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"GINGIVAL CREVICULAR FLUID PROCALCITONIN - A PIVOTAL MARKER IN TYPE II DIABETES WITH CHRONIC PERIODONTITIS"

Periodontology

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ABSTRACT

Aim: To determine the levels of procalcitonin in Gingival crevicular fluid of Type II diabetic patients with chronic periodontitis.
Materials and Method: Study included 24 males and grouped as Group I (Healthy, n=8), Group II (Chronic periodontitis, n=8) and Group III (Chronic periodontitis with type II DM, n=8). Plaque scores, bleeding scores, Probing depth, Clinical attachment loss, RBS and HbA1c were evaluated. GCF procalcitonin levels were analyzed by ELISA.
Results: Significant increase in GCF procalcitonin levels in group III (CP+DM) at $p<0.001$. Periodontal destruction was more pronounced in group III (CP+DM) as assessed by periodontal clinical parameters ($p<0.001$).
Conclusion: GCF procalcitonin reflects the periodontal disease activity in Type II Diabetics with chronic periodontitis and it can be used as a potential marker in these co-morbid conditions.

KEYWORDS

Gingival Crevicular Fluid, Periodontitis, Diabetes Mellitus, Procalcitonin

INTRODUCTION

Periodontitis is a chronic inflammatory disease characterized by inflamed gingiva, bleeding on probing, increased probing pocket depth, clinical attachment loss, pus discharge, and resorption of alveolar bone. It is common in adults affecting approximately 50% of adults and over 60% of over 65 year olds, with severe periodontitis impacting 10–15% of populations[1]. Periodontitis is multifactorial in origin with anaerobic, Gram-negative organisms being the bacterial cause. Environmental, behavioral, and systemic factors also influence the onset, progression, and severity of periodontitis.

Diabetes mellitus is a disease of metabolic dysregulation, primarily of carbohydrate metabolism, characterised by elevated blood glucose levels (fasting blood glucose ≥126mg/dl) that results from defects in insulin secretion (type 1), impaired insulin action (type 2), or both. Type 2 diabetes is more common type than type 1(Constituting 90% of all diabetic cases) Diabetes is associated with a classic group of micro-vascular and macro-vascular complications. Oral complications of diabetes includes alterations in salivary flow, burning mouth, increased incidence of infections, altered wound healing, and increased prevalence and severity of periodontal disease. Periodontal disease is the sixth complication of diabetes mellitus.

Diabetes affects the periodontium through changes in the subgingival microflora, GCF glucose levels, periodontal blood vessels, host response and collagen metabolism[2]. Though periodontally diseased sites in diabetic and non-diabetic individuals harbour similar species, the increased prevalence and severity of periodontitis in diabetes may be due to differences in host response. In Diabetics there is increased GCF glucose [3,4] which causes decreased periodontal ligament fibroblasts which adversely affects the periodontal wound healing[5] and local host response to microbial challenge. Increased thickness of gingival blood vessels and capillaries impair oxygen diffusion and nourishment, altering periodontal tissue homeostasis. Advanced Glycation End products (AGEs) are formed and accumulated in patients with diabetes.

The interaction between AGEs and their receptor (RAGE) leads to increased cellular oxidant stress and activates Nf-kB on monocytes, which causes increased production of pro-inflammatory cytokines (IL-1β, TNF-α and PGE2) which causes severe periodontal destruction. In diabetics, AGEs modified collagen undergoes rapid degradation, together with increased collagenase activity[6] causing changes in collagen metabolism and leads to impaired wound healing.

Procalcitonin (PCT/ProCT), a protein of 116 amino acids with molecular weight of 13kDa, encoded by CALC-I gene. It was discovered 25 years ago as a prohormone of calcitonin produced by the C-cells of thyroid gland and neuroendocrine cells of the lungs. It is intracellularly cleaved by proteolytic enzymes into the active hormone calcitonin. All the PCT formed is converted to calcitonin, so that no PCT enters circulation and its levels in healthy subjects are as low as 10 pg/mL^{-1}. There are no enzymes in the plasma that could breakdown circulating PCT. In sepsis, systemic infection and severe inflammation, the serum levels of PCT increase markedly, attaining values of tens, to hundreds, to thousands-fold that of normal levels (1,00,000pg/mL^{-1}) and this correlates with the severity of the condition. In such conditions, uncleaved PCT is secreted not only by thyroid, but also by other tissues (liver, kidney, pancreas, adipose and white blood cells).[7]

Earlier studies show that PCT is secreted in body fluids other than blood, such as saliva. Salivary ProCT levels were elevated in patients with periodontal disease compared with periodontally healthy controls[8].

Gingival crevice fluid (GCF) is a complex mixture of substances derived from serum, leukocytes, structural cells of the periodontium and oral bacteria. These substances possess a great potential for serving as indicators of periodontal disease

So the aim of the present study was to determine the levels of Procalcitonin in GCF of Type II diabetic patients with chronic periodontitis and to assess whether their levels could be used as a potential marker of periodontal disease activity.

MATERIALS AND METHODS

This study was conducted in the Department of Periodontics, Rajah Muthiah Dental College and Hospital, in association with Medical Biochemistry, Annamalai University, Chidambaram. Research protocol was approved from Institutional Human Ethical Committee (IHEC/0116/2016) and Informed written consent from patients were obtained prior to the initiation of the study.

The study included 24 male subjects in the age group between 25 to 60 years and divided into three groups,

Group I (n=8) included 8 systemically and periodontally healthy subjects (No loss in CAL, PPD 3mm, with no clinical evidence of bleeding on probing and attachment loss).

Group II (n=8) included 8 moderate to severe form of chronic periodontitis patients (PPD ≥4mm; CAL ≥ 3mm, otherwise systemically healthy.

Group III (n=8) included 8 moderate to poorly controlled Type II diabetes mellitus patients with moderate to severe form of chronic periodontitis patients (PPD ≥ 4mm; CAL ≥ 3mm).[9]

Armamentarium

Mouth mirror, Shepherd's hook dental explorer, William's periodontal probe, Face mask, Surgical gloves, BD Vacutainer SST II Advance, BD Vacutainer K2 EDTA 3.6mg tubes, Absorbent filter paper strips (Whatman's No.1) and Micropipettes (figure.1)

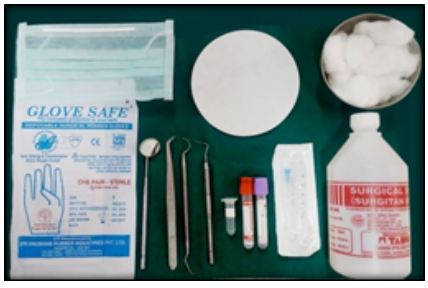

Figure.1 Armamentarium

Clinical examination

Periodontal clinical parameters were recorded **(Plaque index (Silness and Loe 1964)[10],** Gingival Bleeding index **(Ainamo and Bay 1975)[10],** Probing pocket depth **(Carranza) [11](figure. 2)**, Clinical attachment level **(Carranza) [11]**

Figure. 2 Measurement of probing pocket depth with William's periodontal probe

GCF collection

All the subjects gargled vigorously with sterile water to remove loosely adherent debris from the tooth surface and the test site was dried and isolated with cotton rolls or guaze. Three filter paper strips (Whatman no.1) approximately 2.0mm wide and 0.6 mm long were inserted in the crevice of the tooth side by side and left in position for three minutes to absorb all the moisture in the crevice (figure.3).

The central drying strip was then removed and immediately replaced by a collecting strip, 1.0mm wide and 0.6mm long which contained GCF was then transferred to a sterile test tube containing 0.2ml of saline, and transported to the laboratory.

Figure.3 Collection of GCF Samples

ELISA

Samples were analysed by using commercially available Raybio® Human Procalcitonin ELISA Kit using sandwich technique according to the manufacturer's instructions, for the estimation of procalcitonin. This assay employs an antibody specific for human procalcitonin coated on 96-well plate. All the samples and reagents used were brought to room temperature (18-25^{0}C) 100 µl of each standard and the sample were poured into each well. Wells were covered and were incubated for 2.5 hr at room temperature with gentle shaking. 100µl of prepared biotin antibody was added to each well and incubated for 1 hr at room temperature. 100µl of prepared streptavidin was added to each well and incubated for 45 minutes at room temperature with gentle shaking and discarded. 100 µl of Tetramethylbenzidine was added and Incubated for 30 minutes at room temperature. 50µl of 0.2 M sulphuric acid (stop solution) was added to each well and it colour change is observed from blue to yellow, and the intensity of the colour was measured at 450nm immediately.

STATISTICALANALYSIS

Data were analysed using SYSTAT 12 statistical software, Kruskal wallis test was applied to compare the mean between the three groups for age,periodontal parameters (table.1), and Gingival Crevicular Fluid procalcitonin (table.2) Multiple comparison tests for kruskal wallis test was applied to find out the difference between the three groups. Spearman's rho correlation co-efficient was used to find out the relationship between the GCF procalcitonin with that of periodontal parameters and glycemic parameters (table.3)

Table.1 Descriptive statistics and kruskal wallis test value of Age, Periodontal clinical parameters (PI, GBI, PPD and CAL) - Groupwise

	GROUP -I (Healthy)		GROUP-II (CP)		GROUP III (CP+DM)		KRUSKAL WALLIS TEST VALUE	P-VALUE
Para meters	Mean	SD	Mean	SD	Mean	SD		
AGE	33.8	8.20	45.63	4.20	45.88	3.98	6.92	<0.001
PI	0.31	0.07	1.22	0.20	2.07	0.50	20.16	<0.001
GBI	4.26	0.74	63.36	1.96	90.86	6.03	20.50	<0.001
PPD	3.08	0.16	6.14	0.24	7.06	0.49	19.02	<0.001
CAL	-	-	6.64	0.40	7.41	0.29	9.62	0.002

Table.2 Descriptive statistics and Kruskal wallis test value of GCF Procalcitonin levels (pg/ml)-Groupwise

GROUP	MEAN	SD	KRUSKAL WALLIS TEST VALUE	P-VALUE
GROUP I (HEALTHY)	22.11	1.42	20.489	<0.001
GROUP II (CP)	55.26	2.47		
GROUP III (CP +DM	185.38	3.25		

p<0.001

Table.3 Spearman's Rho correlation co-efficient between Periodontal clinical parameters, Glycemic parameters and GCF Procalcitonin

PARAMETERS	PCT-GCF
PI	.886
GBI	.827
PPD	.848
CAL	.908
HbA1c	.681
RBS	.806

**** P <0.01**

RESULTS

Kruskal wallis test showed that, the average age of group III patients (CP+DM) was higher than group II (CP) and group I (healthy). This difference was statistically significant at p<0.001, which means that the prevalence of periodontitis and diabetes increased with age (table.1)

Plaque index, gingival bleeding index and probing pocket depth was found to be increased in group III patients (CP+DM) than group I (

healthy) with statistical difference at p<0.001 (table.1)

GCF procalcitonin levels were increased in group III patients (CP+DM) followed by group II (CP) and healthy, with statistical significance at p<0.001 (table.2)

Spearman's Rho correlation coefficients between Periodontal clinical parameters, glycemic parameters and GCF procalcitonin was found to be positively correlated with statistical significance (p<0.01) (table.3)

DISCUSSION

Diabetes and Periodontitis

Diabetes and periodontitis are common chronic disorders and there is a bi-directional relationship between both the diseases i.e diabetes adversely affecting periodontal health, and periodontal disease adversely affecting diabetic status. Also both have influence on the immune system and inflammatory response of the body and thus negatively impacting each other.

In our study, the periodontal clinical parameters (PI, GBI, and PPD) were found to be increased in CP patients with type II DM (group III) than the healthy group with statistical difference at p<0.001. This is similar to the study by **Campus G et al.,2005** [12] who evaluated the possible association between Type II DM and periodontal disease, they showed that type II DM patients had significant increase in number of teeth with probing depth>4mm (P=0.04), bleeding on probing (P=0.02) and plaque index (P=0.01) when compared to non-diabetic controls. This shows that Type II Diabetics have an increased susceptibility for more severe periodontal disease.

Procalcitonin

Procalcitonin is a prohormone of calcitonin and has various physiological importance, which includes, metabolism of calcium, cytokine network and modulation of nitric oxide synthesis and pain relieving effects. Also Procalcitonin, is a promising marker and prognostic indicator which exhibits greater specificity in identifying patients with bacteremia, sepsis, bacterial meningitis, bacterial super infection, acute pancreatitis and systemic inflammation. A rapid rise in serum ProCT can be seen in active inflammatory condition.

Procalcitonin and periodontitis

Periodontitis is a bacterially induced infection, initiated by Gram negative pathogenic bacteria which release endotoxins which lead to local up-regulation of Procalcitonin. PCT can be observed at various concentrations in different body fluids like saliva. **Meltem karsiyaka hendek 2015** [8]**,** in their study described that the salivary ProCT was significantly higher in patients with generalised aggressive periodontitis followed by chronic periodontitis, gingivitis and lowest in the healthy group at (P<0.05)

Procalcitonin in GCF and Diabetes mellitus

Our study was the first to report the presence of PCT in gingival crevicular fluid and its correlation in Chronic periodontitis patients with type II diabetes. GCF Procalcitonin in group III (CP + type II DM) was 185.38pg/ml and group II patients (CP) was 55.26pg/ml, and in group I (healthy) was 22.11pg/ml, which shows that the expression of procalcitonin in GCF was raised to 8 folds in CP with Type II DM and more than 2 folds rise in chronic periodontitis when compared to healthy group with statistical significance at p<0.001. This can be justified by the increased periodontal destruction occurring in type II DM patients due to endotoxins which further stimulates local release of procalcitonin.

Procalcitonin and Clinical parameters

There was a positive association between the periodontal clinical parameters (PI, GBI,PPD,CAL)and GCF procalcitonin, which was statistically significant at p<0.01 (table.3), which was similar to study by **bassim et al., 2008**[13] in which the Salivary-ProCT levels were correlated with bleeding-on-probing (r = 0.45, p = 0.05), a measure of active inflammation.

Procalcitonin and glycemic parameters

Also, there was a positive association between glycemic parameters (HbA1c and RBS) to GCF procalcitonin, which was statistically significant at p<0.01, which was similar to study by **bassim et al.,2008** [13] in which the Salivary-ProCT levels were correlated with the HgbA1c (r = 0.49, p = 0.03), which was another supportive indication of the underlying connection between the local and systemic inflammatory states of periodontitis and type II diabetes.

CONCLUSION

The results of this study show that, the levels of procalcitonin in GCF samples could be used as a reliable marker of periodontal disease activity in Type II diabetes mellitus patients with chronic periodontitis.

FUTURE DIRECTIONS

- With more number of study samples
- along with Assessment of bacterial bioload
- PCT assessment following interceptive measures (Periodontal therapy)
- To compare the levels of PCT in both Stimulated and Unstimulated pooled saliva in larger population with age and gender matched controls should be carried.

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