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A comparative evaluation of gingival crevicular fluid (GCF) level of interleukin-35 in chronic gingivitis patients and periodontally healthy subjects

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ABSTRACT

Background: IL-35 is an antiinflammatory cytokine. it is a member of the interleukin (IL)-12 family. Regulatory T cells releases IL-35. The aim of this study was to evaluate gingival crevicular fluid (GCF) levels of IL-35 in patients with gingivitis and periodontally healthy subjects.

Methods: GCF samples were obtained from gingivitis patients (n = 15) and periodontally healthy subjects (n = 15). Clinical measurements which were recorded were probing depth pocket depth (PPD), bleeding on probing (BOP), papillary bleeding index (PBI), and modified plaque index (MPI). IL-35 levels in the samples were determined by Enzyme-linked immunosorbent assay (ELISA).

Results: Clinical parameters recorded were significantly higher in the gingivitis group than the healthy group. The GCF IL-35 levels were significantly higher in the healthy group than the gingivitis group.

Conclusion: IL-35 levels were higher in periodontally healthy group as compared to gingivitis group, so it may play a role in suppressing gingival inflammation and maintaining periodontal health.

KEYWORDS : IL-35, gingival crevicular fluid, ELISA, gingivitis

Gingivitis is defined as an inflammation of the gingiva. Several changes in the immune response occur in gingivitis patients like infiltration of the tissues by inflammatory mediators neutrophils, macrophages, B cells, T cells, cytokines and some other mediators. T cells have been found to regulate immune response at the inflammation site by releasing pro-inflammatory and anti-inflammatory cytokines. [1]

Interleukin-12 (IL-12) family contributes to inflammatory response by some processes which are either physiological or pathological. The IL-12 family is linked to the IL-6 cytokine superfamily. IL-12 family includes IL-12, IL-23, IL-27, and IL-35 [2]. IL-35 is inhibitory cytokine generated by regulatory T cells (Treg) populations and it has suppressive effect predominantly. [3]

By arresting mitosis in G1 phase, IL-35 inhibits proliferation of T cells. IL-35 also induces the development of iTr35 cells, a subset of Treg cells. [4] Studies have also demonstrated that IL-35 acts as an immune modulator in variety of disease conditions. IL-35 is highly effective at sites with high inflammation and as an activator of nTreg cells. [5]

Nakijima et al in 2005 stated that in chronic periodontitis increased frequency of T lymphocytes and CD4+CD25+ T cells are present in the inflammatory infiltrate of gingival tissues. Phenotypic markers of Tregs such as Foxp3 were also demonstrated. [6] These results suggest that the regulatory T cells that are found in the chronic lesions must be involved in the modulation of local immune response in chronic gingivitis patients.[1]

Potential markers associated with the severity of disease, and susceptibility of periodontal disease is now a prime area of search and has been receiving considerable attention. Roles of many cytokines have been evaluated in the pathogenesis of periodontal diseases, however the exact role of IL-35 has been studied in very few studies. There is only one study available in literature in which information about plasma, saliva, and gingival crevicular fluid (GCF) levels of IL-35 in periodontally healthy and diseased individuals have been studied. [7]

So, the current analytical study was carried out to with basic aim of comparing the GCF expression levels of IL-35 in periodontally healthy individuals and individuals with gingivitis.

Materials and method

Study Design:

30 systemically healthy individuals were selected for the study.

Study Population:

30 individuals were selected from the outpatient department of Department of Periodontics, Sharad Pawar Dental College, sawangi (M), Wardha. The patients were divided into two groups.

Group A – periodontally healthy group: no attachment loss (AL), probing depth (PD) < 3 mm, bleeding on probing (BOP) scores <20% teeth.

Group B – chronic gingivitis group: no AL, PD <3 mm, and BOP > 20% teeth

Exclusion criteria for the study was patients with history of systemic diseases that could affect the periodontium, patients with history of smoking, pregnant females, h/o periodontal treatment in the past one year, use of any anti-inflammatory and antibiotic drugs in the last 6 months.

An informed consent was signed by the patients and the study protocol was approved by ethical committee of Datta Meghe Institute of Medical Science, Sawangi (Meghe), Wardha. Information concerning their dietary status, mouth cleaning habits, gingival and periodontal status along with other routine clinical data was recorded in the specially designed chart.

Procedure for GCF sampling

GCF samples were taken from two sites with papillary bleeding index (PBI) < 1, PPD < 3,and sites with no BOP in the healthy group;

from two sites with PBI >2, PPD >3, and sites with positive BOP in the gingivitis group. Maxillary sites were selected for sampling so as to prevent saliva contamination. Micro pipettes were used to collect GCF samples. Cotton rolls were used to isolate sampling site to prevent contamination by saliva. The GCF samples from the selected sites were placed into polypropylene tube and freezed at – 70 degree Celsius. The readings were converted to microliters by reference to the standard curve. All GCF samples were stored at – 70 degree Celsius until the laboratory analyses.

Clinical measurements

The subjects were clinically evaluated by Papillary bleeding index (PBI) by Muhlemann, [8] modified plaque index (MPI) by Turskey, Gilmore and Glickmann, [9] Bleeding on probing (BOP), Probing pocket depth (PPD), All measurements were performed by a single calibrated examiner (LS).

Measurement of IL-35 in GCF

ELISA kit was used to measure IL-35 levels. Phosphate-buffered saline (PBS) was used to dilute GCF samples by 300 mL. 100 microliters of dilution of standard blank was added to the wells. 2 hours at 37 degree Celsius temperature was used to incubate plate and then aspirated. After that, 100 mL of detection reagent A and 100 mL reagent B was added and then incubated for 1 hour and 30 minutes at 37 degree Celsius, respectively, along with addition of 90 mL substrate solution, and the plate was incubated for 15 to 25 minutes at 37 degree C. Stop solution of 50 microliter was then added, plate was read in an ELISA reader. The diluted absorbance values of IL-35 (picograms per microliter) in GCF was then multiplied by the dilution agent volume (300 mL PBS). The IL-35 concentrations in a unit volume (picograms per microliter) was then determined by multiplying the GCF IL-35 diluted absorbance values by 300 mL (to account for the dilution) and dividing by the GCF volume (microliters).

Statistical analysis

For all the clinical parameters and IL-35 levels the mean and standard deviation values were calculated. Standard statistical methods were used to analyze mean data. Data were expressed as means and standard deviations. The statistical difference between the groups was tested using Kruskal Wallis ANOVA test, Mann-Whitney U test. Values were considered significant when $P < 0.05$.

Results

Table 1 presents the clinical parameters in the periodontally healthy and gingivitis groups. Clinical parameters were significantly higher in the gingivitis group than the healthy group ($P < 0.001$). Volume of GCF was higher in the gingivitis group than the healthy group. Table 2 shows the GCF levels of IL-35. The GCF concentration of IL-35 was significantly higher in the healthy group than gingivitis groups ($P < 0.05$). (Healthy group = 68.90 pg/micro litre, gingivitis group = 35.02 pg/micro litre)

Discussion

IL-35 has anti-inflammatory properties and is a member of IL-12 superfamily. IL-35 has shown to inhibit proliferation of T cells by arresting mitosis in G1 phase. IL-35 also induces the development of iTr35 cells, a subset of Treg cells. [4] Studies have also demonstrated that IL-35 acts as an immune modulator in variety of disease conditions. IL-35 is highly effective at high inflammation sites and as a potent activator of nTreg cells. [5]

The present study was carried out with the basic aim of comparing the GCF expression levels of IL-35 in periodontally healthy individuals and individuals with chronic gingivitis.

As per our information after evaluating the literature, this is the first study comparing the levels of IL-35 in patients with chronic gingivitis with that of periodontally healthy individuals. However, There are three other studies in the literature evaluating the relationship of IL-35 and chronic periodontitis.

Kalburgi et al (2013) [10] evaluated the levels of IL-35 mRNA in gingival tissues of healthy subjects and chronic periodontitis and aggressive periodontitis patients. It was found that the level of IL-35 mRNA was higher in the chronic periodontitis group as compared to that of the healthy group. Mitani et al. measured the concentration of IL-35 in GCF and gingival tissue expression of EBI3 and IL-12A (heterodimers of IL-35) in healthy subjects and chronic periodontitis patients. The expression of EBI3 and IL-12A in gingival tissues was found to be significantly higher in the chronic periodontitis group than the healthy group. The findings of the present study are consistent with these results, however in the present study the levels of IL-35 are compared between healthy and chronic gingivitis subjects and GCF samples were used as compared to that of gingival tissue used in previous studies.

The study which finds the many similarities with the present study was carried out by Koseglu et al in the year 2016. They evaluated the levels of IL-35 in plasma, saliva and gingival crevicular fluid (GCF) in periodontally healthy individuals, gingivitis patients and chronic periodontitis patients. In this study mean GCF IL-35 level in healthy individuals was 63.19 pg/micro liter, 33.02 pg/micro liter in gingivitis patients and 20.57 pg/micro liter in chronic periodontitis patients. The results in our study are comparable to study by Koseglu. [7]In present study the mean GCF IL-35 levels in healthy individuals were 68.90 pg/micro liter and 35.02 pg/micro liter in healthy individuals.

When these findings are considered it can be stated that the increased level of IL-35 may resolve the inflammation in periodontitis patients. In a study conducted by Wirtz et al.,mice with experimental inflammatory bowel disease were treated with recombinant IL-35. Inflammatory bowel disease and rheumatoid arthritis are chronic inflammatory diseases, as is gingivitis. They found that IL-35 significantly reduced the development of experimental bowel disease. This could indicate that IL-35 has an important role in the control of intestinal immune response. Recombinant IL-35 injections were shown to preserve mice from rheumatoid arthritis. IL-35 may be used an alternative treatment method for inflammatory diseases in the future. [12] These correlations support the hypothesis that the level of IL-35 increases in inflammation sites.

An important limitation of this study is, higher IL-35 levels characterizes healthy periodontal and gingival conditions, which demonstrate it's the anti-inflammatory action. However, IL-35 is not the only anti-inflammatory cytokine and there are other anti-inflmmatory cytokines such as IL-6, Il-4, IL-16. Although IL-35 is the new anti-inflammatory cytokine to be used as a predictor of future disease site. However, the results might alter due to changes in the concentration of other anti-inflammatory cytokines cannot be denied.

Table 1

Comnaprison of mean Clinical Parameters between periodontally healthy group and gingivitis group

Clinical Parameters		Healthy	Gingivitis
PPD	Full mouth	1.3 mm	1.7 mm
	Sampled site	1.5 mm	1.9 mm
PBI	Full mouth	0.73	1.45
	Sampled site	0.77	1.67
PI	Full mouth	0.66	1.58
	Sampled site	0.75	1.72
BOP		8.1 %	50.3%

Table 2

Comparison of mean IL-35 levels in periodontally healthy group and gingivitis group

Mean IL-35 levels	Healthy group	Gingivitis group
	68.90 pg/micro litre	35.02 pg/micro litre

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