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EVALUATION OF PENTRAXINS 3 IN CHRONIC PERIODONTITIS PATIENTS BEFORE AND AFTER THE TREATMENT

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Dr. Varghese Mathew[1], Dr. Sheeja Varghese[2], Dr. M Sankari[3], Dr. N D Jayakumar[4]

Co-Authors: [2,3,4]Department of Periodontia, Saveetha Dental College, Chennai, Tamilnadu, India
Corresponding Author: Dr.Varghese Mathew[1], Department of Periodontia, Saveetha Dental College, Chennai, Tamilnadu, India. E-Mail: varmat14@gmail.com

Abstract

Background: *Pentraxins are a super family of evolutionarily conserved proteins considered as markers of inflammation .They are produced on exposure to pro - inflammatory stimuli like TNF -α, IL-1ɓ and even microbial moieties. Periodontitis is an inflammatory condition initiated by gram negative organisms which cause an up regulation of pro -inflammatory mediators which in turn amplifies the production of PTX3, an acute phase protein. Since it has an extra hepatic synthesis unlike its counterpart CRP, PTX3 is used as a marker to assess the disease activity in periodontitis patients.* ***Materials and Methods:*** *A total of 30 patients were divided into three groups, Group I 10 periodontally healthy subjects, Group II 20 patients with moderate to severe chronic periodontitis, Group III same as group II one month after receiving Scaling and Root Planing. Clinical parameters were recorded and gingival crevicular fluid (GCF) samples were collected from each subject for measuring PTX3 levels at baseline, and 1 month after treatment.* ***Results:*** *In all evaluation periods, there was statistically significant difference in each of the studied clinical parameters and PTX3 level between Group I and Group II. There was also statistically significant reduction in each of the studied clinical parameters and PTX3 level between Group II and Group III.* ***Conclusions:*** *Tissue PTX3 values can be considered as an inflammatory marker for chronic periodontitis. However further interventional studies with a larger sample size and longer follow up are required to use PTX3 as a true diagnostic marker for periodontal diseases.*

Key words: *Pentraxins, Periodontitis, Gingival crevicular fluid, CRP*

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INTRODUCTION

Periodontal disease is a multifactorial infectious disease; the main cause of periodontal disease is the presence of periodontal microorganisms. It is an inflammatory condition that involves microbial dental plaque, genetic factors and environmental factors. [1]

The progression and severity of disease are determined by the host immune response.[2] The majority of tissue destruction in periodontitis results from the host's inflammatory processes. The mediators produced as a part of host response that contribute to tissue destruction include cytokines, prostaglandins, matrix metalloproteinase (MMPs) and acute -phase proteins (APP). The pro inflammatory cytokines like interleukin 1beta (IL1β) and Tumor necrosis factor alpha (TNFα) play a key role in the initiation, regulation and perpetuation of immune response resulting in vascular changes and migration of effector cells such as neutrophils.

Acute phase proteins are a class of proteins whose plasma concentrations increase (positive acute -phase proteins) or decrease (negative acute -phase proteins) in response to inflammation. Pentraxins, also known as pentaxins are an evolutionary conserved family of proteins that belong to the group of acute phase proteins. They are classified into "short" PTXs (CRP and SAP) and "long" PTXs (PTX3).[3, 4]

PTX3 is a recently discovered long pentraxin in the PTX super family. PTX3 blood levels are low in normal conditions <2 ng/mL and increase rapidly (peak at 6 –8 h) and dramatically (200 – 800 ng/mL) during inflammatory and infectious conditions.[4] Plasma concentration of PTX3 was found to be 2.1ng/ml (range 0.57 to 48.18 ng/ml) in subjects with active and 0.63ng/ml (range 0.00 to 1.64ng/ml) in subjects with inactive Takayasu arteritis.[5] Therefore PTX3 can be considered as a marker during the active stage of diseases. PTX3 were found to be higher in localized specimens of various inflammatory conditions like arthritis, vasculitis, psoriasis, arteritis, and asthma; suggestive of its synthesis by the local inflammatory cells.[6, 7, 8, 9]

PTX3 levels were also found to be higher for periodontal diseases.[10] A recent study evaluated the level of PTX3 in GCF of periodontitis patients before and after scaling and root planing and adjunctive application of TTO (TEA TREE OIL).[11]

As PTX3 levels are increased during the early stages of inflammation and the data from current literature on the levels of PTX3 in periodonttis are mostly cross sectional. The aim of the current study was to compare the levels of Pentraxin 3 among healthy and chronic periodontitis subjects before and after non surgical periodontal therapy and to correlate it to clinical parameters.

MATERIALS AND METHODS

The study population consisted of 40 participants attending the outpatient section of Department of Periodontics, Saveetha Dental College and Hospital, Chennai. The study was carried out from August 2014 to September 2014. The study groups comprised of Group I,10 periodontally healthy subjects, Group II, 20 patients with moderate chronic periodontitis, Group III same as group II one month after receiving Scaling and Root Planing. The participants were classified into chronic periodontitis and healthy group in accordance with the AAP criteria (American Academy of Periodontology) 1999.[12] Written informed consent was obtained from those who agreed to participate in the study. Ethical clearance was obtained from the Institutional Ethics committee and Review board. The Ethical clearance number obtained was "SRB/SDMDS12PER2". The power of the study was determined to be 95%.

Participants of this study were of the age group of 30-55 years and included both genders. In case of Generalized Chronic periodontitis, participants with ≥20 teeth were included with a pocket depth of ≥ 5mm. The diagnosis was reconfirmed separately by four examiners and the ambiguous cases were

excluded. Controls had a healthy periodontium with no gingival inflammation (pocket depth ≤3mm and CAL=0). Individuals with the history of any autoimmune diseases or any other systemic diseases which can alter the course of the periodontal disease, use of tobacco in any forms, use of medications like antibiotics, anti-inflammatory drugs or a history of periodontal therapy in the preceding 6 months and pregnant/lactating mothers were excluded from the study patient underwent a full mouth periodontal probing and charting.

The clinical parameters assessed were Plaque Index (PI), Sulcular bleeding index, Probing Pocket Depth (PD) and Clinical attachment level (CAL). PD and CAL were measured using a UNC 15 periodontal probe. The clinical examination and site selection for procurement of sample was done by a single examiner (V.M). Six sites of all the teeth were examined and only the site with the deepest pocket was selected for sample procurement. After making the subjects sit comfortably in an upright position on the dental chair. Without touching the marginal gingiva, supragingival plaque was removed to avoid contamination and blocking of the capillary pipette. GCF was collected by placing 1 -3µl calibrated volumetric micro capillary pipettes obtained from SIGMA Aldrich Chemical Company, USA. By placing the tip of the pipette extracrevicularly for 5 -20 min, a standardized volume of 1µl GCF was collected using the calibration on the micropipette from each test site. The test sites, which did not express standard volume (1µl) of GCF and micropipette contaminated with blood and saliva, were excluded.

SRP was performed for periodontitis subjects at the same appointment after GCF collection. After 4 weeks GCF was collected from the same site of these subjects. The GCF collected was transferred to ependroff tubes containing 100µl of phosphate buffer solution and stored at -70ºC until the time of assay.GCF was collected from healthy subjects using the same method.

Assay Procedure

This assay employs the quantitative sandwich enzyme immunoassay technique using Human pentraxin3/TSG-14 Immunoassay obtained from Research and Development Systems, USA. Catalogue no. DPTX30. A streptavidin -coated plate was incubated with biotinylated monoclonal antibody specific for PTX3. Plates were washed and 20µl of pretreated standards and samples were added to the wells. Any PTX3 present was bound by the immobilized biotinylated antibody. After washing away the unbound substances, an enzyme -linked conjugate specific for PTX3 was added to the wells. Following a wash to remove any unbound conjugate, a substrate solution was added to the wells and colour developed in proportion to the amount of PTX3 bound. The colour development was stopped and the intensity of the colour was measured. The minimum detectable dose (MMD) of PTX3 ranged from 0.007 – 0.116 ng/ml and the mean MDD (sensitivity) was 0.025 ng/ml. The intra -assay precision was 3.8 to 4.4% and the inter-assay precision was 4.1 to 61.1%.

Statistical analysis

Statistical analysis was done using SPSS Version 17 software. Independent sample t test was used to compare the mean pentraxin 3 values between the Group I and Group II. Mann Whitney test was used to compare between Pre and Post treatment mean values in Group II subjects. The correlation between GCF PTX3 with clinical parameters was analyzed using the Spearman rank correlation test. $p < 0.05$ indicated statistical significance.

RESULT

Descriptive statistics of the parameters in the study population are shown in Table 1. PI: mean plaque index, SBI: mean Sulcular bleeding index, PD: mean Probing depth, CAL: mean Clinical attachment level, PTX3: mean Pentraxin 3 Levels, Group I: healthy group, Group II: Chronic periodontitis group, Group III Chronic periodontitis group after treatment. Groups depicts the male to female ratio, average age of the patient, mean plaque index, mean sulcular bleeding index, mean probing

depth and mean clinical attachment level of study participants.

The results indicate (Table 2) that PTX3 levels were higher in Group II (4.60±3.79ng/ml) than Group I (0.19±0.2ng/ml). The mean differences between the groups were also statistically significant (p<0.001). PI: mean plaque index, SBI: mean Sulcular bleeding index, PD: mean Probing depth, CAL: mean Clinical attachment level, PTX3: mean Pentraxin 3 Levels, Group I: healthy group, Group II: Chronic periodontitis group. The table 5.2 summarizes the mean probing depth, the mean plaque index, the mean bleeding index, the mean clinical attachment level and the mean PTX3 levels of the Group I(healthy group) and Group II(chronic periodontitis group).

	Sex (males : females)	Age (years; mean± SD)	PI (mean± SD)	SBI (mean± SD)	PD(mm ; mean± SD)	CAL (mm; mean± SD	PT X3 (ng/m l; mean± SD)
Group I	6:4	37.9 ± 0.69	0.87 ± 0.20	0.82 ± 0.38	1.92 ± 0.33	2.11 ± 0.48	0.19 ± 0.21
Group II	11:9	42.4 ± 1.30	1.89 ± 0.27	2.53 ± 0. 82	4.13 ± 0.66	5.10 ± 0.86	4.60 ± 3.79
Group III	11:9	42.4 ± 1.30	0.92 ± 0.20	0.97 ± 0.26	2.41 ± 0.51	2.90 ± 0.70	1.05 ± 1.20

Table 1: Descriptive Statistics of Baseline Parameters in the Stud y Population

	Probing Depth (Mean±SD)	Plaque Index (Mean±SD)	Bleeding Index (Mean±SD)	Clinical Attachment Level (Mean±SD)	PTX3 (Mean±SD)
Group II	4.136±0.66	1.89±0.27	2.53±0.36	5.10±0.86	4.60±3.79
Group III	2.41±0.51	0.92±0.20	0.97±0.26	2.90±0.70	1.05±1.20
't' Value	9.57	11.524	13.395	10.189	3.92
'P' Value	<0.001*	<0.001*	<0.001*	<0.001*	=0.001*

* Statistically significant (P<0 .05)

Table 2: Comparison of mean PTX3 levels and clinical parameters between Group I and Group II

The PTX3 levels (Table 3) showed a significant reduction in Group III subjects (1.05±1.20ng/ml) 4 weeks after scaling and root planing. The mean difference was statistically significant (p= 0.001).

Spearman's correlation was used to analyse the correlation between PTX3 levels and both the individual and full mouth clinical parameters.

Among Group II, a positive correlation of PTX3 values were found with individual clinical attachment level, individual probing depth, individual plaque index and sulcular bleeding index though not statistically significant.

	Probing Depth (Mean±SD)	Plaque Index (Mean±SD)	Bleeding Index (Mean±SD)	Clinical Attachment Level (Mean±SD)	PTX3 (Mean±SD)
GroupII	4.136±0.66	1.89±0.27	2.53±0.36	5.10±0.86	4.60±3.79
GroupIII	2.41±0.51	0.92±0.20	0.97±0.26	2.90±0.70	1.05±1.20
't' Value	9.57	11.524	13.395	10.189	3.92
'P' Value	<0.001*	<0.001*	<0.001*	<0.001*	=0.001*

Table 3: Comparison of mean PTX3 levels and clinical parameter among Group II and Group III pre and post treatment

DISCUSSION

Periodontal disease is a multifactorial infectious disease; although the main cause of periodontal disease are the presence of periodontal microorganisms, subsequent progression and disease severity are considered to be determined by the host immune response[13, 14, 15] Pentraxin 3 is the first long pentraxin to be identified11 and is produced by a variety of cells like the dendritic cells, endothelial cells, fibroblasts and neutrophils.[16, 17]

In the neutrophils, it is stored in ready to use form in the lactoferrin rich specific granules.[18] Pentraxin 3 is suggested to play an important role in the innate resistance against pathogens, regulation of inflammatory reactions and the clearance of apoptotic cells.[19, 20] Plasma levels of PTX3 are raised in inflammatory conditions associated with an array of disease states, ranging from infections to autoimmune to degenerative disorders.[21]

PTX3 levels were also found to be higher for periodontal diseases**.** PTX3 levels were found to be higher in tissue and saliva of aggressive periodontitis subjects compared to chronic periodontits subjects[22]. An animal study by Keles et al in 2012 on experimental periodontitis model of rats found increased level of PTX3. A recent study had assessed the level of PTX3 in chronic periodontitis subjects before and after SRP with local application of Tea Tree Oil (TTO). The results showed a significant decrease in the level of PTX3 in GCF post SRP with TTO.

To the best of our knowledge no studies have yet assessed the level of PTX3 in GCF of periodontitis subjects pre and post non surgical periodontal therapy (NST) and compared it to healthy subjects. Hence the aim of the present study was to estimate the level of pentraxin 3 in GCF of periodontitis subjects before and after NST and correlate the levels of PTX3 with clinical parameters.

In our study we chose GCF samples as it was a non invasive method to assess PTX3 levels pre and post SRP. GCF was collected using micro capillary pipettes to avoid non -specific attachment of the analyte (PTX3) to filter paper fibers, which can lead to a false reduction in detectable PTX3 levels.[23, 24]

PTX3 levels in our study were estimated to be 4.31±3.81ng/ml for Group II (diseased group). The samples were obtained from sites with deepest probing depth. The significantly higher levels of PTX3 could be attributed to the release of cytokines at tissue -injury sites. Neutrophils arrive early at sites of injury and infection. Studies have demonstrated that peripheral neutrophils from periodontitis patients exhibit hyper-reactivity following stimulation by cytokines.[25] These neutrophils

represent a reservoir of prestored PTX3 that are ready for rapid release. Neutrophils store PTX3 into specific granules and release it in response to inflammatory signals.

The PTX3 levels for Group II (chronic periodontitis) were 4.60±3.79 ng/ml and that of Group III (post treatment) was 1.05±1.20ng/ml. This showed a significant decrease in the PTX3 levels post treatment (p=0.001) which was similar to prior study. The significant reduction in PTX3 levels post treatment observed in our study could be due to the reduction in inflammation leading to reduced number of neutrophils. Previous studies that estimated the levels of oral neutrophils in periodontitis patients before and after SRP found significant reduction.[26, 27]

The limitations of the current study are the limited sample size of 20 subjects in the disease groups as a higher sample size could have given a statistically significant positive correlation. The follow up of periodontitis subjects were done 1 month after scaling and root planing. The high levels of PTX3 seen in few subjects in the periodontitis group could be due to any subclinical inflammation or allergy or any infection not reported by the subjects and the GCF flow at the time of sample collection. Low PTX3 levels found in GCF sample of one subject with periodontitis may be because of the inactive diseased sites.

CONCLUSION

The significant reduction in the levels of PTX3 before and after SRP underlines its use as an inflammatory marker. It thus helps to understand and evaluate the progression of the disease and the response to the periodontal treatment administered during the course of management of periodontitis.

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