

# INTERLEUKIN-8 AND INTERLEUKIN-1 $\alpha$ LEVELS IN THE GINGIVAL CREVICULAR FLUID OF PERIODONTITIS PATIENTS AND THEIR CORRELATION WITH PERIODONTAL POCKET DEPTH AND BLEEDING ON PROBING

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## ABSTRACT

**OBJECTIVE:** To investigate the gingival crevicular fluid (GCF) levels of interleukin (IL)-8 and IL-1 $\alpha$  in gingivitis and periodontitis sites in the same periodontitis participant, as well as their correlation with periodontal pocket depth (PPD) and bleeding on probing (BOP).

**METHODS:** This cross-sectional study was conducted at School of Dental Sciences, Universiti Sains Malaysia, from April 2019 to December 2021. GCF samples were collected from gingivitis ( $\leq 3$  mm) and periodontitis ( $\geq 5$  mm) sites of 30 periodontitis participants. In addition, the periodontal parameters (PPD and BOP) of the related sites were recorded. Enzyme-linked immunosorbent assay was used to determine IL-8 and IL-1 $\alpha$  levels in GCF. The correlations of IL-8 and IL-1 $\alpha$  in GCF levels with PPD and BOP were determined by the Spearman correlation test.

**RESULTS:** Mean PPD for 180 teeth was  $2.5 \pm 0.3$  mm and  $5.6 \pm 0.4$  mm in Gingivitis and Periodontitis respectively  $< 0.001$ . Median (IQR) BOP (%) was 21 (19-26) and 80 (70-90) in Gingivitis and Periodontitis respectively  $< 0.001$ . Median (IQR) IL-8 levels were 8.4 (1.8-25.9) pg/ml and 10.6 (2.3-18.3) pg/ml in patients with gingivitis and periodontitis respectively ( $p = 0.965$ ). Median (IQR) IL-1 $\alpha$  levels were 32.9 (17.5-70.8) pg/ml and 33.74 (15.1-45.9) pg/ml in patients with gingivitis and periodontitis respectively ( $p = 0.413$ ). No significant correlations was observed between interleukins & PPD and IL-8 and IL-1 $\alpha$  with BOP at gingivitis and periodontitis sites.

**CONCLUSION:** Both IL-8 and IL-1 $\alpha$  are present in the GCF of gingivitis and periodontitis sites, with no significant differences. Additionally, the levels of these interleukins are apparently unrelated to PPD or BOP.

**KEYWORDS:** Gingival Crevicular Fluid (MeSH); Intra-subject study (Non-MeSH); Interleukin-8 (MeSH); Interleukin-1alpha (MeSH); Gingivitis (MeSH); Periodontitis (MeSH).

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## INTRODUCTION

Periodontitis is a chronic inflammatory disease of periodontal tissue that manifests itself in the contribution of a variety of factors. Its occurrence is due to the oral microbiome's complicated interaction with the immunological response of the vulnerable host, which results in the destruction of the supporting oral structure.<sup>1,2</sup> In periodontitis, damage occurs in reaction to subgingival

bacteria, either directly or indirectly via inflammatory mediators that might result in tissue destruction.<sup>3</sup> Additionally, periodontitis can have a negative effect on other systemic diseases, such as rheumatoid arthritis, and diabetes.<sup>2</sup> Around half of the world's population is affected by periodontal disease.<sup>3</sup> Whereas, severe periodontitis effected almost 10.8% of global population in 2010.<sup>5</sup>

Cytokines are proteins that are

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required for the proper functioning of the body's immune system. During chronic inflammation, cytokines attract and retain pro-inflammatory cells.<sup>3</sup> Although bacteria cause periodontal disease, the intensity of immunoinflammatory destruction of the periodontal tissues is determined by the host immune response.<sup>2</sup> Cytokines are among the important components of the body's immune response, which are also critical for establishing inflammatory milieu and tissue destruction.<sup>6</sup>

IL-8 promotes neutrophil activation and chemotaxis, which can contribute to periodontal tissue damage.<sup>7,8</sup> Additionally, it stimulates osteoclastogenesis and inhibits osteoprotegerin synthesis in osteoblasts and stromal cells, which may aid in the development of periodontitis.<sup>2</sup> In periodontitis patients, gingival crevicular fluid (GCF) levels of IL-8 were reported to be significantly higher as compared to healthy patients. Intra-subject analyses of the IL-8 in GCF levels reveal contradictory results, with healthy sites exhibiting significantly higher levels than diseased sites.<sup>9</sup> Whereas, others observed increased IL-8 levels in diseased sites.<sup>10</sup>

IL-1 $\alpha$  is another pro-inflammatory cytokine that has been shown to enhance inflammation and alveolar bone resorption. Additionally, IL-1 $\alpha$  promotes prostaglandin E2 and matrix metalloproteinase production, which contribute to connective tissue and bone loss in periodontitis.<sup>2</sup> Several

studies on healthy and periodontitis subjects have demonstrated a correlation between the severity of periodontal disease and IL-1 $\alpha$  level in GCF.<sup>11,12</sup> To our knowledge, no study has compared GCF levels of IL-1 $\alpha$  in gingivitis and periodontitis sites of the same subjects. However, IL-1 $\beta$  expression was lesser in gingivitis lesions in comparison to periodontitis lesions within periodontitis subjects.<sup>13</sup> Furthermore, several studies reported that in periodontitis sites, IL-1 $\beta$  levels in GCF were substantially higher than healthy sites in the same subjects.<sup>9,14</sup>

There were limited studies comparing IL-8 and IL-1 levels in GCF between gingivitis and periodontitis locations in the same patients. Most studies reported the comparison of these cytokines levels in periodontitis subjects and those having healthy periodontium. However, these observational studies can be challenged since the individuals' GCF cytokines may be influenced by factors such as immunological response, dietary habits, and lifestyle decisions such as alcohol or smoking use.<sup>15</sup> Thus, the current study was designed to determine the GCF levels of IL-8 and IL-1 $\alpha$  in gingivitis and periodontitis sites within the same subject, as well as their correlation with periodontal parameters.

## METHODS

This cross-sectional study was conducted at Universiti Sains Malaysia between April 2019 and December 2021. The study enrolled a total of 30 participants ranging in age from 35 to 65 years who matched the following inclusion criteria: (1) having at least 20 teeth, (2) having 50% of teeth with a PPD  $\geq$  5mm, (3) having greater than 30% horizontal alveolar bone loss, (4) not undergone any periodontal

treatment in the last six months, (5) not having systemic disease, (6) not having taken any systemic antibiotic or other medication in the previous three months. In addition, any pregnant or lactating females were not included in this study. Prior to the start of the study, the protocol was explained to the participants, and their written consent was taken. Due to the potential heterogeneity of the samples, it is difficult to perform an accurate power calculation in these situations. Hence, we used a minimum of 30 gingivitis and 30 periodontitis sites in accordance with the standard practice of estimating a parameter with at least 30 samples or more.<sup>16</sup> Prior to the start of the study, the protocol was explained to the participants, and their written consent was taken.

This research was granted ethical approval from Universiti Sains Malaysia's Human Research Ethics Committee (USM/JEPeM/15100370), in (January 2016 to March 2019) and the research was conducted in accordance with the Helsinki Declaration of 1975, as revised in 1983.

Periodontal parameters measurements such as PPD, and bleeding on probing (BOP) were determined throughout the dentition by a single examiner using the University of North Carolina periodontal probe (Hu-Friedy, Chicago, IL, USA). PPD was measured to the closest mm as the distance from the crest of the gingival margin to the base of the pocket on six surfaces of teeth, which includes mesiobuccal, distobuccal, mesiolingual, distolingual, midlingual, and midbuccal.<sup>17</sup> BOP was measured as a percentage of sites that bled within 30 seconds of probing.<sup>18</sup> The GCF was collected from three teeth with gingivitis sites (PPD  $\leq$  3mm) and three with periodontitis sites (PPD  $\geq$

5mm). The cotton rolls were used to isolate the selected teeth for sample collection. Following that, a sterilized curette was used to remove supragingival plaque. GCF was absorbed for 30 seconds from gingival crevices using paper strips (Oraflow Inc, New York). For every new sample, a different site was selected. A separate centrifuge tube was used for each paper strip, and all tubes were placed at -80 degrees Celcius until needed.

Prior to ELISA, GCF samples were taken from the freezer and thawed. Three paper strips from gingivitis and three from periodontitis sites were pooled in two separate centrifuge tube and then eluted overnight at 4° C with 500  $\mu$ l phosphate-buffered saline (R & D, systems, USA). After centrifuging samples at 400g for 4 minutes, strips were removed and the supernatant stored at -20°C for future use.

IL-8 and IL-1 $\alpha$  levels were analyzed by ELISA using commercially available kits (R & D systems, USA) according to the manufacturer's instructions. The optical density was determined using the microtiter plate reader at a wavelength of 450 nm and then computed using a standard curve. The duplicate readings of optical density for each standard and sample were averaged. The results were reported as the total amount of IL-8 and IL-1 $\alpha$  expressed in pg/ml.

The statistical analyses were performed on IBM SPSS version 25 (SPSS Inc., Chicago, IL, USA). The normality of data distribution was determined by the Shapiro-Wilk test. The Mann-Whitney U-test was used to determine whether there was a statistically significant difference between groups. The Spearman Correlation test was utilized to determine the relationship between IL-8, IL-1 $\alpha$  and periodontal parameters.

**TABLE I: GINGIVAL CREVICULAR FLUID LEVELS OF IL-8 AND IL-1 $\alpha$  IN GINGIVITIS AND PERIODONTITIS GROUP**

Interleukines	Status	n	Median (pg/ml)	IQR	Z statistic	P Value <sup>a</sup>
IL-8	Gingivitis	30	8.4	1.8-25.9	-0.044	0.965
	Periodontitis	30	10.6	2.3-18.3		
IL-1 $\alpha$	Gingivitis	30	32.9	17.5-70.8	-0.819	0.413
	Periodontitis	29	33.74	15.1-45.9		

n = number of participants. Significant level was set to 0.05 ; a=Mann Whitney test

All statistical analyses used a significance level of 0.05.

## RESULTS

Out of 30 periodontitis subjects, 17 (56.7%) were males and 13 (43.3%) were females, ranging in age 31-62 years. Mean Periodontal Pocket Depth for 180 teeth was  $2.5 \pm 0.3$  mm and  $5.6 \pm 0.4$  mm in Gingivitis and Periodontitis respectively ( $p < 0.001$ ). Median (with inter quartile range: IQR) for bleeding on probing (%) was 21 (19-26) and 80 (70-90) in Gingivitis and Periodontitis respectively ( $p < 0.001$ ).

IL-8 and IL-1 $\alpha$  were detected in all gingivitis and periodontitis GCF samples within the range of 0.09 to 211.7 pg/ml and 4.25 to 262.1 pg/ml, respectively. Median (IQR) IL-8 levels were 8.4 (1.8-25.9) pg/ml and 10.6 (2.3-18.3) pg/ml in patients with gingivitis and periodontitis respectively ( $p = 0.965$ ). Median (IQR) IL-1 $\alpha$  levels were 32.9 pg/ml and 33.74 pg/ml in patients with gingivitis and periodontitis respectively (Table I).

IL-8 and IL-1 $\alpha$  in GCF were investigated to establish their relation to the periodontal pocket depth at gingivitis and periodontitis sites. Although weak correlations between interleukins and PPD were observed at gingivitis and periodontitis sites, they were not statistically significant (Figure 1). Similarly, IL-8 and IL-1 $\alpha$  were also found not to be statistically significantly correlated with BOP at gingivitis and periodontitis sites (Figure 2).

## DISCUSSION

In this study, periodontitis sites had a slightly higher GCF level of IL-8 than gingivitis sites. However, this difference was not statistically significant. Additionally, the GCF level of IL-8 in gingivitis and periodontitis sites was not significantly correlated with periodontal parameters such as PPD and BOP. This is consistent with the previous studies, which demonstrated that the GCF level of IL-8 was not significantly different between healthy and diseased sites<sup>10,19</sup> and was also not dependent on the gum's bleeding status.<sup>20</sup> Additionally, the GCF level of IL-8 was shown to be not significantly different across the various ranges of periodontal pocket depth evaluated, which were  $< 4$ , 4 to 6, and

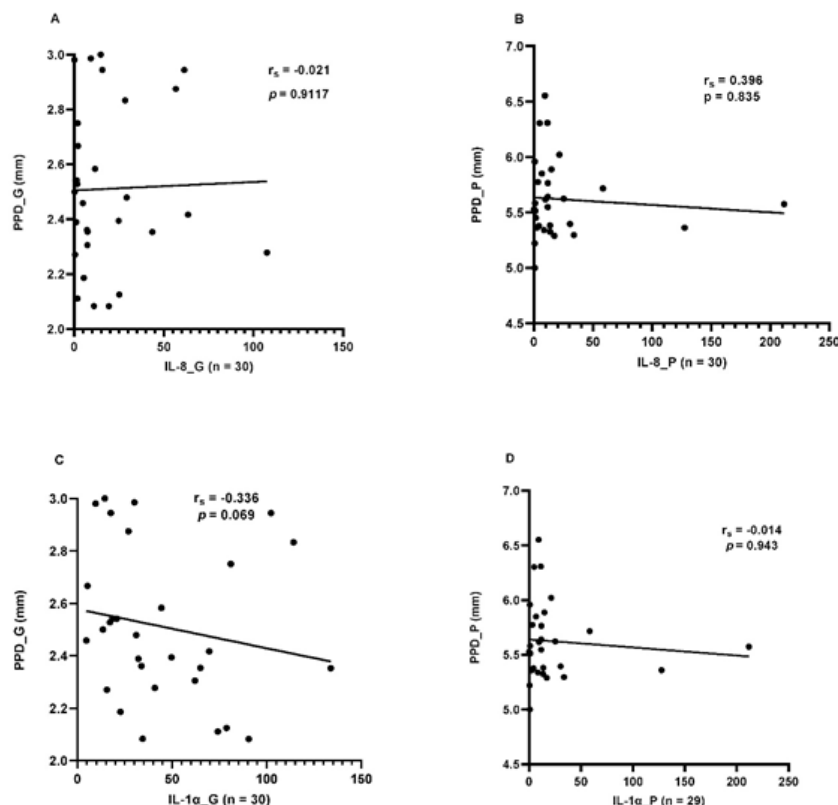


Figure 1: Scatterplot showing the correlation between PPD and GCF levels of IL-8 and IL-1 $\alpha$  at gingivitis and periodontitis sites.  $r_s$ =Spearman coefficient, G=Gingivitis, P=periodontitis, n=number of sites. Significant level was set to 0.05.

$> 6$  mm.<sup>21</sup> In contrast, it has also been observed that the IL-8 level in GCF is significantly higher in periodontitis sites than in healthy sites.<sup>22</sup> Moreover, a positive correlation between IL-8 levels and periodontal parameters such as PPD and BOP was observed.<sup>14</sup> Interestingly, the GCF level of IL-8 was also significantly lower in disease sites than in healthy sites, with a significant negative correlation with PPD and CAL.<sup>9</sup>

This study also demonstrated that the GCF level of IL-1 $\alpha$  was not significantly different between gingivitis and periodontitis sites and was also not correlated with PPD or BOP. Although we couldn't find any intra-subject studies comparing gingivitis and periodontitis sites on IL-1 $\alpha$ , an intra subject study on IL-1 $\beta$  expression was performed on gingivitis and periodontitis tissue and found a higher level of IL-1 $\beta$  in periodontitis lesion.<sup>13</sup> Several studies compared healthy (instead of gingivitis) and periodontitis sites on IL-1 $\alpha$  and IL-1 $\beta$ . In line with our

findings, the GCF concentration of IL-1 $\beta$  was not significantly different between healthy and diseased sites.<sup>7</sup> Furthermore, no significant correlation between IL-1 $\alpha$  and IL-1 $\beta$  level in GCF were found at periodontitis sites and pocket depth or bleeding index.<sup>23</sup> Additionally, the periodontal tissue levels of IL-1 $\beta$  at periodontally stable sites did not differ significantly from those at periodontitis sites.<sup>24</sup> However, there were also contradictory findings that reveal the GCF levels of IL-1 $\alpha$  and IL-1 $\beta$  were significantly elevated at diseased sites.<sup>19,22</sup> and significantly correlated with periodontal parameters such as PPD and BOP.<sup>9,14</sup>

In periodontitis patients, periodontal tissues become inflamed in general, not just at periodontitis sites, but also at gingivitis and clinically healthy sites. This may explain why GCF pro-inflammatory protein concentrations were found to be comparable among periodontal condition sites in patients with periodontitis.<sup>10</sup> IL-8 is a potent chemoattractant for Neutrophils and

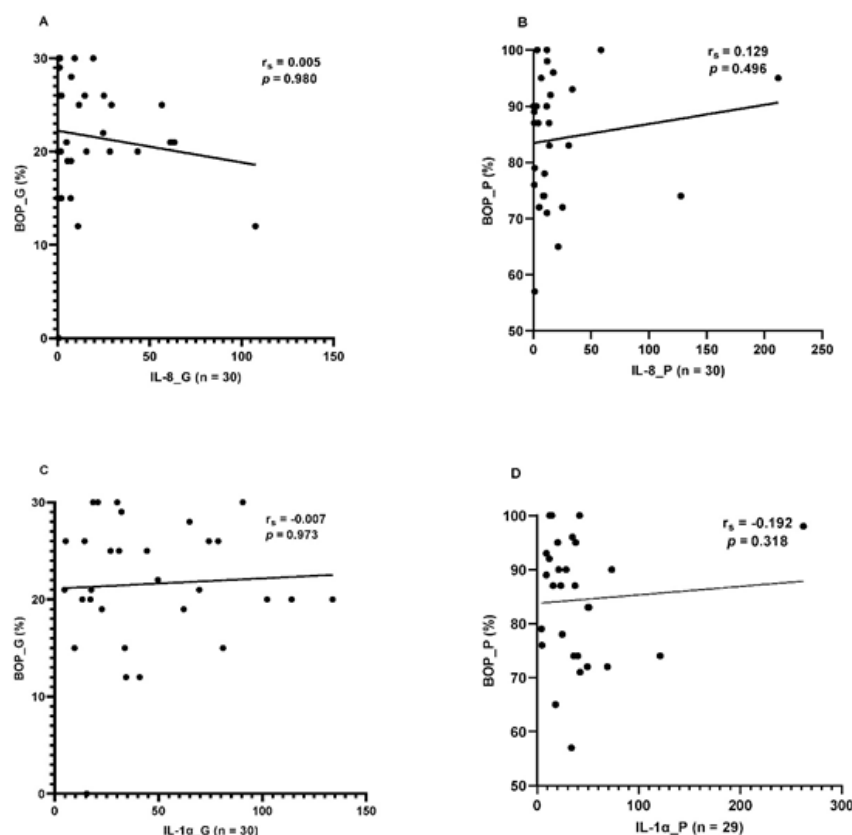


Figure 2: Scatterplot showing a correlation between BOP and GCF level of IL-8 and IL-1 $\alpha$  at gingivitis and periodontitis sites.  $r_s$ =Spearman coefficient, G=Gingivitis, P=periodontitis, n=number of sites. Significant level was set to 0.05.

antigen-presenting cells, which protect healthy gingiva that is constantly exposed to antigenic insult.<sup>20</sup> Additionally, IL-8 has been shown to boost the host immune response to gram-negative bacteria,<sup>25</sup> which are a common component of bacterial plaques. These findings suggest that IL-8 has a protective role for periodontal tissue, explaining their presence in healthy and diseased sites. Additionally, IL-1 $\beta$  has been demonstrated to promote normal gingival tissue turnover,<sup>13</sup> implying that their presence does not always indicate the severity of inflammation.

In comparison to the inter-subject study, this study investigated intra-subject levels of GCF interleukins, which has the advantage of controlling for individual factors associated with interleukin release either locally or systematically. Specifically, this study design can eliminate the influence of human factors such as habits, lifestyles, and immunity status. However, the

downside of this study design is that we cannot compare healthy and diseased sites since healthy sites are hard to locate in periodontitis subjects. As a result, this study was developed to compare the amounts of interleukins in gingivitis and periodontitis sites.

### Clinical Significance

This study provides data on the levels of IL-8 and IL-1 in gingivitis and periodontitis sites within the same patient, which can aid in the understanding of the role of these interleukins in the progression of periodontal diseases.

### Strength of Study

This study adds a fundamental understanding of the role of IL-8 and IL-1 $\alpha$  in periodontal diseases. By examining intrasubject interleukin levels, this study eliminates potential confounding variables that may contribute to the course of periodontal disease in different individuals, such as

lifestyle habits, oral hygiene, and immunological response.

## CONCLUSION

Within the limitations identified in this study, it can be concluded that IL-8 and IL-1 $\alpha$  are present in the GCF of gingivitis and periodontitis sites, with no significant difference in their levels. Also, there are no significant correlations between these interleukin levels with PPD or BOP. Although this study confirmed the role of IL-8 and IL-1 $\alpha$  in the inflammatory process, further research into their roles in periodontal tissue destruction in periodontitis is required.

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## AUTHOR'S CONTRIBUTION

Following authors have made substantial contributions to the manuscript as under:

**MT:** Conception & study design, acquisition, analysis and interpretation of data, drafting the manuscript, approval of the final version to be published

**WNSS:** Study design, acquisition of data, drafting the manuscript, approval of the final version to be published

**RAA:** Conception & study design, acquisition, analysis and interpretation of data, drafting the manuscript, critical review, approval of the final version to be published

*Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.*

## CONFLICT OF INTEREST

Authors declared no conflict of interest

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## DATA SHARING STATEMENT

Research data are not shared



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