Evaluation of Serum CXCL2 in Health, Periodontitis and Type 2 Diabetes Mellitus with Periodontitis

Anirudh B. Acharya[1]*, Noopur Sahu[2], Srinath Thakur[3], Betul Rahman[4], Nadia Khalifa[5]

1. Department of Preventive and Restorative Dentistry, College of Dental Medicine, University of Sharjah, Sharjah, U.A.E.
2. Private Practice, Rourkela-769009, India.
3. Department of Periodontics, I.T.S. Dental College, Ghaziabad-201206, India
4. Department of Preventive and Restorative Dentistry, College of Dental Medicine, University of Sharjah, Sharjah, U.A.E.
5. Department of Preventive and Restorative Dentistry, College of Dental Medicine, University of Sharjah, Sharjah, U.A.E.

Abstract

CXCL2 is an important chemokine produced by inflammatory cells, which has a principal role in chemotaxis of neutrophils and immune responses. The objective of this study was to detect, estimate and compare the serum levels of CXCL2 in health, periodontitis and type 2 diabetes mellitus with periodontitis which has not been reported in the literature.

Forty-five participants were equally divided as Group 1(15 systemically and periodontally healthy subjects), Group 2(15 systemically healthy individuals with periodontitis) and Group 3(15 periodontitis patients with type 2 diabetes mellitus) after controlling for sources of bias. Periodontal and glycemic parameters were recorded. Serum was obtained from all participants. The levels of CXCL2 were estimated using a commercially available ELISA kit.

CXCL2 levels in serum was highest in Group 1, followed by Group 2 and lowest in Group 3. A statistically significant difference was observed between Groups 1 and 2, and Groups 1 and 3. This investigation indicates lower serum CXCL2 concentrations in disease contrary to the literature which generally report elevated levels of this chemokine. Adiposity and neutrophil behaviour may influence CXCL2 expression. More studies are required to confirm a definitive association of CXCL2 in periodontitis and type 2 diabetes mellitus.

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Introduction

Periodontitis(PD) is a complex multifactorial, inflammatory disease of polymicrobial etiology, principally caused by specific pathogen-associated molecular patterns and microbial virulence factors and characterized by progressive destruction of the periodontal connective tissue and alveolar bone.[1,2]

By way of response, the host produces mediators to these pathogens that include numerous cytokines and chemokines. These mediators seem to play a significant role in the inflammatory/immunological pathways leading to the destruction of periodontal tissues. Chemokines are proteins that induce chemotaxis of concerned cells, and are necessary because their expression is responsible for the activity of different cells and leukocytes in health and diseases. CXCL2 (also known as macrophage inflammatory protein-2 alpha (MIP-2α), growth-regulated protein beta (Gro-β), Gro Oncogene-2 (Gro-2) and cytokine-induced neutrophil chemoattractants-3 (CINC-3) is a chemokine belonging to the CXC chemokine family. CXCL2 binds to its receptor CXCR2 and has several biological effects. It is released by a variety of cells, including epithelial cells, fibroblasts, mast cells, monocytes, macrophages and exerts its action during inflammation, healing and other angiogenic processes.[3-6]

Neutrophils are recruited by the periodontal tissues through CXCR2 that has two ligands, CXCL1 and CXCL2. The junctional epithelium of mice has demonstrated expression of both ligands, but evidence exists about oral bacteria influencing an elevated expression of

*Corresponding author:
Assistant Professor Dr. Anirudh B. Acharya
Department of Preventive and Restorative Dentistry
College of Dental Medicine, University of Sharjah
Sharjah, U.A.E.
E-mail: abacharya@gmail.com

CXCL2 in the epithelium of gnotobiotic mice.[7] A couple of investigations[8,9] have revealed that CXCL2 can regulate endotoxin-induced transmigration and extravascular tissue accumulation of leukocytes and osteoclastogenesis. CXCL2 expression is higher in rheumatoid arthritis,[10] hypertension,[11] obesity,[12] oral cancer[13] and diabetes mellitus[14] and the association of these systemic conditions with periodontitis is well established. Hence, it can be hypothesized that CXCL2 may be involved in the pathophysiology of periodontal disease as well.

PD and type 2 diabetes mellitus (T2DM) are considered chronic low-grade inflammatory conditions that share the collective actions of inflammatory mediators on a common pathological platform; an altered expression of CXCL2 in serum may be an ancillary factor contributing to the pathogenesis of PD and T2DM.[15-17]

To the best of our knowledge no studies have evaluated the serum levels of CXCL2 in PD and T2DM together. Therefore, this novel investigation aimed to detect, estimate and compare the serum levels of CXCL2 in health, PD and T2DM with PD (T2DM+PD) and to discern a possible association between PD and T2DM through CXCL2.

Materials and methods

This project 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 prior to commencement of the investigation. The study abided with the World Medical Association's Declaration of Helsinki.

The source of the sample was the above mentioned institution and associated hospital. Volunteers were subjected to an initial screening and only those whose triglycerides < 150 mg/dL, total cholesterol< 200 mg/dL, low density lipoprotein< 100 mg/dL, high density lipoprotein within 40-60 mg/dL and basal metabolic index< 30 were recruited to fulfil the exclusion and inclusion criteria to control for potential sources of bias. The exclusion criteria were: individuals diagnosed with systemic diseases or who were immunocompromised that would be detrimental to take part in the study; tobacco abusers; pregnant/menopausal women; subjects indicated for prophylactic antibiotics preceding dental procedures or medication which could cause prolonged bleeding; individuals who had undertaken any periodontal treatment in the recent three months or who were on an anti-inflammatory or/and antibiotic drug treatment before the investigation. The inclusion criteria were: individuals with a minimum of twenty natural teeth who were diagnosed with moderate-severe PD (Stage II-IV, following the recent classification of periodontal diseases);[18] 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; people with T2DM were required to have been diagnosed with it for at least 1 year (no co-morbid conditions/diabetic complications) with glycated hemoglobin (HbA1C) ≥7.5 and random blood sugar (RBS) ≥200 mg/dl at the time of sample collection. The T2DM group of participants were on prescribed insulin/oral hypoglycemic agents, having had no major modifications in diabetes therapy during the past year.

Forty-five participants (males and females), aged 18-65 years were consequently enrolled after screening and in compliance with the aforementioned inclusion and exclusion criteria. A systematic medical and dental history was compiled and the participants were divided into three groups based on clinical examination and laboratory investigations. Group 1 comprised 15 systemically healthy participants; Group 2 included 15 systemically healthy participants with PD; Group 3 completed the sample with 15 individuals having T2DM with PD. Periodontal clinical variables included oral hygiene index-simplified (OHI-S),[19] gingival index (GI),[20] plaque index (PI),[21] bleeding on probing (BOP-as a percentage),[22] PPD, CAL and radiographic evidence of bone loss documented by a single examiner. Estimation of HbA1C and RBS was done by the concerned department and personnel.

Procedure for sample collection: Two ml of blood was withdrawn by venepuncture from the antecubital fossa (after disinfection of the area) using a 2ml regular syringe with a 20-gauge needle. The blood sample was allowed to clot at room temperature for 10-15 minutes and then centrifuged at 2700 rpm for 10 minutes. The serum thus obtained was collected in Eppendorf

tubes and stored at –80 ℃ till the assay procedure. Samples were assayed as per the manufacturer's instructions for estimating the levels of CXCL2 using a commercially available ELISA kit [Krishgen Biosystems, Mumbai, India], which was conducted in the associated laboratory. The data obtained were statistically analysed.

Statistical analysis: The SPSS-23 (IBM, Armonk, NY, USA) software was used for the statistical analysis. The data were expressed as mean ± standard deviation (SD) and the normality of distribution of the measured parameters was tested using the Kolmogorov-Smirnov test. Applicable statistical tests (One-way ANOVA, Kruskal-Wallis ANOVA, Mann-Whitney U-test, Tukey's multiple post-hoc, Karl Pearson's correlation) were used based on the normality of distribution for the various intragroup and intergroup comparisons. The probability value was set as $p < 0.05$.

Results

Twenty-one males and twenty-four females with a mean age of 43.21 (SD ± 2.65) constituted the forty-five participants. Table 1 depicts the group differences of the variables including demographics. The pair-wise comparisons of the three groups are shown in Table 2.

Parameter	Group 1	Group 2	Group 3	*p*-value
Sex (M/F)	7/8	6/9	8/7	0.416
Age	40.32±8.05	43.71±7.23	45.65±5.25	0.629
OHI-S	0.60 ± 0.26	3.59 ± 0.83	4.03 ± 1.10	0.001*
PI	0.45 ±0.16	2.11±0.31	2.394±0.41	0.001*
GI	0.39±0.16	2.11±0.39	2.77±0.35	0.001*
PPD	2.35±0.39	6.26±0.73	6.95±0.73	0.001*
CAL	1.35±0.39	6.86± 0.50	7.23± 0.70	0.001*
BOP	0.15±0.11	0.94±0.09	0.96±0.07	0.001*
HbA1C	4.35±0.48	5.21±0.18	8.94±0.75	0.001*
RBS	108.68±13.97	115.76±11.46	269.32±21.03	0.001*
CXCL2 (pg/ml)	1557.43± 201.93	544.23±53.82	352.50±86.32	0.001*

Table 1. Demographic data and Mean ±SD values of the parameters and differences between the three groups.

OHI-S: Oral Hygiene Index-Simplified, PI: Plaque Index, GI; Gingival Index, PPD: Probing Pocket Depth, CAL: Clinical Attachment Loss, BOP: Bleeding On Probing, HbA1C: Glycated hemoglobin, RBS: Random Blood Sugar
* statistically significant ($p<0.05$)
Note: Kruskal-Wallis ANOVA for OHI-S, PI, GI, BOP, CXCL2
One-Way ANOVA for PPD, CAL, HbA1C, RBS

	OHI-S	PI	GI	PPD	CAL	BOP	HbA1C	RBS	CXCL2
G1 vs G2	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0013*
G1 vs G3	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0002*
G2 vs G3	0.2372	0.0649	0.0075*	0.1714	0.0136*	0.9174	0.0001*	0.0001*	0.2717

Table 2. Pair-wise comparison of the three groups with the parameters.

G1: Group 1, G2: Group 2, G3: Group 3, vs: versus, OHI-S: Oral Hygiene Index-Simplified, PI: Plaque Index, GI; Gingival Index, PPD: Probing Pocket Depth, CAL: Clinical Attachment Loss, BOP: Bleeding On Probing, HbA1C: Glycated hemoglobin, RBS: Random Blood Sugar
* statistically significant ($p<0.05$)
Note: Mann-Whitney U-test for OHI-S, PI, GI, BOP, CXCL2
Tukey's multiple post-hoc test for PPD, CAL, HbA1C, RBS

Parameters		OHI-S	PI	GI	PPD	CAL	BOP	HbA1C	RBS	CXCL2
OHI-S	r-value									
	p-value									
PI	r-value	0.864								
	p-value	0.0001*								
GI	r-value	0.799	0.812							
	p-value	0.0001*	0.0001*							
PPD	r-value	0.549	0.630	0.595						
	p-value	0.0340*	0.0120*	0.0190*						
CAL	r-value	0.487	0.564	0.524	0.928					
	p-value	0.0650	0.0290*	0.0450*	0.0001*					
BOP	r-value	0.581	0.630	0.432	0.787	0.809				
	p-value	0.0230*	0.0120*	0.1070	0.0001*	0.0001*				
HbA1C	r-value	0.621	0.904	0.776	0.833	0.793	0.881		0.899	
	p-value	0.0211*	0.0001*	0.0218*	0.0001*	0.0001*	0.0001*		0.0001*	
RBS	r-value	0.883	0.734	0.741	0.904	0.661	0.924	0.917		
	p-value	0.0001*	0.0001*	0.0249*	0.0001*	0.0261*	0.0001*	0.0001*		
CXCL2	r-value	-0.961	-0.814	-0.786	-0.376	-0.352	-0.429	-0.887	-0.932	
	p-value	0.0001*	0.0001*	0.0010*	0.1680	0.1980	0.1100	0.0001*	0.0001*	

Table 3. Group 3 Correlations by Karl Pearson's test.

G1: Group 1, G2: Group 2, G3: Group 3, vs: versus, OHI-S: Oral Hygiene Index-Simplified, PI: Plaque Index, GI; Gingival Index, PPD: Probing Pocket Depth, CAL: Clinical Attachment Loss, BOP: Bleeding On Probing, HbA1C: Glycated hemoglobin, RBS: Random Blood Sugar.
*statistically significant ($p<0.05$)

Oral hygiene (by OHI-S), oral biofilm deposits (by PI) and gingival status (by GI) were as expected, significantly worse in both the diseased groups compared with health; no significant difference was found between PD and T2DM+PD, except for the GI score. PPD and CAL were significantly higher in diseased groups when compared with the healthy sample. Likewise, the bleeding score was highest in T2DM+PD group, followed by the PD patients and least in the healthy individuals. In a nutshell, all the clinical periodontal and the glycemic parameters assessed in this study were significantly higher in the diseased groups when compared to the healthy group. The serum concentrations (in pg/ml) of CXCL2 was highest in health, lower in the PD group and lowest in the T2DM+PD group. A significant difference was observed between Groups 1 and 2, and Groups

1 and 3, but none between Groups 2 and 3. A negative correlation was found between CXCL2 and all the parameters in the healthy subjects and PD patients, but most were not significant. However, significant correlations were noted in T2DM+PD for all variables except probing depths, attachment loss and bleeding (Table 3).

Discussion

Inflammatory markers have the potential to provide an insight to a possible link between oral and systemic health that may help in the prognostication of periodontal disease and clarify the pathogenic mechanisms involved. To elucidate the effect of periodontal disease as a source of systemic inflammatory burden, the literature reports variations in the levels of numerous biomarkers in health and disease.[23-26]The aim of this study was to detect, estimate and compare the serum levels of CXCL2 in systemically and periodontally healthy participants; systemically healthy participants with PD; and T2DM individuals with PD.

Results of this study reveal the presence of CXCL2 in the serum of all the samples. However, its concentrations were significantly higher in healthy participants when compared to patients with periodontitis (with, and without T2DM). A negative correlation between CXCL2 and all the variables in Group 3 is noteworthy.

CXCL2 expression has been reported and studied in the periodontal tissues. Miyauchi et al., found an increased expression of CXCL2 in the junctional epithelium in response to LPS stimulation, complemented by a local increase in PMNs.[25] Similarly, another group of researchers reported that the junctional epithelium expresses high levels of CXCL2 and undergoes neutrophil infiltration, because of minimal inflammation induced by commensal bacteria.[27] Commensal bacteria selectively upregulates the expression of CXCL2 in the junctional epithelium, via the MyD88 signalling pathway that correlates with the increased neutrophil recruitment.[7] Macrophages and β-cells in diabetic mice produced chemokines CXCL1 and CXCL2, recruiting CXCR2-expressing neutrophils from blood to the pancreatic islets leading to diabetes.[14] Hence, it can be inferred that CXCL2 and other chemokines have a role in neutrophil migration[28] in the periodontal tissues in response to bacterial stimulation and in diabetes, which may lead to a systemic elevation of their levels. Yet, it has been postulated that an inadequate neutrophil migration because of a decreased expression of CXCR2 is also possible.[29] This could explain why our results do not point to an increased CXCL2 expression in the blood circulation in PD and T2DM+PD, considering that both PD and T2DM are characterized by dysregulated immune responses.

As for the serum levels of CXCL2 which has been investigated in various systemic conditions, studies have shown heterogenous results. Dong Q et al., reported higher serum CXCL2 levels in esophageal squamous cell carcinoma patients compared to healthy controls. The levels of CXCL2 in healthy controls ranged between 0 pg/ml to 2781 pg/ml.[30] In comparison to our results, the serum levels of CXCL2 in the healthy group fell within the that same reported range. A 3-fold higher serum CXCL2 concentration in obese patients when compared to lean subjects of the same age has been observed by other authors.[12] It has also been stated that adipocytes are a source of chemokines and apart from hyperglycemia, obesity contributes to raised chemokine concentrations.[31] As our sample did not include obese individuals (to minimize bias)[32], it is implied that lower levels of CXCL2 in this study are plausible for acceptance in the diseased conditions. To further justify our results, a recent study by Ai et al., reported higher serum levels of CXCL2 in healthy controls when compared to neuromyelitis optica patients though statistically insignificant,[33] which is similar to our observations. It has been inferred that advanced glycosylation end products (AGEs) encourage continued stimulation of neutrophils which reduces their responses and probably decrease the expression of chemokines.[34] This adds credence to our study results noting lower concentrations of CXCL2 in T2DM+PD.

However, generally, the literature supports increased serum concentrations of CXCL2 in diseased states which is contrary to the findings of our study. To the best of our knowledge, no investigations have evaluated the serum levels of CXCL2 in PD with T2DM. Hence, direct comparisons cannot be made with the literature. Also, the difference in serum CXCL2 concentrations between the PD group and T2DM+PD group was statistically insignificant and therefore a confirmatory association between

PD, T2DM and serum levels of CXCL2 cannot be stated with certainty. Hence, we suggest that further longitudinal studies with a larger sample size including other parameters are needed to corroborate our findings for understanding the role of CXCL2 as an inflammatory marker in the pathogenesis of PD and T2DM.

Conclusions

CXCL2 is detectable in the serum of health, PD and T2DM+PD. Serum levels of CXCL2 was lower in periodontitis (with and without type-2 diabetes mellitus) when compared to health. As the results are not in agreement with majority of studies in the literature which attribute to CXCL2 a pro-inflammatory role in various systemic conditions and as there are no studies in relation to its levels in periodontitis, definitive conclusions cannot be made, but this study opens avenues to further investigate CXCL2 in longitudinal studies, and confirm its potential role in periodontitis and type 2 diabetes mellitus.

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Declaration of Interest

The authors report no conflict of interest.

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