Assessment of gingival crevicular fluid levels of IL33 and sST2 in periodontitis patients with stage I-III disease

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Abstract

Introduction: Patients with chronic periodontitis exhibit significant interleukin-33 (IL-33) expression in their periodontal tissue. However, it is debatable whether chronic periodontitis patients with gingival crevicular fluids (GCFs) have increased levels of IL-33. The ligand of the ST2 (suppression of tumorigenity) receptor is IL-33. The Toll-like receptor/IL-1R superfamily includes the ST2 receptor, which exists in two distinct versions; soluble ST2 (sST2), which is secreted, and ST2L, as a transmembrane form. In addition to measuring clinical parameters and correlating them with the levels of IL33 and sST2 in periodontitis patients, the goal of this study was to evaluate the levels of these two biomarkers in the GCF of patients with (stage I, II, and III) periodontitis relative to healthy controls.

Objectives: The present study aimed to evaluate the levels of IL33 and sST2 in the GCF of individuals with periodontitis (stages I, II, and III) compared to those in healthy controls, and to assess clinical parameters and compare them to the sST2 and IL33 levels in patients with periodontitis.

Materials and Methods: A total of 162 participants participated in the present study. Clinical measurements were made for the probing pocket depth (PPD), plaque index (PI), clinical attachment level (CAL), and bleeding on probing (BOP). The GCF was collected from each patient. To measure IL33 and sST2 levels, enzyme-linked immunosorbent assays (ELISAs) were used.

Results: When comparing the periodontitis group to the healthy control group, the levels of IL33 and sST2 were greater ($P < 0.001$). Only in the group with stage II periodontitis was there a significant association between PPD and sST2, whereas no significant correlation was detected between IL33 and periodontal markers.

Conclusion: Compared to those of healthy controls, the GCF of periodontitis patients exhibited increased levels of IL33 and sST2. This finding implies that the pathogenesis of periodontitis and periodontal health are both influenced by IL33 and sST2.

Key point

When the ST2 (suppression of tumorigenity) receptor is bound to interleukin-33 (IL-33), the IL-33/ST2L signaling cascade results in the transcription of inflammatory genes, which triggers the release of inflammatory cytokines/chemokines and an immune response. In contrast to ST2L, soluble ST2 (sST2) binds to IL-33 with great vigor. Consequently, the interaction between IL-33 and sST2 blocks the IL-33/ST2L system.
The tissue-derived nuclear cytokine interleukin-33 (IL-33) is related to the IL-1 family and is expressed in fibroblast-like, epithelial and endothelial cells during inflammation and homeostasis. It serves as an alert system (alarmin) in which immune cells carrying the ST2 receptor (IL-1RL1) are produced in response to cells or damage to tissue (7).

IL-33 has been suggested to have three possible roles in periodontal disease; acting as a systemic cytokine, acting as a chemoattractant, and acting as an alarmin. Mast cells are activated upon recognizing IL-33, releasing chemokines, cytokines, proteases, prostanoids, and histamines that attract neutrophils to the site of infection. Accordingly, it was proposed that the secretion of IL-33 and inflammatory mediators causes osteoclast activation by increasing the production of receptor activator of nuclear factor κB (NF-κB) ligand (RANKL) and decreasing the production of osteoprotegerin (8). The soluble ligand for IL1r1 (also called ST2), a member of the IL-1 superfamily, is IL-33. Two splice variants of ST2 are known to exist; membrane-bound form (ST2) and soluble form (sST2). The sST2 does not signal and functions as a decoy receptor, resulting in free IL-33 sequestration. The ST2 improves the functioning of innate lymphoid cell type 2, regulatory T cells (Tregs), Th2 cells, and mast cells by activating the MyD88/NF-κB signaling pathway (9).

Furthermore, there is conflicting and little evidence available on the amount of IL-33 in periodontitis patients’ oral fluids. Decreased (10) and increased (11) GCF levels and unaltered (10-13) levels in saliva were recorded while contrasting those with periodontitis with those who had healthy periodontia. Periodontitis and elevated blood levels of sST2 have been linked (14). Recently, a pilot investigation showed that periodontitis is linked to increased GCF ST2 levels (15). The literature lacks sufficient research examining the connection between periodontal disease, sST2, and IL-33. To distinguish between periodontal health and disease and between stages I through III periodontitis, the current study compared sST2 and IL-33 levels and examined whether there was a correlation between these biomarkers and clinical criteria.

**Objectives**

The objective of the present study was to evaluate the IL33 and sST2 levels in the GCF (stages I, II, and III) of periodontitis patients in comparison to those of healthy controls, in addition to assessing clinical parameters and comparing them to the sST2 and IL33 levels in patients with periodontitis.

### Materials and Methods

#### Study design

From February to May 2023, 162 patients were chosen in accordance with exclusion and inclusion criteria. In-depth study information was provided to participants, who were also requested to complete a questionnaire that included their dental and medical history, as well as background information. Periodontal clinical parameters, including the plaque index (PI), clinical attachment level (CAL), probing pocket depth (PPD), and bleeding on probing (BOP) were assessed. Each participant provided a sample of GCF. All participants were separated into two study groups: a control group (40 individuals in good periodontal health) and a study group (41 individuals with stage I periodontitis, 40 individuals with stage II periodontitis, and 41 individuals with stage III periodontitis). The periodontitis group was classified as having a buccal/oral CAL greater than 3 mm with pocketing identified at more than two teeth or an interdental CAL equal to or greater than two in nonadjacent teeth. Periodontal health was characterized as BOP <10%, PPD ≤ 3 mm, and intact periodontium (no probing attachment loss) (2). Patients who had medical conditions, such as cerebrovascular disease, coronary heart disease, immunologic disorders, diabetes, hypertension, pregnant or nursing females, females taking contraceptive pills, smokers, alcoholic patients, and those who had antibiotic/anti-inflammatory drugs during the past 3 months were excluded. Patients with a previous history of any extensive periodontal therapy for the preceding 6 months or who were currently receiving ongoing periodontal treatment were excluded.

#### Clinical assessment

The examination did not include wisdom teeth. The CAL, PPD, full mouth BOP, and full mouth PI were the clinical parameters examined for the whole existing dentition. With the exception of plaque scores, where four surfaces (lingual, distal, buccal, and mesial) were evaluated, a whole mouth examination was conducted using the UNC-15 periodontal probe at six locations per tooth (distolingual, buccal, lingual, mesiobuccal, distobuccal, and mesiobuccal). Periapical radiographs were obtained for the worst periodontitis location in each patient to validate staging.

#### Examiner alignment

The clinical parameters (CAL, PPD, BOP and PI) of ten participants were evaluated concurrently by the researcher and an experienced practitioner. The interexaminer calibration was evaluated utilizing the intraclass correlation coefficient (ICC). High levels of agreement were observed across all parameters, with respective values of 0.756, 0.860, 0.833, and 0.782. The same periodontal parameters from ten participants were double-measured by the researcher for intraexaminer calibration. The degree of agreement
was nearly perfect for all parameters, with values of 0.915, 0.886, 0.940, and 0.860, respectively (16).

**Sample collection**

Brill’s approach, which entailed placing and holding a paper strip for 30 seconds after noticing a small amount of resistance, was used to collect GCF samples (17). GCFs were taken from the two sites affected by the most severe periodontitis in the research groups and two control groups at the distobuccal and mesiobuccal locations. Prior to sampling, an electronic scale (Sartorius-BL210S, Göttingen, Germany) was used to weigh each 1.5 mL. Eppendorf tube containing 300 µL of phosphate-buffered saline (PBS). The initial weight was the combination of the added paper strips, PBS, and Eppendorf tube (18). A sterile curet was conducted to eliminate all of the supragingival plaque, and air spray drying was used to dry the sample site. After being inserted into the pocket or sulcus until a minor resistance was noticed, then the paper strip (Oralflow Inc., New York, America) was used for 30 seconds. Blood-contaminated strips were thrown away (19). Within 30 minutes of GCF collection, the strips were weighed again after being reloaded into the same tube. Using differential weighting, the volume of the GCF was determined (20). Approximately 0.5 to 2.4 µL of GCF was collected. At -20 °C, the samples were frozen until the IL-33 and sST2 assay phases after being centrifuged for 20 minutes at 3000 RPM.

**IL-33 and sST2 measurements**

Using enzyme-linked immunosorbent assay kits (Cloud-Clone Corp, Houston, USA), the concentrations of IL33 and sST in the GCF supernatant were determined. The assay sensitivity of the kit described above was less than 0.065 ng/mL for sST2 and less than 6.4 pg/mL for IL33. The assays were performed according to the manufacturer’s instructions. Based on the measured concentrations of sST2 and IL33, the total levels of GCF sST2 and IL33 were estimated.

**Statistical analysis**

For the data analysis, the data were presented and described, and the Statistical Package for Social Science (SPSS version 22) (Chicago, USA, Illinois) was used. For nominal variables, the mean and standard deviation (SD) were utilized. Furthermore, for inferential statistics, the Levene test, Pearson’s correlation (r), and interclass correlation coefficient (ICC) were conducted. The Shapiro–Wilk test was applied to assess the normality of the distribution of the quantitative variables. One-way analysis of variance (ANOVA) with the Games–Howell post hoc test was also performed. The p-value below 0.05 was regarded significant.

**Results**

The Shapiro–Wilk test was applied to establish the normal distribution of each variable in the study. The variables included the GCF levels of sST2 and IL33, periodontal parameters obtained from the GCF collection site, and demographic variables (Table 1). The GCF IL-33 and sST2 concentrations in the periodontitis groups were significantly greater than those in the control group, and a $P < 0.001$ indicates a significant difference between the study groups (Tables 2 and 3). In terms of the relationship between IL33 and periodontal clinical parameters, Table 4 shows that no significant relationships ($P > 0.05$) were detected between the GCF IL33 concentration in the periodontitis groups and periodontal clinical parameters (CAL/PPD/BOP/PI). Table 5 indicates that a significant correlation was detected between sST2 and PPD only in the group with stage II periodontitis, based on the correlation between periodontal clinical parameters and sST2.

**Discussion**

The current cross-sectional research compares and examines the GCF levels of IL33 and sST2 between patients with stage I to stage III periodontitis and those whose periodontium is in good clinical health. In this investigation, the levels of GCF IL33 in periodontal disease patients were considerably greater than those in periodontal disease patients were considerably greater than those in

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PI, Plaque index; CAL, Clinical attachment level; PPD, Probing pocket depth; BOP, Bleeding on probing; ST2, Suppression of tumorigenity; IL-33, Interleukin-33.
healthy periodontal conditions. These outcomes align with other research (21,22). Contrary to our findings, the levels of salivary IL-33 were unchanged in periodontitis patients (10-12). One explanation for this discrepancy is the population in the study. Compared to patients with mild or moderate periodontitis, patients with more severe periodontitis may have a more pronounced IL-33-dependent inflammation, which explains our findings that, in comparison to individuals with periodontally healthy gums, a greater number of patients with periodontitis had detectable or elevated IL-33 levels.

Malcolm et al showed that, in comparison to those of sham-infected controls, the periodontal tissues of mice infected with Porphyromonas gingivalis expressed more IL-33. Additionally, they reported that, in comparison to healthy tissues, gingival tissues from chronic periodontitis patients had greater expression of ST2 and IL-33. They concluded that, when a bacterial infection occurs, IL-33 participates in the acceleration of bone loss in a RANKL-dependent manner (23). These outcomes concurred with earlier research on animals (24).

Several diseases, including asthma (25), cardiovascular diseases (6), inflammatory bowel disease, atopic dermatitis, rheumatoid arthritis (26), and systemic lupus erythematosus (27), have been linked to IL-33. The findings demonstrated that, in comparison to healthy periodontal conditions, the mean sST2 value in the GCF was significantly greater in periodontal disease patients. These outcomes align with previous research (15, 28). Numerous inflammatory and immunological diseases,
such as pneumonia, rheumatoid arthritis, diabetes, cancer, cardiovascular events, and periodontitis, are related to sST2 levels (15, 29-32). Our findings of increased GCF sST2 levels in periodontitis patients are in line with the findings of a cross-sectional study showing a correlation between periodontitis and elevated serum sST2 levels (14). The exact mechanism underlying the association between sST2 and periodontitis is still unknown. Since sST2 is a decoy receptor for IL-33, it prevents the protective effect of IL-33/ST2L signaling against cardiovascular events (29,33-35).

However, upon binding to ST2L, IL-33 initiates NF-kB and mitogen-activated protein kinase activation, worsening inflammation (36). The innate and adaptive immune systems may become activated by IL-33 in response to epithelial infection or injury, and depending on the situation, IL-33 may have an anti- or proinflammatory effect (27,37).

Conclusion
Overall, IL33 and sST2 GCF levels were investigated in periodontitis stages (I-II-III). The findings indicated that in comparison to healthy controls, patients with periodontitis had greater levels of GCF sST2 and IL33.

Limitations of the study
Patients who had diabetes or who smoked or who also had periodontitis were excluded from the current study. In addition, gingivitis and stage IV periodontitis were not included in the current study. Furthermore, ELISA is a technically sensitive and demanding procedure that could be associated with the possibility of reporting false positive and false negative results.

Authors' contributions
Methodology: Samar A. Abood.
Investigation: Samar A. Abood.
Funding acquisition: Samar A. Abood.
Formal analysis: Samar A. Abood.
Data curation: Samar A. Abood.
Conceptualization: Samar A. Abood.
Project administration: Samar A. Abood.
Resources: Samar A. Abood.
Software: Samar A. Abood.
Supervision: Ayser N. Mohammed.
Validation: Ayser N. Mohammed.
Visualization: Samar A. Abood.
Writing–original draft: Samar A. Abood & Ayser N. Mohammed.
Writing–review & editing: Samar A. Abood & Ayser N. Mohammed.

Ethical issues
The ethical committee of the College of Dentistry/University of Baghdad follows the guidelines of Helsinki and Tokyo for humans (reference no.734 on 1/12/2022), which approved the study's protocol. In addition to receiving comprehensive information about the study, participants were required to fill out a questionnaire with specifics about their dental history, medical history, and personal background. Ethical issues (including plagiarism, data fabrication, and double publication) have been completely resolved by the authors.

Conflicts of interest
The authors declare that they have no competing interests.

Funding/Support
No specific commercial or public grant from a nonprofit organization was given for this research.

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