

## Original Research Article

### Response of salivary interleukin-6 to non-surgical periodontal therapy in patients with periodontitis. A sub-Saharan experience.

#### Abstract

**Background:** Periodontitis is a multifactorial infection and inflammatory disease caused by an interplay between bacterial plaque and host immune response. Its pathogenesis is associated with a rise in pro-inflammatory cytokine levels. However, the use of salivary cytokine is not popular in determining the outcome of non-surgical periodontal therapy. The study aimed to assess how salivary IL-6 level responds to non-surgical periodontal therapy (NSPT) among patients in sub-Saharan Africa.

**Methodology:** In this prospective study, 49 patients with periodontitis (Group A) and 49 participants without periodontitis (Group B) were included. Baseline measurements of oral hygiene index (OHIS), bleeding on probing (BOP), probing pocket depth (PPD), number of sites with  $PPD \geq 4$  mm, and clinical attachment loss (CAL) were taken and compared to measurements at 3 months post non-surgical periodontal treatment (NSPT). Additionally, unstimulated whole saliva samples were collected before and after treatment to determine the salivary level of IL-6 using enzyme-linked immunosorbent assay (ELISA). The study used bivariate and multivariate analyses to assess the response of salivary IL-6 to NSPT. Results were considered statistically significant if  $P < 0.05$ .

**Results:** Ninety-eight participants, ranging in age from 19 to 58 years old with a mean age of  $32.55 \pm 10.11$  years, were included in the study. Both groups were comparable in terms of age, education, and socio-economic status, with a male-to-female ratio of 1:2.1. Group A had a statistically significant ( $p=0.001$ ) higher concentration of salivary IL-6 ( $17.41 \pm 3.39$

pg/ml) than Group B ( $7.05 \pm 1.37$  pg/ml) at the baseline. After treatment, Group A showed a noticeable improvement in all periodontal parameters and a decrease in the concentration of salivary IL-6. However, the correlation between the percentage change in the concentration of salivary IL-6 and the percentage change in PPD, CAL, and the number of sites with  $PPD \geq 4$  after NSPT was not statistically significant.

**Conclusion:** The concentration of IL-6 in saliva significantly decreased in participants with periodontitis after non-surgical periodontal treatment.

**Keywords:** Non-surgical periodontal therapy, Periodontitis, Salivary IL-6.

## INTRODUCTION

Periodontal diseases are pathological conditions affecting the gingival tissue and periodontal attachment apparatus.<sup>1</sup>Inflammatory periodontal disease has traditionally been divided into two categories; gingivitis and periodontitis.<sup>1,2</sup> Gingivitis is the milder form; it is reversible and can be defined as the inflammation of gingival tissues in the absence of attachment loss.<sup>1,2</sup> The more severe form is periodontitis which may result to alveolar bone loss and ultimately tooth mobility, abscess formation and tooth loss. Periodontitis is a major dental disease having a global impact.<sup>1-3</sup> It is a common infection affecting 15-20% of the grown-up populace worldwide.<sup>4</sup> Its prevalence in sub-Saharan Africa ranges from 19% to 90%.<sup>5</sup> Akpata<sup>6</sup> in a national review reported that the prevalence of periodontitis is about 15-58% in persons aged 15 years and above in Nigeria. The management of periodontal disease includes; control of risk factors, non-surgical and surgical periodontal procedures.

Recently, the medical literature has been inundated with evidence associating the status of periodontal health with several systemic conditions.<sup>7,8</sup> The systemic reflection of periodontal inflammation is being measured through the indices of some molecular biomarkers detectable in body fluids.<sup>9,10</sup> It is increasingly recognised that periodontitis is associated with systemic inflammation, and serum C-reactive protein (CRP) levels decrease after periodontal therapy.<sup>9</sup> The serum levels of IL-6, IL-8 and TNF- $\alpha$  have been observed to be elevated in chronically inflamed gingival tissues and in the gingival crevicular fluid (GCF) from patients with periodontitis.<sup>10</sup> The host's oral and systemic production of pro-inflammatory cytokines has led researchers to explore the relationship between periodontitis and systemic diseases and suggest a possible bidirectional link between periodontal diseases and multiple chronic diseases.<sup>10</sup> In periodontal diseases, bacterial antigens and other cytokines (IL-1 $\beta$ , TNF- $\alpha$ ) trigger the local production of IL-6.<sup>11,12</sup>

IL-6 is a multifunctional pluripotent pro-inflammatory cytokine that possesses a wide range of activities.<sup>13</sup> It regulates the immune response, haematopoiesis and the acute phase response.<sup>11,13,14</sup> In normal healthy subjects free of inflammation, plasma IL-6 concentrations are typically quite low, in the range of 0.2-7.8 pg/ml but can exceed concentrations of 1600 pg/ml in sepsis.<sup>15</sup> "Under healthy conditions, there is no evidence of a serum-saliva correlation for IL-6, and therefore, salivary IL-6 is likely to reflect inflammatory processes locally in the mouth".<sup>16</sup>

Salivary biomarker analysis can provide adjunctive information for health care professionals alongside traditional oral clinical examination regarding diagnosis, need for treatment and predicting/monitoring the treatment response.<sup>17</sup> Previous similar studies<sup>18-20</sup> used serum and gingival crevicular fluid (GCF) to quantify IL-6 in assessing the outcome of non-surgical periodontal therapy. Balli et al.<sup>19</sup> also found GCF IL-6 to be increased in patients with periodontal disease in contrast to orally healthy individuals, with an ensuing decrease following non-surgical periodontal therapy. Collection of serum and GCF is often not convenient for the patient and requires a professional to obtain such samples. Collection of salivary samples is more convenient, practical, rapid and non-invasive.<sup>16</sup> It requires neither professional technique nor specific materials compared to GCF and serum. Saliva represents a pooled sample from all periodontal sites providing an overall assessment of periodontal disease and health at the subject level.<sup>21</sup> However, whole saliva can be affected by local and systemic conditions.<sup>22</sup>

Traditionally, both diagnosis and assessment of the prognosis of periodontal diseases are based on clinical measures including bleeding on probing (BOP), probing pocket depth (PPD), clinical attachment loss (CAL) and radiographic assessment. However, these measures are of limited use for assessing current disease activity or predicting disease progression and

the patient's response to treatment.<sup>23</sup> Current approaches to periodontal diagnosis, including advanced microbiologic investigations (selective and non-selective culture methods, immunoassay, DNA probe, enzyme assay, polymerase chain reaction, DNA-DNA hybridisation), genomics, proteomics and biochemical analysis have been shown to provide the clinician with the information not available through traditional means.<sup>23</sup> Recently, there has been an increasing interest in using additional diagnostic/prognostic oral fluid biomarkers, such as alkaline phosphatase and matrix metalloproteinase (MMP)-8 in the management of periodontal disease.<sup>24,25</sup> The application of saliva-based diagnostic approaches will help to predict the susceptibility of patients, early identification of susceptible patients, response to treatment and the use of more specific prevention/treatment strategies for high-risk and low-risk patients.

Most of the studies exploring the association between cytokines and inflammatory periodontal disease were non-African and cross-sectional in design.<sup>1-4,26</sup> There are only a few prospective studies in this emerging area of periodontal research and these are mostly inconclusive.<sup>22,26,27</sup> Therefore, this study aimed to assess how salivary IL-6 levels respond to non-surgical periodontal therapy in our environment. This study will provide evidence for the possible use of salivary inflammatory biomarkers to monitor the progression of periodontal diseases and assess the outcome of non-surgical periodontal treatment. If proven relevant, the use of point-of-care testing of salivary biomarkers may augment the traditional method of using clinical parameters in the assessment of periodontal diseases.

## **MATERIALS AND METHOD**

The study was part of a larger project assessing the link between oral and systemic health. It was a prospective and comparative study conducted among patients attending the Periodontology clinic at the Dental Centre of UPTH. The participants were recruited into the study between January and May 2019. Approval for the study was obtained from the

Research and Ethics Committee of the University of Port Harcourt Teaching Hospital, Port Harcourt, Rivers State (Protocol number: UPTH/ADM/90/S. II/VOL.XI/353). The minimal risk associated with the study was explained to the study participants, and the investigation and intervention were at no cost to the study participants.

An equal number of cases and controls were selected and matched for age and gender. The inclusion criteria include; the presence of at least 20 natural teeth, Patients between 18 and 60years (adults)<sup>18</sup>, Patients with no established systemic diseases (such as Diabetes mellitus, hypertension, or rheumatic arthritis that can increase the level of inflammatory cytokines and also affect the outcome of non-surgical periodontal therapy) or suspicious with such diseases and confirmed by investigations, non-smokers, no periodontal treatment in the last 6 months, non-intake of antibiotics in the last 6-8 weeks. Patients who have not taken any medication known to affect the serum or salivary level of inflammatory markers e.g., NSAIDs and immunosuppressant, and willingness to participate with the ability to give informed consent. The sample size was calculated from a previous similar study using the formula for comparison of two groups when the endpoint is quantitative data ( $N = 2SD^2 (Z_{\alpha/2} + Z_{\beta})^2 / (d)^2$ )<sup>28</sup> and was found to be 45 for each group.

### **Sample Groups and Sampling Method**

#### **Group A- Participants with periodontitis**

A total number of 54 participants with gingival BOP > 10%, PPD ≥ 4mm and CAL ≥ 2mm at ≥ 2 non-adjacent sites<sup>29</sup> and who met other inclusion criteria were selected into this group using the systematic random sampling. The first participant was randomly selected from the sampling frame (daily register of adult patients visiting the dental clinic), subsequently, every patient meeting the inclusion criteria at a sampling interval of four (4) was recruited until the desired sample size was attained.

#### **Group B- Periodontally healthy control**

**Comment [M1]:** Please specify the category of periodontal disease as the latest classification (2017)

A total number of 54 periodontally healthy participants (with BOP < 10%, PPD ≤ 3mm)<sup>29</sup> from the hospital community meeting the matching criteria (age and gender) were recruited into this group using systematic random sampling.

### **Standardisation and Calibration of the Investigator**

Intra-examiner reliability was calculated by comparing 2 measurements (with an interval of one week) of PPD and CAL performed on ten patients with periodontitis not related to the study. Kappa statistics showed acceptable reliability with coefficients = 0.93 for PPD and 0.81 for CAL. The reliability testing also served as the pilot test for the data-collecting instrument.

### **Procedure for Data Collection**

Data were collected using self-developed structured questionnaires and clinical oral examination. The oral hygiene status before and after treatment was assessed using the simplified oral hygiene index (OHI-S)<sup>30</sup> and the periodontal status was recorded on a modified periodontal chart. All clinical measurements (BOP, PPD, and CAL) were taken using a UNC-15 graduated periodontal probe from six sites per tooth on all the teeth present in the patient's mouth except the last molars. The periodontal parameters were taken at baseline and 3 months post-treatment. CAL of 1-2 mm is considered mild; moderate: 3-4 mm; while ≥ 5 mm is severe periodontitis.<sup>4,29</sup> The participant's mean percentage of gingival BOP, mean PPD, percentage of sites with PPD ≥ 4mm and mean CAL were calculated from the periodontal chart. The outcome of treatment was defined as the difference between the pre- and post-periodontal parameters.

Comment [M2]: Stage? Grade?

Unstimulated whole expectorated saliva was collected from each participant in the two groups at baseline and 3 months post-treatment. Participants were asked not to eat, drink or use saliva stimulators such as chewing gum and mint for at least one (1) hour before giving the sample.<sup>16,23</sup> The study participants were asked to rinse their mouth with tap water, tilt their

head forward and then gently spit about 2 to 5mls of unstimulated saliva for 5 minutes into plain sterile tubes while seated in an upright position without swallowing. Collected samples were immediately placed in an ice bag and taken to the chemical pathology laboratory of UPTH where they were placed in a refrigerator at -20°C until analysed.<sup>16</sup> The salivary samples were analysed within 7 months of collection and discarded after the analysis.

Concentrations of salivary IL-6 were determined using an enzyme-linked immunosorbent assay kit (Human IL-6 ELISA Kit (Sensitivity: 1.03 pg/ml, standard curve range: 2 pg/ml-600 pg/ml); Bioassay Technology Laboratory, Shanghai, China). The procedure was done according to the manufacturer's directions by a laboratory technologist who was blinded to the salivary samples of the different groups by using codes on the collected samples known only to the principal investigator.

The study participants were given oral hygiene instructions to motivate them on oral hygiene measures (such as brushing techniques and interdental flossing). The clinical procedure (scaling and subgingival root planing) for Group A participants was done by the lead researcher using both manual and ultrasonic scaling instruments. The treatment regimen did not include antibiotics treatment.<sup>31,32</sup> Participants were reviewed after one week to reinforce the post-operative instructions, and at three (3) months to record their periodontal parameters in the periodontal chart after treatment. Group B received only oral prophylaxis (routine scaling and polishing only) after oral examination.

Data were analysed using the IBM Statistical Package for Social Sciences version 25.0 (IBM SPSS Statistics, Armonk New York). Tables and charts were used for data presentation appropriately. Numerical variables were presented using means and standard deviation. Results were expressed in frequency and percentages for the categorical variables (age groups, sex, educational level, socio-economic status, and severity of periodontitis). A chi-square test with

**Comment [M3]:** Why there is no recall for group B, one week after the base line to reinforce the oral health instructions and reassess how they perform brushing and flossing?

Fishers' exact correction, paired t-test, independent t-test and Pearson's correlation analysis was done. The statistical significance level was set at  $p < 0.05$ .

## RESULTS

A total of 108 participants were recruited for the study comprising 54 participants in each group. However, 98 (49 in each group) participants completed the study. The mean age of the 98 participants was  $32.55 \pm 10.11$  years with an age range of 19-58 years, while the male-to-female ratio was 1:2.2. The mean age  $\pm$  SD for Group A and Group B were  $33.83 \pm 10.14$  and  $31.26 \pm 10.0$  respectively. The difference in the mean age between the two groups was not statistically significant ( $p = 0.210$ ).

Most of the study participants were in the 30-39 age group and had a tertiary level of education with class 2 socio-economic status. There was no statistically significant difference ( $p > 0.05$ ) in the age groups, gender distribution, educational status, and socioeconomic status (SES) between the two groups (Table I).

**Comment [M4]:** Please check the numbers as in "abstract" part it is different (1:2.1)

**Table I: Sociodemographic data of study participants.**

Sociodemographic variables		Group A (n=49)	Group B (n=49)	$\chi^2$ , p-value
Age groups	20-29	14(28.6)	17(34.7)	1.03, 0.793 <sup>#</sup>
	30-39	23(46.9)	19(38.8)	
	40-49	7(14.3)	9(18.4)	
	50-59	5(10.2)	4(8.2)	
Gender	Male	16(32.7)	15(30.6)	0.05, 0.828 <sup>#</sup>
	Female	33(67.3)	34(69.4)	
Educational status	Primary	1(2.0)	0(0.0)	1.39, 0.49 <sup>#</sup>
	Secondary	8(16.3)	6(12.2)	
	Tertiary	40(81.6)	43(87.8)	
Socio-economic status	Class 1	8(16.3)	7(14.3)	1.76, 0.623 <sup>#</sup>
	Class 2	24(49.0)	20(40.8)	
	Class 3	0(0.0)	0(0.0)	
	Class 4	2(4.1)	1(2.0)	
	Class 5	15(30.6)	21(42.9)	
Age in years, mean $\pm$ SD		33.83 $\pm$ 10.14	31.26 $\pm$ 10.0	0.210 <sup><math>\beta</math></sup>
Age range		20 – 58	19 – 55	

$\chi^2$  = Chi-square value, <sup>#</sup>Fisher's exact p-value,  <sup>$\beta$</sup> independent t-test

### **The response of periodontal parameters and salivary IL-6 to non-surgical periodontal treatment**

Table II shows the results of the paired samples t-test performed to determine the response of clinical periodontal parameters and salivary IL-6 level to non-surgical periodontal therapy after three months in Groups A and B. It also shows the inter-group comparison using the independent t-test. There was a significant decrease in all clinical periodontal parameters and salivary IL-6 in the participants with periodontitis (i.e. both Groups A and B) at three months post-therapy compared to baseline values.

The OHIS significantly decreased from the baseline values in Groups A and B at follow-up. It reduced from  $2.77 \pm 0.71$  to  $0.83 \pm 0.36$  in Group A and  $0.74 \pm 0.28$  to  $0.29 \pm 0.12$  in Group B. The mean difference in OHIS between the two groups at 3 months post-therapy was statistically significant ( $p = 0.001$ ).

At 3 months post-treatment, the mean percentage of sites with gingival BOP had significantly decreased from 37.80% to 9.82% in Group A and from 6.67% to 3.28% in Group B. The mean difference in baseline and post-treatment values between the two groups was statistically significant ( $p < 0.001$ ). The mean PPD significantly reduced from  $4.44 \pm 0.34$  mm to  $3.03 \pm 0.36$  mm in Group A. Also, the percentage of sites with periodontitis ( $PPD \geq 4$  mm) significantly decreased from 11.57% to 5.57% in Group A.

Post-operatively, the concentration of salivary IL-6 significantly ( $p < 0.001$ ) reduced from  $17.41 \pm 3.39$  pg/ml to  $7.47 \pm 2.91$  pg/ml in Group A. Likewise, it significantly decreased from  $7.05 \pm 1.37$  to  $6.07 \pm 1.01$  pg/ml in Group B. The concentration of salivary IL-6 was significantly higher in Group A compared to Group B at follow-up,  $t(59.3) = 3.17$ ,  $p = 0.002$ .

**Table II: Means ( $\pm$ SD) of clinical periodontal parameters and salivary IL-6 for both Groups A and B at baseline and follow-up visits.**

Variables		Group A	Group B	Between groups <i>p</i> -values
OHIS	Baseline	2.77 $\pm$ 0.71	0.74 $\pm$ 0.28	< 0.001*
	3 months	0.83 $\pm$ 0.36	0.29 $\pm$ 0.12	< 0.001*
	IgP	< 0.001*	< 0.001*	
Bleeding on probing (%)	Baseline	37.82 $\pm$ 9.13	6.67 $\pm$ 1.55	< 0.001*
	3 months	9.82 $\pm$ 2.38	3.28 $\pm$ 0.81	<0.001*
	IgP	< 0.001*	< 0.001*	
Probing pocket depth (mm)	Baseline	4.44 $\pm$ 0.34		
	3 months	3.03 $\pm$ 0.36		
	IgP	< 0.001*		
Percentage of sites with PPD $\geq$ 4mm	Baseline	11.57 $\pm$ 4.48		
	3 months	5.57 $\pm$ 3.86		
	IgP	< 0.001*		
Clinical attachment loss (mm)	Baseline	3.58 $\pm$ 0.53		
	3 months	2.17 $\pm$ 0.48		
	IgP	0.024*		
Salivary IL-6 (pg/ml)	Baseline	17.41 $\pm$ 3.39	7.05 $\pm$ 1.37	< 0.001*
	3 months	7.47 $\pm$ 2.91	6.07 $\pm$ 1.01	0.002*
	IgP	< 0.001*	< 0.001*	

\*Statistically significant ( $p < 0.05$ ) IgP = Intragroup *p*-value

An independent t-test was performed to compare the mean percentage change in periodontal parameters and salivary IL-6 in Group A and Group B after NSPT (Table III). The change in OHIS observed in Group A compared to that observed in Group B after three months of NSPT was statistically significant ( $p = 0.001$ ). Likewise, the change observed in the percentage of sites with gingival BOP in Group A ( $73.27 \pm 6.67$ ) was significantly higher than in Group B ( $49.23 \pm 11.84$ );  $t(50.41) = 20.73$ ,  $p < 0.001$ . The percentage change in concentration of salivary IL-6 ( $57.69 \pm 10.99$ ) achieved in Group A over 3 months was significantly higher than that achieved in Group B ( $10.37 \pm 27.58$ ),  $t(83.58) = 23.47$ ,  $p = 0.001$ .

Reduction in periodontal parameters and concentration of salivary IL-6 observed after NSPT was higher in Group A compared to Group B.

**Table III: Comparison of the mean percentage change in periodontal parameters and salivary IL-6 after NSPT in groups A and B.**

Variables	Group A	Group B	t	df	p-value
OHIS	$70.60 \pm 8.93$	$55.62 \pm 26.39$	3.76	58.84	$<0.0001^*$
Bleeding on probing	$73.27 \pm 6.67$	$49.23 \pm 11.84$	12.38	75.64	$<0.0001^*$
Probing pocket depth	$31.49 \pm 7.55$				
Total percentage of sites with $PPD \geq 4$	$54.03 \pm 26.03$				
Clinical attachment loss	$38.78 \pm 13.05$				
Salivary IL-6	$57.69 \pm 10.99$	$10.37 \pm 27.58$	11.15	62.87	$<0.0001^*$

Data are presented as Mean  $\pm$  SD

\*Statistically significant ( $P < 0.05$ )

### Correlation of salivary IL-6 and clinical periodontal parameters

Pearson's correlation coefficients were computed to determine the relationship between the concentration of salivary IL-6 and clinical periodontal parameters at baseline (Table IV). The correlation between baseline OHIS, percentage of sites with gingival BOP, and the level of IL-6 in saliva was positive, high, and statistically significant; (OHIS:  $r = 0.869$ ,  $p\text{-value} = <0.0001$ ) (% BOP:  $r = 0.902$ ,  $p\text{-value} = <0.0001$ ). Also, the correlation of salivary IL-6 with the percentage of sites with  $\text{PPD} \geq 4$  mm was positive, moderate, and statistically significant,  $r = 0.442$ ,  $p\text{-value} = 0.001$ . However, the linear relationship between IL-6 in saliva and mean  $\text{PPD}/\text{CAL}$  was positive, weak, and not statistically significant.

**Table IV: The correlation of baseline periodontal parameters and salivary IL-6**

Baseline periodontal parameters		Salivary IL-6
OHIS	r	0.869
	p-value	<0.0001*
	N	98
Mean BOP	r	0.902
	p-value	<0.0001*
	N	98
Mean Probing pocket depth (mm)	r	0.154
	p-value	0.289
	N	49
Mean CAL	r	0.130
	p-value	0.373
	N	49
Mean Total Percentage of sites with $\text{PPD} \geq 4$ mm	r	0.442
	p-value	0.001*
	N	49

r = Pearson's correlation coefficient

\*Statistically significant ( $P < 0.05$ )

Table V shows the correlation between the percentage change in salivary IL-6 and the percentage change in periodontal parameters after NSPT. The relationship between the percentage change in salivary IL-6 and the percentage change in OHIS and gingival BOP was positive, moderate, and statistically significant; (OHIS:  $r = 0.424$ ,  $p\text{-value} = <0.0001$ ) (BOP:  $r = 0.552$ ,  $p\text{-value} = <0.0001$ ). The correlation of the percentage change in concentration of salivary IL-6 with the percentage change in PPD, CAL, and sites with  $PPD \geq 4$  mm was not statistically significant.

**Table V: The correlation of the percentage change in salivary IL-6 and the percentage change in periodontal parameters after NSPT**

Periodontal parameters		Salivary IL-6
OHIS	r	0.424
	p-value	<0.0001
	N	98
Mean BOP	r	0.552
	p-value	<0.0001
	N	98
Mean Probing pocket depth (mm)	r	0.234
	p-value	0.105
	N	49
Mean CAL	r	0.075
	p-value	0.607
	N	49
Mean Total Percentage of sites with $PPD \geq 4$ mm	r	0.033
	p-value	0.820
	N	49

r = Pearson's correlation coefficient

\*Statistically significant ( $P < 0.05$ )

## Discussion

Periodontitis is both a polymicrobial and multifactorial disease where clinical measures, such as BOP, PPD, and CAL reflect the current status and cumulative disease experience but have limited predictive value.<sup>33</sup> The potential application of saliva-based diagnostic tests for periodontal disease represents an exciting new opportunity for chair-side diagnostics based on its non-invasive characteristics. In this study, the response of salivary IL-6 to non-surgical periodontal treatment was assessed. The use of molecular biomarkers like salivary IL-6 can augment the traditional clinical parameters used in assessing and monitoring the progression of periodontal diseases.<sup>17,20,21</sup>

Similar to other studies,<sup>2,31,32,34</sup> there was a high percentage of females in this study with a male-to-female ratio of 1:2.2. This is not surprising since females tend to seek care for their oral health compared to males readily.<sup>31,32</sup> The 67.6% of female participants in the current study is within the range of 52.6 to 73% reported in earlier studies.<sup>2,31,32,34</sup> Moreover, hormonal fluctuations increase the incidence of gingival inflammation in females<sup>31</sup>; this could be responsible for the higher percentage of females in this study.

When the periodontitis group was compared to the periodontal healthy group in the present study; salivary IL-6 was remarkably higher in the group with periodontitis. This corroborates the findings of some studies that reported periodontitis as a low-grade chronic inflammatory disease associated with increased salivary IL-6.<sup>11,35</sup> In contrast; Teles et al.<sup>20</sup> did not observe any significant difference in the mean salivary level of IL-6 between individuals with healthy periodontium and those with periodontitis. Interleukin-6 modulates the response to bacteria in the periodontium and excessive IL-6 response contributes to chronic inflammation by amplifying the inflammatory cascade, activating T-cells and degrading periodontal

tissues.<sup>35</sup>This may, in turn, favour the growth of specific keystone pathogens which could predispose to further tissue damage.<sup>35-37</sup>

The mean baseline levels of salivary IL-6 in participants with periodontitis, and healthy controls reported in this study are within the range of values reported in previous studies.<sup>10,11,38,39</sup>The concentration of salivary IL-6 correlated with an increase in periodontal parameters in this study. This is similar to the observation of Batool et al.<sup>39</sup> in a similar study of patients with periodontitis and healthy controls. Also, Nanakaly<sup>40</sup> in a study of 60 subjects with similar mean age and gender distribution to this study, found the salivary level of IL-6 to be directly proportional with the extent of probing pocket depth. The positive linear relationship between IL-6 and clinical periodontal parameters in patients with periodontitis; supports the hypothesis that IL-6 is likely to be involved in the pathogenesis of periodontitis.<sup>11,13,38</sup> This underscores its role in inducing the differentiation of local osteoclasts that could result in bone resorption, the hallmark of periodontitis progression.<sup>39</sup>The findings in this study also corroborate the findings of Alwan et al.<sup>11</sup> and other similar studies<sup>31,34</sup> where IL-6 increased in the saliva, serum and GCF of patients with periodontitis compared to healthy controls.

In contrast to the finding of Alwan et al.<sup>11</sup> who observed a positive, weak, and significant ( $p = 0.041$ ) correlation between mean CAL and salivary IL-6 in patients with periodontitis; the positive relationship of salivary IL-6 with mean PPD and CAL in this study was not statistically significant. This may be attributed to the small number of patients with moderate to severe periodontitis in this study. These findings suggest that the salivary level of IL-6 may be used to differentiate severe periodontitis from mild and moderate forms of the disease. This also corroborates the previous studies<sup>11,31,39,40</sup> that found IL-6 to have diagnostic and prognostic potentials for the monitoring of periodontal disease.

This study showed that non-surgical periodontal therapy resulted in a significant improvement in all periodontal parameters, as observed in othersimilar studies.<sup>17,25,31,34,41,42</sup>NSPT has been reported to reduce the local as well as the systemic burden of inflammation (i.e., pro-inflammatory cytokines).<sup>31,34,43</sup>Positive participants' cooperation with oral hygiene instructions may have also contributed to this,<sup>25</sup>since the levels of salivary IL-6 significantly reduced in both groups. The mean percentage change in the gingival BOP in this study was comparable to earlier similar studies<sup>25,31,32,34</sup>where improvement in gingival BOP was better in patients with periodontitis at follow-up.Thechange in gingival BOP observed at follow-up among participants with periodontitisin this study was lower thanthose reported in previous studies<sup>31,34</sup> but higher than 21.2% reported byGoncalves et al.<sup>32</sup>The difference in the results reported in the previous studies<sup>25,31,32,34</sup> may be attributed to their inclusion/exclusion criteria, the severity of periodontitis, treatment protocol and different treatment time used for the non-surgical periodontal treatment.<sup>26</sup> Altay et al.<sup>34</sup>included smokers in their study while others<sup>2,25,31,32</sup> similar to the current study did not. Smoking has been found to affect the outcome of both non-surgical and surgical periodontal therapy.<sup>44,45</sup> In addition, treatment protocol in previous studies varies from 2 to 6 appointments for SRP completed between 7 to 14 days.<sup>25,26,32</sup>In this study, SRP was done in one appointment.

This study shows significantly better improvement in the percentage of sites with periodontitis (PPD $\geq$  4mm) in the participants with periodontitis after treatment similar to the study done in Malaysia by Akram et al.<sup>18</sup>but contrasted with Duzagac et al.<sup>46</sup>that found no significant difference.The percentage change in salivary IL-6 correlates positively with the percentage change in CAL in this study, however, it was not statistically significant. The short interval between baseline and post-treatment evaluation of periodontal parameters may have contributed to the findings in this study.<sup>26,47</sup> CAL being a measure of cumulative

periodontal disease may require a long-term prospective study for its treatment outcome relationship with salivary IL-6 to be ascertained.

The positive correlation between the percentage change in concentration of salivary IL-6 and the percentage change in the periodontal parameters in this study after NSPT; emphasizes the possibility of using salivary IL-6 to monitor the treatment response of periodontitis after non-surgical periodontal therapy. However, more longitudinal studies will be needed to determine the validated reference values of salivary IL-6 concentrations for periodontal health and the different subcategories of periodontitis.

#### **Limitations of the study**

The small sample size used in this study and the exclusion criteria (i.e. non-diabetic, non-smoker) limit the possibility of generalizing the findings

#### **Conclusion**

The concentration of IL-6 in saliva significantly decreased in subjects with periodontitis after non-surgical periodontal treatment. However, the correlation between the percentage change in salivary IL-6 and the percentage change in PPD, CAL, and sites with  $PPD \geq 4\text{mm}$  was not statistically significant. Periodontal clinical parameters are necessary for periodontal diagnostics; new approaches to periodontal diagnosis would correctly determine current disease activity, predict sites vulnerable to future breakdown, and assess the response to periodontal interventions. The use of biomarkers could give relevant clinical adjunctive information about the individual's host response levels, thus defining the optimal period between periodontal maintenance visits after active periodontal treatment.

**Consent:** As per international standards or institutional standards, participants gave their consent before participating in the study.

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