Comparison of Level of Pentraxin-3 in Gingival Crevicular Fluid with Chronic Periodontitis in Well Controlled and Uncontrolled Diabetes Mellitus Patients

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Abstract: Background and Aim: Periodontal diseases are characterized by a complex set of biologic interaction between a microbial ecosystem and the host's immune and inflammatory machinery, such interaction play a role in the development of periodontal disease via the release of inflammatory mediators. The usefulness of such inflammatory mediators as biomarkers for Chronic periodontitis in Diabetes Mellitus patients is unknown. In this study, we tested whether gingival crevicular fluid concentration of human Pentraxin – 3; a local inflammatory marker, would be a useful biomarker for detecting Chronic Periodontitis. Methods: 45 patients with chronic periodontitis were divided into 3 groups based on presence / absence of Diabetes mellitus and whether it is well controlled or not. Clinical parameters were recorded. The clinical and radiographic examinations, group allocations, and sampling site selection for all patients were performed. The samples were collected on the subsequent day using microcapillary pipettes. Collected samples were assayed using Enzymes linked immuonosorbent assay. Results: Statistical analysis was done using One Way Anova test, to compare between 3 groups. Independent sample t-test was done for 2 groups comparison. Statistically significant results were found between group A and group C for Russell's periodontal index. Statistically significant results were not found for Pentraxin-3 levels between groups. Conclusion: By this study we can conclude that Pentraxin – 3 in gingival crevicular fluid increases with increase in inflammation irrespective of the presence or absence of the systemic diseases. [M Parmar, Natl J Integr Res Med, 2018; 9(2):31-35]

Key Words: chronic periodontitis, Diabetes mellitus, gingival crevicular fluid, One Way Anova

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Introduction: Periodontal diseases are microbial infections elicitated by a host response with resultant destruction of osseous and soft tissues.[1,2] Periodontal infection permits injurious agents derived from bacteria, or bacteria themselves in its early stage to gain entry into the connective tissue.[3-5] Several acute phase proteins orchestrate the inflammatory cascade in response to the bacteria or endotoxins derived from these periodontal pathogens to bring about a balance between host and inflammatory stimuli. Elevated levels of various Acute Phase Proteins (APPs) in Gingival Crevicular Fluid (GCF) is one of the consequences of these inflammatory reactions.[6,7] APPs are the plasma proteins whose concentration increases or decreases by at least 25% during inflammation. APPs include Creactive proteins (CRP), several coagulation and complement factors, amyloid, transport proteins, and anti proteases enzymes. Both the positive and negative effect of acute and chronic inflammation including chemotaxis, phagocytosis, protection against oxygen free radicals and tissue repair are mediated by these APPs. During infection the concentrations of a number of these APPs in serum and GCF increases rapidly to several hundred folds and throughout the infection, it remains elevated.

In the immunopathology of periodontitis, there have been several proinflammatory APPs. The CRP, plasminogen-activator inhibitor 1, and fibrinogen are the APPs that receive high concentration. Evidence suggests that the Interleukin (IL) -1β and Tumor Necrosis Factor-alpha (TNF-α) are responsible for the destruction of periodontium. IL-1β and TNF α are the potent inducers of a new PTX called Pentraxin-3 as suggested by recent research. Thus the pentraxins promise to be key markers for periodontal disease diagnosis.[1]

For over a century, these Pentraxins are known to researchers as a classic APPs.[2] PTX3 is an evolutionarily conserved, acute phase inflammatory multimeric glycoprotein in the family same as the C-reactive protein (CRP).[8]

It is considered to be a true independent indicator of disease activity. As these are produced by extrahepatic tissues in contrast to CRP.[9] Diabetes mellitus is a chronic metabolic disorder caused by

inherited or acquired deficiency in production of insulin or by ineffectiveness of the insulin produced.[10] The relationship between periodontal diseases and diabetes mellitus has been extensively examined. It is clear from epidemiologic research that diabetes increases the risk and severity of periodontal diseases. Periodontitis is considered as sixth complication of Diabetes Mellitus.[11] Diabetes and periodontal diseases are strongly interrelated and have common pathobiology. During periodontal disease, the inflammatory events may play an important role in the development of insulin resistance and diabetes, and this may probably facilitates the progress of periodontal disease.[12] It is suggested that hyperglycemia through the body's scavenger system against advanced glycation end products could exacerbate inflammatory tissue destruction indirectly and that hyperglycemia through the cell-matrix interactions might impair the biological functions of periodontal connective tissue directly.

It also appears that systemic manifestations result because of many oral bacteria, which are sign ficantly increased in periodontitis. Thus the ability to use acute phase reactants as a measure of inflammation has substantial support.

Methods: The present study was carried out at Department of Periodontics, Goenka research institute of Dental Science, Piplaj Gandhinagar for period of one year. The study population consisted of 45 samples. Almost balanced subjects in respect to gender and age (22 females and 23 males; age range: 23 to 60 years) who attended the Outpatient Section of the Department of Periodontics. Participants who agreed to participate voluntarily, the written informed consent was obtained. From the institution's ethical committee and review boards, the ethical clearances were obtained.

Inclusion Criteria:

1. Patients with chronic periodontitis having probing pocket depth (PPD) of ≥ 4mm.
2. Patients categorized as type 2 Diabetes Mellitus (controlled and uncontrolled based on HbA1C levels. We considered < 7% as controlled and >7% as uncontrolled DM pts)
3. Clinical attachment loss of ≥ 3mm
4. Patients who have not undergone any periodontal surgery for atleast
5. Month prior to sampling.

Exclusion Criteria:

1. History of any antibiotic therapy within 6 months prior to study.
2. History of any systemic disease other than diabetes.
3. Subjects who are pregnant and pre-eclamptic.
4. Subjects with history of smoking and alcoholism.
5. Immunocompromised pateints .
6. Subjects who regularly use mouthwash.

Each subject underwent full-mouth periodontal examination and charting, along with periapical radiographs using the paralleling technique. Based on the gingival index (GI), probing pocket depth (PPD), clinical attachment level (CAL), and radiographic evidence of bone loss, and based on whether patients are diabetic or not, and whether it is under control or not, participants were categorized into three groups:
GROUP A: Chronic periodontitis patients
GROUP B: Chronic periodontitis with well controlled blood sugar level.
GROUP C: Chronic periodontitis with uncontrolled blood sugar level.
Probing Depths and Clinical Attachment Levels were measured.

Site Selection and Fluid Collection: On the first day, the clinical and radiologic examinations, group allocations, and sampling site selection for all patients were performed. To prevent the contamination of GCF with blood associated with probing on the infalmmed site, the samples were collected on the subsequent day.

Per participant in each group, only one site was selected. Sites with PPD ≥ 4 mm were identified using a William's graduated periodontal probe. Among these, the site that showed the highest PPD and signs of inflammation, along with radiographic confirmation of bone loss, was selected for sampling.

On the subsequent day, the area was isolated using cotton rolls to avoid saliva contamination and after gently drying the area, the supragingival plaque was removed. Placing the microcapillary pipette at the entrance of the gingival sulcus and by touching the gingival margin gently, GCF samples were collected(FIG 2). Using the calibration on white color–coded 1- to 5 µL calibrated volumetric microcapillary pipettes, a standardized volume of 1 µL was collected from each group. To ensure atraumatism, maximum

of 10mins was allotted for each sample collection, within this allotted time if the sites did not express any GCF, then those sites were excluded. The micropipettes that were suspected to be contaminated with saliva and/or blood were also excluded.

Immediately, these collected GCF samples were transferred to airtight plastic vials and stored at -70ċ until assayed.

Assay Procedure: By using an enzyme-linked immunosorbent assay (ELISA) kit, the samples were assayed for PTX3 levels according to manufacturer's instructions. The quantitative sandwich enzyme immunoassay technique was employed in the assay procedure. Specific for PTX3, a biotinylated monoclonal antibody was incubated in a streptavidin coated plate. Plates were washed, and then to the wells, the pretreated standards and samples were added. The immobilized biotinylated antibody bounds the PTX3, if it was present. Any unbound substances were washed away and then to the wells an enzyme-linked conjugate specific for PTX3 was added. Any unbound conjugate was removed by washing, and then to the wells, a substrate solution was added. In proportion to the amount of PTX3 bound, color developed. The development of color was stopped, and the color intensity was read on an ELISA reader using primary wavelength as 405nm.

Statistical Analyses: A software program was used to analyse the data. For each parameter, the descriptive statistics (means – SDs) were calculated. Group comparisons for variables were performed by the One-way ANOVA test. In addition, comparisons between two groups were done using the independent sample t-test. $P < 0.05$ indicated statistical significance.

Results: The results of the present study indicate that the mean PTX3 concentration in GCF was highest in group C (7.972ng/mL). The mean Pentraxin 3 concentration increased from group A to group C. However, the difference between the groups was not statistically significant. All the values are rounded off to the nearest whole number as shown in table 1.Further comparison between two groups using the independent sample t-test was carried out. The results showed that the differences were not statistically significant between group A and B as shown in table 2. Whereas statistically significant difference were seen between group A and group C and also between group B and group C with respect to Russell's periodontal index.

Table 1: One Way Anova Test To Compare Between 3 Groups

Groups	Gingival Index (MEAN±SD)	Russell Periodo Ntal (MEAN±SD)	Probin_Depth (MEAN±SD)	Clinical_Attach Ment (MEAN±SD)	Clinical_Attach Ment (MEAN±SD)
A	1.93±0.27	4.4±0.90	6.07±0.96	4.0±1.00	5.70±1.01
B	2.14±0.61	4.58±0.61	6.40±1.24	4.33±1.11	5.81±1.18
C	1.97±0.40	5.08±0.64	6.33±0.97	4.20±0.86	5.97±2.09
P value	0.39	0.52	0.67	0.67	0.88

Table 2: Independent Sample T-Test To Compare Between Group A And Group B

Groups	Gingival Index (MEAN±SD)	Russell Periodo Ntal(MEAN±SD)	Probin_Depth (MEAN±SD)	Clinical_Attach Ment(MEAN±SD)	PentraXin-3 Levels(Mean±SD)
A	1.93±0.27	4.0±1.0	6.07±0.96	4.0±1.00	5.70±1.01
B	2.14±0.61	4.58±0.61	6.40±1.24	4.33±1.11	5.82±1.18
C	1.97±0.40	5.08±0.64	6.33±0.97	4.20±0.86	5.97±2.09
P value	0.23	0.62	0.42	0.77	0.77

Discussion: In the 1990s, the PTX3 was identified and is produced in the peripheral tissues by both resident and innate immune cells in response to inflammatory signals. In response to interleukin (IL)-1β, tumor necrosis factor (TNF)-α, and microbial components, including lipopolysaccharides, this PTX3 can be expressed by leukocytes, dendritic cells, monocytes, and macrophages.[13]

All these cytokines and inflammatory cells are increased during periodontal breakdown, which made this research reasonable and also there are many

systemic manifestation of many oral bacteria and it increases in presence of periodontitis.

In patients with generalized aggressive periodontitis, the PTX3 concentration was higher in gingival tissues compared to CP patients, and these PTX3 levels correlated with clinical parameters positively. Hence, in periodontal disease, the tissue PTX3 level can be considered as a marker of inflammation.[14]

Gumus et al concluded a contrast results on salivary Pentraxin 3 levels in his study that salivary PTX3 levels were same in chronic periodontitis and healthy group of patients, whereas it was higher in aggressive periodontitis patients compared to healthy group.

Measurement of pentraxin 3 level in gcf or plasma may help identify a subset of patients undergoing a process of periodontal breakdown or those who are at higher risk for destructive disease. In contrast to CRP, PTX3 are synthesized by extrahepatic tissues by vascular endothelium at the site of inflammation. Hence PTX3 levels are believed to be the true independent indicators of periodontal disease activity.[15]

In atherosclerotic lesions that involve macrophages, neutrophils, dendritic cells, or smooth muscle cells, the PTX3 is expressed predominantly and it was shown that for inflammatory cardiovascular disease, this PTX3 may be a novel biomarker. PTX3 is produced in response to interleukin-6 by the liver and PTX3 is produced in response to primary inflammatory stimuli by the heart and vasculature. So the authors in this study hypothesized that in cardiac injuries such as myocardial infarction, PTX3 could be an acute phase reactant more closely related than CRP.[16]

Our study comprised of 3 groups. These groups helped this study to evaluate the role of pentraxin3 in controlled and uncontrolled diabetic and non-diabetic patients with chronic periodontitis. Participants within age (23 – 60years) were included to minimize the influence of age factor on the level of pentraxin-3. In this study, to avoid non-specific attachment of PTX3 to lter paper, GCF was collected using micocapillary pipettes. The quantity of biomarker released depends on the intensity of the stimuli. To allow the accurate quantitative estimations of PTX3 with high specificity and sensitivity, the ELISA is used in this present study.

The results of the present study indicate that the concentration of the GCF PTX3 increases in chronic periodontitis patients with uncontrolled diabetes mellitus. The GCF values are more in GROUP C patients than other groups but the difference was not statistically significant.

The PTX3 concentration was detected in previous studies in various chronic inflammatory conditions such as atherosclerosis,[17] and recognized as an early marker for unstable angina[18] and myocardial infarction.[19]

Plasma PTX3 levels were found to be associated with development and progression of diabetic retinopathy and may be a more accurate predictor of diabetic retinopathy development than high sensitivity.[20]

PTX3 is believed to be a independent indicator of disease activity at sites of inflammation and vasculitis. Also PTX3 has antiviral activity against influenza virus.[21] Also Pentraxin-3 in patients with type 2 Diabetes Mellitus is associated with the presence and severity of coronary artery disease(CAD). And hence it is a better biomarker for detection of CAD than CRP.[22]

PTX-3 may represent a useful marker of endothelial dysfunction in early diabetes nephropathy.[23]

Greater amount of pentraxin 3 concentration was seen in group C patients which indicates that the greater the amount of periodontal tissue destruction taking place in uncontrolled diabetes mellitus. From one participant to the other in the different groups, the pentraxin 3 concentration was highly variable. Long-term interventional studies with larger sample sizes should be carried out to exclude interparticipant variations.

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