Estimation of Beta-CTXin gingival crevicular fluid and serum of periodontally healthy individuals and chronic periodontitis patients before and after non-surgical periodontal therapy- An in-vivo study

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Abstract

Objective and Design: Beta Cross-linked C-terminal telopeptide of type I collagen (fragment β- CTX) is newer addition to the list of bone resorption markers in serum. The purpose of our study was to estimate and compare the levels β-CTX in gingival crevicular fluid (GCF) and serum of periodontally healthy individuals and chronic periodontitis (CP) patients before and after nonsurgical periodontal therapy (NSPT).

Materials and Methods: Twenty Five subjects with equal distribution of males and females were divided into two groups i.e Group I: 10 Periodontally healthy individuals, Group II: 15 CP patients aged between 30-50 years. GCF and serum samples were collected at baseline in Group I whereas in Group II GCF and serum samples were collected at baseline and 2 months after NSPT. The levels of GCF and serum β-CTX were quantified using enzyme-linked immunosorbent assay (ELISA). The clinical parameters evaluated were plaque index (PI), gingival index (GI), probing pocket depth (PPD) and clinical attachment level (CAL) and the correlations of β-CTX in GCF and serum with clinical parameters were evaluated.

Results: The mean concentration of β-CTX increased from Group I to Group II. The mean concentration of β-CTX in GCF and serum was reduced after 2 months of NSPT. Furthermore, reduction in β-CTX levels was positively correlated to gain in CAL in GCF.

Conclusion: Periodontal treatment resulted in the reduction of β-CTX levels suggesting that β- CTX can be considered as a potential marker of bone resorption in CP.

Keywords: Collagen, Gingival Crevicular Fluid, C-terminal telopeptide, Chronic Periodontitis, Bone Resorption, Bone turnover Marker.

Introduction

Chronic Periodontitis (CP) is defined as 'an inflammatory disease of the supporting tissues of the teeth caused by specific microorganisms or groups of specific microorganisms, resulting in progressive destruction of the periodontal ligament and alveolar bone with pocket formation, recession, or both.[1] When left untreated, the final outcome is alveolar bone loss and exfoliation of the involved teeth.[2]

During the process of remodelling bone constantly undergoes changes. Through coupling mechanism there is a balance between the amount of bone resorbed by osteoclasts and the amount of bone formed by osteoblasts.[3] Firstly, bone formation takes place which is followed by bone resorption that leads to loss of bone density, both in men and women. Disruption of the bone remodeling cycle will cause Paget's disease, osteomalacia, or osteoporosis.

Wide spread research has been done in the area of the host response biochemical markers of periodontal disease. Various bone resorption markers like Hydroxyproline, cross-linked N- terminal telopeptide of type I collagen (fragment NTx), bone sialoprotein, tartarate resistant acid phosphatase, pyridinoline are found useful in this regard.

Cross-linked C-terminal telopeptide of type I collagen (fragment β-CTX) is newer addition to the list of bone resorption markers in serum.[4]

Osteoclasts are multinucleated cells which digest organic bone matrix rich in collagen fibres. Hence, the direct indicators of bone resorption are the fragments of bone collagen produced by osteoclastic activity.[5]

Majority of the organic matrix of bone consists of type I collagen. This is a helical protein that is cross linked at both the C-terminal and N-terminal ends of the molecule. On the C-telopeptide end, two fragments have been characterized and include 1) ICTP (cross-linked carboxyterminal telopeptide of type I collagen. 2) CTX (C-terminal crosslinked telopeptide of type I collagen) with a linear eight amino acid sequence (EKAHDGGR). CTX is generated by cathepsin K activity. The CTX epitope consists of an aspartyl-glycine motif that is prone to spontaneous isomerisation and racemisation giving rise to four isoforms; the alpha-aspartic acid converts to the beta form as the bone ages giving rise to β-CTX.

β-CTX which is a carboxy terminal telopeptide is exceptional because it is derived from degradation of mature collagen thus representing a true indicator for the resorptive process. Detection of such a molecule in gingival crevicular fluid (GCF) might lead to the development of a marker directly related to tissue breakdown in periodontitis. Serum levels of β-CTX were found to be elevated in post-menopausal osteoporosis,[4] rheumatoid arthritis,[7] and pagets disease.[8]

Till date, no studies have been done to evaluate and compare the levels of β-CTX in GCF and serum in periodontal health and CP patients. Hence, this study was designed to estimate and compare the GCF and serum β-CTX levels in periodontally healthy individuals and CP patients before and after Non-Surgical Periodontal Therapy (NSPT).

Materials and Methods

The study population comprised of 25 subjects attending the Outpatient Department, Department of Periodontology, Dr. D.Y. Patil Dental College and Hospital, Dr. D Y Patil Vidyapeeth, Pimpri, Pune. Subjects were matched to eliminate age and sex as confounding factors. The study was approved by the institutional ethics committee, Dr. D Y Patil Vidyapeeth, Pune. The procedure was explained and a written informed consent was obtained from the participants prior to the study.

The inclusion criteria was subjects in the age group of 30-50 years; presence of at least 14 natural teeth, subjects with CP were selected based on clinical parameters like Probing Pocket depth (PPD), Clinical Attachment Level (CAL), Gingival Index (GI), Plaque index (PI),[9] Subjects with normal serum calcium and phosphate levels.[10]

The exclusion criteria included patients with systemic diseases, smokers, pregnant, lactating and postmenopausal female subjects, patients on anti-inflammatory drugs, bisphosphonates, alendronates, patients having received antibiotic therapies during the previous 3 months, hormone replacement therapy, Vitamin D, and calcium supplements. Subjects who have received periodontal therapy within preceding six months were also excluded from the study.

Subjects were categorized into two groups based on PPD, CAL, PI (Silness & Loe 1964), GI (Loe & Silness 1963) and radiographic evidence of bone loss on Orthopantomography (OPG).

Group I: 10 Periodontally healthy subjects Pl<1, GI<1, PPD ≤3mm, CAL=0

Group II at baseline: 15 chronic periodontitis patients (PI ≥1, GI ≥1, PPD ≥5 mm, CAL ≥3 mm, evidence of bone loss on radiograph).[9]

Group II (2 months after NSPT): Subjects of Group II at baseline treated with scaling and root planing.

Clinical procedure

In Group I and Group II all the clinical parameters were recorded at baseline. On subsequent day samples were collected early morning under fasting state[11] GCF and blood for serum samples were collected and stored at -70ºC until the time of assay. In Group I, due to absence of active inflammation, samples were collected from multiple sites by pooling of GCF to ensure adequate amount for the study. In Group II subjects sites with ≥3mm of CAL showing highest attachment loss, PPD≥5mm along with radiographical conformation of the bone loss, were assigned for sampling. From each test site, a standarized volume of 1 µL was collected using white colour- coded 1-5µL calibrated volumetric microcapillary pipettes (Sigma Aldrich). Two millilitres of blood was collected from the ante cubital fossa by venipuncture using a 20-gauge needle with a 2ml syringe. Blood sample were allowed to clot at room temperature and after 1 hr was centrifuged at 3,000rpm for 5mins to separate serum component. GCF and serum samples were stored at -70ºC until the assay. In Group II thorough full mouth supra and subgingival scaling and root planing was performed. Patients were recalled at two months for follow up. Full mouth periodontal reassessment was carried out after which GCF and blood for serum samples were collected again and stored at -70ºC until the time of assay. Estimation of β-CTX levels of GCF and serum was carried out with the help of Enzyme Linked Immunosorbent Assay [ELISA] (Human beta crosslaps) with sensitivity of 5.52 ng/l procured from Assay Biotechnology Laboratory, Shanghai, CHINA.

Statistical analysis

All analysis was done in the R statistical software environment (Foundation for Statistical Computing Vienna, Austria). Normality assumptions were tested using the Shapiro-Wilk normality test and visual inspection of QQ plots. Non-parametric Mann–Whitney–Wilcoxon (MWW) tests were used to assess for significant differences in demographic and clinical parameters between Group I & Group II (α= 0.05, two-sided). Descriptive statistics of the outcome parameters β-CTX (GCF) and β-CTX (Serum) were computed and similarly compared between group I & II using Non-parametric Mann–Whitney–Wilcoxon (MWW) tests(α= 0.05, two-sided) and the outcome parameters β-CTX (GCF) and β-CTX (Serum) at T_0 and $T_1$and their change (by subtracting the value at $T_1$from the value at T_0) were tested using the Shapiro-Wilk normality test and visual inspection of QQ plots. Comparisons of both the levels at T_0 and T_1 were assessed Wilcoxon's singed-rank test for paired samples (α= 0.05, two-sided). Spearman's correlation tests were performed to test for significant association of baseline levels of β-CTX (GCF) with β-CTX (Serum) and to test for significant association between the magnitudes of change in levels of β-CTX (GCF) with β-CTX (Serum) with the magnitudes of change in the clinical measures of periodontitis severity (PPD, CAL) from T_0 to T_1(α= 0.05). P values<0.05 were considered significant.

Results

Demographic data

The demographic data of age and gender in study groups are summarized in Table 1. There was no statistically significant difference in mean age and gender distribution between the groups. Table 2 presents the mean PI, GI, PPD, CAL in Group I and Group II at baseline. There was a statistically significant difference between all clinical parameters in both the Groups at baseline. Table 3 presents the mean PI, GI, PPD, CAL in Group II at baseline and at 2 months after NSPT. There was a significant decrease in PI, GI, PPD, CAL in Group II after therapy.

Laboratory findings

Fig. 1 presents mean β-CTX in GCF in Group I and Group II at baseline. The mean β-CTX values in GCF of Group I and II at baseline was 11.99 ± 2.70 ng/l and 15.41 ± 3.66 ng/l respectively which showed statistically significant difference between the groups (P < 0.020). Fig. 2 presents mean β-CTX in serum in Group I and Group II at baseline. The mean β-CTX values in serum of Group I and II at baseline was 10.26 ± 5.15ng/l and 14.59± 4.83ng/l

respectively, which showed statistically significant difference between the groups (P < 0.028). Fig. 3 presents mean β-CTX in GCF in Group II at baseline and 2 months after NSPT. The mean β -CTX values for Group II in GCF at baseline was 15.41 ± 3.66 ng/l whereas at 2 months of NSPT, β-CTX value was reduced to 12.79 ± 3.98 ng/l showing reduction of 2.62 ± 4.87 ng/l which was statistically significant (P < 0.035). Fig. 4 presents mean β-CTX in serum in Group II at baseline and 2 months after NSPT. The mean β-CTX values for Group II in serum at baseline was 14.59 ± 4.83ng/l whereas at 2 months of NSPT, β-CTX value was reduced to 13.22 ± 5.59ng/l showing reduction of 1.37 ± 4.02 ng/l which was not statistically significant (P>0.302).

Correlation analysis

Table 4 shows correlation between the change in levels of β-CTX (GCF) and β-CTX (Serum) with change in clinical measures of periodontitis severity (PPD, CAL) from T_0 to T_1 in Group II. Change in β-CTX (GCF) Versus Change in CAL was statistically significant (P<0.015).

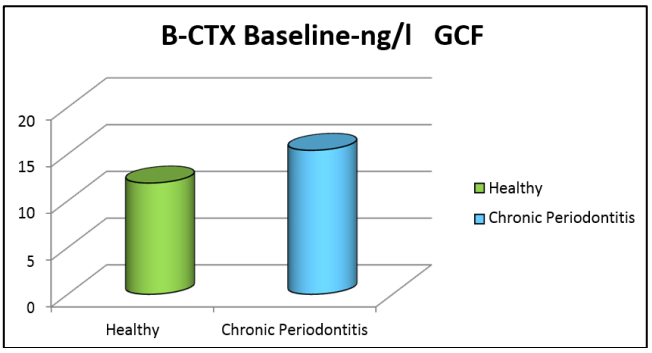

Fig. 1: Bar diagram showing comparison of Beta-CTX in GCF in Group I and Group II at baseline

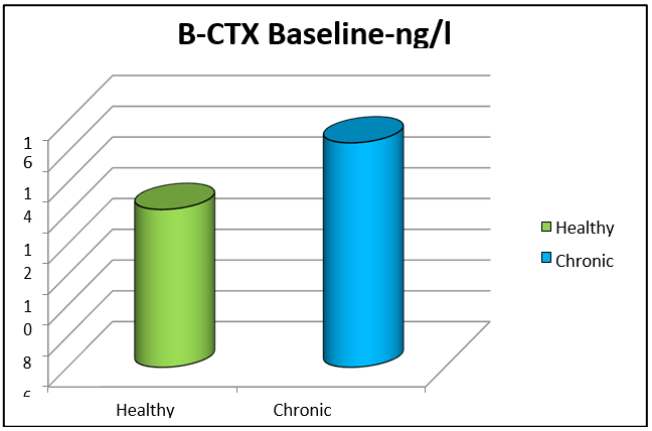

Fig. 2: Bar diagram showing comparison of Beta-CTX in serum in Group I and Group II at baseline

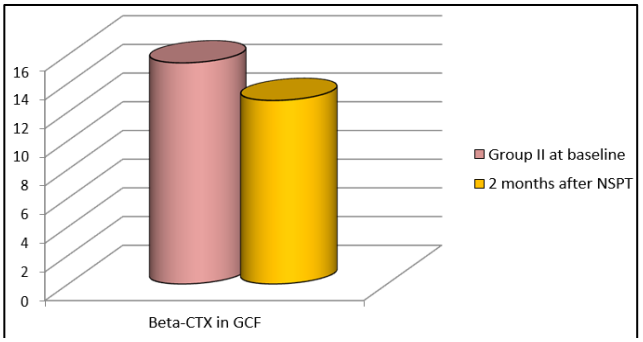

Fig. 3: Bar diagram showing comparison of Beta-CTX in GCF in Group II at baseline and 2 months after NSPT

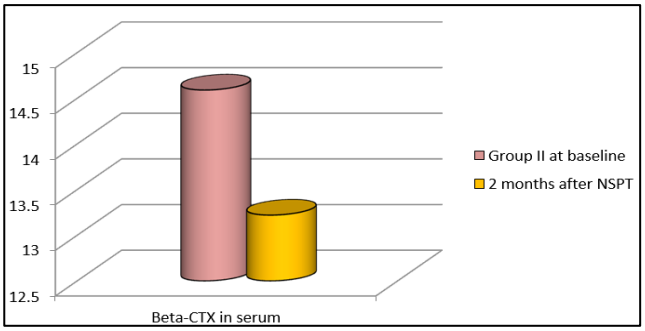

Fig. 4: Bar diagram showing: Comparison of Beta-CTX in serum in Group II at baseline and 2 months after NSPT

Discussion

Bone destruction caused by proteolytic enzymes such as matrix metalloproteinases (MMPs) and cathepsin K, breaks down type I collagen due to which, there is release of cross-linked telopeptides into the circulation (serum, saliva, urine) as stable fragments, such as pyridinoline cross-linked carboxyterminal telopeptide of type I collagen (ICTP),[12] C-terminal type I collagen telopeptide (CTX).[13] Along with understanding of bone degradation process, proper recognition of sites with periodontal disease progression or sites at risk of future deterioration is essential.[14] A biochemical marker definite for bone degradation would help in the differentiation of gingival inflammation from active periodontal damage.

Bone turnover markers consists of markers of bone resorption and formation which display osteoclast and osteoblast action respectively. Formation of amino and carboxy-terminal telopeptides (NTx, pyridinoline cross-linked carboxyterminal telopeptide of type I collagen (ICTP) and CTX) into the blood stream is caused by degradation of type I collagen by osteoclast.

CTX is released into the blood stream during degradation of type I collagen mediated by osteoclast, which constitutes >90% of organic matrix of bone. CTX is derived from α1-chain C-telopeptide of type I collagen that has undergone aging-associated peptide chain rearrangement (β -isomerization).[15] β-CTx serves as a definite marker for the degradation of mature type I collagen. Increased serum concentrations of β-CTx have been found in patients with osteoporosis,[4] rheumatoid arthritis,[7] pagets disease.[13]

Table 1: Demographic distribution of the study groups

	Group I N=10	Group II N=15	P Value
Age			
Mean ± SD	38.3±6	39.13±6.479	0.8669
Gender			
Male	4	7	1
Female	6	8	

Table 2: Comparison of PI, GI, PPD, CAL in Group I and Group II at baseline

Parameter	Group I		Group II		P Value
	Mean	SD	Mean	SD	
PI	0.57	0.20	1.95	0.25	**<0.0001***
GI	0.56	0.09	2.01	0.40	**<0.0001***
PPD	2	0	5.75	1.03	**<0.0001***
CAL	0	0	5.89	0.93	**<0.0001***

Table 3: Comparison of PI, GI, PPD, CAL at Group II baseline and 2 months after NSPT

Parameter	Group II baseline (T_0)		2 months after NSPT (T_1)		Change (T_0-T_1)	P Value
	Mean	SD	Mean	SD		
PI	1.96	0.25	1.00	0	0.96(0.25)	**0.0004***
GI	2.02	0.40	0.99	0.06	1.02(0.35)	**0.0008***
PPD	5.75	1.03	4.70	0.95	1.04(0.59)	**0.0007***
CAL	5.89	0.93	4.85	0.94	1.03(0.44)	**0.0007***

Table 4: Spearman's correlation β-CTX GCF and serum levels with clinical parameters

	Spearman's correlation Coefficient (rho value)*	P value
Change in B-CTX (GCF) Versus Change in PPD	0.143	0.611
Change in B-CTX (GCF) Versus Change in CAL	0.613	0.015*
Change in B-CTX (Serum) Versus Change in PPD	-0.298	0.280
B-CTX (Serum) Versus Change in CAL	0.200	0.474

ICTP is the most studied marker. However, study done by Talonpoika JT et al (1994),[16] found measurable levels of ICTP in GCF suggesting that a smaller proportion of ICTP can have its source from soft tissue breakdown making it less specific to bone resorption. Serum NTx has been described as a strong marker of osteoclastic collagen cleavage.[17] However, a study by Gursoy UK et al(2013),[18] showed very low levels of NTx in saliva. This is attributed to its higher thermal denaturation rate in comparison with that of ICTP or CTX at physiological temperature of 35-37 degrees in saliva. In the assessment and monitoring of bone metabolic disorders, CTX is considered one of the most definite and sensitive markers of bone resorption.

In our study GCF samples were collected because most of diagnostics currently focus on the use of GCF, which is an exudate that can be obtained from gingival sulcus or periodontal pocket. As GCF traverses the inflamed tissue, it carries molecules that are involved in the destructive process.[19] To assess the disease state GCF offers one of the most accessible entries in the body.[20] In current study serum samples were collected because β-CTX is a systemic bone resorption marker.

In current study early morning fasting GCF and serum samples were taken. It is said that early morning (between 7.30-9.30 am)[21] fasting samples diminishes the circadian variation of biochemical bone resorption markers.

Current study showed that mean concentrations of β-CTX in GCF and serum increased progressively from healthy to chronic periodontitis subjects. This increase in the levels of β-CTX is due to the osteoclast-mediated degradation of type I collagen that takes place during bone loss seen in chronic periodontitis. The results of the present

study are in accordance with that of Pellegrini G et al (2008),[22] who stated that during active resorption of bone as a result of advanced disease, CTX is released into the periodontal tissues, gathers in GCF, where it could be evaluated to assess disease activity and severity. According to a study by Miricescu D et al (2014),[23] CTX levels are increased in patients with chronic periodontitis. Elevated levels of β-CTX in GCF and serum compared to healthy group suggests that β-CTX may be considered one of the possible bone resorption marker.

The mean β-CTX values for Group II in GCF and serum at baseline was higher than in Group II after 2 months of NSPT. Decreased values of β-CTX both in GCF and serum in Group II after 2 months of NSPT may represent early positive changes on the bone.[24] In current study single episode of NSPT did not have significant effect on reduction in levels of β-CTX on serum as it is a systemic bone resorption marker.

In current study there is no correlation of baseline levels of β-CTX (GCF) with β-CTX(serum) in Group I and Group II which indicates that the β-CTX levels of GCF is both qualitatively and quantitatively distinct from that of serum. The reasons could be, the turnover rate of β-CTX is higher at the local site from periodontal tissues in response to inflammation than that of serum, the variability of β-CTX levels in GCF and serum could be attributed to the role of β-CTX in different stages of disease process at the time of collection of GCF and serum samples. Overall, the change in β-CTX levels with the change in clinical parameters in Group II was not statistically significant, except with clinical attachment level demonstrating a statistically significant relationship in GCF. The reduction in β-CTX levels in GCF from baseline to 2 months of NSPT correlated with the gain in CAL levels as shown by the Spearman's rank correlation test. For periodontal disease progression, the presence of previous attachment loss has been identified as a primary risk factor.[25] Changes in the clinical attachment level afford a better indication of the degree of periodontal destruction (or gain) compared to PPD.[26]

The increase in β-CTX levels in chronic periodontitis group and subsequent reduction after NSPT, showed that β-CTX levels changes according to the magnitude of bone resorption in disease and after treatment. One limitation of this study is the small sample size evaluated. Further longitudinal studies with larger sample size should be carried out to confirm the findings of the study and better understand the role of β-CTX in chronic periodontitis.

Conclusion

Within the limitations of the study, β-CTX can be successfully estimated in GCF and serum of chronic periodontitis patients and its levels subsequently reduced after NSPT suggesting that the marker responds to therapy. Thus, it can be considered as a potential bone resorption marker of chronic periodontitis. Further longitudinal prospective studies must be carried out to affirm these findings and understand the possible role of β-CTX in pathogenesis of alveolar bone destruction in chronic periodontitis.

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Conflict of interest

None.

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