RESEARCH ARTICLE

Interleukin-33 Level in Biological Fluids for Periodontitis Patients in Al-Najaf City, Iraq

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ABSTRACT

Background: The study's purpose was to compare the levels of interleukin-33 (IL-33) in gingival crevicular fluid (GCF), saliva, and serum of periodontal disease patients and periodontally healthy subjects, as well as the relationship between these levels in biological fluids and age.

Methods: In this study, there were 88 participants: 60 stable, non-smoking persistent periodontitis cases and 28 systemically and periodontally healthy, non-smokers. Both participants had their GCF, entire saliva, and serum samples were taken. IL-33 was measured using enzyme-associated immunosorbent assay (ELISA) kits.

Results: The GCF IL-33 concentration had significantly higher in the periodontal diseases group than in the stable group ($p = 0.01$). GCF and serum IL-33 concentration significantly positively correlated with age. There was a significantly positively correlation between G C F and serum concentrations of IL-33.

Conclusions: The IL-33 appears to play a part in periodontal disease pathogenesis. They may be used as diagnostic biomarkers in saliva or GCF for chronic periodontitis.

Keywords: Gingival Crevicular fluid, Interleukin-33, Serum, Saliva, Periodontitis.

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INTRODUCTION

Periodontal disorder is a major public health problem that affects people all over the world. The prevalence of the periodontal disease has risen dramatically in recent decades, and a substantial body of evidence suggests that it has a clear connection to systemic diseases.[1] Periodontitis is a multifaceted disease caused by the host's reaction to dysbiotic biofilm, which results in the degradation of supporting tissues.[2] Despite the fact that dysbiosis of a local bacterial population causes local inflammatory reaction over through the host's immune response causes osteoclastic behavior and alveolar bone loss.[3]

Cytokines are important regulators of homeostasis and inflammatory response, acting in the first process of immune responses to microbes and triggers at barrier positions and connecting tissue cells with lymphocytes and accessories cell types.[4] Some cytokines, when produced and released, help the immune system, while others cause cell death and disease progression.[5]

The only methods for diagnosis are clinical periodontal and radiographic results, along with patient history.[6] However, in some cases, these parameters do not provide convincing evidence; Consequently, research host immune system is underway to find early diagnostic methods for this disease.

The evaluation of the host response is a more sophisticated form of detecting periodontal disease. This involves using biochemical and immunologic approaches to determine the degree of specific and non-specific mediators as part of the individual response to periodontal infection. The levels of these cytokines can be measured in saliva, GCF and serum.[7]

The IL-33 is a comparatively recent cytokine from the IL-1 superfamily which is produced by epithelial and endothelial cells, and it has been identified as a T helper 2 immune response inducer. IL-33 binds to the ST2 ligand-receptor.[8] Gingival epithelial and connective tissue cells in the periodontium produce IL-33.[9] IL-33, which could also serve as an alarmin.[10] Gingival epithelial cell death during periodontal tissue damage result in the production of IL-33, which serves as an early warning sign for tissue damage. ICAM-1 expression becomes strongly induced by IL-33 on endothelium, and close conjunction expression is decreased, enabling inflammatory mediators to extravasate. IL-33 induces 11 RANKL expression and bone resorption in murine gingival cell osteoclasts.[9]

In both stable and inflammatory situations, the immune response processes and cytokine networks in periodontal tissue are not well understood.[3] Previous studies' findings are mixed and inconclusive, given the function of IL-33 in chronic

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inflammation and periodontal tissue destruction. As a result, the study aims to measure GCF, serum, and saliva levels and look into the local and systemic effects of IL-33 in people with periodontitis. In addition, the age of patients with this cytokine level and the association between them.

MATERIALS AND METHODS

Study Population

During the months of April to October 2019, all patients in this study visited the outpatient periodontics department at the College of Dentistry, University of Kufa and Al-Kafeel University in Najaf, Iraq. Each participant signed a written informed consent document. The institutions' ethical committee gave their approval.

There were 60 periodontitis patients in total. (38 men and 22 women; age, 21 to 70 years) and 28 periodontally healthy volunteers (12 males and 16 females, age range (21 to 65) years). Individuals with diabetes, pregnant or nursing women, smokers, Anyone who had taken antibiotics or periodontal medicine in the prior three months was not included in the study. A single calibrated clinician performed a clinical periodontal test on all patients, and samples were categorized as chronic periodontitis patients if they showed a minimum of two sites with a clinical probing depth ≥ 4 mm and clinical attachment loss ≥ 4 mm.

Gingival Crevicular Fluid (GCF) Sampling

GCF specimens were obtained using filter paper strips (Periopaper, Oraflow Inc., USA). After cautiously inserting paper strips into the gingival sulcus/crevice until light friction was sensed, they were put in place for thirty seconds. To prevent mechanical injury, caution was taken. Strips were discarded that were tainted with blood. Following gingival fluid collection, the two-period paper strips per collection site that absorbed GCF from each patient were pooled and directly taken to tubes for microcentrifuges containing 200 µL buffer solution and deposited frozen at - 80°C for later study.[12,13]

Saliva Sampling

Whole saliva was taken by expectoration into plastic tubes on the day GCF samples were taken. The saliva samples were clarified for 10 minutes using an 800gx centrifuge, then aliquoted and processed at -80°C until used in the assay.[14]

Serum Sampling

Each participant in this study provided five milliliters of venous blood. The serum separation was performed for approximately 15 minutes by centrifugation at 3000 rpm. The serum was then collected in the sterile Eppendorf tube, and the serum was isolated, aliquoted, and stored at a temperature of -80°C before ELISA proceeded.

Assessment of IL-33 level in GCF, Saliva and Serum Samples

According to the manufacturer's instructions, commercial human enzyme-linked immunosorbent Assay (ELISA) Kits (Elabscience, USA) were used to test IL-33 levels in GCF, saliva, and serum. For IL-33, the lower recognition threshold was 15.63 pg/mL. The optical density of the samples was read at 450 nm and compared with the standards. Concentrations of IL-33 in the GCF samples were estimated using the standard curve.

STATISTICAL ANALYSIS

All statistical studies were carried out using the SPSS v.24 software. For all variables that followed the normality of the distribution, the Software t- independent test was used, and non-parametric tests were used for variations that did not meet the normality of the data. At a p-value of 0.05, this is statistically important. The Spearman's correlation test had been used to search for associations between the levels of IL-33 in clinical samples and the age of the participants. [15]

RESULTS

Participants' Characteristics

Descriptive statistics of the study population are shown in Table 1. Between the sample classes, there was a significant difference in sex distribution (p = 0.001). The CP group's average age was higher than the stable control group's; however, no significance was observed (p = 0.690), which were 41.82 ± 12.459 years and 40.68 ± 12.356 years, respectively.

GCF, Saliva, and Serum Levels of IL-33 Cytokines

The levels of IL-33 in GCF, saliva, and serum samples are given in Table 2. significant differences are shown between GCF, saliva, and serum in the concentration of IL-33 within the same group, whether periodontitis patients or healthy subjects(p = 0.0001 and p = 0.007 respectively). Additionally, this study records a significant increase in the GCF level of IL-33 in patients (p = 0.01) compared to the healthy control group. Although there is an increase in IL-33 in saliva and serum, no significant difference is observed between patients and the healthy control group (p = 0.071 and p = 0.812, respectively).

Table 1: Descriptive statistics of study population

Parameters		*Control N=28*	*Patients N= 60*	*p-value*
Age(Mean ± SD)		40.68 ± 12.356	41.82 ± 12.459	0.690
Sex No. (%)	Male	12 (42.9)	38 (63.3)	0.001*
	Female	16 (57.1)	22 (36.7)	

*Significant differences at p ≤ 0.05 between patients and control.

Table 2: IL-33 level in serum, saliva, and GCF in patients and healthy control group

	Patients n = 60	*Control n = 28*	
IL-33	*Median (IQR)*	*Median (IQR)*	*p-value*
IL-33 in serum	27.27 (20.86–78.11)	31.23 (23.71–79.38)	0.812
IL-33 in saliva	59.10 (23.36–109.23)	36.92(23.46–59.36)	0.071
IL-33 in GCF	112.46 *(77.41–193.05)	65.55(46.49–119.60)	0.01
p-value	0.0001	0.007	

*Significant differences at p ≤ 0.05 between patients

Table 3: The correlation between levels of IL-33 in biofluids and age

Parameters		*Age*	*IL-33 in GCF*	*IL-33 in saliva*	*IL-33 in serum*
IL-33 in serum	Correlation Coefficient	.66**0	.314* 0	.207 0	1
	p-value	.0001 0	.014 0	.112 0	
IL-33 in saliva	Correlation Coefficient	.216 0	0.253	1	.
	p-value	.098 0	.052 0		
IL-33 in GCF	Correlation Coefficient	.371**0	1		
	p-value	.0040			

*.The correlation is significant at the 0.05 level

**. The correlation is significant at the 0.01 level

GCF and serum IL-33 concentration significantly correlated with age (r = 0.371, p = 0.004 and r = 0.66, p = 0.0001 respectively). There was a significantly positive correlation between GCF and serum concentrations of IL-33(r = 0.314, p = 0,01). While, there was a weak, positive relationship between the concentration of IL-33 in saliva and its concentration in serum and GCF, which did not reach statistical significance (Table 3).

DISCUSSION

The increase and activation of many cytokines during the development of periodontal disease is well known.[16,17] Suppose our understanding of the immune response in the onset and development of disease improves. In that case, the identification of cytokines with unique functions typically leads to the advancement of cytokine-targeting medicines.[18]

Since the IL-1 family of cytokines is beneficial in degenerative diseases, including periodontitis and rheumatoid arthritis.[19] This study investigates, quantify and compares the concentration of IL-33 in serum, saliva, and GCF between periodontitis patients and periodontally systemically healthy subjects. In the present study, only GCF IL-33 levels significantly differed between periodontitis patients and healthy groups (Table 2).

When a cell is injured or dies, IL-33 is released, serving as an "alarmin" to warn the immunity to tissue destruction, triggering the differentiation of Th17, Th2 and Treg cells, and affecting wound healing of injured tissues.[20,21] The death of gingival epithelial cells may cause the release of IL-33 during periodontal tissue destruction, which functions as an alert, augmenting the Th2 response while also acting as an anti-inflammatory.[10] However, IL-33 development is stimulated in periodontal disease as a result of high levels of pro-inflammatory cytokines produced in response to bacterial infection, and osteoclastogenesis may be favored.[22,23] IL-3324 also triggers the recruiting of RANKL-expressing B and T cells. This may explain why the concentration of IL-33 in periodontitis patients is higher than in healthy people.On the contrary, Awange (2014) IL-33 has been proposed to perform a pro-inflammatory function in periodontal disease. He demonstrated that oral keratinocytes produce IL-33 and that *P. gingivalis*, a periodontal pathogen, can trigger increased intracellular expression of this cytokine. Differences in the function of IL-33 in periodontal pathologies, however, may be due to tissue regulation differences. Furthermore, since cytokines work in "networks," IL-33's role can be influenced by its interactions with other cytokines and chemokines as well as the tissue in which it is expressed.[25] Lapérine *et al*. (2016) reported that IL-33 overexpression in gingival epithelial cells is linked to chronic periodontitis and may trigger RANK-L, implying that IL-33 can function as an extracellular alarmin with pro-inflammatory properties in chronic periodontitis by perpetuating bone resorption caused by *Porphyromonas gingivalis* infection.[9]

The results of previous research on IL-33 in periodontal disease by a variety of authors have been somewhat contradictory. In vitro studies confirmed the central function of IL-33 in periodontitis.[26-29] The results of human studies have been indecisive at best. Clinical human experiments evaluating IL-33 concentrations in periodontal stable and illness have been published in the literature. Gümüş et al. (2017) found that salivary concentrations of IL-33 are higher in chronic periodontitis groups. However, the IL-33 level in sera was similar.[6] In contrast to a stable population, IL-33 concentrations in peri-implantar crevicular fluid were significantly higher in the peri-implantitis and peri-implant mucositis groups.[30] IL-33 levels in peri-implant fluid from mucositis sites were found to be unaffected by the degree of bone resorption around implants or teeth.[31] Tarrad et al. (2018) concluded that IL-33 plays a role in the pathogenicity of periodontitis, which reported that IL-33, in both GCF and saliva, was significantly higher in chronic periodontitis patients when compared to the stable control individuals.[32] Pai B and Pradeep results indicated that the means differed significantly in plasma and GCF concentrations of IL-33 among patients with chronic or aggressive periodontitis than from healthy controls, and they suggested that IL-33 offers a useful biomarker of periodontal disease, as it can differentiate between healthy and diseased periodontal sites.[33] Ballambettu *et al.* detect a significant IL-33 concentration in GCF and plasma in periodontitis patients compared to healthy control subjects.[34] When Medara et al. looked at longitudinal variance in 15 cytokines in relation to periodontitis, they found treatment-related decreases in salivary IL-23, IL-17A,, IFN, TNFand IL-33 level.[35] Askian *et al*. found a high level of salivary IL-33 before treatment and a decrease after non-surgical periodontal treatment, implying that IL-33 is linked to the seriousness of the periodontal disease and the degree of non-surgical periodontal treatment success. As a result, IL-33 could be a promising therapeutic target in the future for moderate chronic periodontitis (MCP).[36]

Other results that have been documented in most human studies partly agree with what was reached in this study.[37-40] The findings on IL-33 levels in GCF contradicted those of Kurşunlu *et al*. who found that in the chronic periodontitis, generalized aggressive periodontitis and gingivitis groups, IL-33 concentrations in GCF were less than in the stable community.[39] The findings contrasted with Papathanasiou *et al.*,

who found no IL-33 in the GCF of patients with chronic periodontitis.[38] Besides this, Awang (2014) confirmed that serum IL-33 levels were below the detection threshold. IL-33 was also not found in any of the saliva or GCF samples.[25] Moreover, despite the fact that Buduneli *et al*. found IL-33 in the plasma, GCF, and saliva of periodontitis patients and healthy controls, they concluded that the individuals with chronic periodontitis had substantially lower concentrations of IL-33 in GCF, despite the fact that GCF total levels, salivary, and plasma concentrations of IL-33 were comparable in the study groups.[37] Sağlam *et al*. compared the levels of IL-33 in patients with chronic periodontitis, gingivitis, and controls in their plasma, saliva, and gingival crevicular fluids. When compared to the control group, gingivitis and CP groups had higher total amounts of GCF IL- 33. The level of IL-33 in the GCF correlated with clinical parameters as well, however, GCF IL-33 concentrations were significantly lower in the chronic periodontitis group than in the healthy control subjects. Also, Both groups had similar amounts of IL-33 in saliva and plasma.[40]

In this study, the higher IL-33 concentration in GCF relative to serum and saliva may increase local IL-33 output due to increased vasculature in diseased periodontal tissues or periodontal tissue destruction.[41-44] In patient groups, the higher IL-33 serum concentration may be due to an overflow into the blood circulation from the periodontal tissues since this study involved systemically stable participants.

GCF component analysis provides a precise indication of the relationship between individual metabolic variance and disease status.[45] As a result, in the absence of obvious changes in soft tissue parameters, the concentration of IL-33 may be a true predictor of periodontal disease development.

Based on this finding and other studies, it appears that the method for determining IL-33 levels in biological fluids will vary depending on the ELISA kit used in the analysis. Since different companies manufacture different IL-33 assay kits with different minimum detection limits, the efficiency of different kits varies; making comparisons and combining data from different studies is difficult. This is true for nearly every cytokine studied in periodontology so far. Ethnic variations between research groups, differences in the form and severity of periodontal disease, age, sample preparation, clinical sample storage conditions, sample size, and genetic and environmental factors are all possible explanations.

CONCLUSIONS

Results of this research showed a significant increment in IL-33 level in GCF compared with the level of IL-33 in saliva and serum in periodontitis patients, in addition when comparing patients with healthy controls. These results indicate a considerable role for IL-33 in the pathogenicity of periodontitis as a local warning to the occurrence of periodontal tissue breakdown, which helps stimulate other cytokines' response to the site of infection.

Further study of the periodontal-specific cytokine network and the host immune response is warranted. Development in this field may result in the advancement of tissue-specific treatment technologies, lowering the risk of periodontal disease for patients and the community and the effect of local periodontal disorder on systemic diseases.

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