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ORIGINAL ARTICLE

Comparison of Oncostatin M in Patients with Chronic Periodontitis with and without Diabetes

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ABSTRACT

Objective: To compare the Oncostatin M (OSM) concentrations in tissues of patients with chronic periodontitis with and without diabetes. **Material and Methods:** Sixty-four subjects visiting the dental outpatient department were categorized as "healthy" (Group 1), "periodontitis" (Group 2), and "diabetes with periodontitis" (Group 3) groups. The clinical oral examination included assessment of plaque, gingivitis, probing depth, clinical attachment level. Blood glucose was assessed for group 3 patients. OSM concentration in the tissues was assessed using ELISA in all groups. **Results:** The mean OSM was 0.02 ± 0.04 pg/mg in the healthy group, 0.12 ± 0.09 pg/mg in the chronic periodontitis group and 0.13 ± 0.10 pg/mg in the diabetes-periodontitis group. A significantly higher mean OSM was seen in Group 2 and Group 3 than Group 1. The amount of OSM positively correlated with probing depth and clinical attachment level. **Conclusion:** Periodontal disease causes a rise in Oncostatin M, independent of the diabetic status. Expression of OSM in the gingival tissues can serve as an inflammatory marker.

Keywords: Diabetes Mellitus; Periodontitis; Gingiva; Inflammation; Cytokines.

Introduction

Microorganisms on the tooth surface and gingival sulcus initiate periodontitis by triggering the host response to produce pro-inflammatory cytokines, which cause the breakdown of connective tissue and bone. Literature hints at diabetes mellitus increasing the incidence and occurrence of periodontitis. Periodontal health may be adversely affected by impaired glycemic control, immune dysfunction to bacterial challenge, oxidative stress, and increased inflammatory cytokine presence in diabetes [1]. Hyperglycemia can potentiate several inflammatory cytokines in the gingival sulcular fluid [2]. Similarly, periodontitis enhances systemic oxidative stress and acute phase reactants such as C-reactive protein. Therefore, persistent chronic inflammation in periodontal tissues can affect the control of diabetes [3].

Oncostatin M (OSM), a cytokine of the IL-6 family, is produced by immune cells such as T cells, neutrophils, monocytes, and dendritic cells in response to tissue injury [4]. It potentiates the actions of IL-1/TNF-α to augment "RANKL" production, promoting bone destruction [5]. In addition, it is known to induce changes in endothelial cells, which results in increased vascular permeability [6]. Previous studies have reported the association of OSM with chronic diseases like rheumatoid arthritis [7], inflammatory bowel disease [8], cancer [9], insulin resistance in Type 2 diabetic patients [10], and patients with chronic periodontitis [11].

Clinical studies in humans have often utilized body fluids like serum [11,12], gingival crevicular fluid [13], and saliva [14] for estimating OSM. However, studies on OSM concentration in human tissues are scant [14]. Hence, determining cytokine function at the site of disease would provide appropriate and accurate information on local tissue activity. Moreover, literature on OSM among people with diabetes is limited. Due to growing evidence of an inter-relationship between diabetes and periodontitis, it is worthwhile evaluating the OSM concentrations in patients having chronic periodontitis with and without diabetes. Hence, our study aimed to compare the tissue concentrations of OSM among patients with "chronic periodontitis", "diabetes with chronic periodontitis", and healthy controls. The null hypothesis was that there would be no significant difference in the mean OSM in different patients with chronic periodontitis with and without diabetes and healthy controls.

Material and Methods

Study Design and Ethical Clearance

A cross-sectional study was conducted among the patients visiting the outpatient department of periodontics during the period 2014 to 2016. The institutional ethics committee of Kasturba Hospital and Kasturba medical college approved the study protocol (IEC: 733/2014). Informed consent was obtained from all the participants.

Sampling

We screened 313 patients keeping in view of the inclusion and exclusion criteria. Sixty-six patients were eligible, out of which only 64 people consented to be part of the study. We excluded patients with aggressive periodontitis, uncontrolled diabetes, or systemic diseases other than type 2 diabetes mellitus, tobacco users, periodontal therapy or medication in the past six months, pregnancy, lactation, and those undergoing hormone replacement therapy.

The sixty-four recruited patients were categorized into three groups. The healthy group (Group 1; n=22) consisted of participants with at least 20 natural teeth, probing depth ≤ 3mm, gingival index score ≤ 1,

no clinical attachment loss. The chronic periodontitis group (Group 2; n=21) consisted of participants with probing depth ≥ 5mm, clinical attachment loss of ≥ 3mm. Finally, the diabetes-periodontitis group (Group 3; n=21) consisted of Type 2 diabetes mellitus with HbA1c ranging from 5.5 - 7%, probing depth ≥ 5mm and clinical attachment loss ≥ 3mm.

Clinical Oral Examination

A single trained examiner recorded the gingival index (GI) [15], plaque index (PI) [16], probing depth (PD), and clinical attachment level (CAL). Additionally, Group 3 patients were also evaluated for Glycated hemoglobin (HbA1c), fasting blood glucose (FBG), and postprandial blood glucose (PPBG) levels.

Sample Collection

Gingival tissue samples were obtained from groups 2 and 3 as part of the periodontal treatment protocol (curettage, periodontal surgery, crown lengthening or extraction). In addition, in Group 1 individuals, gingival tissue samples were obtained if indicated / referred for treatments such as crown lengthening or gingivectomy.

Sample Analysis

The samples were placed into coded Eppendorf vials containing 0.5ml phosphate buffer solution (pH 7.4) and stored at -80 degrees C. Collected tissues were weighed in Sartorius BT 124 S analytical balance, followed by tissue homogenization by the manual stirring of each sample for 2-3 minutes. The stir was washed for each sample to avoid tissue contamination. Next, vortexing was performed at 300 rpm for 10 minutes using HeidolphTM Shaker Vibramix 110, followed by centrifugation at 2900 rpm (659 g force) in a cooling centrifuge (Model: C-30BL, REMI laboratory instruments, Mumbai, India) for 20 minutes for supernatant collection.

The ELISA kit [ELISA protocol: (Biospes-Chongqing Biospes Co., Ltd, China)] was used to determine OSM concentration in the tissues. Standard preparation followed the manufacturer's protocol (Biospes-Chongqing Biospes Co., Ltd, China). The components of the kit were brought to equilibrium at room temperature for 15-30 minutes. Ten standard wells were set on the pre-coated plate. A standard curve was plotted by denoting serial dilutions of one known concentration of the analyte across a range of concentrations around the expected 'unknown' concentration. The protein concentrations of patient samples were recorded at 450 nm in the ELX 800 MS microplate reader and determined. Interpolation relied on an appropriately constructed curve.

Statistical Analysis

The statistical analysis for all the data was performed using SPSS version 18 software (SPSS Inc. Released 2009, Version 18.0, Chicago, USA). A p-value of <0.05 was considered statistically significant. The Chi-square test compared the sex distribution among the three study groups. ANOVA with posthoc Tukey test, Independent samples t-test and Kruskal Wallis ANOVA with posthoc Dunn test were used to compare continuous variables (age, gingival index, and plaque index, PD, CAL, OSM) among the three groups. Pearson and Spearman correlation coefficients were used to evaluate the correlation between OSM and other variables.

Results

Sixty-four individuals participated in the study. No significant differences existed in sex distribution among the three groups (p=0.218) (Table 1). The healthy group comprised 40.9% females and 59.1% males; the periodontitis group had 66.7% females and 33.3% males, while the diabetic-periodontitis group had 47.6% females and 52.4% males. Patient ages ranged from 23 to 65 years with a mean of 45.16 ±10.97. The mean age was significantly more in groups 2 (48.33 ± 9.18) and three (52.95 ± 7.65) compared to group 1 (37.55 ± 10.16) (Table 1).

Table 1. Comparison of demographic data, clinical indices, and Oncostatin M among the groups.

Variables	Group 1 N (%)	Group 2 N (%)	Group 3 N (%)	p-value	Post-hoc Test
Male	13 (59.1)	7 (33.3)	11 (52.4)	0.218	-
Age (Mean and SD)	37.55±10.16	48.33±9.18	52.95±7.65	<0.001	Group 2, 3 >Group 1[+]
PI (Mean and SD)	0.92±0.23	1.37±0.35	1.51±0.21	<0.001	Group 2, 3 >Group 1[+]
GI (Mean and SD)	0.93±0.19	1.4±0.31	1.6±0.27	<0.001	Group 3 >2 >1[+]
PD (Mean and SD)	-	5.02±0.98	5.52±0.72	0.065	-
CAL (Mean and SD)	-	4.29±0.83	4.57±0.59	0.212	-
Oncostatin M(pg/mg) (Mean and SD)	0.02±0.04	0.12±0.09	0.13±0.10	<0.001	Group 2, 3 >Group 1[‡]

[#]Chi-square test; [+]ANOVA with post-hoc Tukey's test; [++]Independent sample t-test; [‡]Kruskal Wallis ANOVA with post-hoc Dunn's test.

Clinical and Biochemical Parameters

Significant differences existed in mean plaque and gingival index among the groups (p<0.001 and p<0.001), with mean plaque highest in Group 3. The post hoc test showed that groups 2 and 3 showed higher mean plaque scores than group 1. Similarly, the gingival index progressively increased with the lowest values in Group 1, followed by Group 2, and the highest mean gingival index in Group 3, with a significant difference between the groups. The mean PD and CAL showed no significant differences between Groups 2 and 3 (p=0.065 and 0.212, respectively) (Table 1). Among those in Group 3, the mean fasting blood glucose level was 121±18.0 mg/dl; mean glycated hemoglobin was 6.53± 0.08 %, while the postprandial blood glucose level was 161± 35.0 mg/dl.

Comparison of OSM Concentrations and its Relationship with Other Variables

The mean OSM was 0.02 ± 0.04 pg/mg in Group 1, 0.12 ± 0.09 pg/mg of tissue in Group 2, and 0.13 ± 0.10 pg/mg of tissue in Group 3. There was a significant difference in the mean OSM amounts within the three groups (p<0.001). The posthoc test showed that the mean OSM amount in Groups 2 and 3 was higher than Group 1. However, no significant difference between Groups 2 and 3 was noticed (Table 1). A correlation of age with OSM was carried out, which showed a weak positive correlation between age and protein (OSM) (r=0.262; p=0.037). Age-wise comparisons showed that older age groups had higher OSM amounts than younger aged individuals (Table 2) did.

Table 2. Comparison of Oncostatin M in age stratified patient groups.

Variable	Age Groups <35 [A] Mean ± SD	36-50 [B] Mean ± SD	51-65 [C] Mean ± SD	p-value	Post-hoc test
Protein (pg/mg)	0.05 ± 0.09	0.10 ± 0.10	0.11 ± 0.09	0.035 *	A < C

*Significant at p<0.05.

The plaque and gingival indices in the three groups did not correlate with OSM. Group 3 demonstrated a moderately strong correlation of OSM with probing depth (r=0.719) and a fair correlation with clinical attachment level (r=0.448). In Group 2 also, OSM showed a fair correlation with probing depth, which

was significant (r=0.425). OSM did not show a significant correlation with the biochemical parameters (FBG, PPBG, glycated hemoglobin) within group 3 (Table 3).

Table 3. Correlation of Oncostatin M with clinical and biochemical parameters.

Variables	Oncostatin M					
	Group 1		Group 2		Group 3	
	CC	p-value	CC	p-value	CC	p-value
Plaque Index	0.181	0.419	0.078	0.736	0.164	0.478
Gingival Index	0.037	0.87	0.148	0.521	0.195	0.398
Probing Depth	-	-	0.425*†	0.037	0.719*‡	0.04
Clinical Attachment Level	-	-	0.176	0.432	0.448*†	0.023
Fasting Blood Glucose	-	-	-	-	0.26	0.6
Postprandial Blood Glucose	-	-	-	-	0.153	0.153
HbA1c	-	-	-	-	0.063	0.785

CC = Correlation Coefficient; †Fair correlation; ‡Moderately strong correlation; *Correlation is significant at the 0.05 level (2-tailed).

Discussion

Studies have indicated the potential role of OSM in various chronic inflammatory conditions [7,8]. Our study evaluated the OSM concentration in chronic periodontitis with and without diabetes. We observed increased amounts of OSM in chronic periodontitis with or without diabetes. This was similar to the study by Jones et al. [14], who reported elevated OSM in the gingival epithelium, and immune cell infiltrates of the gingival connective tissues of periodontitis patients. Hosokawa et al. reported that OSM could induce expression of CXCL10 and ICAM-1 in human gingival fibroblasts cells. These agents promote Th1 cell infiltration and retention in diseased tissues. Similar effects might explain OSM presence in periodontitis sites intensifying the disease [17].

Furthermore, the elevation of OSM in the periodontitis groups may be attributed to the effects of periodontal pathogens in the subgingival environment. *T. denticola*, a prevalent bacterium in deeper pockets, stimulates OSM to release from oral neutrophils and macrophages by degranulation and de novo synthesis [14]. The elevated oral neutrophil OSM correlated with the presence of *T. denticola* [14]. Therefore, we can infer that OSM quantity can rise during inflammatory changes seen in the local tissue environment, irrespective of the diabetic status.

Lin et al. reported OSM in the GCF in 22/31 healthy sites and 30/31 sites with periodontitis. An increase in OSM concentrations was evident with increasing severity of disease. Healthy sites showed a range of 68 to 7534 pg/ml, while mild periodontal disease ranged from 93 to 5321 pg/ml. Moderate periodontitis sites showed 56 to 6429 pg/ml values, and severe periodontitis cases ranged from 521 to 2961 pg/ml [18]. Lu et al. [19] reported OSM values of 0–4.77 pg/site in healthy sites, while mildly diseased sites had 0–5.1 pg/site, and moderate disease sites showed OSM from 0.85–6.60 pg/site, and approximately 1.31–7.80 pg/site in severely diseased sites. Thorat et al. [12] reported 66.15±28.10 pg/ml in healthy individuals compared to 128.33±22.96 pg/ml in gingivitis and 726.65±283.56 pg/ml in periodontitis. Similarly, higher amounts of OSM in the saliva of patients with periodontal disease (62 pg/ml) than healthy individuals (23 pg/ml) has been reported [14]. Therefore, we construe that the quantity of OSM intensifies with increasing periodontal tissue destruction.

The OSM concentration from our study samples differed considerably from the previous research. The differences could be attributed to the differently sourced samples (tissue *vs.* GCF), amount of disease or age of the patients. Nevertheless, the OSM concentration shows an increased concentration in chronic periodontitis with and without diabetes.

OSM showed a weak positive correlation with the age of the patients. However, the older age groups showed higher amounts of OSM protein than younger individuals. This could be attributed to the fact that periodontitis is generally seen in elderly patients and the same is reflected in the age distribution among the three groups.

OSM showed a positive correlation with periodontal parameters. Thorat et al. [12] also reported a correlation between GCF OSM and the periodontal parameters. Gingival tissue biopsies of periodontitis patients have shown a more significant proportion of the pro-inflammatory type of macrophages. These 'M1' type of macrophages aggravate periodontal inflammation by secreting M1 cytokines such as IL-6, TNF- alpha and IL-12 [20]. 'M2' macrophages involved in healing are also found in periodontal tissues. The various periodontal microbes (*P. gingivalis*, *T. forsythia*, *T. denticola*) activate these two types of macrophages, instigating OSM release [14]. Furthermore, diabetes is associated with hyper-responsive macrophages [21].

We observed that diabetes had minimal effect on OSM expression in patients with chronic periodontitis. However, significant elevated OSM concentrations were seen in patients with chronic periodontitis. Few studies are available on the significance of OSM in the periodontal environment. The present study adds to the existing information asserting the local effects of Oncostatin M as an immune modulator, independent of the influence of the systemic status. The focus of the research was to elucidate the presence of OSM in the gingival tissues.

A limitation of this study is its cross-sectional study design, where temporal association could not be established. Further, OSM may act through either OSM receptor I or II [4]. This may also limit the interpretations made in our study as different signaling pathways may be activated based on the type of receptor involved, enabling a varied OSM response affecting the environmental conditions. Future studies should consider these aspects with a focus on peri-implant disease severity. Therapeutic agents that target OSM can also be an area for further research.

Conclusion

Elevated levels of Oncostatin M were seen in chronic periodontitis patients. It could be a potential marker of localized periodontal inflammation.

Authors' Contributions

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All authors declare that they contributed to critical review of intellectual content and approval of the final version to be published.

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None.

Conflict of Interest

The authors declare no conflicts of interest.

Data Availability

The data used to support the findings of this study can be made available upon request to the corresponding author.

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