Serum and Salivary Levels of CXCL16 In Health and Periodontitis: A Comparative Preliminary Investigation

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Abstract

CXCL16 is a chemokine associated with disease, but no clear relationship has been reported in periodontal disease. The objectives of this study were to detect, estimate, and compare the levels of CXCL16 in saliva and serum and to find out if the saliva and serum levels are comparable in healthy individuals and periodontitis patients.

Thirty participants were divided into two groups: Group 1[G1(n=15)] were healthy (periodontally and systemically); Group 2 [G2(n=15)] were periodontitis patients (systemically healthy). Unstimulated whole saliva and serum were collected from each participant. Periodontal clinical variables were evaluated and levels of CXCL16 in saliva and serum of each participant were estimated using a commercially available Enzyme-Linked Immunosorbent Assay (ELISA) kit. The data were statistically analyzed. CXCL16 was detected in all the participants. Significant differences in CXCL16 levels were observed between the healthy and periodontitis groups.

The levels of CXCL16 were found to be significantly higher in the healthy group, although other reports in the literature suggest that the levels are higher in inflammatory conditions. Saliva may be a reliable source for the estimation of this chemokine.

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Introduction

Periodontitis is an inflammatory disease affecting the connective tissue attachment and the supporting bone around teeth.[1] The initiation and progression of periodontitis are dependent on microorganisms that can cause disease and the presence of virulence factors. Bacterial infection and host response to pathogenic infection are critical.

Periodontal disease results from the activation or inactivation of various inflammatory mediators which include cytokines, chemokines, and growth factors. The mechanism of the molecular network in the pathogenesis of periodontal disease is challenging to understand as research in this area is ongoing and new data and concepts are inundating our comprehension.

The literature also indicates a systemic relationship of periodontal disease. The association between systemic diseases and periodontal diseases such as an increase in the incidence of cardiovascular diseases,[2] adverse pregnancy outcomes,[3] chronic pulmonary disorders,[4] rheumatoid arthritis,[5] and carcinoma [6] have been established. One of the methods by which these relationships have been established is by associating biomarkers of interest in serum.

The network of inflammatory mediators in local infections such as periodontal disease as well as systemic diseases involves almost all cell types in the body.[7] However, it is unclear as to

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which cell types and mediators are definitively related to pathogenesis of periodontal disease and a systemic association. Chemokines, one of which is CXCL16 have a role to play in the pathogenesis of disease. The interaction between chemokines and their receptors is thought to be due to the migration of leukocytes into inflamed tissue.

Leucocytes migrate due to induction by chemokines and based on conserved cysteine motifs,[8] they are grouped as either type C, CC, CXC or CX3C. The major types of chemokines are secreted as small, soluble molecules; while there being two exceptions, i.e., fractalkine and CXCL16, which are expressed as membrane-bound molecules on the cell surface. This membrane-bound CXCL16 causes firm binding of cells that express CXCR6,[9] which is a unique receptor for CXCL16. Membrane-bound CXCL16 is made up of a C-terminal chemokine domain and a glycosylated mucin-like stalk which has a single helical transmembrane domain.[10] CXCL16 is a chemotactic pro-inflammatory mediator associated with several systemic inflammatory diseases and is considered an inflammatory biomarker. Many biomarkers have been identified from various body fluids. Among these saliva and blood are the most studied body fluids which contain reliable biomarkers to detect disease. Saliva is an informative body fluid that contains an array of analytes (protein, mRNA, and DNA) that can be used as biomarkers for clinical applications, and translation and blood profile provide a systemic expression of the biomarker's association with a local infection such as periodontal disease.[11,12]

Therefore, the objective of this study was to detect, estimate, analyze quantitatively, and compare the salivary and serum levels of CXCL16 in health and periodontitis, and to find out if salivary levels can be compared with serum levels to potentially use saliva as a non-invasive source of CXCL16 potential biomarker of periodontal disease.

Materials and Methods

This cross-sectional study was conducted in the Department of Periodontics, of the concerned institution. The institution's ethical committee provided the clearance for the research proposal. An informed consent was taken from all the participants before the commencement of the investigation. The study abided by the World Medical Association's Declaration of Helsinki.

Based on *a priori* the sample size was calculated as follows: standard deviation (SD) in group 1=2.85, SD in group 2=2.39, mean difference between group 1 and group 2 =2.68, effect size=1.02290076335878, α error=5%, power=80%, two-sided, number (n)=15 in each group, with the sample size formula being $n=2S^2 (z_{1-\alpha}+z_{1-\beta})^2/d^2$, where $z_{1-\alpha}$= z value for level (1.96 at 5% α error or 95% confidence), $z_{1-\beta}$ = z value for level (0.842 at 20% β error or 80% power), d=margin of error(=2.68), S=pooled SD= (S1+S2)/2.

Thirty volunteers of both sexes, aged 30-55 years were recruited. The inclusion criteria were individuals with a minimum of twenty natural teeth who were diagnosed with moderate-severe periodontitis (PD) (Stage II-III, following the recent classification of periodontal diseases);[13] having probing pocket depth (PPD) ≥5 mm; clinical loss of attachment (CAL) ≥2 mm with bone loss more than 15% of the root length as evidenced by radiographic imaging. The exclusion criteria were individuals with systemic diseases/immunocompromised; tobacco abusers; pregnant/lactating/menopausal women; subjects who would require prophylactic antibiotics preceding dental treatment, on an anti-inflammatory and/or antibiotics or any other medication which could affect bleeding and individuals who had periodontal treatment in the past three months. Participants were selected for each group after a thorough and precise case history recording that included the patient's chief complaint, clinical examination, and evaluation. The same operator carried out clinical measurements. All the participants were subjected to the recording of Gingival Index (GI),[14] Plaque Index (PI),[15] Bleeding on Probing (BOP)-the number of positive findings was then expressed in percentage of the number of gingival margins examined.

A UNC-15 (Hu-Friedy® Manufacturing Inc., Chicago, IL, USA) periodontal probe was used for assessing the probing pocket depth (PPD)-measurements were made at six different sites of each tooth present: mesio-buccal, mid-buccal, disto-buccal, mid-lingual, disto-lingual, and lingual). The clinical attachment loss (CAL) was measured as the distance between the cementoenamel junction and apical end of the

probe in millimetres for each tooth at six sites mesio-buccal, mid-buccal, disto-buccal, mid-lingual, disto-lingual, lingual. The CAL was not measured for the teeth with calculus or cervical caries or without a clinical tooth crown. Based on these inclusion and exclusion criteria and clinical examination, the participants were divided into two groups. Group 1(G1): 15 systemically and periodontally healthy participants and, Group 2 (G2): 15 systemically healthy periodontitis patients.

Serum and saliva samples were obtained from all the volunteers. Five ml of blood was collected by venepuncture using a 20-gauge needle from the antecubital fossa. The serum was then extracted from the blood. Ten ml of unstimulated whole saliva was collected in sterile tubes. The serum and saliva samples were stored at –80°C till the assay procedure. The samples were then assayed for levels of CXCL16 by using a commercially available ELISA kit (Krishgen Biosystems, Mumbai, India) as per the manufacturer's instructions.

Statistical analyses of the variables were performed based on the normality of distribution using the Kolmogorov-Smirnov/Shapiro-Wilk tests, after which appropriate tests such as the one-way/two-way ANOVA, Tukey's multiple post-hoc and Karl Pearson's correlation tests were applied. The probability value was set as $p < 0.05$. The IBM-SPSS-22 (IBM-SPSS, Armonk, NY, USA) software was employed for the analyses.

Results

Thirty systemically healthy participants grouped as 15 periodontally healthy individuals and 15 periodontitis patients were included in the study. The raw data was tabulated and expressed as mean and SD and are depicted in (Table1). The CXCL16 levels (ng/ml) in the saliva of the healthy group, the saliva of the periodontitis group, the serum of the healthy group, serum of the periodontitis group had the lowest and highest values of 5.8 and 18.4, 6.33 and 16.8, 3.8 and 13.9, 3.5 and 11.34, respectively.

	G1	G2	*p*-value
Sex (M/F)	8/7	9/6	-
Age	42.28±(4.67)	44.61±(3.89)	-
PI	0.46±(0.20)	1.55±(0.16)	0.0001*
GI	0.43±(0.22)	1.53±(0.11)	0.0001*
PPD	1.25±(0.14)	5.97±(0.79)	0.0001*
CAL	1.25±(0.14)	6.57±(0.73)	0.0001*
BOP	0.12±(0.03)	0.69±(0.09)	0.0001*
CXCL16 in saliva	11.71±(3.84)	10.14±(2.95)	0.0001*
CXCL16 in serum	8.30±(2.71)	6.94±(2.40)	0.0001*

Table 1. Demographic and Mean (±SD) values of the clinical parameters and significant differences by One-Way ANOVA between the three groups. G1: Group 1(health), G2: Group 2(periodontitis), PI: Plaque Index, GI; Gingival Index, BOP: Bleeding On Probing, PPD: Probing Pocket Depth, CAL: Clinical Attachment Loss.
* statistically significant ($p<0.05$)

The two-way ANOVA showed a statistically significant difference between saliva and serum levels of CXCL16 in the two groups but not between health and periodontitis (Table 2). Pair-wise comparison by Tukey's multiple post-hoc procedures revealed statistically significant differences in CXCL16 levels between healthy saliva and serum, periodontitis saliva and serum (Table 3).

	Degrees of freedom	F-value	*p*-value
G1 and G2	1	3.51	0.06
Saliva and serum	1	17.93	0.0001*

Table 2. Comparison of CXCL16 by two-way ANOVA. G1: Group 1(health), G2: Group 2(periodontitis).
* statistically significant ($p<0.05$)

G2 Saliva vs G2 Serum	0.02*
G1Saliva vs G2 Saliva	0.49
G1 Serum vs G2 Serum	0.60
G1 Saliva vs G2 Serum	0.0005*
G1 Serum vs G2 Saliva	0.35

Table 3. Pair-wise comparison of CXCL16 in saliva and serum of G1 and G2 by Tukey's multiple post hoc procedures.G1: Group 1(health), G2: Group 2(periodontitis).
* statistically significant ($p<0.05$)

In addition to these, the Pearson's correlation test and a multiple regression analysis were employed. It is to be noted that no statistically significant correlations or regressions were observed regarding CXCL16 (Tables 4-8).

Parameters		CXCL16 (ng/ml)	PI	GI	PPD	CAL	BOP
CXCL16 (ng/ml)	R_S	-					
	p-value	-					
PI	R_S	0.28	-				
	p-value	0.31	-				
GI	R_S	0.24	0.57	-			
	p-value	0.38	0.02*	-			
PPD	R_S	0.15	0.03	0.26	-		
	p-value	0.58	0.88	0.34	-		
CAL	R_S	0.15	0.03	0.26	1.00	-	
	p-value	0.58	0.88	0.34	0.00*	-	
BOP	R_S	0.01	0.34	-0.05	-0.24	-0.24	-
	p-value	0.96	0.20	0.83	0.37	0.37	-

Table 4. Correlations among CXCL16, PI, GI, PPD, CAL and BOP in G1 saliva by Spearman's rank correlation. G1: Group 1(health), PI: Plaque Index, GI; Gingival Index, PPD: Probing Pocket Depth, CAL: Clinical Attachment Loss, BOP: Bleeding On Probing, R_S: Spearman's Rank.
* statistically significant (p<0.05)

Parameters		CXCL16 (ng/ml)	PI	GI	PPD	CAL	BOP
CXCL16 (ng/ml)	R_S	-					
	p-value	-					
PI	R_S	-0.02	-				
	p-value	0.91	-				
GI	R_S	0.29	-0.06	-			
	p-value	0.28	0.82	-			
PPD	R_S	0.03	0.10	0.09	-		
	p-value	0.90	0.71	0.73	-		
CAL	R_S	0.05	0.08	0.05	0.93	-	
	p-value	0.84	0.77	0.84	0.0001*	-	
BOP	R_S	-0.32	0.14	-0.04	0.02	-0.03	-
	p-value	0.23	0.59	0.88	0.93	0.90	-

Table 5. Correlations among CXCL16, PI, GI, PPD, CAL and BOP in G2 saliva by Spearman's rank correlation. G2: Group 2(periodontitis), PI: Plaque Index, GI; Gingival Index, PPD: Probing Pocket Depth, CAL: Clinical Attachment Loss, BOP: Bleeding On Probing, R_S: Spearman's Rank.
* statistically significant (p<0.05)

Parameters		CXCL16	PI	GI	PPD	CAL	BOP
CXCL16	R_S	-					
	p-value	-					
PI	R_S	-0.17	-				
	p-value	0.52	-				
GI	R_S	-0.08	0.57	-			
	p-value	0.77	0.02*	-			
PPD	R_S	0.35	0.03	0.26	-		
	p-value	0.19	0.88	0.34	-		
CAL	R_S	0.35	0.03	0.26	1.00	-	
	p-value	0.19	0.88	0.34	0.0001*	-	
BOP	R_S	0.14	0.34	-0.05	-0.24	-0.24	-
	p-value	0.59	0.20	0.83	0.37	0.37	-

Table 6. Correlations among CXCL16, PI, GI, PPD, CAL and BOP in G1 serum by Spearman's rank correlation.G1: Group 1(health), PI: Plaque Index, GI; Gingival Index, PPD: Probing Pocket Depth, CAL: Clinical Attachment Loss, BOP: Bleeding On Probing, R_S: Spearman's Rank.
* statistically significant (p<0.05)

Parameters		CXCL16	PI	GI	PPD	CAL	BOP
CXCL16	R_S	-					
	p-value	-					
PI	R_S	0.22	-				
	p-value	0.42	-				
GI	R_S	-0.18	-0.06	-			
	p-value	0.51	0.82	-			
PPD	R_S	0.07	0.10	0.09	-		
	p-value	0.79	0.71	0.73	-		
CAL	R_S	0.01	0.09	0.04	0.95	-	
	p-value	0.96	0.72	0.87	0.0001*	-	
BOP	R_S	0.20	0.14	-0.04	0.02	-0.04	-
	p-value	0.45	0.59	0.88	0.93	0.86	-

Table 7. Correlations among CXCL16, PI, GI, PPD, CAL and BOP in G2 serum by Spearman's rank correlation. G2: Group 2(periodontitis), PI: Plaque Index, GI; Gingival Index, PPD: Probing Pocket Depth, CAL: Clinical Attachment Loss, BOP: Bleeding On Probing, R_S: Spearman's Rank.
* statistically significant (p<0.05)

Clinical Parameters	Estimates	SE	*p*-value	OR	95% CI for OR	
					Lower	Upper
PI	-0.58	1.67	0.72	0.56	0.02	14.74
GI	-1.57	1.73	0.36	0.21	0.01	6.15
PPD	-0.62	1.35	0.64	0.54	0.04	7.51
CAL	1.13	1.30	0.38	3.11	0.25	39.28
BOP	-0.22	3.38	0.94	0.80	0.00	603.47

Table 8: Multiple logistic regression analysis of CXCL16 with clinical parameters. PI: Plaque Index, GI; Gingival Index, BOP: Bleeding On Probing, CAL: Clinical Attachment Loss, PPD: Probing Pocket Depth, SE: Standard Error, OR: Odds Ratio, CI: Confidence Interval.
* statistically significant (p<0.05)

Discussion

The complexity of the host response at the cellular and molecular level to putative periodontal microbiota has resulted in enormous research to understand the pathogenesis of periodontal diseases.

The literature has a large volume of information regarding various biomarkers and their behavior in periodontitis. However, the interaction between these molecules and the networking of the signaling mechanisms are not well understood in entirety. This has led investigators to study the potential of these inflammatory mediators in the periodontal tissues, the gingival crevicular fluid, saliva, and serum. The inflammatory mediators include C-Reactive protein, matrix metalloproteinases, immunoglobulins, macrophage inflammatory protein-1 alpha, monocyte chemoattractant protein-1, leptin, adiponectin, cytokines such as the interleukins and TNF-α, as well as several chemokines, amongst others. By and large, most studies have pointed to a variation of these proteins in disease when compared with health.

CXCL16 has shown a contributive function in diseases such as colitis,[16] SLE,[17] rheumatoid arthritis,[18] angina,[19,] and atherosclerosis,[20] where its level was varying. At the outset, it has to be noted that expression of CXCL16 may be controlled by cytokines in periodontal disease.[21] Furthermore, Hosokawa et al., in 2009 demonstrated that CXCL16 induced HGF proliferation, which implies its role in periodontally diseased tissues.[22]

The present study had the objective of detecting and quantifying a chemokine CXCL16 in a cross-sectional setting involving healthy participants and periodontitis patients. CXCL16 was estimated in saliva and serum to obtain some information regarding the role of CXCL16 in periodontal pathogenesis.

About biomarkers in saliva, Miller et al.,[23] reported IL-1β, MMP-8, and OPG to be higher in periodontitis. MIP-1a and MCP were found to be significantly increased in gingivitis and periodontitis compared with health according to Nisha et al.[24] Rangbula et al.,[25] in their study observed salivary IgA, IL-1β, MMP-8 in periodontitis patients were significantly higher than in the periodontally healthy subjects and a relationship with clinical variables like PI, GI, PPD, CAL, and BOP was observed. Another study by Al Sabbagh et al.,[26] involving salivary MIP-1a, OPG, C-telopeptide pyridinoline cross-links of type I collagen and b-C-terminal type I collagen telopeptide concluded that these biomarkers could discriminate health from periodontal disease based on their elevated levels. In the present investigation assessing CXCL16, the salivary levels were found to be higher in health as compared with periodontitis.

When considering the inflammatory mediators in the serum of periodontally diseased individuals to provide insight into the systemic inflammatory response, numerous studies have concluded that a significant variation exists in the levels of the mediators that were investigated. For example, Pradeep et al.,[27] reported higher levels of serum oncostatin M in periodontitis with a positive correlation to PPD and CAL which decreased after non-surgical therapy, when compared with a healthy group. Significant differences between health and periodontitis were found in serum leptin, IL-6, and CRP levels by Shimada et al.,[28] which was associated with periodontal clinical variables. The concentration of these serum biomarkers in periodontitis decreased after non-surgical therapy. Ide et al.,[29] concluded from their research that serum fibrinogen, C-reactive protein, sialic acid, TNF-α, IL -6, and -1β did not vary significantly between a diseased state and that of health. Teles et al.,[30] examined serum levels of IL-6, TNF-α, adiponectin, leptin, resistin, and vitamin D and they concluded that therapy to bring a diseased periodontium to health did not have any influence on the serum levels of the biomarkers assessed, although a significant effect was seen in clinical and microbiological variable. This is noteworthy because in our current study, from a cross-sectional point of view, the CXCL16 levels in serum were higher in health when compared with periodontitis, having no statistical significance, mirroring the estimates in saliva.

Studies involving a comparison of both saliva and serum have been undertaken. Detzen et al.,[31] evaluated soluble CD163 (sCD163) in serum and saliva which was increased in periodontitis and correlated positively. Purwar et al.,[32] estimated leptin in the saliva and serum of periodontitis patients. Their observations indicated a significantly lower concentration of salivary leptin in periodontitis as compared with health and vice versa for the serum leptin concentrations. The aforementioned literature

points out the contrasting results inferred based on the study designs and settings as well as the molecules evaluated. CXCL16 in the present study is higher in health as compared with periodontal disease in both saliva and serum. A statistically significant difference in the levels of CXCL16 between saliva and serum in health and periodontitis, as well as healthy saliva and serum of periodontitis was observed.

A clearer explanation would have been possible if some of the cytokines that control the expression of CXCL16 were also evaluated in this study. The observations of this study, where serum and salivary CXCL16 was lower in periodontitis as compared with health reflects the results of the investigation by Sheikine et al.,[19] in angina pectoris, who observed higher CXCL16 in healthy controls. They have suggested lower levels of CXCL16 in disease to have an athero-protective role. According to Day et al.,[33] CXCL16 is a chemokine that is constitutively expressed and involved in steady-state cell trafficking and homeostatic control as demonstrated in their study on bronchial epithelial cells and their observation that there was no difference in the CXCL16 concentrations between health and inflammatory lung disease. Hence, it is our guarded hypothesis that lower levels of CXCL16 observed in periodontitis in the current study could indicate a similar inference, i.e., it being periodontal-protective in an inflammatory condition or constitutively expressed in a healthy state.

Limitations of the study

Our study may have the drawback of less power owing to a relatively small sample size. Also, a large variation in the individual levels of CXCL16 in ng/ml could have presented the current observations. It is a matter of conjecture if CXCL16 has a potential role as a biomarker of periodontal disease. This study does not demonstrate a clear role for CXCL16 in periodontitis as its levels were not significantly corroborating in saliva and serum and no forthcoming correlations as compared with health.

Conclusion

The literature reveals many studies assessing the role of CXCL-16 in systemic diseases, where the levels of CXCL-16 were significantly higher in diseased conditions as compared with health, except in a few. To the best of knowledge, this study is a first of its kind where the levels of CXCL-16 in saliva and serum were investigated. As the results are not in agreement with the majority of the studies in the literature which attribute a pro-inflammatory role for CXCL-16 in systemic diseases, the current report suggests that CXCL-16 should be studied further to validate its potential role in a local inflammatory disease such as periodontitis, and also explore the association with other systemic diseases.[34-36]

Declaration of Interest

The authors report no conflict of interest.

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