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Salivary IL-33 and sST2 levels in relation to *TLR2* rs111200466 polymorphism and periodontitis

Mustafa Yilmaz[1,2] | Qiushui He[3,4] | Esra Demir[5] | Johanna Teräsjärvi[3] | Ulvi Kahraman Gürsoy[1]

[1]Department of Periodontology, Institute of Dentistry, University of Turku, Turku, Finland

[2]Department of Periodontology, Faculty of Dentistry, Biruni University, Istanbul, Turkey

[3]Institute of Biomedicine, Research Centre for Infections and Immunity, University of Turku, Turku, Finland

[4]InFLAMES Research Flagship Centre, University of Turku, Turku, Finland

[5]Department of Periodontology, Faculty of Dentistry, Bezmialem Vakif University, Istanbul, Turkey

Correspondence
Mustafa Yilmaz, Department of Periodontology, Faculty of Dentistry, Biruni University, Istanbul, Turkey.
Email: myilmaz@biruni.edu.tr

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Abstract

Objectives: Toll-like receptor-2 (TLR2) signalling pathway is involved in the regulation of interleukin (IL)-33 and its receptor suppression of tumorigenicity-2 (ST2). This study aimed to compare salivary IL-33 and soluble ST2 (sST2) levels of periodontitis patients with those of periodontally healthy individuals in relation to their *TLR2* rs111200466 23-bp insertion/deletion polymorphism within the promoter region.

Materials and Methods: Unstimulated saliva samples were collected, and periodontal parameters were recorded from 35 periodontally healthy individuals and 44 periodontitis patients. Non-surgical treatments were applied to periodontitis patients, and sample collections and clinical measurements were repeated 3 months following therapy. Salivary IL-33 and sST2 levels were measured with enzyme-linked immunosorbent assay kits, and *TLR2* rs111200466 polymorphism was detected by polymerase chain reaction.

Results: Elevated salivary IL-33 ($p = 0.007$) and sST2 ($p = 0.020$) levels were observed in periodontitis patients, in comparison to controls. sST2 levels declined 3-months following treatment ($p < 0.001$). Increased salivary IL-33 and sST2 levels were found to be associated with periodontitis, with no significant relation to the *TLR2* polymorphism.

Conclusion: Periodontitis, but not *TLR2* rs111200466 polymorphism, is associated with elevated salivary sST2 and possibly IL-33 levels, and periodontal treatment is effective in reducing salivary sST2 levels.

KEYWORDS
genetics, inflammation, interleukins, periodontal debridement, periodontal diseases, toll-like receptors

1 | INTRODUCTION

Toll-like receptor-2 (TLR2) is a transmembrane pathogen recognition receptor that can induce innate and adaptive immune responses against invading microorganisms (Anwar et al., 2013). TLR2 gets stimulated by pathogen-associated molecular patterns and regulates alarmin cytokines such as interleukin (IL)-33 (Nile et al., 2010). IL-33 is an IL-1 like cytokine that is secreted by a variety of cells as a response to cell injury (Cohen et al., 2015). It is the only ligand of the IL-1 receptor-like 1, known as suppression of

tumorigenicity-2 (ST2). There are two isoforms of ST2: the transmembrane form (ST2L) and the soluble form (sST2; Griesenauer & Paczesny, 2017). IL-33 functions by binding a complex composed of ST2L and IL-1 receptor accessory protein. Binding of IL-33 to ST2L initiates the TLR/myeloid differentiation primary response gene 88 (MyD88)/nuclear factor kappa-B (NF-κB) signalling pathways and enhances T-helper 2 functions (Cayrol & Girard, 2018; Cohen et al., 2015; Griesenauer & Paczesny, 2017). As IL-33 is primarily expressed by epithelial and endothelial cells, it is thought to have a crucial role in mediating host responses in infectious and inflammatory disorders (Milovanovic et al., 2012). Soluble ST2 (sST2), on the other hand, acts as a decoy receptor and restrains IL-33/ST2L activity by sequestering IL-33 (Pascual-Figal & Januzzi, 2015). The production of sST2 is induced by lipopolysaccharide stimulation and inflammatory cytokines via an NF-κB dependent pathway, which is considered a defence mechanism to balance the immune response under such circumstances (Mildner et al., 2010). sST2 levels are upregulated and related to disease severity in a variety of infectious and inflammatory conditions such as Covid-19, hepatitis, sepsis, asthma, diabetes, cardiovascular events and cancer (Aimo et al., 2019; Bajwa et al., 2015; Bergis et al., 2016; Brunner et al., 2004; Gao et al., 2015; Lin et al., 2016; Oshikawa et al., 2001; Sanchez-Marteles et al., 2021).

Periodontitis is an infectious-inflammatory disease of the tooth-supporting tissues that is mediated by a disrupted immune-inflammatory response to a pathogenic biofilm (Könönen et al., 2019). Elevated serum levels of sST2 have been associated with periodontitis (Torrungruang et al., 2019), and two pilot studies recently demonstrated increased gingival and peri-implant crevicular fluid levels of ST2 in periodontitis and peri-implant diseases, respectively (Navya et al., 2022; Ozgur et al., 2022). However, there is no prior research on salivary sST2 levels of periodontitis patients or, naturally, on the effect of periodontal treatment on salivary sST2 levels. Moreover, the available information on the IL-33 levels in the oral fluids of periodontitis patients is limited and contradictory: Both decreased (Buduneli et al., 2012) and increased (Sağlam et al., 2017) levels in gingival crevicular fluid (GCF), and unaltered (Buduneli et al., 2012; Medara et al., 2020; Sağlam et al., 2017; Selman et al., 2021) levels in saliva were reported when comparing periodontitis patients to periodontally healthy individuals. Our knowledge on salivary IL-33 levels following periodontal treatment is also very scarce (Medara et al., 2020).

Polymorphisms at the pattern recognition receptor genes are related to numerous diseases, including infections, dermatitis, inflammatory bowel disease and cancer (Broen et al., 2012; de Koning et al., 2012; Gursoy et al., 2016; Henckaerts et al., 2007; Oh et al., 2009; Royse et al., 2017; Schneider et al., 2016; Schröder & Schumann, 2005). *TLR2* promoter −196 to −174 insertion(ins)/deletion(del) (rs111200466) polymorphism has been associated with thrombocytopenia during sepsis, schizophrenia, human immunodeficiency virus infection, gastric cancer and hepatitis C virus-related hepatocellular carcinoma (Aflouk et al., 2021; de Matos Lourenço et al., 2020; De Re et al., 2019; Jiang et al., 2022; Laplana et al., 2020). Recent studies demonstrated that individuals with the variant genotypes ins/del or del/del of the *TLR2* polymorphism have higher mRNA expression of *TLR2* gene than those with an ins/ins genotype (de Matos Lourenço et al., 2020; Proença et al., 2015). Indeed, *TLR2* rs3804100, rs1898830 and rs5743708 single nucleotide polymorphisms, which lead to variations in the amino acid sequence of TLR2, have been related to periodontal tissue destruction (Shan et al., 2020; Takahashi et al., 2011). Recently, *TLR2* rs111200466 deletion was revealed to be associated with altered serum IL-33 levels in children (Teräsjärvi et al., 2023). Based on this background information, we hypothesised that there is an association between periodontitis and elevated salivary IL-33 and sST2 levels, and this could be related to the presence of *TLR2* rs111200466 polymorphism. Therefore, our aims were (1) to compare salivary IL-33 and sST2 levels of patients with periodontitis with those of periodontally healthy individuals before and after periodontal treatment and (2) to explore the possible associations between salivary IL-33 and sST2 levels and periodontitis and *TLR2* rs111200466 polymorphism.

2 | MATERIALS AND METHODS

2.1 | Patient recruitment

The study's accordance with ethical principles was approved by the Ethics Committee of Biruni University's Medical Faculty (2015-KAEK-75-23-01, 2023). All participants evaluated in the current study have been recruited between March 2021 and August 2021 as part of a previous project (initial ethical approval: 2015-KAEK-43-19-27, 2018), where we investigated macrophage-related immune-inflammatory signals in periodontitis (Yilmaz, Demir, Gürsoy, Firatli, & Gürsoy, 2023). The participants were informed about the expansion of our study and their consent was obtained. Thirty-five periodontally healthy individuals with <10% full-mouth bleeding probing and no sites with probing depth > 3 mm (15 smokers) and 44 stage III/IV grade C periodontitis patients with at least two bleeding pockets of 6–10 mm (18 smokers) were evaluated (Papapanou et al., 2018). The exclusion criteria were as follows: systemic diseases and conditions such as diabetes mellitus or cardiovascular disease with potential effects on the host response and periodontal status; former or casual smokers; <15 teeth present; periodontal therapy within a year before the initiation of the study, anti-inflammatory or antibiotic use in the last 3 months; contraceptive use; pregnancy or lactation. Individuals who consumed ≥5 cigarettes per day for more than a year were considered smokers, whereas never smokers were considered non-smokers.

2.2 | Sample collection, clinical examinations and periodontal treatment

Saliva samples were obtained by asking the participants to expectorate into plastic tubes in the morning at the dental office,

at least 2 h after the person had last consumed anything other than water and before the clinical recordings. All samples were promptly placed and stored at −80°C until they were transferred to the University of Turku in Finland with dry ice for biochemical analysis. The average storage time of saliva samples was 18–24 months.

After the collection of saliva samples, plaque index (PI), probing depth (PD), clinical attachment level (CAL) and bleeding on probing (BoP) scores were recorded from six sites of each tooth at baseline. Periodontitis patients received non-surgical treatment that was completed in 1 day (full mouth scaling and root debridement) by combining power (Variosurg™, NSK) and hand instruments (American Eagle Instruments) following sample collection and clinical measurements. The treatment was conducted in two sessions with a brief rest between them. As part of the initial project, sample collection and clinical measurements were repeated and oral hygiene was reinforced at 2, 6, 12 and 24 weeks and supragingival scaling was performed at 6, 12 and 24 weeks. The current study confines the analysis to the clinical measurements and salivary samples obtained only at baseline and 3 months following treatment. The rest of the samples were not analysed in this study.

2.3 | Detection of salivary IL-33 and sST2 levels and *TLR2* rs111200466 polymorphism

Detection of salivary IL-33 and sST2 levels were conducted by a highly experienced technician together with the first author of this manuscript (M.Y.). To avoid possible run to run variation, both samples (before and after treatment) from the same study subject were analysed on the same plate. The samples were thawed and centrifuged for 10 min at 5°C with a spinning speed of 500×*g*. IL-33 and sST2 levels were measured with Human IL-33 (E-EL-H2402) and Human sST2 (E-EL-H6082) enzyme-linked immunosorbent assay (ELISA) kits (Elabscience Biotechnology) according to the instructions of the manufacturer in duplicates. The detection ranges of IL-33 and sST2 were 15.63–1000 pg/mL and 0.31–20 ng/mL, respectively. The IL-33 and sST2 kits had a sensitivity of 9.38 pg/mL and 0.19 ng/mL, respectively. Both kits had intra- and inter-kit coefficients of variation that were less than 10%. The values below the limit of detection (LOD) were substituted with half of the lowest limit of detection.

The pellets from the saliva samples were used for genomic DNA extraction. The genomic DNA was extracted with the E.Z.N.A.® Blood DNA extraction kit (Qmega Bio-Tek) according to the manufacturer's protocol for body fluids. DNA concentrations were determined by a spectrophotometer (NanoDrop 2000, Thermo Scientific). Insertion and deletion of 23 bp at −196 to −174 of *TLR2* promoter was detected with polymerase chain reaction (PCR; Teräsjärvi et al., 2023). The PCR products were visualised by gel electrophoresis, and a single band at 286 bp was judged as wild type and a single 264-bp band was judged as homozygous variant, whereas the heterozygous variant had two bands of 286 and 264 bp as described by Tahara et al. (2007).

2.4 | Statistical analysis

SPSS v 27.0.1.0 (IBM) was used for the statistical analysis. Shapiro Wilk test and Q–Q plots revealed that the salivary IL-33 and sST2 levels were skewed. The distributions of smoking, gender and *TLR2* polymorphisms among groups (periodontitis vs. periodontally healthy) were assessed with chi-square test. The comparisons of age, clinical parameters and analyte levels between periodontally healthy participants and periodontitis patients were performed with Mann Whitney-*U* test. The clinical parameters and analyte levels in periodontitis patients at baseline and those at 3 months following treatment were compared with each other using Wilcoxon signed rank test. Two groups were formed according to the *TLR2* rs111200466 genotype (1. ins/ins; 2. ins/del & del/del) and separately evaluated in periodontally healthy participants, in periodontitis patients at baseline and in periodontitis patients following treatment. A linear regression analysis was conducted to evaluate the associations between the analyte levels and periodontal status, unadjusted and adjusted for age, smoking and *TLR2* polymorphism. The associations between the analyte levels with the *TLR2* polymorphism were also assessed with linear regression analysis, unadjusted, and adjusted for age, smoking and periodontal status. $p < 0.05$ was considered statistically significant.

3 | RESULTS

A total of 79 individuals (35 control, 44 periodontitis) were evaluated. Smoking status ($p = 1.000$) and gender ($p = 0.096$) were equally distributed among groups. The median age of periodontitis patients was higher than that of periodontally healthy participants ($p < 0.001$; Table 1). The frequencies of the examined genotypes were as follows: four individuals (11.4%) with del/del or ins/del and

TABLE 1 The distribution of age, gender, the percentage of smokers, and *TLR2* genotypes in relation to periodontal status.

	Periodontitis ($n = 44$)	Periodontally healthy ($n = 35$)	*p*
Age	42.7 ± 9.9	35.1 ± 7.8	**<0.001**
Male %	45.5%	28.6%	0.096
Smoker %	40.9%	42.9%	1.000
TLR2 polymorphism			
ins/ins	75%	88.6%	0.107
ins/del or del/del	25%	11.4%	

Note: *p* value in bold represents a statistically significant difference ($p < 0.05$).

31 individuals (88.6%) with ins/ins genotype in the periodontally healthy group; 11 individuals (25%) with del/del or ins/del and 33 individuals (75%) with ins/ins genotype in the periodontitis group (Table 1).

As expected, periodontal parameters (full-mouth mean scores of PI%, BoP%, PD and CAL) of the periodontitis group when compared to those of healthy participants (each $p<0.001$), as well as the parameters at baseline when compared to those at 3 months among periodontitis patients (each $p<0.001$) demonstrated statistically significant differences (Table 2). A thorough breakdown of the clinical parameters can be found in our previous publication reporting salivary macrophage-related proteins in the same population (Yilmaz, Demir, Gürsoy, Firatli, Loimaranta, & Gürsoy, 2023).

IL-33 levels were below the LOD in 28 healthy participants and in 22 periodontitis patients at baseline, and in 20 periodontitis patients 3 months after periodontal therapy. sST2 levels were below the LOD in four healthy participants and in nine periodontitis patients at baseline, and in 16 periodontitis patients after periodontal therapy. At baseline, IL-33 ($p=0.007$) and sST2 levels ($p=0.020$) of periodontitis patients were higher than those of periodontally healthy participants. While the sST2 levels of periodontitis patients declined following therapy ($p<0.001$), the change in IL-33 levels was not statistically significant ($p=0.417$; Table 2).

IL-33 and sST2 levels did not differ between groups formed according to the *TLR2* genotypes in each periodontal status and at each time point in periodontitis patients (Table 3). Salivary IL-33 and sST2 levels were found to be associated with the periodontal status (IL-33: unadjusted $p=0.018$, adjusted $p=0.046$; sST2: unadjusted $p<0.001$ and adjusted $p<0.001$). The associations between IL-33 and sST2 levels and *TLR2* polymorphism were found to be not statistically significant (Table 4).

4 | DISCUSSION

To the best of our knowledge, our study is the first to show that salivary sST2 and potentially IL-33 levels are associated with periodontitis but not with *TLR2* rs111200466 polymorphism (ins/del or del/del). In the present study, both IL-33 and sST2 levels were higher in periodontitis patients when compared to healthy individuals, and sST2 levels declined following initial periodontal treatment, while IL-33 levels were not affected by treatment.

TLR2 is expressed in gingival epithelial cells and can activate inflammatory cytokines and antimicrobial peptides when induced by molecular patterns of periodontal pathogens (Ding & Jin, 2014). TLR2 activation can trigger the local release of IL-33, which then activates ST2 receptors (Huang et al., 2020). *TLR2* −196 to −174 ins/del polymorphism has been shown to upregulate TLR2 expression in gastric and colorectal tissues (de Matos Lourenço et al., 2020; Proença et al., 2015), and *TLR2* gene polymorphisms have the potential to change salivary immune-regulating proteins (Gürsoy et al., 2022). Based on these prior studies, we expected that *TLR2* rs111200466 polymorphism would alter salivary IL-33 and sST2 levels, but this hypothesis was rejected.

According to our results, IL-33 levels are increased in periodontitis, but treatment is not effective in reducing these levels. However, since IL-33 was not found in a significant portion of healthy participants (80%) and in nearly half of the periodontitis patients, these

TABLE 2 Periodontal parameters and salivary IL-33 and sST2 levels in relation to periodontal status.

	Periodontitis (T0) ($n=44$)	Periodontitis (T1) ($n=44$)	Periodontally healthy ($n=35$)	*p* periodontitis (T0) vs. Periodontally healthy	*p* periodontitis (T0) vs. periodontitis (T1)
PI (%) mean ± SD	81.6 ± 18.7	45.1 ± 19.9	13.3 ± 11.8	**<0.001**	**<0.001**
BoP (%) mean ± SD	71.4 ± 22.5	20.8 ± 11.2	2.2 ± 1.8	**<0.001**	**<0.001**
PD mean ± SD	3.8 ± 0.7	3.2 ± 0.7	1.6 ± 0.2	**<0.001**	**<0.001**
CAL mean ± SD	4.1 ± 0.7	3.2 ± 0.7	1.6 ± 0.3	**<0.001**	**<0.001**
Number of pockets with PD ≥4 mm	72.3 ± 24	29.2 ± 20	0		**<0.001**
Number of pockets with PD ≥6 mm	24.5 ± 16.1	7 ± 9	0		**<0.001**
IL-33 (pg/mL) median (Q1–Q3)	11.8 (7.8–42.7)	16.7 (7.8–42.0)	7.8 (7.8–7.8)	**0.007**	0.417
sST2 (pg/mL) median (Q1–Q3)	1428 (499–3352)	675 (155–1333)	749 (677–995)	**0.020**	**<0.001**

Note: *p* values in bold represent statistically significant differences ($p<0.05$).

Abbreviations: T0, baseline; PI, plaque index; BoP, bleeding on probing; PD, probing depth; CAL, clinical attachment loss; SD, standard deviation; T1, 3 months after treatment; Q1–Q3, 25–75th percentile.

TABLE 3 Salivary IL-33 and sST2 levels in individuals with and without *TLR2* rs111200466 deletion.

	Periodontitis (T0) ($n=44$)			Periodontitis (T1) ($n=44$)			Periodontally healthy ($n=35$)		
	TLR2 rs111200466, ins/ins ($n=33$)	*TLR2* rs111200466, del/ins or del/del ($n=11$)	*p*	*TLR2* rs111200466, ins/ins ($n=33$)	*TLR2* rs111200466, del/ins or del/del ($n=11$)	*p*	*TLR2* rs111200466, ins/ins ($n=31$)	*TLR2* rs111200466, del/ins or del/del ($n=4$)	*p*
IL-33 (pg/mL) median (Q1–Q3)	21.6 (7.8–42.6)	7.8 (7.8–7.8)	0.562	18.8 (7.8–42.1)	7.8 (7.8–7.8)	0.237	7.81 (7.81–7.81)	7.81 (7.81–7.81)	0.299
sST2 (pg/mL) median (Q1–Q3)	1650 (272–3300)	1034 (520–3912)	0.860	685 (155–1282)	563 (155–1403)	0.637	767 (677–995)	695 (285–1032)	0.452

Abbreviations: T0, baseline; T1, 3 months after treatment; Q1–Q3, 25–75th percentile.

results should be carefully interpreted. Comparably, Papathanasiou et al. (2014) were unable to detect IL-33 in any of the GCF samples they collected from sites with different clinical statuses: healthy, gingivitis or periodontitis. In light of previous research and current findings, it can be concluded that IL-33 is generally present in low quantities in oral fluids. On the other hand, sST2 levels were mostly above the lowest limit of detection in both healthy participants and periodontitis patients, with an increase in undetectable levels after periodontal treatment reflecting the decline in sST2 activity following treatment.

sST2 levels have been linked to a variety of immune and inflammatory conditions, including cardiovascular events, cancer, rheumatoid arthritis, diabetes, pneumonia, and finally, periodontitis (Bergis et al., 2016; Lin et al., 2016; Navya et al., 2022; Pascual-Figal & Januzzi, 2015; Watanabe et al., 2015). Our findings regarding the elevation of sST2 levels in periodontitis are consistent with a recent cross-sectional study, where an association between elevated serum sST2 levels and periodontitis was demonstrated (Torrungruang et al., 2019). However, the mechanism behind the link between periodontitis and sST2 is yet to be revealed. sST2 is a decoy receptor for IL-33, inhibiting IL-33/ST2L signaling that has a protective effect against cardiovascular events (Bergis et al., 2016; Seki et al., 2009). On the other hand, binding of IL-33 to ST2L results in NFκ-B and mitogen-activated protein kinase activation and thus can exacerbate inflammation (Kakkar & Lee, 2008). Following injury or infection in the epithelium, IL-33 can signal the innate and adaptive immune systems and, depending on the condition, it can have pro- or anti-inflammatory effects (Miller, 2011; Pichery et al., 2012). IL-33 levels have been found to be increased in gingival tissues of patients with periodontitis and have been associated with inflammation in periodontal tissues and with bone loss via the RANKL-dependent pathway (Malcolm et al., 2015). Previous studies demonstrated elevated serum and GCF IL-33 levels; however, salivary IL-33 levels were found to be unaltered in periodontitis, which is not in line with our findings (Buduneli et al., 2012; Medara et al., 2020; Sağlam et al., 2017). This difference can be attributed to the studied populations; more severe periodontitis may be associated with a more significant IL-33-dependent inflammation than mild or moderate periodontitis cases, which can explain our results regarding the fact that more periodontitis patients had detectable/elevated IL-33 levels when compared to periodontally healthy participants. Interestingly, in one of the mentioned studies, the researchers reported decreasing salivary IL-33 levels following treatment, although IL-33 levels at baseline were similar between periodontitis patients and periodontally healthy individuals (Medara et al., 2020). The rapid inactivation of IL-33 by oxidation regulates ST2-dependent inflammation by limiting the range and duration of its effect (Cohen et al., 2015). Therefore, increased salivary IL-33 levels suggest a disruption in this inactivation mechanism, which may lead to the enhanced inflammation observed in periodontitis.

In the present study, serum levels of sST2 were not measured and thus, the potential correlation between local and systemic sST2 levels has not been assessed, which can be considered a limitation of

TABLE 4 Unadjusted and adjusted associations of salivary IL-33 and sST2 levels with periodontitis and *TLR2* rs111200466 polymorphism.

	Periodontal status		*TLR2* rs111200466 polymorphism	
	Unadjusted	Adjusted for age, smoking and *TLR2* rs111200466 polymorphism	Unadjusted	Adjusted for age, smoking and periodontal status
IL-33	B = 14.739; β = 0.266; **$p = 0.018$**	B = 13.400; β = 0.241; **$p = 0.046$**	B = 4.241; β = 0.060; $p = 0.597$	B = 8.511; β = 0.121; $p = 0.276$
sST2	B = 1199; β = 0.384; **$p < 0.001$**	B = 1305; β = 0.418; **$p < 0.001$**	B = −31.15; β = −0.008; $p = 0.945$	B = 372,3; β = 0.094; $p = 0.347$

Note: *p* values in bold represent statistically significant differences ($p < 0.05$).

our study. Moreover, it can be beneficial to include gingival crevicular fluid in future research to gain insight into the effects of the sST2/IL-33 axis on site-level pathology and healing potential following treatment. In fact, one prior study reported elevated ST2 levels in crevicular fluid of periodontitis patients, but that study was conducted in a small sample size due to its pilot nature and the impact of periodontal treatment on ST2 levels was not assessed (Navya et al., 2022). Another drawback of our study is that we used self-reporting rather than chemical analysis of exposure biomarkers to assess smoking status. To note, the data from both smokers and non-smokers was pooled and the statistical analysis was carried out adjusting for smoking in order to prevent any possible impact of smoking on our findings. The high number of samples with IL-33 levels below the limit of detection should also be kept in mind before coming to any firm conclusions. On the other hand, we think that the small number of samples with detectable IL-33 levels, especially among healthy individuals, is attributable to the likelihood that IL-33 is present in very small concentrations in oral secretions, as was mentioned above, and is not evidence of sample deterioration. The storage time of the samples has possibly a negligible effect on our findings, since various salivary proteins are considered to be stable for at least 2 years, which we discussed in our previous publication (Shields et al., 2019; Yilmaz, Demir, Gürsoy, Firatli, Loimaranta, & Gürsoy, 2023). Finally, the small sample size in our study should be considered; further studies on other polymorphisms of *TLR2* and on different genes coding TLRs in larger populations can be helpful to reveal the genetic aspect of salivary IL-33 and sST2 levels in periodontitis.

Within the limitations of our study, salivary sST2 and IL-33 levels are not dependent on the *TLR2* rs111200466 polymorphism. Salivary sST2 levels are associated with periodontitis, and non-surgical periodontal treatment is effective in reducing sST2 levels. These results corroborate the potential involvement of the IL-33/ST2 pathway in periodontal pathogenesis. Increased salivary sST2 levels in periodontitis patients may be associated with the host's effort to balance IL-33/ST2L activity. Our findings, suggesting preliminary evidence for an association between salivary IL-33 and periodontitis, need further evaluation. The functions of the IL-33/ST2 pathway and soluble ST2 in periodontal aetiology and repair of the gingiva following periodontal therapy may be better understood with additional research in individuals with variable disease severity.

AUTHOR CONTRIBUTIONS

Mustafa Yilmaz: Conceptualization; investigation; writing – original draft; formal analysis. **Qiushui He:** Conceptualization; writing – review and editing; methodology; funding acquisition. **Esra Demir:** Investigation; writing – review and editing. **Johanna Teräsjärvi:** Investigation; writing – review and editing. **Ulvi Kahraman Gürsoy:** Conceptualization; writing – review and editing; supervision; formal analysis; funding acquisition.

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CONFLICT OF INTEREST STATEMENT

All authors have no conflicts of interest to disclose.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Mustafa Yilmaz https://orcid.org/0000-0002-2005-1417
Esra Demir https://orcid.org/0000-0002-7442-1778

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