Caspian Journal of Dental Research

Original Article

The effect of periodontal therapy on IL-17 and IL-23 in Gingival Crevicular Fluid (GCF) of patients with severe periodontitis

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Abstract

Introduction: Cytokines are the important factors in the progression of periodontal diseases. The aim of this study was to find out the effect of the first phase of the periodontal therapy on the amount of existing IL-17 and IL-23 in gingival crevicular fluid in patients with severe periodontitis and in the control group.

Methods: In this clinical trial intervention study,after purifying the parts which were under consideration in 22 patients with severe periodontitis, gingival crevicular fluid was gathered using periopaper located within the gingival sulcus, Then, the first phase of periodontal therapy was carried out and sample taking from the same parts was conducted after 4 weeks. The same phase was carried out on 24 healthy patients (control group). All patients were selected from the Department of Periodontology, Tehran Islamic Azad University Dental Branch. Since the data did not have a normal distribution, therefore, nonparametric tests were used for comparing the groups (Mann-Whitney U Test). A $p \leq 0.05$ is considered as significant.

Results: The results showed that there was a meaningful and significant difference between the IL-17 viscosity before ($p<0005$) and after ($p<005$) the therapy and IL-23 viscosity before ($p<0001$) and after ($p<001$) the therapy between the two groups.

Conclusions: According to the results of the present study, we can conclude that IL-17 and IL-23 have a significant role in the pathogeneses of periodontal disease.

Keywords: Severe periodontitis, IL-17, IL-23

[DOR: 20.1001.1.22519890.2013.2.1.2.5]

[DOI: 10.22088/cjdr.2.1.32]

تاثیرفازاول درمان پریودنتال برمیزان اینترلوکین های ۱۷و۲۳ مایع شیار لثه ای بیماران دارای پریودنتیت شدید

مقدمه: سایتوکاین ها فاکتورهای مهمی درپیشرفت بیماری های پریودنتال هستند. هدف ازاین مطالعه تعیین تاثیرفازاول درمان پریودنتال برمیزان اینترلوکین های ۱۷و۲۳ مایع شیارلثه ای بیماران دارای پریودنتیت شدید و گروه کنترل می باشد.

مواد و روش ها: پس ازپرکردن فرم رضایتنامه بیماران واجد شرایط وارد مطالعه شده ومایع شیار لثه ای ۲۲ بیمار دارای پریودنتیت و ۲۲ بیمارسالم (گروه کنترل) توسط periopaper جمع آوری شد. ۴ هفته پس از انجام فاز اول درمان پریودنتال شامل جرم گیری و آموزش بهداشت دوباره نمونه گیری انجام شده و برای انجام تست ELISA به آزمایشگاه فرستاده شد.

یافته ها: نتایج نشان دادند که میزان اینترلوکین ۱۷ قبل ($p<0005$) و بعد از درمان پریودنتال ($p<005$) و اینترلوکین ۲۳ قبل ($p<0001$) و بعد از درمان($p<001$) بین دو گروه تغییر می یابد.

نتیجه گیری: براساس یافته های این مطالعه می توان نتیجه گرفت که اینترلوکین های ۱۷و۲۳ نقش مهمی درپیشرفت بیماری پریودنتال دارند.

واژگان کلیدی: پریودنتیت شدید، اینترلوکین ۱۷، اینترلوکین ۲۳

[DOR: 20.1001.1.22519890.2013.2.1.2.5]
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Introduction

Periodontitis is the inflammation of tooth supporting tissues caused by a special group of microorganisms; it can lead to the progressive degeneration of periodontal ligament and alveolar bone loss, gingival recession or both by pocket formation, following the stimulation of the immune-system products or lack of therapy. Bacterial products can also cause tissue degeneration by affecting the immune system. Periodontal evaluation and treatment results are usually performed by clinical and radiographic examinations.

Investigating factors like pocket depth (PD), bleeding on probing (BOP) and the height of the alveolar bone crest are the common ways of evaluating periodontitis. The value of the present criteria has been questioned due to the recent progress in the pathogenesis of periodontal disease and the identification of immunologic factors. Some of the new methods of diagnosis are finding specific microorganisms in GCF, evaluating the nature of host response in serum, GCF, saliva, etc.

The products of the inflammatory response, including PMN cells, appear in the gingival crevicular fluid; the periodontium cells are influenced by pathogenic factors like cytokines, prostaglandins and periodontal pathogenic bacteria (1). Cytokines are the important factors in the progression of periodontal disease. The sources of cytokines are monocytes, macrophages and PMNs; they are the important parts of the defense system against bacterial lipopolysacaride and have important role in activating macrophages and differentiating CD8 and CD4.

It is generally agreed that the balance control between CD8 and CD4 (T-helper1 & T-helper2) plays a central role in the development of immunity in periodontal diseases. Th1 and Th2 products cause cellular and hemoral responses, respectively. In primary periodontal lesions, the Th1 cells slightly increase while advanced lesions tend to shift to Th2 cells (2). IL-17 is the product of Th1 and IL-23 is the product of macrophages and dendritic cells (3).

Considering the relationship between the two interleukins, it is expected that following the periodontitis progression, their rates change, as well. IL-23 is a heterodimer consisting of a single 19Kd (p19) chain paired with p40 protein of IL-12. This interleukin is produced by macrophages and dendritic cells in response to microbial infections. IL-23 receptors exist on T cells and natural killer cells (NKCs). Recent studies have shown that IL-23 has a significant role in resistance against some gram negative bacteria like klebsiella pneumonia. Stimulation and differentiation of T cells is a way through which IL-23 affects autoimmune responses.

IL-17 includes a family of 6 cytokines which are structurally interrelated. Some of these factors can cause tissue degeneration and hypersensitivity reactions and some others have significant role in resisting bacterial infections. The structure of these cytokines and their receptors is not the same and is different. IL-17A, F is one of the most specific members of this group and is produced by different cells including T-helper 1, 2 and T cell $CD4^{+}$. The distinction and durability of this interleukin depends on TGFß, IL-23 and cytokines that are generated during the immune response (IL-6).

IL-17 A, F affect endothelial cells and macrophages and lead to the generation of IL-1, TNFβ and other chemokines. It also causes the generation of hematopoietic cytokines which stimulate the production of neutrophils. It seems that interleukin 17, generated by T-cells, is an important index of tissue destruction in autoimmune diseases (3).

In the study of Takashi et al. it is mentioned that the amount of IL-17 in GCF of the patients with acute periodontitis increases (4); however, in the study of Vernal et al, it is said that the amount of this interleukin decreases under the same conditions (5). Considering the disagreements and lack of sufficient studies, particularly in Iran, the aim of present study was to compare the change in the amounts of IL-23 and IL-17 in the gingival crevicular fluid of patients with acute periodontitis and healthy people before and after the first phase of periodontal therapy.

As mentioned before, according to the relationship between IL-23 and IL-17, the null hypothesis is that, the amount of interleukins increases in periodentitis which is an inflammatory process; also, following the first phase of periodontal therapy, the amount of interleukins decreases along with gingival inflammation reduction.

Methods

In this clinical trial, intervention study, data collection was performed by a dentistry student under the superivision of a periodontologist. In order to avoid

bias at the time of conducting the final tests, the samples were coded so that neither the operator, nor the observer would be aware of the nature of the samples under study.

For gathering GCF samples, the subjects were selected from the patients referred for dental treatment to the Dental School of Islamic Azad University Tehran and Shahid Beheshti Dental School in 2008-2009; treatment was carried out by selecting the available samples to enter into the study after obtaining their consent (by signing the enclosed letter of consent) for participating in the study.

The eligible cases included the patients who had generalized severe periodontitis diagnosed by measuring CAL (clinical attachment loss) and examining BOP (bleeding on probing). In the 25 patients with a minimum of 15 teeth including at least 10 posterior teeth; probing was performed on the 6 points of each tooth: mesiobuccal, distobuccal, mesiolingual, lingual and distolingual. In more than 30 areas, clinical attachment loss≥5 positive result of the diagnostic test BOP, was diagnozed as severe chronic periodontitis. In control group, periodontally healthy persons without any clear and stable gingival inflammation with CAL<1 and positive BOP were selected.

The exclusion criteria were patients that used antibiotics and immunosuppressive drugs (cyclosporine A, corticosteroids) and NSAIDS within the last 6 months, smoking during the recent 3 months, drug addiction within the last 3 months, diabetes, pregnancy and systemic diseases affecting the periodontium. Two GCF samples of all cases were gathered from the sites with the following features:

Case group: CAL≥5 mm in patients with severe periodontitis (area of posterior teeth)

Control group: CAL<1 and without any periodontal inflammation in healthy persons (BOP negative)

Moreover, the samples were not gathered from the restored the teeth, teeth with prosthesis restorations, decayed teeth, teeth with occlusal interventions (premature contact in the centric or lateral and protrusive occlusion) and hopeless teeth.

The members of the control group were equal to the case group in number and were selected from the persons referred to the university without periodontal disease. The age and sex of the cases were matched in case and control groups. In order to gather GCF, the selected sites were isolated; saliva and gingival plaques were removed by a rubber cup and a scaler. The periostrips were placed inside the gingival sulcus of the teeth for 30 seconds. The strips with visible signs of blood or saliva were excluded from the study. Then, the periostrips were placed in a microtube and first kept under -20^{0c}.

After collecting the required number of samples, 200µl BPs was added to all microtubes and after that, they were centrifuged at 10000 rpm for 30 minutes; then, the periostrips were removed and the solutions were kept at -70°C until ELISA test were performed. Then, the first phase of periodontal therapy (scaling & root planning and oral hygiene control) was performed and the cases were called for re-sampling after 4 weeks. The said steps for gathering GCF from the areas of taking the first samples were repeated.

After making the required coordination with the laboratory in Shahid Beheshti University, the samples were forwarded for ELISA test and the amount of interleukins 17 and 23 was determined. The data obtained from the laboratory findings were the basis for statistical analyses. After performing Tukey test, it was found that the variables did not have a normal distribution. Therefore, the nonparametric tests were used for comparing the groups (Mann-Whitney U test). Spearman correlation coefficient test was used to determine the correlation between the variables. The criteria for statistical tests were considered according to the p-value≤0.05.

Results

In table 1, descriptive statistical analyses were provided for age, CAL, PD, IL-17 and IL-23 before and after the treatment and the correlation between the following in the case (chronic periodontitis) and control groups, respectively.

The mean IL-17 saturation in case group was 30.27, and 17.29 for control group before the treatment and there was a significant difference between the two groups (p<0.005). The mean IL-17 saturation after the treatment was 27.66 for case, and 19.6 for control group. There was a significant difference between the two groups before and after the treatment (p<0.005).

The mean IL-23 density was 33.77 and 14.08 before treatment and 28.93 and 18.52 after treatment for the case and control groups. Moreover, there was a significant difference between IL-17 and IL-23

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saturation before and after therapy in the case group ($p<0.05$).

However, such a significant difference was not observed in the control group. In case group, there was a significant correlation between CAL and age ($p<0.05$), CAL and IL-17 saturation before therapy ($p<0.005$), IL-17 and IL-23 before therapy ($p<0.005$) and IL-23 saturation before and after therapy ($p<0.005$). However, the correlation between CAL and age was negative and positive in other cases. In control group, there was only a negative correlation between CAL and IL-17 saturation before therapy ($p<0.05$) and no significant statistical correlations existed among the variables of the control group (figure 1, 2).

Table 1. Descriptive statistical analyses for age, CAL, PD, amount of IL-17 and IL-23 before and after therapy

Indices Variables	Number	Minimum	Maximum	Mean	Standard deviation
Age (Case group)	22	45	62	51.45	5.12
Age (Control group)	24	40	55	48.75	4.00
CAL* (Case group)	22	5	7	5.77	0.51
CAL (Control group)	24	0	1	0.42	0.41
PD** (Case group)	22	3	7	5.14	1.16
PD (Control group)	24	1	3	1.83	0.70
IL-17 before therapy (Case group)	22	0.40	1.60	0.93	0.48
IL-17 before therapy (Control group)	24	0.38	1.60	0.54	0.24
IL-17 after therapy (Case group)	22	0.30	0.70	0.40	0.12
IL-17 after therapy (Control group)	24	0.3	0.4	0.32	0.03
IL-23 before therapy (Case group)	22	64.00	290.00	113.00	64.37
IL-23 before therapy (Control group)	24	59.00	89.00	64.25	8.29
IL-23 after therapy (Case group)	22	39.00	62.00	53.41	9.32
IL-23 after therapy (Control group)	24	39.00	59.00	45.71	8.06

*CAL: Clinical Attachment Loss
** PD: Pocket Depth

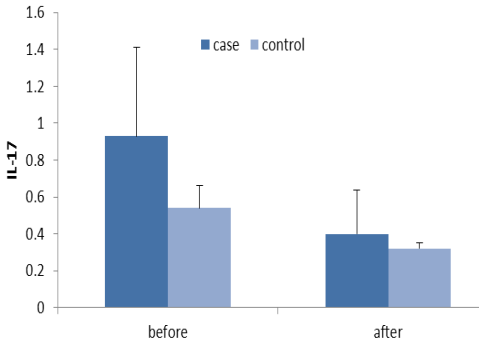

Figure 1. Level of IL-17 before and after treatment

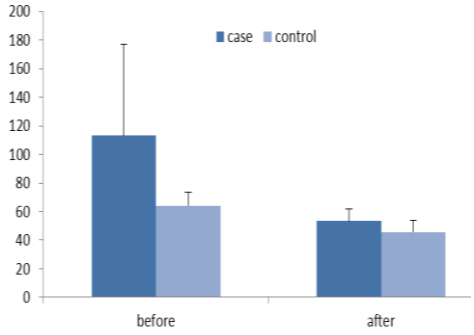

Figure 2. Level of IL-23 before and after treatment

Discussion

The present research revealed that IL-17, and IL 23 amounts are significantly higher in cases with moderate to severe chronic periodontitis. Vernal et al. demonstrated an increase in IL-17 amounts in GCF samples and the surface fluid of gingival cells culture in chronic periodontitis (5). Honda et al. mentioned that the expression of IL-17 and IL-23 in periodontitis cases is relatively higher and they suggested a new idea on periodontal disease pathogenesis considering the role of T cells (6). Ohyama et al. said that IL-17, and IL-23 expressions in periodontal lesions, particularly the regions adjacent to bone loss were significantly higher than the norml sites and they concluded that the activity and stimulation of Th17 cells could be observed in periodontal inflammatory lesions (7). In

the study of Cardoso et al. in 2009, an increase in expression and presence of IL-17, 23, 6, IL-1β and TGF-β in chronic periodontitis was reported. Therefore, they considered an important role for Th17 cells in periodontal disease pathogenesis (8).

Evidently, there is a great compatibility between the findings and results obtained from the mentioned studies. We also found that the presence of IL-17 and IL-23 (as the most important cytokines inducing T cells distinction) was considerable.

Avani et al. mentioned that an increase in IL-18 amounts in GCF was seen in periodontal cases while IL-17 was not observed in any of the cases under study. Therefore, contrary to IL-17, IL-18 as an appropriate inflammatory index, was considered important (9). The difference between the findings and results gained from the mentioned study could be due to the following reasons: a) Difference in laboratory methods: in the mentioned study, GCF samples were gathered by Microcapillary pipettes on the gingival sulcus entry while periopaper or periostrips were used in our study. Furthermore, considering the fact that at least 50µl of samples would be needed for each ELISA test, the method of providing at least 100µl (for measuring IL-17, 18) out of the 1µl volume was not specified in the mentioned study) and since the mentioned study was carried out in India, racial differences might also be significant.

We also found that even after the therapy, the density difference in the case group remains significantly higher than the control group. Moreover, we specified that the difference between IL-17 density before and after therapy and IL-23 density before and after therapy in the case group was statistically meaningful. According to the available sources, no studies have been conducted on the effect of therapy in IL-17 or IL-23 density changes after therapy; thus, studies exclusively performed on the effect of periodontal therapy on other cytokines are presented below.

Thunell et al. expressed that primary periodontal therapy would lead to a decrease in the density of IL-1α, β, IL-2, 3, 6, 7, 8, 12 and CCL5(10). Lopes et al. reported that following root planning and scaling in chronic periodontal cases, no meaningful difference for IL-1β density was seen before and after therapy (11).

As mentioned before, in the present study, different cytokines, preferably those associated with IL-17, were studied and hence, they would have a significant role in the specific immune section. In a study conducted by Lopes etal. only IL-1β was studied IL-1β is produced by almost all inflammatory cells and it has a remarkable role in severe (and not chronic) inflammation. The study of Thunell et al. is similar to our study in the way how cytokines play a role in specific defense and provide Th1 responses (like IL-2, 10). Tsai et al. Reported that IFN-δ density reduction and IL-4 density increase were seen following non-surgical primary periodontal therapy (12).

Although different cytokines are studied in the said study, it might be similar to our study in a way that a kind of reduction in Th1 activity and Th2 cells activity increase is referred to a reduction in IFN-δ density reduction (one of the most important Th1 cytokines) and IL-4 density increase (as one of the most important Th2 cytokines). This implies the change in Th cells balance. We also found that IL-17 and IL 23 densities reduce following therapy.

In addition, in the present study, it was found that there is a meaningful correlation between CAL and IL-17 density before treatment in the control group ($p<0.05$). In the case group, IL-17 and IL 23 densities are meaningfully correlated before and after therapy ($p<0.005$). Since there were no similar articles available in this field, the findings could not be compared with any other studies.

Conclusion

According to the results of the present study, the first phase of periodontal therapy can decrease the amount of inflammatory cytokines and therefore plays a significant role in periodontitis treatment, bone loss reduction and gingival tissue recovery.

Acknowledgments

The authors are grateful to Shahid Beheshti Immunology Institute for performing ELISA tests.

Funding: Personal expenses were used for this study.
Conflict of interest: There is no conflict of interest.

[DOR: 20.1001.1.22519890.2013.2.1.2.5]
[DOI: 10.22088/cjdr.2.1.32]

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[DOR: 20.1001.1.22519890.2013.2.1.2.5]
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