

Original Research Article

Evaluation of Interleukin-23 in Periodontal Health and Disease

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ABSTRACT

Background: Periodontitis is a chronic inflammatory disease that causes tooth-supporting tissues destruction. It has been found that IL-23 is increased at sites suffering from clinical attachment loss, indicating that IL-23 may have a role in the progression of periodontitis.

Objective: This study aim was to evaluate the level of IL-23 in gingival crevicular fluid and blood in the studied groups (localized aggressive periodontitis, chronic periodontitis, gingivitis and controls) selected from Umm Al-Qura University.

Materials & Methods: Forty systemically healthy female subjects were included in this study and divided into 4 groups; 10 subjects with localized aggressive periodontitis, 10 subjects with chronic periodontitis, 10 subjects with gingivitis and 10 control subjects. Gingival crevicular fluid and blood were obtained from all subjects and evaluated using enzyme-linked immunosorbent assay (ELISA).

Results: IL-23 level was statistically significantly higher in gingival crevicular fluid and blood of chronic periodontitis group compared with the control group, gingivitis group and localized aggressive group.

Conclusion: There is a statistical significant increase in the IL-23 concentration of gingival crevicular fluid and blood in chronic periodontitis. Therefore, IL-23 may have a role in the pathogenesis of chronic periodontitis.

Key words: Gingival crevicular fluid, IL-23, Chronic periodontitis, localized aggressive periodontitis.

INTRODUCTION

Gingivitis is a gingival inflammation that doesn't affect the connective tissue attachment. ⁽¹⁾ Plaque accumulation at the gingival sulcus or close to it causes gingivitis. ⁽²⁾ Periodontitis is a chronic infectious inflammatory disease. ⁽³⁾ It can be classified into chronic and aggressive. ⁽⁴⁾ Chronic periodontitis is a periodontal tissue inflammation that is caused by the presence of microorganisms in the dental plaque. Some forms of chronic periodontitis remain stable for years, but some forms keep progressing, which may lead to tooth loss.

⁽⁵⁾ Aggressive periodontitis is a type of periodontal disease characterized by its rapid progressive nature that occurs in clinically healthy individuals. Aggressive periodontitis occurs earlier in life compared to chronic periodontitis and shows a more rapid attachment loss and bone destruction. ⁽⁶⁾ The host response to bacteria occurs according to the nature and control of innate and adaptive immunity. T-cells and B-cells control the adaptive immune response, which is important for the production of antibodies. B-cells collaborate with

activated CD4⁺ T-helper cells to produce antibodies, which are used as a part of the humoral immunity. T-helper-cell population has different cell subsets. ⁽⁷⁾ One of the subsets is T-helper type 1 (Th1) which is characterized by the production of interleukin-12 and interferon gamma. ⁽⁸⁾ Interleukin (IL)-12 is a heterodimeric proinflammatory cytokine made of a p35 light chain and a p40 heavy chain. IL-12 is considered as an important regulator of cell-mediated immunity because it helps in the differentiation of (Th1) cells and evidence shows that IL-12 has a therapeutic effect in infectious diseases and tumors. ⁽⁹⁾ The other T-helper-cell subset is Th2 cells, which manifests in humoral immunity and cause periodontal lesions progression by producing specific interleukins. ⁽⁸⁾ There is another subset of T-helper cells called Th17, which produce IL-17 with the presence of IL-23. Th17 has a potential role in the pathogenesis of cell-mediated tissue damage that could be caused by autoimmunity or immune responses against microbial infection. IL-23 is a part of IL-12 family. It is a heterodimer, which is made up of a p40 subunit and a p19 subunit. The p19 subunit is similar to p35 subunit that is found in IL-12 and the p40 subunit is identical to IL-12 p40 subunit. ⁽¹⁰⁾ Because of these similarities in their structure, it was believed that IL-23 has the same role as IL-12 in regulating Th1-mediated immune responses. However, currently it is clear that IL-23 and IL-12 have different roles on the cell mediated immunity. ⁽⁹⁾ IL-23 is secreted by vigilante dendritic cells and macrophages after they encounter bacteria. IL-23 drives Th17 cells, which produce a proinflammatory cytokine (IL-17). ⁽⁸⁾ IL-17 is involved in inflammation as well as protection of periodontitis. ⁽¹¹⁾ Bone homeostasis is affected by inflammation; during periodontal inflammation osteoprotegerin/RANKL ratio decreases and the protective effect diminish during periodontitis. Interleukin-17 stimulates RANKL that's why it's known to have osteoclastogenic properties. Interleukin-17 can also induce

destruction of both connective tissue and the underlying bone by inducing matrix metalloproteinases expression. B cells differentiate into antibody-secreting plasma cells, which are considered a major source of RANKL during periodontitis. Interleukin-17 increases the chance of bone destruction because it enhances B cells survival and proliferation. ⁽¹¹⁾ The IL-23 and Th17 pathways have been involved in the pathogenesis of rheumatoid arthritis. ⁽¹⁰⁾ Rheumatoid arthritis is considered a good model for chronic periodontitis since it has similar patterns of soft and hard tissue destruction to chronic periodontitis. ⁽¹²⁾ It has been found that patients with periodontal disease have elevated levels of IL-17 and Th17 compared to patients with healthy periodontal tissues. ⁽³⁾ It has also been noticed that increased levels of IL-23 were found in sites with clinical attachment loss, which indicate the potential role of IL-23 in the progression of periodontitis. Ohyama et al. hypothesized that the IL-23-induced Th17 pathway plays an important role in periodontal pathology and examined the expression of cytokines, and related molecules, in periodontal lesions and control sites. The result showed that IL-23 was expressed at significantly higher levels in periodontal lesions than in control sites. These results suggest that the IL-23-induced Th17 pathway is stimulated in inflammatory periodontal lesions. ⁽¹⁰⁾ Allam et al. took biopsies from oral mucosa as well as the coronal and bottom regions of chronic periodontitis and analyzed them using immunohistochemistry, immunofluorescence, flow cytometry and real-time PCR. Taken together, this study suggested that IL-17-producing T cells predominate at severe inflammatory sites of chronic periodontitis and that the amount of Th17 cells in the chronic periodontitis tissue is directly related to the number of IL-23p19-producing Mo-like cells. ⁽¹³⁾ Zhao et al. investigated the levels of Th17/Th1/Th2 cytokines in GCF from 30 chronic periodontitis patients before and after treatment using ELISA. This study results

suggested that Th17 cells play a destructive role in the immune balance of periodontitis and the effect of Th1 cells is not significant while Th2 cells have a protective effect. ⁽¹⁴⁾ Cificibasi et al. investigated serum and GCF levels of IL-17, and IL-23 before and after nonsurgical periodontal therapy in generalized aggressive periodontitis (GAP) patients compared to healthy controls. IL-17 and IL-23 levels were measured by ELISA. IL-23 levels in serum decreased significantly at 3 months as a result of the therapy. Significant reductions were also observed in GCF IL-17 and IL-23, levels at 3 months after therapy. However, the GCF levels of IL-17 and IL-23 in GAP patients were still higher than those in the controls at 3 months. A significant decrease in the local and systemic levels of IL-17 and IL-23 based on the therapy might indicate the role of these mediators in tissue destruction in periodontal tissues. ⁽¹⁵⁾ Himani et al. evaluated the levels of IL-23 in GCF of systemically healthy subjects with healthy periodontium, gingivitis and chronic periodontitis. IL-23 concentration was measured using ELISA. Their results showed that the highest mean IL-23 concentration in GCF was found in patients with chronic periodontitis and the lowest was in patients having a healthy periodontium. These results suggested that the increase of IL-23 concentration in GCF was proportional to the amount of periodontal tissue damage. As the IL-23 concentration in GCF is directly proportional to the severity of the periodontal damage, it can be speculated that IL-23 has a possible role in the pathogenesis of periodontal disease. ⁽⁸⁾ Dutzan et al. examined the mRNA expressions of IL-17 and IL-23 using real-time reverse transcription-polymerase chain reaction in gingival biopsies. Samples that were collected from chronic periodontitis patients showed a significant over expression IL-17, and IL-23p19 that was detected in periodontal disease affected tissues compared to healthy gingival tissues. ⁽¹⁶⁾ Lester et al. evaluated the concentration

of IL-23, IL-17 using ELISA within gingiva from normal sites and sites of chronic periodontal disease. Their results showed that the gingival concentrations of IL-23 and IL-17 were significantly greater at moderate and severe CAL sites than at normal sites. These results suggested that the IL-23/IL-17 immune response might be an important factor in the chronic nature of the disease. ⁽¹⁷⁾ Cardoso et al. investigated the mRNA expression in gingival and alveolar bone samples from healthy patients and patients with chronic periodontitis. Their results showed elevated levels of IL-17 and IL-23 mRNA and protein in diseased tissues as well as the presence of Th17 cells in gingiva from patients with periodontitis. Moreover, IL-17 and the bone resorption factor RANKL were abundantly expressed in the alveolar bone of diseased patients, in contrast to low detection in controls. ⁽¹⁸⁾ This study aimed to evaluate the level of IL-23 in GCF and blood from localized aggressive periodontitis, chronic periodontitis, gingivitis and healthy controls.

MATERIALS AND METHODS

Subjects: Forty systemically healthy females were selected from Umm Al-Qura University, Dental Teaching Hospital.

Inclusion criteria: All subjects were matched regarding the age (20 – 40 years) and presented with at least 15 teeth (excluding third molars) and were free from any systemic diseases.

Exclusion criteria: pregnancy, lactation, current smoking, and smoking within the past five years, periodontal or/and antibiotic therapies in the previous six months, use of mouth rinses containing antimicrobials in the preceding two months, systemic conditions that may affect the progression of periodontal disease and long-term administration of anti-inflammatory and immunosuppressive medications.

A written informed consent was obtained from all subjects.

Study Groups:

This study included 40 subjects who were divided into 4 groups.

10 subjects with localized aggressive periodontitis (LAGP), 10 subjects with chronic periodontitis (CP), 10 subjects with gingivitis (G) and 10 healthy controls (C).

- LAGP group was presented with a pocket depth (PD) 6 mm and clinical attachment level (CAL) 4 mm, and familial aggregation (subjects were asked if they had at least one of their family members presenting or with a history of periodontal diseases).
- CP group was presented with (PD) ≥ 5 mm and (CAL) ≥ 3 mm.
- G group was presented with generalized gingival inflammation with bleeding on probing and no attachment loss.
- C group was presented with clinically healthy gingiva with zero plaque index (PI), (CAL) = 0 mm, and (PD) ≤ 3 mm.

Clinical examination: All subjects were examined and the periodontal condition was assessed based on the following parameters: plaque index (PI), ⁽¹⁹⁾ bleeding on probing (BOP), probing depth (PD) and clinical attachment level (CAL) by a single investigator.

GCF Samples Collection:

One examiner performed all the clinical and radiological examination and sampling site selection. The selection of test site for GCF sample collection was based on the site that exhibited the worst inflammatory manifestations (in patients with gingivitis) and the site with the greatest level of attachment loss (in chronic periodontitis and localized aggressive periodontitis cases) along with radiographic evidence of alveolar bone loss. Supra gingival plaque was removed with cotton pellets and the isolation was obtained with cotton rolls prior to sample collection. GCF samples were collected from two sites into the pocket for 30 seconds using standard

perio papers (Oraflow Inc., Smithtown, NY, USA). Any blood contaminated perio paper was excluded. The collected GCF was immediately transferred to an airtight plastic vial and stored at -20°C until the time of ELISA.

Blood Sample Collection:

Venous blood was collected from all subjects. Serum was isolated by low-speed centrifugation and stored at -20°C until the time of ELISA.

Determination of IL-23:

Samples were analyzed for IL-23 using commercially available human enzyme-linked immunosorbent assay (ELISA) kits. Analyses were performed according to the manufacturer's protocol.

Statistical Analysis: Data was analyzed using SPSS version 17. The comparison between means was tested using t-test and one-way ANOVA test. Pearson correlation was used to check the correlation between IL-23 levels and clinical parameters. All the statistical analyses were performed considering $P < 0.05$ to be significant.

RESULTS

The clinical parameters of the study population are summarized in Table 1. IL-23 mean level in GCF and serum are shown in Table 2. The results of the ANOVA test showed that the difference in levels of IL-23 amongst the GCF and serum groups was statistically significant at $P < 0.05$ (Table 2). IL-23 level in GCF was statistically higher in patients with CP (107.005 ± 53.50 pg/mL) than those in C (27.26 ± 19.74 pg/mL) or G (42.57 ± 4.47 pg/mL) or even LAGP (37.81 ± 17.31 pg/mL).

IL-23 levels in serum were statistically significantly higher in CP group (89.98 ± 76.19 pg/mL) than those in C (35.35 ± 17.22 pg/mL) or G (25.70 ± 20.91 pg/mL) or LAGP (26.24 ± 21.11 pg/mL).

IL-23 level in GCF had a statistical significant positive correlation with PI in CP group and with BOP in LAGP (Table 3). IL-23 level in serum had a statistical significant

positive correlation with CAL in CP group (Table 3). Pairwise comparison showed that IL-23 level in GCF and serum was

statistically significantly different between C and CP, G and CP and between CP and LAgP (P < 0.05) (Table 4).

Table 1. Full mouth clinical parameters of all groups.

	Controls	Gingivitis	Chronic	Localized aggressive
PD(mm)	1.20±0.42	2.90±0.32	5.60±0.70	6.30±0.48
CAL(mm)	0	0	3.40±0.52	4.40±0.52
BOP(%)	1.20±0.67	21.83±11.46	58.75±26.49	34.91±11.92
PI(%)	0	18.48±7.31	49.90±14.35	5.86±2.06

Values are given as mean ± SD.

Table 2: IL-23 level in GCF and serum in all studied groups.

Controls	Gingivitis	Chronic	Localized aggressive	(P-value)
GCF	27.26±19.74	42.57±4.47	107.005±53.50	0.000*
Serum	35.35±17.22	25.70±20.91	89.98±76.19	0.004*

* Significance level P < 0.05.

Table 3 Pearson's correlation coefficient test comparing IL-23 level in GCF and serum with the clinical parameters of all groups.

		Controls	Gingivitis	Chronic	Localized aggressive
PD	GCF	0.43	-0.01	0.38	0.47
	Serum	-0.42	0.22	0.62	-0.01
CAL	GCF	---	---	0.27	-0.32
	Serum	---	---	0.70*	0.19
BOP	GCF	0.22	-0.08	0.378	0.71*
	Serum	0.09	-0.31	0.40	-0.49
PI	GCF	---	-0.05	0.87**	-0.59
	Serum	---	0.19	-0.30	0.54

* Correlation is statistically significant at the 0.05 level (2-tailed).

** Correlation is statistically significant at the 0.01 level (2-tailed).

Table 4. Pair wise comparison of all groups.

GCF	Serum
C Vs G	C Vs G
G Vs CP*	G Vs CP*
C Vs CP*	C Vs CP*
C Vs LAgP	C Vs LAgP
LAgP Vs G	LAgP Vs G
LAgP Vs CP*	LAgP Vs CP*

*Significance level at P < 0.05.

DISCUSSION

Periodontitis is a chronic infectious inflammatory disease. (20) The existence of periodontopathic bacteria in combination with high levels of proinflammatory cytokines and low levels of inflammation inhibitory cytokines and other factors causes the progression of periodontal disease. (20) Cytokines are released during periodontitis as a result of lymphocyte infiltrating the periodontal lesion, which eventually causes periodontal tissue damage. (3) IL-23 is secreted by vigilante dendritic cells and macrophages after they encounter bacteria. IL-23 drives Th17 cells, which produce a proinflammatory cytokine (IL-17). (8) IL-17 stimulates RANKL and because of that, it is known to have osteoclastogenic properties. IL-17 can also induce destruction of both

connective tissue and the underlying bone by expressing matrix metalloproteinases. (11)

IL-23 levels in GCF and serum were statistically significantly higher in CP than C or G and this is in accordance with the results obtained by Himani et al, (8) Ohyama et al (10) and Lester et al (17) Some studies examined mRNA expressions of IL-23 like Dutzan et al. (16) and Cardoso et al. (18) these studies showed a significant over expression of IL-23 in periodontal disease affected tissues compared to healthy gingival tissues. Allam et al. suggested that the amount of Th17 cells in the CP tissue is directly related to the number of IL-23p19-producing Mo-like cells. (13) The present study didn't show a significant difference in IL-23 level in GCF and serum between C and LAgP. Our findings are in accordance with Borch et al. who found no significant difference between C and generalized aggressive periodontitis in the release of IL-17. (21) And in accordance with Ay et al. who investigated IL-17 level in the GCF of healthy and generalized aggressive periodontitis by ELISA, IL-17 levels were not significantly different between their

groups. ⁽²²⁾ Conversely, Cifcibasi et al. showed increased levels of IL-23 in serum and GCF of generalized aggressive periodontitis patients compared to those in healthy controls. ⁽¹⁵⁾ And Shaker and Ghallab investigated IL-17 level in GCF of healthy, chronic periodontal disease and generalized aggressive periodontal disease by ELISA, their study showed a significant increase in the IL-17 level in the generalized aggressive periodontal disease. ⁽²³⁾ This controversy could be explained by the very low concentration of IL-23 in the samples. The population in this study is different than the previous studies, therefore racial variations could explain the different response.

Also, the influence of subject's gender has not been eliminated since our subjects included only females. Also, the type of patients chosen to participate in this study who represented good general health and they were free from any systemic disease and had not taken antimicrobials, anti-inflammatory or immunosuppressive medications and had not performed periodontal therapy in the previous six months or used mouth rinses. Also, pregnancy, lactation, current smokers were excluded from this study. These strict criteria for patient's selection could also account for the non-significant difference in IL-23 level.

Moreover, during periodontal inflammation the proinflammatory and anti-inflammatory cytokines balance shifting affect the intensity, duration, and resolution of inflammation. ⁽²³⁾

There was a statistically significant positive correlation in IL-23 GCF level with PI in CP group and with BOP in LAgP. A statistically significant positive correlation was also found in IL-23 serum level with CAL in CP group.

Also, the variety of IL-23 level among the patients in each group can be explained by the differences in the disease stage at the time of sample collection since the assay method could not determine

whether the inflammation was progressing or in remission.

However, the current findings suggested that IL-23 has a role in the periodontal destruction of chronic periodontitis, further investigation with a larger population and sensitive methods are required to clarify the specific contribution of IL-23 to the pathogenesis of aggressive periodontal diseases.

CONCLUSION

There is a statistically significant increase of IL-23 level in chronic periodontitis. Therefore, IL-23 may have a role in the pathogenesis of chronic periodontitis.

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