[5] IL-29 is a cytokine produced by cells infected with viruses and has marked antiviral protection in a wide variety of cells.[22] Many studies have already proven the presence of herpesviruses in periodontal pockets of chronic and aggressive periodontitis.[5,6] The active role of herpesvirus in periodontal diseases is debatable and not been established consistently. Shivaprasad *et al*. hypothesize that elevated levels of IL-29 in periodontitis might be because of the induction of IL-29 production by the increased presence of herpesviruses in periodontal pockets compared to the healthy periodontium.[15] It might be the mechanism of the host to counteract the presence of herpesviruses.

According to the results of this study, IL-29 expression in the gingival tissue increased in aggressive and chronic periodontitis. The amount of IL-29 production in aggressive periodontitis was higher than chronic periodontitis and healthy periodontal tissue. Furthermore, IL-29 levels correlated positively with clinical parameters such as PPD, CAL, and gingival index.

Previous researches showed that herpesviruses such as human cytomegalovirus and Epstein–Barr viruses exist in periodontal pockets of chronic and aggressive periodontitis patients and were found more in periodontal pockets of aggressive forms.[15,23] Notably, herpesviruses are present (in less number) even in clinically healthy patients with shallow sulcus depth.[24]

Shivaprasad *et al*. (2015) evaluated the relationship between the amount of IL-29 in the gingival crevicular fluid (GCF) and its gene polymorphism in patients with chronic and aggressive periodontitis. They observed a high concentration of this in the gingival fluid of patients with aggressive periodontitis.[15]

An increase in the amount of IL-29 in aggressive periodontitis may be due to a multiplication in the frequency of herpesvirus in periodontal pockets in patients with aggressive periodontitis compared to chronic periodontitis.[5,25,26] A previous study had shown that IL-4 and IL-13 could be playing an important inhibitory role in inflammation-induced periodontitis.[27]

Shivaprasad *et al*. said that IL-29 inhibits the production of IL-13 and can be result in reduced inhibitory role of IL-13 in inflammation of periodontitis. In contrast, a view is expressed that IL-13 inhibition will contribute to the induction of B-cell response and will play an active role in maintaining periodontal health.[15]

In a study done by Shivaprasad and Pradeep, IL-6 levels in GCF of aggressive periodontitis patients were more than that of chronic periodontitis patients and least in the healthy group.[28] Furthermore, a study done by Rosalem *et al*. showed elevated levels of IL-8 in GCF of aggressive periodontitis patients compared to that of chronic periodontitis.[29] Increased IL-29 levels in aggressive periodontitis patients might increase the production of pro-inflammatory cytokines such as IL-6 and IL-8 which is lower in chronic periodontitis patients and least in participants with healthy periodontium.

Due to the high level of IL-29 production in aggressive periodontitis compared to chronic periodontitis and healthy periodontal tissue in this study, it can be said that this cytokine may be effective in the etiopathogenesis of aggressive periodontitis.

CONCLUSION

According to the results of this study, IL-29 expression was increased in the gingival tissue in aggressive and chronic periodontitis. Epithelial cells of the pocket wall and connective tissue cells can produce IL-29. IL-29 local expression in aggressive periodontitis is higher than the chronic periodontitis and healthy groups, which could suggest the role of IL-29 in the etiopathogenesis of aggressive periodontitis.

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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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