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Effect of Non-surgical Periodontal Therapy on the IL-21 levels in Gingival Crevicular Fluid of Patients with Periodontal Health and Disease. A Clinico-biochemical Study

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ABSTRACT

Aim: To investigate the effect of non-surgical periodontal therapy on the levels of IL-21 in gingival crevicular fluid of patients with chronic gingivitis and periodontitis.

Materials and methods: Gingival crevicular fluid samples were collected from chronic gingivitis (n=12), chronic periodontitis (n=12) and controls (n=10). Clinical parameters like Plaque index, Gingival index, Gingival bleeding index, Probing depth and Relative attachment level were recorded at baseline and at the end of six weeks. Patients with chronic gingivitis and periodontitis received non-surgical periodontal treatment (Scaling and Root planing). IL-21 was quantified through an Enzyme-linked Immunosorbent Assay. Data was expressed using the x^2, student t and Mann- Whitney U tests.

Results: The baseline levels of IL-21 in GCF was significant in chronic gingivitis ($p<0.05$) and highly significant in chronic periodontitis patients ($p<0.001$) when compared to controls. Non-surgical periodontal therapy was found to significantly reduce the levels of IL-21 in chronic Periodontitis compared to gingivitis.

Conclusion: With increase in severity of the periodontal destruction from chronic gingivitis to chronic periodontitis a substantial increase in the concentration of IL-21 was observed ($p<0.05$). Non-surgical periodontal therapies have significant impact on its levels and were well correlated with clinical parameters of periodontal tissue destruction.

INTRODUCTION

Periodontal disease results from destruction of periodontal tissues provoked by stimulation of bacterial challenge to host immune-inflammatory response [1,2]. Bacterial plaque is the primary etiological factor for periodontitis, but the majority of destruction of the periodontal tissues concludes from a sequence of immune-inflammatory reactions [3]. Resulting cellular activation collectively contribute to tissue destruction and bone resorption with the release of inflammatory mediators like cytokines, chemokines etc. [4]. These cytokines have been grouped as Th1, Th2, Th17 and T regulatory (Treg) based on their expression pattern and effects on target cells or tissues [5].

Classically, periodontal disease has been explained by Th1/Th2 pathway [6]. Th1 cells are associated with a protective response against bacterial infection whereas Th2 cells predominate in the advanced period of the disease suggesting their role in the destruction and progression of periodontal lesions [7]. Alternatively, host response to pathogenic bacteria has been described by IL-23/IL-17 pathway [8]. This alternative pathway occurs when bacteria induce synthesis of IL-23/17 rather than IL-12, which plays an important role in theinitiation and maintenance of inflammatory response. IL-23/17 pathway as compared to classical pathway has much fine-tuned cellular immune response that bridges the innate and adaptive arm of the immune response [9].

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IL-21 is principally composed by activated T cells which facilitate IL-21 to modulate the acquired and innate immunity [10]. IL-21, a type-I cytokine structurally appears similar to IL-2, IL-4, and IL-15 proteins. IL-21 compete in the immunity against tumor cells [11] and chronic viral infections [12] and with the advancement of immune inflammatory diseases in various organs systems [13,14].

Therefore from the above observation, the aim of present study is to evaluate the role of GCF IL-21 levels in health, disease and post nonsurgical periodontal therapy (NSPT) and further correlate it with the clinical parameters of periodontal tissue destruction.

CLINICAL RELEVANCE

Scientific Rationale for Study

Interleukin-21 (IL-21), an autocrine regulatory factor of Th17 cells plays a key role in inhibiting the function of Th1 and regulatory T cells (Treg).

Principal Findings and Practical Implications

IL-21 in GCF was found multifold times high in periodontal inflammation compared to periodontal health and it was found there was a significant impact on the levels of IL-21 following non-surgical therapy. This study signifies the role of IL-21 as a biomarker for periodontal destruction.

MATERIALS AND METHODS

Patients

A total of thirty four patients (19 males and 15 females, aged 20-60 years) were consecutively enrolled over a six month period (April 2014 to September 2014) from the outpatient department of periodontology, Krishnadevaraya College of Dental Sciences, Bangalore, and Karnataka. Ethical clearance for the study was obtained from the institutional ethical committee affiliated to Rajiv Gandhi University of Health sciences (02-D012-36773). The study was conducted in accordance with the ethical principles described in the Declaration of Helsinki 2008. Procedure of the study was explained and informed written consent was obtained from all the participants before their inclusion in the study. 24 patients having diseased periodontium were grouped into two test groups, i.e., 12 chronic gingivitis and 12 chronic periodontitis patients. 10 healthy individuals were included as control. Inclusion criteria were: patients having more than or equal to 14 functional teeth, systemically healthy patients who had not received any form of surgical and non-surgical periodontal therapy or received antibiotics or non-steroidal anti-inflammatory therapy within the past 6 months of the study.

Chronic gingivitis was defined as having probing depth (PD) less than or equal to 4 mm, relative attachment loss (RAL) less than or equal to 3 mm and more than to 25% sites with gingival bleeding present (BOP) [15].

Chronic periodontitis was defined as having probing depth more than or equal to 5 mm, RAL more than or equal to 8 mm, with more than or equal to 10% sites with BOP positive and evidence of bone loss determined radiographically [16]. Patients who volunteered with no evidence of periodontal disease determined by the absence of increased PD or AL were considered as healthy control group.

CLINICAL MEASUREMENT

Pocket depth (PD), [17] assessments of gingival bleeding i.e., gingival index (GI) [18], dichotomous measurement of supra-gingival plaque accumulation i.e., plaque index (PI) [19], and bleeding on probing (BOP) [20] to the base of the crevice were measured. Recording of clinical parameters were done for all the teeth excluding 3rd molar, on all the six sites for each tooth.

GCF Samples Collection and Analysis

In all three groups, GCF samples were collected at baseline and 6 weeks post treatment. Site selection was based on the highest score recorded for single site in the oral cavity. A single site in each subject that showed increased inflammatory manifestations (chronic gingivitis) and the highest RAL level (chronic periodontitis) was selected for gingival crevicular fluid collection. We selected a single site in each subject to avoid pooling of samples from multiple sites, as periodontitis is a site specific disease. Prior to the collection of GCF samples supragingival plaque was removed with cotton pellets avoiding contact with marginal gingiva. Standard paperstrips were carefully inserted to a depth of approximately 2 mm, into the sulcus/pocket for 30 seconds for collection of GCF. The strips from the selected sites were placed immediately into individual microcentrifuge tubes containing 200 µL of phosphate buffer solution. The tube containing the periopaper strips were vortexed and homogenized for 30 seconds and then centrifuged at 12,500 rpm at 4°C for 5 minutes for eluting the protein embedded in the strips. The samples were stored at -80°C until further analysis.

A calibrated appliance Periotron was used to quantify the GCF sample volume. Readings obtained were converted to actual volume (µL) by reference to a standard curve. A blank gingival fluid collection (perio-col) strip was placed between the periotron fluid meta-sensors and the instrument was adjusted to display a reading of zero. A microlitre syringe was used to accurately deliver

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0.25-1.25 µL fluid (distilled water) to perio-col strip. The strips were immediately placed between the periotron sensors. The periotron score volume displayed the known volume of fluid recorded. This step was repeated three more time with 0.25 µL of test fluid and the average score recorded. The above step was repeated using volume of 0.5, 0.75, 1.0, 1.25 µL, and in every instance the mean periotron value calculated and recorded. Once all the score were obtained, a standard curve was computed with known fluid volume (X-axis) and periotron score (Y-axis). In a similar way GCF volumes (from health, chronic gingivitis and chronic perodontitis) from study patients were obtained automatically with periotron score. The interpolation from standard caliberation graph gave volume of fluid.

ELISA

The kit used monoclonal antibody MT 21.3 biotin and human recombinant IL-21 standard (captured antibody). ELISA reader (spectramax 190 Absorbance microplate reader; molecular devices; Sunnyvale, CA, USA) with 450 nm as elementary wavelength was used to measure the absorbance of the substrate. Conversion of the absorbance readings obtained, into definite volume (pg/mL) were performed using standard reference curve. The protein concentration at each site (pg/mL) were determined by dividing the total amount of IL-21 (pg) by gingival crevicular fluid volume (µL) and subsequently the (pg/µL) values were converted into (pg/mL).

Periodontal Treatment Protocols

Clinical parameters and collection of GCF samples for all patients was done at baseline. Following, all patients received thorough oral hygiene instructions; a full mouth supra-gingival scaling was done. Non-surgical periodontal therapy for group III was performed in 2-3 appointments.

STATISTICAL ANALYSIS

Proportions were compared using Chi-square test (χ^2) test of significance. Normality of data was tested using Shapiro-Wilk test. A student t-test was performed to determine pre and post treatment difference values. One way analysis of variance (ANOVA) was used to test the difference between the groups. Comparison of the biochemical and clinical parameters were performed using Kruskal-Wallis non-parametric test. $P<0.05$ was considered as statistically significant.

RESULTS

Clinical parameters of patients with chronic gingivitis, chronic periodontitis and healthy volunteers included in this study are summarised in **Table 1**. The healthy control group exhibited significantly lower values in all clinical periodontal measurements. There was statistically difference in age and sex between the groups. Highly significant ($p<0.05$) difference with a higher percentage of sites with plaque, gingival index (GI) and gingival bleeding index (GBI) as well as PD were observed in chronic gingivitis and periodontitis when compared to control group. After nonsurgical periodontal treatment highly significant difference was noted in all clinical parameters (PI, GI, BOP) except PD in group II (chronic gingivitis) **(Table 2)**, whereas in group III (chronic periodontitis) clinical parameters PI, GI, PD, BOP and RAL showed highly significant difference **(Table 3)**. These differences were indicated through improved clinical symptoms.

IL- 21 levels in gingival tissues from periodontal sites of chronic gingivitis and Periodontitis patients were found to be significantly higher than in gingival tissues from periodontal sites of healthy individuals. Before non-surgical periodontal therapy the levels of IL- 21 in chronic gingivitis patients was 3.5 fold more than the control group and 28 folds higher in chronic periodontitis patients when compared to healthy individuals **(Table 4)**. After treatment, the levels of IL-21 in GCF were down-regulated in both the test groups. This highly significant decrease in the levels of IL-21 was noted in group III chronic periodontitis patients through their improved clinical response. In group II patients, preoperative and post-operative difference in IL-21 was non-significant. In group III, when comparison was done between the levels of IL-21 pre and post operatively a highly statistical reduction was observed ($p<0.001$) **(Table 5)**.

Table 1. Clinical characteristics of test and control group.

Charateristics	Chronic periodontitis (n=12)	Chronic gingivitis (n=12)	Healthy volunteers (n-10)
Age (mean year)	44.4 ± 4.42	30.0 ± 2.58	25.2 ± 2.12
Female %	50	32	50
PI %	2.14 ± 0.35	1.81 ± 0.46	0.31 ± 0.06
GI%	2.24 ± 0.35	1.71 ± 0.46	0.31 ± 0.04
GBI%	40.1 ± 6.95	37.6 ± 3.52	8.30 ± 0.98
PD (mm)	6.75 ± 1.13	2.50 ± 0.52	1.60 ± 0.51
RAL(mm)	11.42 ± 1.44	---	---

n: no. of patients; PI: Plaque index; GI: Gingival index; GBI: Gingival bleeding index;
PD: Probing depth; RAL: Relative attachment level

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Table 2. Periodontal clinical parameters of chronic gingivitis pre and post non-surgical therapy.

Clinical parameters	Before treatment	After treatment
Plaque index (PI)	1.81 ± 0.59	0.51 ± 0.43*
Gingival index (GI)	1.71 ± 0.46	0.84 ± 0.53*
Gingival bleeding index (GBI)	37.63 ± 3.57	26.6 ± 2.04
Probing depth (PD)	2.50 ± 0.52	2.25 ± 0.45*
Highly statistically significant i.e., p<0.001; NS-non significant		

Table 3. Periodontal clinical parameters of chronic periodontitis pre and post non-surgical therapy.

Clinical parameters	Before treatment	After treatment
Plaque index (PI)	2.14 ± 0.35	1.54 ± 0.32*
Gingival index (GI)	2.24 ± 0.35	1.60 ± 0.29*
Gingival bleeding index (GBI)	40.1 ± 6.95	27.5 ± 2.70 (NS)
Probing depth (PD)	6.75 ± 1.13	4.33 ± 0.98*
Relative attachment level (RAL)	11.42 ± 1.40	9.50 ± 1.24*
Highly statistically significant i.e., p<0.001; NS-non significant		

Table 4. Mean IL-21 levels in chronic periodontitis, chronic gingivitis and healthy patients.

Characteristics	Chronic Periodontitis	Chronic Gingivitis	Healthy
IL-21	64621.667 ± 11411.219	7865.700 ± 1288.868	2245.900 ± 771.718

Table 5. Effect of scaling and root planning on IL-21 levels in chronic periodontitis and chronic gingivitis patients.

Characteristics	Pre treatment (IL-21)	Post treatment (IL-21)	P value
Chronic Gingivitis	7865.700 ± 1288.868	7215.34 ± 1125.553	p=0.247(NS)
Chronic Periodontitis	64621.667 ± 11411.219	7390.2 ± 1982.429	p<0.001*

DISCUSSION

IL-21 is a proinflammatory cytokines expressed by activated Th1 and Th17 cells (proinflammatory lineage) functions via receptor IL-21R, a type I cytokine receptor [21]. IL-21 expressed at site of inflammation in varying quantities is mainly secreted by activated CD4+ T cells and natural killer T cells (NKT). The activities of IL-21 have effects on both lymphoid and myeloid lineages [22]. IL-21 is a helper cytokine that orchestrate a potent innate response. It has effect on leukocyte subsets, such as antigen presenting dendritic cells and phagocytic macrophages to a lesser extent compared to T cells, NK cells and B cells. IL-21 regulates IgE production and eosinophillic requirements [23]. IL-21 activates the JAK/signal transducer and activator of transcription (STAT) pathway [24,25]. Two other major pathways have been associated with IL-21 signalling: mitogen activated protein kinase (MAPK) and phosphoinositol-3 kinase (PI-3K), thus interlinked to cellular functions [26].

IL-21 plays a pivotal role in the pathogenesis of various inflammatory systemic diseases like inflammatory bowel disease [27], rheumatoid arthritis [28] and colitis [29]. Th1/Th2/Th17 paradigm has offered an explanation of periodontal pathogenesis on the principle of periodontal disease activity dictated by a complex interplay between the host immune system and the periodontal pathogens. Thus IL-21 plays a paramount role in inflammation and its overproduction leads to complication of the inflammatory response intensifying tissue damage. Since rheumatoid arthritis and periodontitis has been associated with one another [28] increasing evidence has led to the hypothesis that dysregulation of IL-21 signalling pathway during microbial infection may be a determinant periodontal disease activity. This has given way for several studies to elucidate the role of IL-21 cytokine in the pathogenesis of periodontal disease in subjects with & without systemic involvement. There are few observational studies [30-34] and one interventional study regarding the role of IL-21 cytokine in periodontal disease progression [35]. However, to date no similar study on the role of IL-21 in periodontal health & disease has been performed and we have considered investigating the role of IL-21. The discovery of Th17 related cytokine IL-21 in patient with chronic periodontitis has however given way to a new research area for the pathogenesis of many inflammatory diseases where IL-21 plays a paramount role in inflammation [35].

In the our study, IL-21 levels in chronic periodontitis was found to be 28 folds more than the healthy controls and 8 folds more than in chronic gingivitis. We have shown the expression of IL-21 levels in chronic gingivitis, chronic periodontitis and have correlated its level with clinical parameters before and after scaling and root planning with healthy controls. The results have demonstrated that IL-21 was down regulated ($p<0.05$) in both test groups following non-surgical therapy.

These results were similar to the findings that GCF IL-21 levels were over expressed in patients with untreated chronic periodontitis suggesting their role in tissue destruction depicting chronic periodontal disease [26,32]. Non-surgical periodontal treatment is the most basic and effective method for majority of periodontal patients. After non-surgical periodontal therapy, a statistically significant reduction was observed in the IL-21 levels and in all the clinical parameters (PI, GI, GBI, PD, RAL) implying that this cytokine might play a role in the development of periodontal inflammation. This is the first study on the effect of non-

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surgical periodontal therapy on the expression of IL-21 in chronic gingivitis patients. After treatment in chronic gingivitis group, on re-examining the patients 6 weeks later, clinical parameters (PI, GI, GBI) except PD showed statistically highly significant reduction. We found IL-21 levels in chronic periodontitis was drastically reduced following scaling and root planning and was also demonstrated by improved clinical response suggesting its major role as destructive cytokine in periodontal disease progression. All the clinical parameters including PI, GI, GBI, PD, RAL were decreased with those in pre-treatment, consistent with the change in IL-21 levels. Results showed positive co-relation of IL-21 with the clinical parameters (PI, GI, GBI, PD, and RAL) in chronic periodontitis group.

Currently there is only one interventional study on the effect of non-surgical periodontal treatment on GCF IL-21 levels in chronic periodontitis patients [2]. There are very few observational studies of IL-21 levels in saliva of patients with chronic periodontitis [33,34]. On the basis of our result we can deduce that non-surgical periodontal therapy down regulated the expression of Th17 related cytokine (IL-21) thus contributing to the relief of periodontal inflammation.

Hence within the limitations of our study it can be summarized that with the variations in periodontal inflammation, there is a significant increase in the local concentration of IL-21 in gingival crevicular fluid. In our study, intervention therapy was found to be beneficial in both the test groups. It can be postulated that greater the extent of periodontal destruction, the higher the gingival crevicular fluid IL-21 concentration that signifies the proinflammatory role of IL-21.

CONCLUSION

Significant increase in the concentration of IL-21 in gingival crevicular fluid is observed with increase in periodontal destruction. Non-surgical periodontal therapy aided in decrease of GCF IL-21 levels in clinical gingivitis and chronic periodontitis. However further longitudinal study; elucidating the molecular mechanisms of IL-21 in the inflammatory process in periodontal tissues, with larger sample size would be required to validate the role of IL-21 as a biomarker for periodontal destruction.

Conflict of Interest and Source of Funding

The study received no financial support. We declare that there is no conflict of interest concerning the contents of the study. This study has been self-supported.

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