Original Research Article

Effect of phase I therapy on salivary carboxy terminal telopeptide of type I collagen in chronicperiodontitis patients

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Abstract

Background: Salivary biomarkers are extensively studied in various fields of dentistry. Carboxy terminal telopeptide of type I collagen (CTX), a degradation product that is a marker of bone resorption is released into tissues during the periodontal disease process and reaches the saliva via GCF. Changes in salivary CTX levels can be used to detect and monitor periodontal disease activity.
Aim of the study: The present study aimed to analyze the effect of phase I therapy in chronic periodontitis subjects by evaluating the salivary CTX levels and to compare and correlate the salivary CTX levels with clinical parameters.
Materials and methods: This study was conducted in Aravind Dental Hospital. (for 3 months). Salivary CTX levels were determined in patients with chronic periodontitis, n=25 (study group), and healthy controls (n=20) using Enzyme-linked Immunosorbent Assay (ELISA). The salivary CTX levels were compared and correlated with clinical parameters namely PI, GBI, PPD, CAL before and after phaseI therapy.
Results: There was a statistically significant increase in salivary CTX levels in the study group when compared to the control group ($p<0.01$), and these levels reduced significantly after treatment. A positive correlation was shown between the clinical parameters and salivary CTX levels but the correlation was not significant in both groups ($p>0.05$).
Conclusion: In the present study, there was a significant difference in salivary CTX levels between the groups, and a significant decrease in these levels was observed after phase I therapy in the study group. This signifies that the detection of CTX in saliva may be useful to detect individuals at risk, periodontal disease activity, and its response to periodontal therapy.

Key words

Carboxy terminal telopeptide of type I collagen (CTX), Bone resorption, Markers, Chronic periodontitis.

Introduction

The presence of active periodontal disease with continuing attachment loss threatens the oral health, comfort, and function of the patient. If the disease activity could be determined, therapeutic measures may be fashioned for individual patients. Recently, the field of Periodontics has advanced dramatically in different ways to assess sites and individuals with active disease [1]. Proteolytic enzymes play a major role in the destruction of periodontium during the disease process. These enzymes (collagenases, proteases, aminopeptidases, etc.) and their breakdown products are considered as markers of periodontal disease activity [2]. During the initial phase of bone resorption, the pH at the site is acidic. As a result, cathepsin K (cysteine protease) released from the osteoclasts attacks the type I collagen at multiple sites, including several sites in the helical region. CTX is generated by cathepsin K, but ICTP is destroyed by this enzyme. ICTP is generated by the action of MMP's such as MMP – 9 and MMP – 12 [3]. Thus ICTP is termed as CTX – MMP. CTX is considered as the most specific and sensitive marker to monitor metabolic bone diseases and physiological bone turnover. It can be detected from samples by Enzyme-linked Immunosorbent Assay (ELISA) using highly specific monoclonal antibodies. Unstimulated, whole saliva represents a pooled sample of all periodontally diseased sites. Hence, saliva was used to analyze the level of this biomarker in generalized chronic periodontitis patients [4].

Materials and methods

This study was conducted in Aravind Dental Hospital (for 3 months). Salivary CTX levels were determined in patients with chronic periodontitis, n=25 (study group), and healthy controls (n=20) using Enzyme-linked Immunosorbent Assay (ELISA). The salivary CTX levels were compared and correlated with clinical parameters namely Plaque index (PI), Gingival bleeding index (GBI), Probing pocket depth (PPD), Clinical attachment level (CAL) before and after phaseI therapy.

Inclusion criteria

- Systemically healthy subjects.
- Age: 30 – 40 years.
- Gender: Both males and females.
- Patients with generalized chronic periodontitis with a minimum of 20 teeth.

Exclusion criteria:

- History of any systemic illness.
- History of systemic antibiotics or anti-inflammatory drugs during the past 6 months.
- Pregnancy, lactation, and menopause.
- Patients who smoke or use tobacco in any form.
- History of any periodontal treatment during the past 1year.

The selected subjects were divided into 2 groups based on the following criteria: Group I – Control group (n=20) Group II – Study group (n=25). The present study is an interventional study. Saliva samples were collected at baseline in both the groups and after Phase, I therapy in group II. Hence group II was further classified into Group II A – before Phase I therapy Group II B – after Phase I therapy. In addition, subjects should meet the following criteria: Should have good oral hygiene throughout the periodontal treatment. Absence of any lesions in the oral cavity. Clinical Attachment Level was measured from the Cemento – Enamel Junction (CEJ) to the base of the pocket in millimeters using William's Periodontal Probe. Three measurements were made on the buccal aspect and three on the lingual aspect of each tooth – a total of six sites per tooth (Mesiobuccal, Midbuccal, Distobuccal, Mesiolingual, Midlingual, and Distolingual). If

the gingival margin is located on the anatomic crown, the level of the attachment was determined by subtracting from the probing pocket depth, the distance from the gingival margin to the CEJ. If both were the same, the loss of attachment was calculated to be zero. If the gingival margin coincides with the CEJ, the loss of attachment was calculated as equaling the probing pocket depth. If the gingival margin is located apical to the CEJ, the loss of attachment was greater than the probing pocket depth and therefore the distance between the CEJ and the gingival margin was added to the PPD. Subjects were selected randomly, with no discrimination in age and sex between both groups. Each subject underwent periodontal examination and charting using the clinical parameters (gingival bleeding index, plaque index, probing pocket depth, clinical attachment level). Orthopantamogram was taken for Group II subjects. Radiographic bone loss was recorded dichotomously (presence or absence). Bitewing radiographs (posterior) were taken to confirm the bone loss. No delineation was done for Group II based on the extent of bone loss. 2ml of unstimulated whole saliva was collected at baseline from Group I and II. Oral hygiene instructions were given. Phase I therapy was performed for Group II subjects. Patients were re-evaluated after 1 month using the same clinical parameters and 2ml of unstimulated whole saliva sample was collected from Group II B subjects after phase I therapy. Salivary CTX were estimated in the samples using Enzyme-Linked Immunosorbent Assay.

Statistical analysis

The statistical analysis was done using the computer software program SPSS (Statistical Package for Social Sciences) version 17. Mean and Standard Deviation were estimated for different variables in each group. Mean values were compared between the two groups by using *Student's independent t-test. Paired t-test* was used to compare the mean values within the same group. *Chi-square test* was done to compare the gender distribution between the two groups. Pearson's correlation coefficient was used to analyze the correlation between the clinical parameter and salivary CTX level. In the present study, *P-value <0.05* was considered as the level of significance.

Results

Table - 1 shows the comparison of gender distribution between the study and control group. The males constituted 45.8% in Group I and 54.2% in Group II. Females constituted about 42.9% in Group I and 57.1% in Group II. There was no statistically significant difference in the distribution of the gender between the groups. The mean Plaque Index score in Group I was 0.26 ± 0.22 and 2.58 ± 0.15 in Group II which was statistically highly significant (p<0.01**). A positive correlation was observed between Plaque Index and salivary CTX level in Group I and Group II but the correlation was not significant.(p>0.05) The gingival bleeding index was significantly increased in the Group IIA (96.22±2.54) as compared to the control group (15.57 ± 6.06with p-value < 0.01**, which reduced to 21.79 ± 7.25 following phase I periodontal therapy with a p <0.01** The gingival bleeding index showed a positive correlation with salivary CTX in group I, IIA and IIB but the correlation was not significant. (p>0.05) The PPD was significantly increased in the study group (6.27 ± 0.93mm) as compared to the control group (2.84 ± 0.53mm) with p-value <0.01** The probing depth reduced considerably following treatment to 3.07 ± 0.68 mm (p-value <0.01**) but not to the level of the control group (p-value <0.01**) Linear correlation was observed between probing depth and salivary CTX levels in all the groups but the correlation was not significant. In the study group, a statistically significant difference was found between group II-A (6.93 ± 0.64mm) and group II-B (4.22 ± 0.52mm) values with p-value <0.01**. Since the loss in the clinical attachment level in the control group was absent the comparisons with that group was highly significant. A positive correlation was observed between CAL and salivary CTX but the correlation was not significant.

Table – 1: Clinical correlation parameters group I and group II A.

	GROUP	MEAN	S.D	P-VALUE
PI	I	0.26	0.22	
	II A	2.58	0.15	0.000**
GBI (%)	I	15.57	6.06	
	II A	96.22	2.54	0.000**
PPD (MM)	I	2.84	0.53	
	II A	6.27	0.93	0.000**
CAL (MM)	I	0.00	0.00	
	II A	6.93	0.64	0.000**

**** < 0.01 – HIGHLY SIGNIFICANT *0.05-0.01 – SIGNIFICANT $ >0.05 – NOT SIGNIFICANT**

Table – 2: Comparison of salivary CTX between group I andgroup II A.

	Group I		Group IIA		P-value
CTX (pg/ml)	**Mean**	**S.D**	Mean	S.D	
	38.39	6.22	152.98	16.74	0.000^{**}

The mean salivary CTX levels were 152.98±16.74 pg/ml in the study group and 38.78±6.22 pg/ml in the control group. There was a high statistically significant difference in salivary CTX levels between the study and the control group. A highly significant difference ($p<0.01$**) was observed on comparing the salivary CTX level between Group II subjects before (152.98 ±6.22 pg/ml) and after (80.90±19.90 pg/ml) therapy. Comparison of salivary CTX level between Group I (control group) and Group II (study group) after phase I therapy was also highly significant ($p<0.01$**). It implies that the levels did not reduce to the level of the controls after phase I therapy ** < 0.01 – highly significant *0.05-0.01 – significant $ >0.05 – not significant (**Table – 2**).

Discussion

Diagnostic testing has been a great challenge in periodontology, as the disease has a multifactorial etiology and its progression is characterized by periods of quiescence interspersed with episodes of acute destruction. Most importantly the diagnostic measures (probing depth, bleeding on probing, radiographic assessment) used for assessing periodontal disease status provide information about the past disease and fail to diagnose the current disease status [5]. Moreover, they are not reliable for identifying individuals with progressing disease activity. Biomarkers of disease activity facilitate earlier detection of disease and help in monitoring therapy outcomes [6]. Recently, salivary markers have been investigated for the assessment of periodontal disease activity and its response to treatment. Markers of collagen degradation such as pyridinoline, deoxypyridinoline, ICTP, and CTX are being used to analyze the normal and pathologic processes in bone. In the literature, pyridinoline cross-linked carboxyterminal telopeptide of type I collagen (ICTP) is reported to be more specific for bone resorption and is considered a good tool for assessing metabolic bone diseases like osteoporosis, rheumatoid arthritis, and bone metastases [7]. There are only a few studies about CTX about periodontal diseases. Miller et al analyzed the salivary biomarkers of periodontal disease and hypothesized that during active resorption of bone, CTX is released into the periodontal tissues, collected in GCF, and is transferred to the whole saliva. Hence saliva can be used to assess the disease activity and to monitor periodontal therapy [8]. Although GCF is site-specific, the whole saliva represents a pooled sample of all periodontally involved sites and is

potentially useful in detecting individuals at risk. Stimulated whole saliva is less suitable for diagnostic applications because the foreign substances used to stimulate saliva tend to modulate the fluid pH and stimulate the water phase of saliva secretion, resulting in dilution in the concentration of proteins of interest. In the present study, unstimulated whole saliva was collected from both the groups at baseline and in Group II after phase I therapy [9]. Owing to diurnal and dietary influences on CTX levels, saliva samples were collected from the study subjects in the morning hours following an overnight fast. CTX markers in serum and salivary samples are reported to be stable at -20^0C Therefore the collected samples were stored at -20^0C.In the present study, CTX was detected in all saliva samples from patients with chronic periodontitis and healthy controls (detection limit of the kit used in the present study – 44.7 pg/ml) [10]. In the present study, salivary CTX was increased in the study group (mean –38.39 pg/ml) when compared to the control group (mean – 152.98 pg/ml) with a highly significant difference ($p<0.01$). This signifies that the cathepsin K enzyme has taken part in the degradation of type I collagen and carboxyterminal telopeptide of type I collagen (CTX) has been released into periodontal tissues which have reached the saliva via GCF and have been detected by ELISA [11]. The clinical parameters namely the plaque index and gingival bleeding index, probing depth, clinical attachment level were correlated with salivary CTX levels in the control and study group. A positive correlation was observed between the clinical parameters and salivary CTX level in all the groups but the correlation was not significant. This might be probably due to the low sample size (n=45) in this study [12]. On comparing the mean values of the clinical parameters, a highly significant difference was shown before and after phase, I therapy in the study group. Also, a positive correlation was observed between the clinical parameters and salivary CTX levels after phase I therapy. This signifies that the inflammation was reduced after therapy and gain in the clinical attachment level was obtained [13]. On the evaluation of salivary CTX levels in the study group before and after phase, I therapy highly significant difference ($p<0.01$) was observed. However, the salivary CTX levels after phase I therapy were not reduced to the level of controls. This signifies the role of the host response to periodontal treatment which varies from patient to patient [14]. Also the observation period after phase I therapy could have been prolonged, which could have resulted in further reduction of salivary CTX levels. Moreover, evaluation of CTX levels after the surgical phase of periodontal therapy could have resulted in levels closer to the control group. Hence further studies can be conducted to evaluate salivary CTX levels after the surgical phase of periodontal therapy [15].

Conclusion

CTX was detected in the saliva of chronic periodontitis and healthy control subjects. Salivary CTX level was significantly increased in chronic periodontitis subjects as compared to healthy controls. Positive correlation was shown between the clinical parameters and salivary CTX levels in both groups but the correlation was not significant. Significant reduction in salivary CTX levels was seen after phase I therapy in the chronic periodontitis group but did not reduce to the level of healthy controls.

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