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Diagnostic Accuracy of GCF IL33 and sST2 For Periodontitis Stage I, II, and III

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ABSTRACT:

IL33 was highly expressed in the periodontal tissue of patients with chronic periodontitis. However, there is a debate regarding whether there is an elevation in the level of IL33 in gingival crevicular fluid (GCF) of patients with chronic periodontitis. As a member of the Toll-like receptor/IL-1R superfamily, interleukin-33 serves as the ligand for the ST2 receptor. The ST2 receptor consists of two isoforms: ST2L, which is a form that bridges the cell membrane, and soluble ST2 (sST2), which is a form that is released into the surrounding environment. The objective of this study was to evaluate the accuracy of diagnosis of IL33 and sST2 in distinguishing between various stages of periodontitis (I, II, and III) and periodontal health. A total of 162 participants were involved in the study, and GCF was obtained from each individual. The IL33 and sST2 levels were detected using an enzyme-linked immunosorbent assay (ELISA). All the data showed statistical significance in differentiating, except for the IL33 GCF level between stage II and stage III periodontitis, where the result was not statistically significant. Based on this investigation, GCF IL33 and sST2 showed a high level of sensitivity and specificity in distinguishing between periodontal health and periodontitis.

Keywords: periodontitis, GCF, IL33, sST2.

1. INTRODUCTION:

Periodontal diseases are a group of chronic oral inflammatory conditions that occur due to an imbalance in the microbial community and the response of the host's immune system (Vos et al., 2017). The hallmark of periodontitis is host-mediated, microbially-associated inflammation that causes periodontal attachment loss (Tonetti et al., 2018). Biomarkers can serve as a basis for identifying periodontal disease at an early stage, predicting how it will develop in the future, and evaluating the effectiveness of treatment. This, in turn, can facilitate improved treatment planning and prognosis (Gul et al., 2020).

Clinical parameters are exceptionally efficient methods for monitoring the health and disease status of the majority of patients, most likely due to their favorable response to conservative periodontal care principles, Continuous disturbance, and decrease of the bacteria present in

the gingiva and below the gingival line (Tonetti et al., 2018). However, there are limitations/drawbacks when diagnosing periodontitis based on the clinical parameters. Clinical parameters depend highly on the examiner's manual dexterity, experience, and practice (Tonetti et al., 2018).

Oral fluids are a promising diagnostic medium that contains important markers for periodontal inflammation. Salivary diagnostics has experienced a notable increase in interest and attention in recent years. Alternatively, an uncomplicated and non-invasive technique involving an oral rinse was implemented to gather oral polymorphonuclear leukocytes (PMNs) (Rijkschroeff et al., 2016). In recent decades, using biomarkers in oral fluids to diagnose periodontal disease has become popular (Nsaif and Hassan, 2023, Mohammed et al., 2022, YOSUF, 2019, Abdul-Mounther et al., 2017). Multiple research studies have examined various biomarkers in the circulation and gingival crevicular fluid (GCF) (Aljuboori and Mahmood, 2020). These studies were conducted with the assumption that chronic inflammation of the periodontium results in a long-lasting inflammatory state throughout the body (Zekeridou et al., 2019). Interleukin-33 (IL-33) is a nuclear cytokine derived from tissues and is a member of the IL-1 family. It is highly expressed in fibroblast-like cells, endothelial cells, and epithelial cells throughout the processes of homeostasis and inflammation. It serves as an alarm signal (alarmin) that is secreted in response to tissue damage or cell injury in order to alert immune cells that express the ST2 receptor (IL-1RL1) (Cayrol and Girard, 2018).

Three functions of IL-33 concerning periodontal disease have been suggested: alarmin, chemoattractant, and systemic cytokine. Once mast cells identify IL-33, they get activated and release histamines, prostanoids, proteases, leukotrienes, chemokines, and cytokines that draw neutrophils to the site of infection. The secretion of IL-33 and inflammatory mediators has been proposed to cause activation of osteoclast by raising the production of RANKL and lowering the production OPG (Cordero da Luz et al., 2014). Il1rl1, sometimes referred to as ST2, is a constituent of the IL-1 superfamily, and its sole identified ligand is IL-33. ST2 exists in two distinct splice types: sST2, a soluble form that functions as a decoy receptor by sequestering IL-33 without initiating any signaling, and ST2, a membrane-bound form that triggers the MyD88/NF-κB signals to enhance the activities of Th2 cells, regulatory T cells (T reg), innate lymphoid cells type 2 and mast cells (Griesenauer and Paczesny, 2017).

Furthermore, the existing data regarding the levels of IL-33 in periodontitis patients' oral fluids is both inadequate and conflicting: Both increased (Sağlam et al., 2017) and decreased (Buduneli et al., 2012) GCF levels and unaltered (Buduneli et al., 2012, Medara et al., 2020, Sağlam et al., 2017, Selman et al., 2021) salivary levels were seen when contrasting patients with periodontitis to individuals with healthy periodontium. Periodontitis and elevated serum levels of sST2 have been linked (Torrungruang et al., 2019); a recent pilot study showed that levels of ST2 in the gingival crevicular fluid were higher in individuals with periodontitis (Navya et al., 2022). The existing literature lacks sufficient research that has adequately examined the diagnostic accuracy of IL33 and sST2 in distinguishing between various stages of periodontitis (stage I, II, and III) and periodontal health.

2 MATERIALS AND METHODS:

2.1 Study design:

Between February and May 2023, a total of 162 subjects were selected based on specific inclusion and exclusion criteria. The participants were given an extensive array of details regarding the research endeavor and were subsequently required to complete a survey encompassing their personal characteristics, medical background, and dental records. Gingival crevicular fluid was obtained from each participant as a sample. The College of Dentistry/University of Baghdad's ethical committee adheres to the Helsinki and Tokyo criteria for human research (reference no. 734, dated 1/12/2022), which has accepted the study's protocol. The participants were separated into two groups: a control group consisting of 40 periodontally healthy persons and a study group composed of 41 patients with periodontitis with stage I, 40 patients with periodontitis with stage II, and 41 patients with periodontitis with stage III.

Periodontitis groups were defined as having interdental clinical attachment loss (CAL) of two or more in nonadjacent teeth or buccal/oral CAL of 3 mm or more with pocketing higher than 3 mm observed in at least 2 teeth. Periodontal health is characterized by a probing pocket depth (PPD) of 3mm or less, bleeding on probing (BOP) rate of less than 10%, and the absence of any loss of attachment during probing (Tonetti et al., 2018). Individuals who are diagnosed with medical conditions such as hypertension, diabetes mellitus, coronary heart disease, cerebrovascular disease, immunologic disorders, females who were pregnant or nursing, females who were using contraceptive pills, smokers, alcoholics, had taken antibiotics or anti-inflammatory drugs within the previous three months, had undergone extensive periodontal therapy within the previous six months, or were currently undergoing active periodontal treatment were all excluded from the study.

2.2 Periodontal parameters and clinical examination:

The full mouth plaque index (PI), bleeding on probing (BOP), probing pocket depth (PPD), and clinical attachment loss (CAL) were assessed for all teeth present in the mouth. A comprehensive oral examination was conducted using a periodontal probe (UNC-15) to assess six specific sites on each tooth (mesiobuccal, buccal, distobuccal, mesiolingual, lingual, distolingual), except for plaque scores, which were evaluated on four surfaces (mesial, buccal, distal, lingual). The clinical assessment did not encompass the evaluation of wisdom teeth.

2.3 Sample Collection:

The GCF samples were collected using Brill's technique, which involved inserting a paper strip until a minor resistance was detected and then maintaining it in place for thirty seconds (Gul et al., 2022). GCF was obtained from the two worst periodontitis-affected sites in the study groups and two sites from mesiobuccal and distobuccal areas in the control group. Prior to sampling, the weight of each Eppendorf tube (1.5ml) containing 300 µl additional phosphate buffer saline (PBS) was measured using an electronic scale (Sartorius-BL210S, Göttingen, Germany), with the initial weight being (Eppendorf tube, PBS, and added paper strips weight) (Figueredo et al., 2017). After removing all supragingival plaque with a sterile curet, the sample site was dried using an air spray. A paper strip manufactured by Oraflow Inc.® in New York, America, was inserted into the sulcus or pocket until a slight resistance was observed. The strip remained in place for 30 seconds. Strips contaminated with blood were discarded (Barros et al., 2016). After the gingival crevicular fluid collection, the

strips were placed in the same tube, and their weight was measured again within 30 minutes of collection. The GCF's volume was calculated using differential weighting (Griffiths, 2003). The amount of GCF collected was around 0.5 to 2.4 µL. The samples were subjected to centrifugation at a speed of 3000 revolutions per minute for a duration of 20 minutes. They were then stored in a frozen state at a temperature of -20 degrees Celsius until the IL33 and sST2 assay phase.

2.4 IL33 and sST2 measurement:

The GCF supernatant was analyzed for IL33 and sST2 concentration using Enzyme-linked immunosorbent assay kits from Cloud-Clone Corp and MyBioSource, USA. The kit described above demonstrated an assay sensitivity of less than 6.4 picograms per milliliter for IL33 and less than 0.127 nanograms per milliliter for sST2. The assays were conducted following the manufacturer's guidelines. The total level of GCF IL33 and sST2 was determined by measuring the concentration of IL33 and sST2.

2.5 Statistical analysis:

The data was described, analyzed, and presented using the Statistical Package for Social Science (SPSS version -22, Chicago, Illinois, USA). The Receiver Operating Characteristic Curve (ROC) was utilized to assess the diagnostic capacity as discrimination or differentiation is altered (Obuchowski et al., 2004).

3 RESULTS:

Table 1 presents data on plaque index (PI), bleeding on probing (BOP), probing pocket depth (PPD), and clinical attachment loss (CAL) for individuals with stage I, II, and III periodontitis.

Table 2 presents the diagnostic sensitivity and specificity of GCF IL33 and sST2 utilizing relative operating Characteristic curve analysis. All of the results showed statistical significance in differentiating GCF IL33 levels except that between stage II and stage III periodontitis, which did not yield a significant result.

Table (1): periodontal parameters for healthy control and stage I, II, and III periodontitis.

	PI	BOP	PPD	CAL
ctrl	5.724±3.758	5.655±1.995		
Stage I	24.054±17.061	32.071±20.137	4.003±0.019	1.925±0.182
Stage II	29.285±20.409	32.410±16.846	4.128±0.237	3.658±0.574
Stage III	31.693±16.099	32.005±19.406	4.459±0.375	4.173±0.855

Ctrl: periodontal health. All values are presented as mean ± standard deviation.

Table (2): GCF IL33 and sST2 sensitivity, specificity, and cut-off points.

Vars.	Comparison	AUC		P value	Optimal cutoff point	Sensitivity	Specificity
IL 33	Control X Stag I	0.654	Sufficient	**0.017**	132.035	58.8	62.5
	Control X Stage II	0.815	V.good	**0.000**	107.78	80	55
	Control X Stage III	0.870	V.good	**0.000**	141.20	85.4	65
	Stage I X Stage II	0.693	Sufficient	**0.003**	132.47	70	41.5
	Stage I X Stage III	0.731	Good	**0.000**	187.132	73.2	65.9
	Stage II X Stage III	0.520	Bad	0.755	229.275	53.7	47.5
	Control X PD	0.780	Good	**0.000**	136.67	71.3	65
ST2	Control X Stag I	0.338	Not useful	**0.002**	3.296	31.7	41.3
	Control X Stage II	0.961	Excellent	**0.000**	1.855	95	80
	Control X Stage III	0.996	Excellent	**0.000**	3.085	100	99.5
	Stage I X Stage II	0.831	v.good	**0.000**	2.66	82.5	48.8
	Stage I X Stage III	0.966	Excellent	**0.000**	3.916	95.1	85.4
	Stage II X Stage III	0.698	Sufficient	**0.002**	6.820	68.3	60
	Control X PD	0.926	Excellent	**0.000**	1.00	95.9	67.5

AUC: area under the curve, P value: significance level at $p < 0.05$.

4 DISCUSSION:

The aim of this study was to assess the diagnostic potential of IL-33 and sST2 in differentiating between periodontal health and disease, as well as differentiating the severity or stages of periodontitis. The main finding of this study was that the chosen biomarkers exhibited high sensitivity and specificity in differentiating between healthy periodontium and periodontitis. They also succeeded in differentiating stages of periodontitis except for IL-33, which showed a bad sensitivity and specificity value to differentiate between stage II and III, and for sST2, which showed not useful sensitivity and specificity value to differentiate between healthy control and stage I periodontitis.

Staging of periodontitis as a mean to express the severity of this disease has been introduced as a component of the diagnosis statement of the last classification system of periodontal/peri-implant diseases (Tonetti et al., 2018). Diagnostic biomarkers could compensate for the limitations of conventional clinical diagnosis methods, including radiographic images and full-mouth periodontal charting. GCF is a diagnostic tool that assesses inflammation and bone resorption biomarkers in the oral cavity. It is non-invasive, efficient, and easy to use (Aljuboori and Mahmood, 2020). GCF can differentiate between active inflammation areas, predict future tissue damage, and identify early signs of periodontitis. (Sanikop et al., 2012).

The results indicate a higher level of PI in all stages of periodontitis, which aligns with the findings of Asif et al. (2022). They observed an increase in plaque scores from mild to moderate to severe periodontitis, and this can be explained by the dose-response relationship between oral hygiene and periodontitis, where poorer oral hygiene leads to higher plaque levels and, consequently, more damage to the periodontium. (Lertpimonchai et al., 2017). The study's findings revealed that the control group had the lowest mean percentage of bleeding on probing. Nonetheless, its level increased significantly in patients with periodontitis. These findings could result from histopathologic changes causing aberrant gingival bleeding during gingival inflammation. These changes include capillary dilation and engorgement, as well as sulcular epithelial thinning or ulceration. Because the capillaries are inflamed and closer to the surface, and the thinning, deteriorated epithelium is less protective, modest stimulation usually causes capillary rupture and gingival bleeding. (Newman et al., 2018). These findings were consistent with (Saliem, 2016) and (Ali and Mahmood, 2018), which indicated that the percentage of bleeding sites in periodontitis groups was greater than in the control group. The mean values of PPD (probing pocket depth) and CAL (clinical attachment level) were the lowest in the Stage I group. However, there was a considerable increase in their average values from the Stage I group to the Stage III group. The possible causes of these effects are likely related to increased bacterial infiltration and the accumulation of plaque, which subsequently leads to the destruction of the sulcular and junctional epithelium and damage to the adjacent alveolar bone in cases of periodontitis. The findings were consistent with previous studies conducted by (Al-Rawi et al., 2011, Talib and Ahmed, 2016), which also reported higher average values of PPD and CAL in individuals with periodontitis.

Results showed that GCF IL-33 was good in differentiating periodontitis from periodontal health with 0.780 AUC, and it showed the potential of GCF IL-33 to differentiate stages of periodontitis; sensitivity ranges from 53.7% to 85.4%, while the specificity ranges from 41.5% to

65.9%. These results agreed with (Tarrad et al., 2018), who found that the AUC for GCF IL-33 is 0.740. Additionally, GCF sST2 was excellent in differentiating periodontitis from periodontal health, and it showed the potential of GCF sST2 to differentiate stages of periodontitis; sensitivity ranges from 31.7% to 100%, and specificity from 41.3% to 99.5%.

CONCLUSION:

The combination of GCF IL33 and sST2 showed a high specificity and sensitivity in differentiating between periodontal health and periodontitis. IL33 proved ineffective in differentiating between periodontal health and stage I periodontitis and stage II and III periodontitis. sST2 could not distinguish between the state of periodontal health and stage I.

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