Assessment of IL-38 And IL-40 Levels in Gingival Crevicular Fluid in Gingivitis and Stage III Grade C Periodontitis: A Comparative Clinical Study

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KEYWORDS
Gingival Crevicular Fluid, Interleukins, Interleukin-40, Macrophages, Periodontitis

ABSTRACT
Periodontal diseases are infections of the supporting apparatus of teeth caused by microbial agents, and cytokines have an important role in the inflammatory response. The purpose of this study is to measure the concentrations of interleukin-38 (IL-38) and interleukin-40 (IL-40) in the gingival crevicular fluid (GCF) in patients with stage III grade C periodontitis (SIII-GC), gingivitis and those with healthy periodontium. The study included a total of 75 people, 26 PH patients, 26 G patients, and 23 SIII-GC chronic periodontitis patients. Clinical parameters of a patient such as plaque index (PI), gingival index (GI), bleeding upon probing (BOP), probing pocket depth (PPD) and clinical attachment loss (CAL) were analyzed. GCF was collected and measured using the ELISA technique with IL-38 and IL-40 specific antibodies and kits. Interleukin-38 and interleukin-40 of the SIII-GC periodontitis group was significantly higher ($P < 0.05$) than other groups as regards normal gingival crevicular fluid IL-38 and IL-40 levels. However, differences in IL-38 and IL-40 levels were not significant between the two other groups: PH and G ones ($P > 0.05$). Positive correlation was found between GCF derived IL-38 and IL-40 levels with clinical periodontal parameters from sampled areas ($P < 0.001$). The strongest findings of this research indicate that within GCF, both IL-38 and IL-40 levels were elevated as total GCF IL-38 and total GCF IL-40 levels in different patients were high.

1. Introduction

The chronic disease known as periodontitis involves pathogenic inflammatory processes in the tissues that surround teeth. Although pathogens may initiate periodontitis, they do not cause the disease to progress on its own. The progression of the disease greatly depends on the host's immune reaction. In the case of periodontal disease pathogens, periodontium tissues release pro-inflammatory mediators as an immunological response to the insult from these pathogens.[1,2]

Interleukin-38 (IL-38) is an anti-inflammatory cytokine that has been identified recently. Interleukin-38 is produced by immune cells and specifically infiltrates monocytes and macrophages that associate with the resolution of inflammation.[3] It has been previously demonstrated that IL-38 decreased the release of certain pro-inflammatory mediators which may be beneficial in controlling inflammation and preventing otherwise irreparable tissues from being destroyed. It appears that IL-38 may facilitate a few other cytokines in an attempt to steer inflammation and shield target tissues from damage. Hence, IL-38 may be helpful in some inflammatory diseases like periodontitis.[4] IL-38, which is highly expressed in areas of chronic inflammation, is usually elevated in the gingival crevicular fluid (GCF) of individuals with periodontitis indicating an active role in the area of inflammation.[5]

The latest cytokine in the market, IL-40, has attracted interest due to its participation in the regulation of inflammation. IL-40, a product of immune cells such as macrophages and B cells, has immune response modifying and tissue repair assisting activities.[6] Temporary obstruction in Type-2 cells is also seen as IL-40 being associated with immune modulation, recruitment and activation of inflammation site cells.[7] Recent findings indicate that during inflammation, IL-40 might exert its effects on structural tissue of bone and bone marrow, minimizing tissue damage.[8] Increased IL-40 levels were observed in the GCF of patients with periodontitis which emphasizes that it is related to the inflammatory and immune responses in periodontal diseases.[9]

Previous research has looked into the levels of IL-38 and IL-40 in the GCF of patients with periodontitis; however, there have been limited studies that utilize the most recent classification criteria for periodontal diseases. This study intends to evaluate the levels of IL-38 and IL-40 in the GCF of individuals diagnosed with stage III grade C (SIII-GC) periodontitis and gingivitis (G), according to the updated classification system, and to compare these levels with those found in periodontally healthy (PH) individuals.

2. Material and Methods

2.1Research Group and Clinical Evaluation

Between December 2023 and November 2024, a total of 75 volunteers aged 28 to 48 years (mean age = 38.96 ± 5.11) participated in the study. This research was carried out in the Department of Periodontology and received approval from the Ethics Committee of Sri Sai College of Dental Surgery. All participants provided written consent after being informed about the study's purpose and design.

After taking patient histories, an oral examination was performed. Individuals were excluded from the study if they had systemic conditions such as diabetes or immunological disorders that could affect inflammation, were smokers, pregnant, breastfeeding, or had taken any medication that could influence the periodontium in the six months prior, as well as those currently receiving periodontal treatment. The study included volunteers who were free of systemic diseases, non-smokers, and had at least 16 permanent teeth.

All periodontal evaluations were carried out using a manual probe (Williams, Hu-Friedy, Chicago, IL) by a skilled and calibrated periodontist. The plaque index (PI), gingival index (GI), and bleeding on probing (BOP) were measured at four sites. Furthermore, periodontal probing depth (PPD) and clinical attachment loss (CAL) were recorded at six sites per tooth, excluding third molars. A calibration exercise was performed on five patients with periodontitis who were not part of the study, resulting in an intra-examiner reliability kappa coefficient of 0.90 for PPD and 0.88 for CAL.

Following the clinical assessment, participants were divided into three groups according to the 2017 classification system:[10]

1. **PH control group (n = 26):** This group includes individuals with clinically healthy gums on a stable periodontium, characterized by a bleeding on probing (BOP) of less than 10%, a probing pocket depth (PPD) of 3 mm or less, and no attachment or bone loss.
2. **Group G (n = 26):** This group consists of individuals with a BOP of 10% or higher, a PPD of 3 mm or less, and no attachment or bone loss, indicating plaque-induced gingivitis.
3. **SIII-GC periodontitis group (generalized) (n = 23):** Individuals in this group have an interdental clinical attachment loss (CAL) of 5 mm or more, a PPD of 6 mm or greater, and radiographic evidence of bone destruction extending to the middle or apical part of the root, with a maximum loss of 4 teeth due to periodontitis, qualifying them as having stage III periodontitis in 30% or more of the regions assessed. During the diagnosis of periodontitis, it was ensured that the CAL was not due to gingival recession from trauma, tooth decay reaching the cervical area, CAL observed in the distal area of the second molar related to the extraction or misalignment of the third molar, lesions in the marginal periodontium of endodontic origin, or vertical root fractures. The assessment of radiographic bone destruction was based on the tooth exhibiting significant bone loss relative to the root length. If there was rapid bone loss compared to the biofilm, and the percentage of root bone loss divided by age was greater than 1.0, it was classified as grade C.

2.2GCF Sampling Procedure

Gingival crevicular fluid (GCF) samples were collected in the morning, 24-48 hours after the clinical periodontal assessment. Samples were taken from the buccal side of two interdental spaces of single-rooted teeth in each jaw using paper strips (Periopaper; Proflow). For the periodontally healthy control group, GCF was collected from sites that were free of bleeding on probing (BOP) and inflammation. In the gingivitis group, GCF was gathered from areas exhibiting BOP and inflammation but without any clinical attachment loss. For the periodontitis group, GCF was collected from regions with the most significant radiographic bone loss and the highest probing pocket depth (PPD). After plaque removal with a sterile curette, the site was isolated using cotton rolls, air-dried, and the paper strip was placed in the gingival sulcus or periodontal pocket for 30 seconds to absorb the fluid. Any paper strips contaminated with oral fluids were excluded from the study. The collected strips were then placed in sterile tubes and stored at -40°C until analysis.

2.3Measurement of IL-38 and IL-40 levels in GCF

To measure the levels of IL-38 and IL-40 in GCF, enzyme-linked immunosorbent assay (ELISA) kits were used, following the manufacturer’s instructions (Human IL-38 ELISA kit and Human IL-40 ELISA kit-

Elabscience, Texas, USA). Before applying the GCF samples to wells pre-coated with antibodies for IL-38 and IL-40, the kit-provided standards were diluted according to the manufacturer's specifications. After adding the stop solution to each well, absorbance was recorded using a spectrophotometric ELISA reader (Microplate Reader; Biotek, Winooski). The total levels of IL-38 and IL-40 (pg/30s) collected over 30 seconds were quantified, with detection limits of 2.69 ng/mL for IL-38 and 1.06 ng/mL for IL-40.

2.4Statistical Analysis

In this study, we utilized G Power 3.1 software to determine the necessary sample size. Our calculations indicated that a minimum of 20 participants per group was essential to maintain a type I error rate of 0.05, with an effect size of 0.91 and a test power of 80% for a one-way ANOVA analysis. To assess whether the variables followed a normal distribution, we conducted the Kolmogorov–Smirnov and Shapiro–Wilk tests, which informed our choice of statistical methods. We set a critical value of p=0.05. Based on the results, if the p value was less than 0.05, the data indicated a non-normal distribution; conversely, if it was greater than 0.05, the data followed a normal distribution. To compare the groups, we employed the Kruskal–Wallis test, a nonparametric method, due to the non-normal distribution of the data. We analyzed frequency data using the chi-square test. Additionally, we assessed the relationship between clinical parameters and IL-38 and IL-40 levels in GCF through Spearman's rank correlation analysis. All data analyses were conducted using SPSS software (v. 22.0, IBM).

3. Result

Table 1 presents the demographic data and clinical parameters for the 75 participants in the study, detailing both the entire mouth and specific sampling sites. Among the participants, 57.33% were female and 42.67% were male. There were no significant age differences between individuals in the PH, G, and SIII-GC periodontitis groups (P > 0.05). The GI and BOP parameters, assessed for both the entire mouth and the sampling sites, were significantly higher in the SIII-GC periodontitis and G groups when compared to the PH control group (P <0.05). However, these parameters were similar between the SIII-GC periodontitis and G groups. Additionally, the PI, PPD, and CAL parameters were significantly increased in the SIII-GC periodontitis group compared to both the G and PH control groups (P <0.05) (Table 1).

Table 1: The study groups demographic profiles and clinical periodontal parameters.

	PH (n=26)	**G (n=26)**	**SIII-GC (n=23)**
Demographic variables			
Age (years)	38.65±6.24	38.42±4.51	39.82±4.34
Gender (female/male)	18/8	13/13	12/11
Periodontal parameters			
Whole mouth			
PD (mm)	2.85±0.16#‡	3.16±0.25*‡	7.44±0.28*#
CAL (mm)	0‡	0‡	5.18±0.27*#
GI	0.14±0.10#‡	1.98±0.24*	2.08±0.19*
BOP (%)	1.99±0.22#‡	75.82±5.84*	78.18±3.47*
PI	0.42±0.22#‡	1.70±0.33*‡	2.93±0.21*#
Sampling site			
PD (mm)	2.01±0.17#‡	4.00±0*‡	8.95±0.50*#
CAL (mm)	0‡	0‡	5.32±0.32*#
GI	0‡	2.27±0.12*	2.42±0.26*
BOP (%)	0‡	100*	100*
PI	0.76±0.18#‡	2.15±0.50*‡	2.99±0.14*#

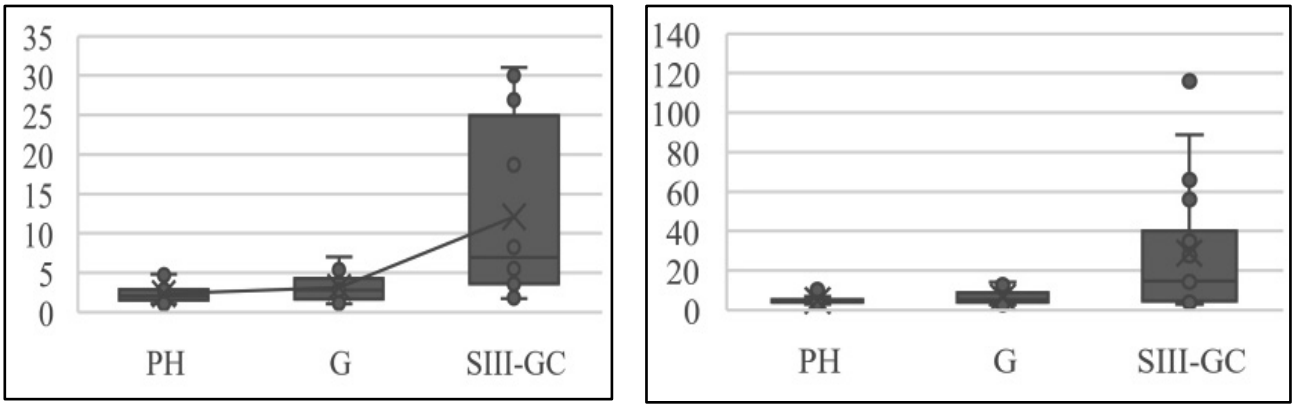
Abbreviations: PH, periodontally healthy; G, gingivitis; stage III grade C periodontitis, SIII-GC; PI, plaque index; GI, gingival index; BOP, bleeding on probing; PD, probing depth; CAL; clinical attachment loss. All data (except gender) are given as mean ± SD.

*Significantly different from PH

Significantly different from G

‡ Significantly different from SIII-GC periodontitis

Figure 1 shows the mean difference in total IL-38 and IL-40 levels in GCF. Total IL-38 and IL-40 levels in GCF were significantly higher in the SIII-GC periodontitis group compared to the G and PH control groups (P < 0.05). However, total IL-38 and IL-40 levels were similar between the PH control group and the G group (P > 0.05).



IL-38(pg/30s) IL-40(pg/30s)

Abbreviations: IL-38, interleukin-38; IL-40, interleukin-40; PH, periodontally healthy; G, gingivitis; SIII-GC, stage III grade C periodontitis.

Figure 1: The total concentrations of IL-38 and IL-40 in the gingival crevicular fluid (GCF) were measured in the PH, G, and SIII-GC periodontitis groups using ELISA.

Table 2. shows the relationship between the total IL-38 and IL-40 levels and the clinical parameters in the whole mouth and sampling site. GCF IL-38 and IL-40 total levels were positively associated with the whole mouth and sampling area clinical periodontal parameters (PI, GI, BOP, PD, and CAL) ($P < 0.001$).

Table 2: Correlations between total GCF IL-38 and IL-40 levels with clinical parameters of study groups

Clinical parameters	IL-38(pg/30s)	IL-40(pg/30s)
Whole mouth		
PI	0.537**	0.631**
GI	0.350**	0.246**
BOP	0.346**	0.293**
PD	0.617**	0.554**
CAL	0.575**	0.534**
Sampling site		
PI	0.580**	0.500**
GI	0.353**	0.298**
BOP	0.327**	0.284**
PD	0.599**	0.567**
CAL	0.585**	0.478**

Abbreviations: IL-38, interleukin-38; IL-40, interleukin-40; PI, plaque index; GI, gingival index; BOP, bleeding on probing; PD, probing depth; CAL, attachment loss. Spearman's rank correlation test. $P < 0.001$. All data are given as correlation coefficients.

4. Discussion

The 2017 reclassification of periodontal diseases established a new framework for assessing disease severity and progression, offering a structured way to understand periodontal health.[11] Our results suggest that IL-38 and IL-40 could be important biomarkers within this framework. The elevated IL-38 levels in SIII-GC cases indicate its role in local inflammatory responses, aligning with previous studies by Becerik et al.[12] and Toyman et al.[13], which found increased pro-inflammatory cytokine levels in periodontitis. The connection of IL-38 with anti-inflammatory properties, despite its elevation in periodontitis, implies that it may help modulate the inflammatory response, potentially preventing significant tissue damage. This underscores its promise as a therapeutic target, particularly in severe cases where excessive inflammation hastens periodontal deterioration.

Likewise, IL-40 was found in higher concentrations in the GCF of patients with SIII-GC periodontitis, reinforcing the idea of its role in bone resorption and immune regulation.[14] Prior research by Takahashi et al. has highlighted IL-40's function in promoting osteoclast differentiation, a key process in bone degradation.[15] The increased IL-40 levels align with other studies suggesting that IL-40 aids immune responses in inflamed tissues and plays a role in maintaining bone and tissue integrity.[16]

The positive correlation found in our study between IL-38 and IL-40 levels and clinical periodontal parameters,

such as plaque index, gingival index, and clinical attachment loss, suggests their potential as clinical biomarkers. This correlation has also been noted in previous research, highlighting the predictive ability of these cytokines in evaluating the severity of periodontal disease.[17] The use of GCF analysis for localized biomarker assessment enhances the specificity of our findings, as indicated in studies focusing on site-specific inflammation markers in periodontal research.[18]

While our results are promising, they are constrained by the sample size and the absence of serum and saliva analysis. Future research should involve larger sample sizes and consider other biological fluids to explore whether IL-38 and IL-40 can serve as systemic markers for inflammation, thereby expanding their diagnostic relevance in periodontal and other inflammatory conditions.

5. Conclusions

IL-38 and IL-40 levels in GCF show promise as markers for differentiating between gingivitis and advanced periodontitis. Their strong correlation with clinical measures highlights their potential in identifying individuals at increased risk for severe periodontitis.

Acknowledgements: No financial support was received.

Conflict of Interests: Nothing to declare.

Financial Disclosure Statement: Nothing to declare.

Human Rights Statement: Consent was obtained from the patient/s and approved for the current study by ethical committee.

Animal Rights Statement: None required.

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