ISSN: 2581-5989
PubMed - National Library of Medicine - ID: 101738774

International Journal of Dental Science and Innovative Research (IJDSIR)

IJDSIR : Dental Publication Service

Available Online at: www.ijdsir.com

Volume – 2, Issue – 2, March - April - 2019, Page No. : 516 - 521

Effect of Phase I periodontal therapy on Interleukin-33 levels in periodontal disease.

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Type of Publication: Original Research Paper

Conflicts of Interest: Nil

Abstract

Background: Interleukin-33 (IL-33) is a novel member of the IL-1 superfamily. It has been identified as a dual function protein and a ligand for ST2 receptor, which plays a pivotal role in osteoclastogenesis. The aim of this study was to evaluate the IL-33 levels in gingival crevicular fluid (GCF) and plasma in subjects with chronic periodontitis, and to evaluate the effect of Phase I or non-surgical periodontal therapy on the GCF and plasma IL-33 levels.

Materials And Methods: 60 subjects (age range: 30–56 years)) were selected and divided into groups based on the gingival index, probing pocket depth, clinical attachment level, and radiologic parameters (bone loss): Group H (30 subjects with healthy periodontium), Group CP-BT (30 subjects with chronic generalised periodontitis), while, Group CP-BT patients after 8 weeks of the treatment (scaling and root planing, SRP) constituted Group CP-AT. GCF samples and plasma samples were collected to estimate the levels of IL -33 using enzyme linked immunosorbent assay kit.

Results: The mean IL-33 concentration in GCF and plasma was higher for Group CP-BT compared to Group H and decreased after non-surgical periodontal therapy in chronic generalised periodontitis group (CP-AT). The difference between them was statistically significant ($p<0.001$).

Conclusion: IL-33 can be considered as an "inflammatory marker" of periodontal disease and health and has the potential to be a therapeutic target in the treatment of periodontal disease.

Keywords: Periodontitis, Gingival Crevicular Fluid, Plasma, Interleukin.

Introduction

Periodontitis is an inflammatory disease that results in the destruction of the periodontal apparatus of the teeth. It is a result of the complex interplay between the microbial factors and the host response. Cytokines are chemical mediators produced by the inflammatory and immune cells useful in mediating inflammation and carrying out specialized functions such as chemotaxis.

Interleukin-33 (IL-33) is a recently identified cytokine included in the IL-1 superfamily, due to its structural homology to other members and its functioning as a ligand to the ST2 receptor, an orphan receptor belonging to the IL-1R family.[1] It has been found to have a dual function: that of an alarmin, which alerts the cells of the innate immune system during an infection or mechanical injury. And secondly, it is an intracellular nuclear factor,

which represses gene expression by facilitating chromatin compaction.[2]

IL-33 has a repertoire of activities, both pro- and anti-inflammatory, in different conditions and diseases. IL-33 is expressed in rheumatoid arthritis (RA) synovium and is elevated in serum and synovial fluid of RA patients.[3,4] IL-33 is produced in response to TNF-α through NF-κB (Nuclear factor – κB) and MAP-K (mitogen activated protein kinase) pathways.[1] IL-33 expression is also up-regulated in intestine from patients with inflammatory bowel disease(IBD)[5] and in inflamed salivary glands from patients with Sjogren's syndrome.[6]

IL-33 is associated with the endothelial cells in the inflamed tissues of patients with rheumatoid arthritis and Crohn's disease, where it is a nuclear factor which regulates transcription.[7] The facts that increased microvasculature is a prominent histological finding in periodontitis and that IL-33 has a role in promoting Th2 responses,[1,8] (such as those in destructive periodontitis), warrant a detailed investigation of its role in periodontitis.

In the light of the above facts, the present study is designed to estimate and compare the concentration of IL-33 in GCF and plasma in periodontal health and chronic generalised periodontitis affected subjects before and after non-surgical periodontal treatment.

Material And Methods

Sixty subjects (n = 60; 31 men and 29 women; age range: 30–56 years) were selected from outpatient pool, Periodontology department , Government Dental College and Research Institute, Bengaluru, Karnataka, India. Ethical clearance for the study was obtained from the institutional ethical committee and review board, Government Dental College and Research Institute, Bangalore and was in accordance with the Declaration of Helsinki 1975, as revised in 2013. Written informed consent was obtained from those who agreed to participate voluntarily in this study. Patients with chronic inflammatory diseases like RA and IBD, respiratory diseases such as chronic obstructive pulmonary disease, asthma and bronchitis, immunodeficiency state like those infected by Human Immunodeficiency virus, pregnancy, giant cell tumours of the bone, coronary heart disease, hypertension, aggressive periodontitis, diabetes mellitus, Nicotine usage, alcoholism and who had taken steroids, contraceptives, anti- inflammatory drugs, antibiotics or any periodontal treatment in the preceding six months were excluded from the study.

Full mouth periodontal examination was done clinically for all subjects along with periapical radiographs using long cone technique. To dierentiate chronic generalised periodontitis subjects from periodontally healthy group radiographic bone loss was recorded dichotomously (presence or absence). No further demarcation was attempted within the chronic generalised periodontitis group based on the extent of alveolar bone loss. Based on the gingival index (GI), [9] pocket-probing depth(PPD), clinical attachment level (CAL) and radiograph evidence of bone loss. Subjects were categorized into three groups. Group H: 60 samples (30 GCF and 30 plasma samples) from 30 healthy subjects with clinically healthy periodontium, with a GI score of 0, a PPD of ≤ 3 mm and CAL of 0, with no evidence of bone loss on radiograph. Group CP: 60 samples (30 GCF and 30 plasma samples) from 30 chronic generalised periodontitis subjects who had signs of clinical inflammation, a GI score of ≥1, a PPD of ≥5 mm and CAL≥1 with radiographic evidence of bone loss. Patients with chronic generalised periodontitis were treated with a nonsurgical periodontal therapy (i.e. scaling and root planing) and GCF samples were collected from the same sites 8 week after the treatment along with plasma samples to constitute Group CP-AT (the after-treatment group).

Selection of site and GCF Fluid collection

Group allocation and sample site selection was performed by an examiner (ARP). All the clinical parameters such as PPD, CAL, and GI were measured using a University of North Carolina-15 periodontal probe (Hu-friedy, Chicago, IL, USA) and the radiographic evaluation conducted by a calibrated examiner (SPB). GCF samples were collected at the subsequent appointment to avoid contamination of GCF with blood associated with the probing of inflamed sites. The sites showing the greatest CAL and signs of inflammation, and bone loss, were selected for sampling in Group CP subjects. To ensure the collection of an adequate amount of GCF, multiple sites were sampled in the periodontally healthy group. The selected site was cleaned first, isolated, and air dried using sterile cotton rolls, and using a Universal curette (Hufriedy, Chicago, IL, USA) the supragingival plaque was removed gently to avoid contamination of the paper strips (Periopaper, Ora Flow Inc., Amityville, NY USA).

The paper strips were placed gently at the entrance of the gingival sulcus/crevice until the light resistance was felt. [10] The GCF sample was collected atraumatically and the absorbed volume in each strip was quantified by an electronic device (Periotron 8000, ProFlow Inc., Amityville, NY, USA) where a digital readout was converted to microliters using a software (MLCONVERT.EXE software version 2.52, Ora Flow, Amityville, NY, USA). Samples that were suspected to be contaminated with blood and saliva were excluded from the study. After collection of the gingival fluid, four periopaper strips/site were pooled, immediately transferred to micro centrifuge tubes containing 400 μL of phosphate buffer saline and stored frozen at -80^0 C for subsequent analysis. Scaling and root planing was performed and oral hygiene instruction were given for Group CP subjects. After 8 weeks, the CP group patients were recalled for collection of after-treatment biofluid samples.

Blood Collection and plasma extraction

Using a 2 ml syringe, 20-gauge needle two millilitres of blood was collected from the antecubital fossa by venepuncture and immediately transferred to EDTA coated vials. The plasma was separated from blood by centrifugation at 3000 rpm for 5 min and was immediately transferred to a plastic vial and stored at -80^0 C until the time of assay.

IL-33 Analysis

The GCF and plasma samples were assayed for IL-33 levels using a highly sensitive enzyme linked immunosorbent assay (ELISA) kit (Human IL-33 DuoSet ELISA CatLog Number: DY3625, R & D systems, USA) according to the manufacturer's instruction and samples were run in duplicate and mean was taken into consideration.

Statistical analysis

Statistical analyses was done using SPSS statistical software. (SPSS version 18.5, Chicago, IL, USA) Power calculations were performed before initiation of study. Based on the power of the study, grouping and sample size was determined at the 95% confidence interval ($p<0.05$). The one way ANOVA test was performed to compare the IL-33 concentration in GCF and plasma between the three study groups. Paired t test was used to determine the difference in IL-33 concentration and clinical parameters in the CP group and CP-AT groups. Pair-wise comparison of IL-33 concentration in GCF and plasma between the Groups H and Group CP and Group H and Group CP-AT was performed using Tukey test. p values < 0.05 were considered statistically significant. The intra group correlation of GCF and plasma concentrations of IL-33 with clinical parameters was performed using Spearman's rho Correlation test. The

mean intra-examiner standard deviation of differences in repeated PPD and CAL measurements was obtained using single passes of measurements (correlation coefficients between duplicate measurements; r = 0.95).

Results

The descriptive statistics of the study population are tabulated as mean±SD. (Table 1) All the collected GCF and plasma samples tested positive for the presence of IL-33. The mean IL-33 concentration in GCF in Group H, Group CP and Group CP-AT were 407.13±46.805 pg/ml, 480.13±54.674 pg/ml and 418.72±48.605 pg/ml, respectively. The mean IL-33 concentration in plasma in Group H, Group CP, and Group CP-AT were 15.72 ± 4.515 pg/ml, 23.21 ± 7.902 pg/ml and 16.25 ± 3.61 pg/ml, respectively. The mean IL-33 concentration in GCF and plasma was observed to be the highest in Group CP and least in Group H.

A statistically significant difference ($p<0.001$) in the mean GCF and plasma concentration of IL-33 was seen between the Groups H and CP but not between the groups H and CP-AT. (Table-1). There was a statistically significant reduction in the mean PPD, CAL and IL-33 concentration in GCF and plasma in the CP group after treatment ($p< 0.001$) (Tables-2 and Tables-3). There was no statistically significant correlation between clinical parameters and IL-33 concentration in GCF and plasma. (Table-4).

Discussion

IL-33 is the newest member of the IL-1 superfamily which has been observed to mediate its biological effects via the ST2 receptor to activate NF-κB and MAP kinases. Also, IL-33 can drive the production of Th2-associated cytokines (i.e., IL-5 and IL-13) from in vitro polarized Th2 cells and can induce the expression of IL-4, IL-5, and IL-13 and promoting severe pathological changes in mucosal organs.[1]

IL-33 plays an important role in inflammatory diseases including hypersensitive diseases like asthma, autoimmune diseases like rheumatoid arthritis, cardiovascular diseases like heart failure and neurodegenerative diseases like Alzheimer's disease.[] IL-33 is abundantly expressed in the synovial membrane and elevated in serum and synovial fluid of RA patients and is reduced following anti-Tumour necrosis factor (TNF) treatment.[3]Fibroblasts in periodontitis are, similarly to in RA, active cells highly important in the inflammatory process via modulation of myeloid cells leading to osteoclast activation and bone destruction. The pro-inflammatory cytokine TNF-α regulates IL-33 expression in synovial and gingival fibroblasts, by a mechanism involving NF-kB and MAPK.[11]

Numerous studies have reported contradictory results in assessing levels of IL-33 in GCF, plasma and saliva in periodontal disease.[12,13,14] Also, there are no reports published in literature till now which have investigated IL-33 concentration in GCF and plasma in periodontal disease before and after non-surgical treatment and compared these levels with healthy subjects. The present study is thus the first to assess the role of Phase I periodontal therapy on IL-33 concentration in GCF and plasma in periodontal disease.

The results of the current study indicate that concentration of IL-33 in GCF and plasma is increased in periodontal disease as compared to health. After treatment by non-surgical periodontal therapy and instituting strict oral hygiene measures the mean concentration of IL- 33 in GCF and plasma in chronic periodontitis group reduced to nearly the same levels as the healthy group.

In the present study the influence of age and gender of the subjects on the IL-33 levels was diminished by including nearly equal number of males and females in each group

and selecting the subjects within the specified age group of 30–56 years. GCF was collected using the absorbent filter paper strips in the study. The advantages of the technique are that it is non-traumatic, quick and easy to use, and allows collection from individual sites.

Koseoglu et al. found in their animal study that the experimental periodontitis group exhibited increased expression of IL-33 and RANKL as compared with the healthy group.[15] Saglam et al. reported that the increase in total amounts of GCF IL-33 may have a role in the pathogenesis of periodontal disease.[16] Thus, the increase in GCF and plasma concentrations of IL-33 from periodontally healthy subjects to chronic generalised periodontitis and decrease after non-surgical periodontal treatment in the present study can be attributed to its proinflammatory properties.

The concentration of IL-33 in our study was higher in GCF than in plasma, which could be explained by local production of IL-33 in diseased periodontal tissues, suggesting that IL-33 levels might serve as a marker for local disease activity. The increase in IL-33 could also be the result of destruction of local periodontal tissues in diseased sites.

To our knowledge this is the first study evaluating the effect of nonsurgical periodontal therapy on IL-33 levels in Indian population. Further multicentric, longitudinal, prospective studies can be carried out along with other alarmins like IL-1α, high-mobility group box protein-1 (HMGB-1) and regulators of osteoclastogenesis such as the Receptor activator of nuclear factor (NF)-kB/ Receptor activator of nuclear factor (NF)-kB-ligand/ Osteoprotegerin (RANK/RANKL/OPG) to confirm the findings of the study and better understand the role of IL-33 as an inflammatory marker in periodontal health and disease.

Conclusion

Within the limitations of the present study, it can be postulated that with increase in the amount of periodontal destruction there is substantial increase in the concentration of IL- 33 both in GCF and plasma. Also, treatment of periodontal disease leads to a proportional reduction in GCF and plasma levels of IL-33. Thus, IL-33 can be considered as potential inflammatory marker of periodontal disease. Furthermore, the finding that the concentrations of IL-33 in GCF are significantly greater than those in plasma samples suggesting a possible local IL-33 synthesis and/or storage within the periodontium. However further multicentric, longitudinal, prospective studies with larger sample size and long-term follow-up are needed to validate IL-33 as an inflammatory marker in periodontal disease as well as its possible therapeutic applications in periodontal health and disease.

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