# EFFECT OF PHASE I THERAPY ON SALIVARY CARBOXYTERMINAL TELOPETIDE OF TYPE I COLLAGEN IN CHRONIC PERIODONTITIS PATIENTS

*A Dissertation submitted in*
*partial fulfillment of the requirements*
*for the degree of*

**MASTER OF DENTAL SURGERY**

**BRANCH – II**
**PERIODONTICS**

**THE TAMIL NADU DR. M.G.R. MEDICAL UNIVERSTIY**

**2010 – 2013**

## CERTIFICATE

This is to certify that **Dr. S. KIRUTHIKA**, Post Graduate student (2010-2013) in the Department of Periodontics, Tamil Nadu Government Dental College and Hospital, Chennai - 600 003, has done this dissertation titled **"EFFECT OF PHASE I THERAPY ON SALIVARY CARBOXYTERMINAL TELOPEPTIDE IN CHRONIC PERIODONTITIS"** under our direct guidance and supervision in partial fulfillment of the regulations laid down by the **Tamil Nadu Dr. M.G.R. Medical University**, Chennai - 600 032 for **M.D.S.,** (Branch-II) **Periodontics** degree examination.

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*"Knowledge is in the end based on acknowledgement."*

LudwigWittgenstein

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# ABSTRACT

**Background:**

Salivary biomarkers are extensively studied in various fields of dentistry. Carboxyterminal telopeptide of type I collagen (CTX), a degradation product which is a marker of bone resorption is released into tissues during 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:**

The aim of the present study is to analyse 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:**

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 phase I therapy.

**Results:**

There was 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. Positive correlation was shown between the clinical parameters and salivary CTX levels but the correlation was not significant in both the 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, the periodontal disease activity and its response to periodontal therapy.



**Key words: Carboxyterminal telopeptide of type I collagen (CTX), bone resorption markers, chronic periodontitis**

## DECLARATION

| | |
|---|---|
| **TITLE OF DISSERTATION** | Effect of phase I therapy on salivary carboxyterminal telopeptide of type I collagen in chronic periodontitis |
| **PLACE OF STUDY** | Tamil Nadu Government Dental College & Hospital, Chennai-600003 |
| **DURATION OF THE COURSE** | 3 Years |
| **NAME OF THE GUIDE** | Dr. Maheaswari Rajendran |
| **HEAD OF THE DEPARTMENT** | Dr. K. Malathi |

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Date: 09 .08.2011

Title of the Work : Effect of phase I therapy on salivary carboxyterminal telopeptide levels in chronic periodontitis patients

Principal Investigator: Dr.S.Kiruthika, IInd Year MDS., PG student,

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The request for an approval from the Institutional Ethical Committee (IEC) was considered for the following on the IEC meeting held on 25.07.2011 at the Principal's Chambers, Tamil Nadu Government Dental College & Hospital, Chennai-3.

**"Advise to proceed with the study"**

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This agreement herein after the "Agreement" is entered into on this day 12.12.2010 between the Tamil Nadu Government Dental College and Hospital represented by its Principal having address at Tamil Nadu Government Dental College and Hospital, Chennai, (hereinafter referred to as, 'the college')

And

Dr. MAHEASWARI RAJENDRAN aged 48 years working as Professor at the college, having residence address at Parvathi Illam No 9, Old No 10, Santhome, Chennai, Tamil Nadu (hereinafter referred to as the 'PG/Research and Principal Investigator')

And

Dr. S. KIRUTHIKA, aged 31 years currently studying as final year post graduate student in the Department of Periodontics herein after referred to as the 'PG/Research student and co-investigator').

Whereas the 'PG/Research student as part of her curriculum undertakes to research on EFFECT OF PHASE I THERAPY ON SALIVARY CARBOXYTERMINAL TELOPEPTIDE OF TYPE I COLLAGEN IN CHRONIC PERIODONTITIS for which purpose the PG/Principal investigator shall act as Principal investigator and the College shall provide the requisite infrastructure based on availability and also provide facility to the PG/Research student as to the extent possible as a Co-investigator

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9. It is agreed that as regards other aspects not covered under this agreement, but which pertain to the research undertaken by the Student Researcher, under guidance from the Principal Investigator, the decision of the College shall be binding and final.
10. If any dispute arises as to the matters related or connected to this agreement herein, it shall be referred to arbitration in accordance with the provisions of the Arbitration and Conciliation Act, 1996.

In witness whereof the parties hereinabove mentioned have on this the day month and year herein above mentioned set their hands to this agreement in the presence of the following two witnesses.

College represented by its Principal  Student Researcher

Witnesses  Student Guide

1.

2.

# CONTENTS

# LIST OF ABBREVIATIONS

| | |
|---|---|
| AAP | American Academy of Periodontology |
| Ala | Alanine |
| Arg | Arginine |
| b-AP | Bone Alkaline phosphatase |
| BOP | Bleeding on Probing |
| CAL | Clinical Attachment Level |
| C-terminal | Carboxyterminal |
| ELISA | Enzyme Linked Immunosorbent Assay |
| GBI | Gingival Bleeding Index |
| GCF | Gingival Crevicular Fluid |
| Gly | Glycine |
| His | Histidine |
| HPLC | High Performance Liquid Chromatography |
| IL | Interleukin |
| kDa | kilo Dalton |
| LPS | Lipopolysaccharide |
| Lys | Lysine |
| MMP | Matrix metalloproteinases |
| N-terminal | Amino – terminal |
| PI | Plaque Index |
| PPD | Probing pocket depth |
| RIA | Radio immunoassay |
| S.D. | Standard Deviation |
| SPSS | Statistical Package for Social Sciences |
| TRACP 5b | Tartrate resistant acid phosphatase 5b |

# LIST OF PHOTOGRAPHS

# LIST OF TABLES

# LIST OF FIGURES

# 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.

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.[86]

During the initial phase 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.[35] Thus, the triple helix becomes more susceptible to further action of other enzymes like MMP's at neutral pH.[2, 37, 85] Two of the degradation product from the C – terminal telopeptide region of type I collagen are[37]:

i. Carboxyterminal telopeptide of type I collagen (CTX)
ii. Pyridinoline cross linked carboxyterminal telopeptide of type I collagen (ICTP / CTX – MMP)[85]

Carboxyterminal telopeptide of type I collagen (CTX) is an octapeptide (Glu – Lys – Ala – His – Asp – Gly – Gly – Arg) found at the cross linking site of the alpha 1 (I chain) chain at the C – terminal telopeptide region of type I collagen.[85]

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. Thus ICTP is termed as CTX – MMP.[37, 85] CTX is considered as the most specific and sensitive marker to monitor metabolic bone diseases and physiological bone turnover.[37]It can be detected from samples by Enzyme linked Immunosorbent Assay (ELISA) using highly specific monoclonal antibodies.[14, 80]

Unstimulated, whole saliva represents a pooled sample of all periodontally diseased sites.[18]Hence, saliva was used to analyse the level of this biomarker in generalized chronic periodontitis patients.

The present study was conducted to determine the effect of Phase I therapy in generalized chronic periodontitis patients by evaluating salivary CTX levels before and after periodontal therapy.

## AIM AND OBJECTIVES

AIM:

The aim of the study is to determine the effect of phase I therapy in generalized chronic periodontitis patients by evaluating the salivary levels of CTX using Enzyme Linked Immunosorbent Assay (ELISA).

OBJECTIVES:

1. To compare salivary CTX levels between generalized chronic periodontitis patients (study group) and healthy controls (control group).
2. To compare salivary CTX levels before and after phase I therapy in study group.
3. To correlate salivary CTX levels with clinical parameters like bleeding on probing, Plaque Index, Probing pocket depth and Clinical attachment level in both the groups.

## REVIEW OF LITERATURE

Bone is a dynamic connective tissue that undergoes continuous remodeling by two counteracting processes, namely bone formation and bone resorption. These processes rely on the activity of osteoclasts (resorption), osteoblasts (formation) and osteocytes (maintenance).[62]

### TYPE I COLLAGEN

Type I collagen comprises 90% of the organic matrix of bone and is the most abundant collagen in osseous tissues.[68] Like other types of collagen, type I collagen also comprises three polypeptide chains (α-chains) which form a unique triple-helical structure. It is a heterotrimer of two α1(I) and one α2(I) chains. The α1 (I) chain is more conserved than the α2 (I) chain.[52] The N (amino) and C (carboxy) terminal telopeptides, do not have a repeating Gly-X-Y structure and do not adopt a triple helical conformation. These account for 2% of the molecule and are essential for fibril formation. The telopeptides are the most immunogenic regions of type I molecule.[49,50]

### CROSS LINKS:

The strength of the collagen fibre depends on the formation of covalent cross-links between the telopeptide and adjacent helical domains of collagen molecules. Type I collagen has four cross-linking sites: one in each telopeptide and two others at the sites in the triple-helical domain at residues 87 and 930.[78]

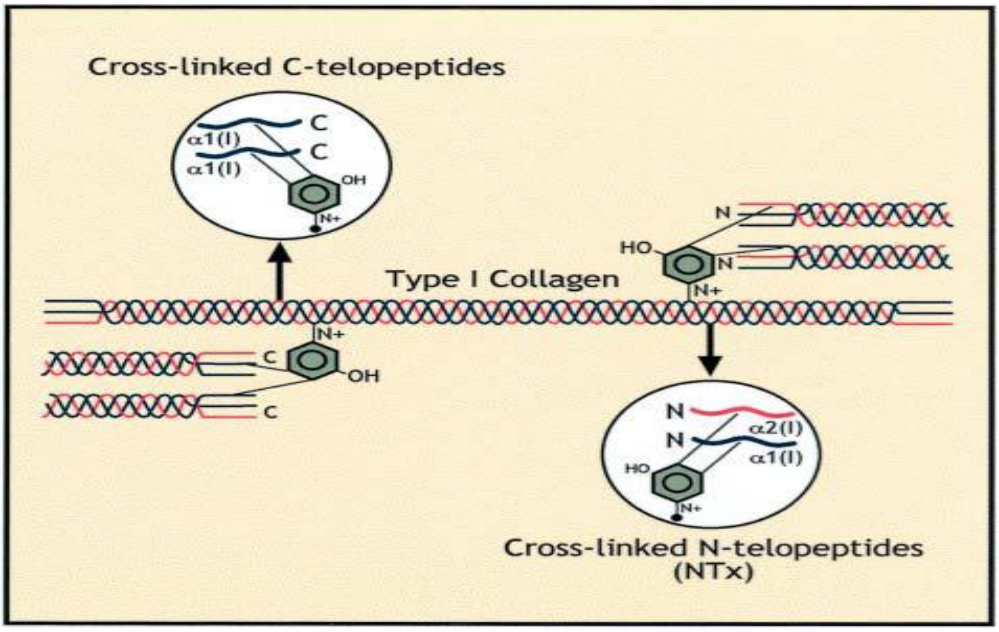



**Figure1. AMINO- AND CARBOXY-TERMINAL TELOPEPTIDES OF TYPE I COLLAGEN (Calvo et al 1996)**

The enzyme needed for forming cross links is lysyl oxidase. The divalent cross links are formed between the collagen telopeptide region and the helical region. These crosslinks can be reduced, hence termed as reducible crosslinks.[87]

The mature cross-linking residues are trivalent 3- hydroxypyridinium residues, lysyl pyridinoline (LP or Dpd or D-Pyr) and hydroxylysylpyridinoline (HP or Pyr). These trivalent cross-links connect together two telopeptide chains and one helical domain in a separate collagen molecule.[20]

An inborn defect with inhibited collagen cross-linking leads to severe diseases, e.g. Ehlers-Danlos syndrome, Marfan syndrome, Menke's disease or X-linked form of cutis laxa.[5]

## RESORPTION OF BONE:

Resorption of bone is accomplished by the multinucleated osteoclast. This cell attaches to mineralized surfaces of the bone and forms a unique resorption area. The ruffled border of the osteoclast is sealed from the cell – surrounding environment by the clear zone. One of the first steps in the resorption sequence is acidification of the ruffled border area. The low pH results in dissolution of the mineral, thus exposing the collagenous and noncollagenous bone matrix.[5,30]

A subsequent enzymatic attack of the matrix constituents leads to the final degradation of the bone. Two types of proteinases may participate at this level: the cysteine proteinases namely Cathepsin K, which acts preferably at acidic pH, and the matrix metalloproteinases (MMP), which acts at neutral pH.[35, 63]

### Cathepsin K

Cathepsin K, a cysteine protease is the major collagen degrading enzyme which is secreted by the osteoclasts. It belongs to a papain superfamily of cysteine proteinases and is highly cell and tissue specific, concentrating in the osteoclasts of the resorbing bone.[22] Cathepsin K is concentrated along the ruffled border of the osteoclasts facing the bone resorption lacunae, and also in vesicles, granules and vacuoles close to the resorption zone.[89] The other bone cells, such as osteoblasts, osteocytes and bone marrow cells, elicit no cathepsin K expression.[61,89] Cathepsins S, B and L are expressed at very low levels by the osteoclasts.[23]

Though cathepsin K is a highly bone-specific enzyme, the different bones contain variable amounts of cathepsin K activity. Long bones and vertebrae

where rapid bone remodeling occurs contain more cathepsin K than the bones with low resorption rate, such as calvariae.[31,38]

Cathepsin K is autoactivated at acidic pH and in sites with elevated temperatur.[7,56] The maximal enzyme activity at pH 5.5 (acidic).[7] Cathepsin K has unique property to cleave collagen both outside the helical region[35] and at multiple regions inside the helix.[9]

**Matrix metalloproteinases:**

MMPs are activated by tissue or plasma proteinases, opportunistic bacterial proteinases or by the uPA/plasmin system.[45,66] Several MMPs participate in collagen degradation and have been identified during bone resorption using different assays. These include the collagenases (MMP–1, - 2, -13, and – 14) and the gelatinases (MMP – 2, MMP – 9).[37]

The predominant MMPs in the pathogenesis of periodontitis are MMP 8 (collagenase 2), MMP 9 (gelatinase B) and MMP 13 (collagenase 3).[43] MMP 9 is highly expressed in the osteoclasts. In vitro experiments have detected maximal activity of MMP 9 at pH of 7.5.[36] MMP 8 and 13 are neutrophil and bone cell derived MMPs respectively. MMP 8 is a strong biomarker candidate for detecting alveolar bone destruction.[43]

## DEGRADATION PRODUCTS OF TYPE I COLLAGEN

Collagen degradation products include pyridinoline, deoxypyridinoline, amino-terminal peptides (N-terminal) and carboxy-terminal (C-terminal) telopeptides. The pyridinium cross-links, pyridinoline (Pyr) and deoxypyridinoline (Dpyr) are trifunctional cross-links that together with other di-, tri-, and tetrafunctional cross-

links stabilize the collagen structure within the extracellular matrix. Pyr is found mainly in bone and cartilage. Dpyr is present mainly in bone and dentin.[33]

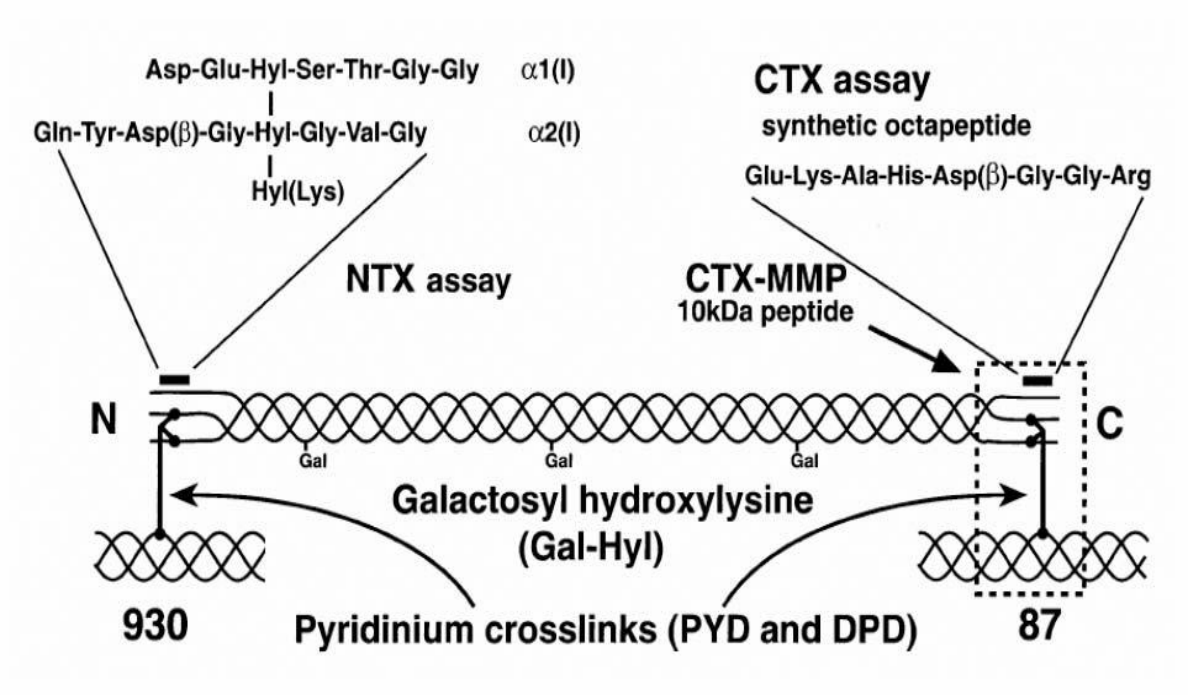



**Figure 2: An illustration of the breakdown products of type I collagen at N and C terminal end[62]**

The critical cysteine proteinase cathepsin K, which is selectively expressed by osteoclasts, is extremely efficient in type I collagen degradation.[36,39] Cathepsin K which is active at acidic pHdigests the organic matrix and releases carboxyterminal telopeptide of type I collagen degradation (CTX) – the eight amino acid sequence. As the pH increases, MMPs exert their activity and 10kDa peptide namely the cross -linked C- telopeptide of type I collagen (ICTP) is released.

Released ICTP can be further destroyed by cathepsin K[37] which can activate an osteoclastic enzyme, tartrate – resistant acid phosphatase type 5b (TRACP5b).[91]

TRACP 5b has been detected in ruffled border of an osteoclast membrane and has an inherent ability to further degrade partly degraded type I collagen products.[44]

**Methods to evaluate the degradation products of type I collagen[62]**

Hydroxypyridinium crosslinks

Pyridinoline (PYD) - HPLC, EIA

Deoxypyridinoline (DPD) - HPLC, EIA

Crosslinked telopeptide:

ICTP (CTX-MMP, carboxyterminal type I collagen telopeptide) - RIA

CTX / βCTX (Linear octapeptide derived from carboxyterminal type I collagen telopeptide) - ELISA

NTX (Aminoterminal crosslinked type I collagen telopeptide) - ELISA

## CARBOXYTERMINAL TELOPEPTIDE OF TYPE I COLLAGEN (CTX)

CTX is an octapeptide with an aminoacid sequence Glu-Lys-Ala-His-Asp-Gly-Gly-Arg. It is cleaved by the action of Cathepsin K enzyme on the crosslinking site of the alpha 1 chain of the type I collagen in carboxy-terminal telopeptide region.[86]

The CTX epitope contains an aspartyl-glycine motif (DG), which can undergo spontaneous isoaspartyl transformation. During the synthesis of new collagen, this motif in the native aspartyl form denoted as α CTX, gets spontaneously

converted to isoaspartyl form (denoted β CTX) during aging of the bone matrix. The isomerization reaction occurs spontaneously in bone under physiological conditions. Quantification of the relative amounts of the newly synthesized α CTX form compared with the age modified β CTX form (α/βratio) can detect the average age of the resorbed bone collagen.[15,16,20.21]

FORMATION OF CTX DURING BONE RESORPTION:

During bone resorption, owing to the low pH at the ruffled border area of the osteoclast, Cathepsin K is released from the osteoclast. Cathepsin K has the ability to generate CTX from insoluble human collagen.[37] The larger Cathepsin K generated fragments are not detected by the antibody designed to recognize an eight amino acid sequence from CTX, and that they become detectable on further fragmentation by MMPs. Once this C – terminal fragments are generated, pH increases and they become liable to the action of MMPs.[37]

Thus a contribution of MMPs to CTX immunoreactivity once initiated by cathepsin K is compatible with the model of osteoclast – mediated resorption proposed by Everts et al. Therefore, it is evident that CTX directly reflects the osteoclastic cathepsin K- dependent matrix degradation.[29]

**ESTIMATION OF CTX USING IMMUNOASSAYS**

**Bonde M *et al*** [64] in **1994** developed an Enzyme linked Immunosorbent Assay (ELISA) for quantifying type I collagen degradation products in urine. The peptide sequence used in this assay (Glu – Lys – Ala – His – Asp – Gly – Gly – Arg) was specific for the C – telopeptide α1 chain of type I collagen. The rationale behind using this sequence is:

- Important region for intermolecular crosslinks
- Proximity of the crosslinking residues
- Compact nature of these molecules protects these peptides from further renal degradation
- Medium obtained from osteoclasts cultivated from bone slices showed the same amino acid sequence
- C telopeptide fragments were reactive to ELISA

He concluded that collagen is the major constituent of bone, hence this peptide sequence is a potential marker for bone resorption.

**Bonde M *et al***[63] in **1997** developed an enzyme linked immunosorbent assay for measuring type I collagen degradation products in serum (S-ELISA) with an isomerised form of an eight amino acid sequence. He concluded that this assay is a sensitive and specific index of bone resorption and can be used to monitor patients with metabolic bone diseases. Fledelius C et al 1997[31] studied the urinary fragments from C-terminal telopeptide of type I collagen. He reported that Asp-Gly site present in type I collagen is prone for isomerisation and the degree of isomerisation increases with increasing age of bone.

Direct competitive immunoassays for the quantification of CTX in urine requires only one CTX chain to react. A lysine residue (K) within the CTX epitope participates in inter- and intramolecular cross-links, joining two CTX epitopes.

| Assay | CTX Fragments Measured |
|---|---|
| αCTX RIA | αCTX, αCTX-αCTX, αCTX-βCTX |
| Urine CTX | βCTX, βCTX-αCTX, βCTX-βCTX |
| Serum CTX | βCTX-βCTX |
| ααCTX ELISA | αCTX-αCTX |

Rosenqvist *et al*. 1998[80] developed a new assay (Serum CrossLaps One Step ELISA) for the determination of serum concentrations of the β-isomerized C-telopeptide of type I collagen. This assay is performed as a monoclonal sandwich assay in a one-step procedure and is based on a monoclonal antibody that exclusively recognizes the isomerized β-aspartate (D) form of the EKAHβDGGR epitope of the α1-chain of human type I collagen.[32]

The above kit was clinically tested by Christgau et al 1998[14] for monitoring anti-resorptive treatment in osteoporosis treatment. He reported that the Serum one step ELISA was equivalent to the urine test that is commonly performed.

Therefore it was confirmed that CTX can be determined in the serum by means of a sandwich assay employing two βCTX specific antibodies, and only molecules containing at least two β CTX chains (β-βCTX) are measured indicating mature (or "aged") bone collagen. In contrast, α-αCTX can be measured by the recently developed ELISA, which is believed to reflect resorption of newly synthesized bone collagen.[16,17]

**ICTP as an index of MMP – DRIVEN COLLAGENOLYSIS:**

Cross linked carboxyterminal telopeptide of type I collagen (ICTP) is generated by the action of MMPs. It requires a trivalent crosslink, including two phenylalanine-rich domains of the telopeptide region of the alpha – 1 chain of type I collagen.[77]

MMPs (MMP 2, 9, 13, 14) generate ICTP from insoluble collagen. ICTP is degraded by cathepsin K. MMPs from peri-osteoclastic cells compensate for inactive cathepsin K by degrading collagen that was demineralised by the osteoclast, and thereby generate ICTP.[37]

**CLINICAL SIGNIFICANCE OF CTX AND ICTP**

Lamster *et al* in 1994[39] concluded in his study that the actual detection of connective tissue-derived molecules may lead to a more accurate assessment of tissue breakdown, because of the tremendous variability of the host response in different individuals. The most immunogenic parts of the type I collagen are the telopeptides and there are several immunoassays for their specific measurement.[49,50]

CTX is considered as the most specific and sensitive under normal remodeling conditions.[79,86] Serum and urinary CTX levels have been reported to be efficient markers of bone resorption in many metabolic bone diseases, characterized by increased osteoclastic resorptive activity, including osteoporosis, Paget's disease.[37]

Serum ICTP is considered as a sensitive marker to detect osteolysis related to bone metastasis from breast, prostate cancers, or multiple myeloma. ICTP has been proven to be a poor marker in certain situations, especially osteoporosis,

where serum ICTP levels are not increased and do not respond to short – term antiresorptive therapy, including bisphosphonates.[37]

Earlier GCF and salivary ICTP levels were considered to predict future alveolar bone loss in periodontitis[85] and marker of advanced periodontitis[42]. Recently Gursoy et al reported that ICTP is a sensitive marker of neoplastic changes of bone and is less sensitive to osteoclasts – mediated degradation which occurs in periodontitis.[42]

FACTORS RESPONSIBLE FOR VARIABILITY AND FLUCTUATIONS IN BONE MARKERS[62,67]

1. Diurnal rhythm - Bone marker levels are usually high in early morning hours and low in the afternoon and evenings. Most pronounced diurnal changes have been reported for CTX.
2. Diet - Serum CTX values are affected by food intake. Therefore samples are collected in fasting state
3. Liver and renal diseases - CTX concentrations are affected in patients with liver and renal diseases.
4. Puberty, Menopause and Pregnancy – Marker concentrations increase in puberty and menopause; decrease in pregnancy.
5. Age: Marker levels are increased in men aged 20-40 yrs and decreased above 50 yrs.
6. Bed rest, exercise and seasonal changes also causes fluctuations in marker levels.

## SALIVA – AS A DIAGNOSTIC MEDIUM

The determination of markers of active disease (diagnostic tests) or prediction of disease (prognostic tests) using a non invasive diagnostic tool can provide accurate information of periodontal disease status.[60]

Saliva is a most valuable oral fluid which is used as a non – invasive diagnostic medium for various medical diagnosis and research procedures. The water content of saliva is approximately 99.5%. The pH of saliva is 5.6 – 7.6. Saliva varies greatly in different individuals and in the same individual under different circumstances.[25]

Whole saliva consists of a mixture of oral fluids, and includes secretions of major and minor salivary glands, constituents of non – salivary origin (GCF), expectorated bronchial secretions, serum and blood cells from oral wounds, as well as bacteria and bacterial products, viruses and fungi, desquamated epithelial cells and food debris.[60]

Saliva has been evaluated as a diagnostic fluid for detecting caries risk[9], periodontitis[15], oral cancer[59], breast cancer[84], salivary gland diseases[46], and systemic disorders such as hepatitis and the presence of human immune-deficiency virus[67] or Hepatitis C virus.[27]

Salivary biomarkers can be categorized as proteomic markers (locally produced proteins of host and bacterial origin), genetic /genomic biomarkers such as DNA and mRNA of host origin, microbial markers (bacteria and bacterial products), steroid hormones and volatile compounds.[51] Degradation products of type I collagen comes under proteomic markers.[60]

**REVIEW OF STUDIES ON SERUM and URINARY CTX:**

**Kushida K *et al*[55]** in **1995** compared marker of bone formation and resorption in post menopausal women and concluded that resorption markers (ALP, BGP, PYD, DPYD, CTX) increased in post menopausal women than formation markers.

**Garnero P *et al*[34]** in **1995** studied the different effects of bisphosphonate and estrogen therapy on free and peptide bound bone cross-links excretion and concluded that bisphosphonate therapy decreased markedly the cross-linked peptides without significant change in free cross-link excretion contrasting with a decrease of both free and peptide-bound cross-links after estrogen therapy.

**Bjarnson *et al*[6]** in **2000** conducted study to evaluate the early response in biochemical markers to predict long term response in bone mass during bone replacement therapy in early postmenopausal women. He reported that serum and urinary CTX after 2 weeks of therapy significantly correlated to 3 year bone mass response.

**Rosen *et al*** [79] in **2000** in his study determined the utility of serum CTX and other markers in monitoring the efficacy of antiresorptive treatment. He concluded that serum CTX assay shows greater utility for assessing anti-resorptive treatment.

**Hosjing DJ *et al*[37]** in **2006** conducted study on biochemical assessment of Paget's disease in bone. He reported that in untreated Paget's disease, the αCTX is raised proportionately more (16-fold) than βCTX (3-fold) and decreases in response to bisphosphonate therapy to a greater extent than βCTX (measured in the sCTX assay).

**Marx R E *et al***[65] in **2007** in his study reported that morning fasting serum CTX marker is a useful tool to assess the risk and time course of oral bisphosphonate induced osteonecrosis of the jaw.

**Garnero *et al***[38] in **2008** in his study reported that measuring α/β ratio if type I collagen helps in predicting fracture risk in post menopausal women.

**Sonia Talwar *et al***[83] in **2011** studied the bone turnover marker in osteoporosis. She reported that CTX responds remarkably to antiresorptive therapies. Serum ICTP is insensitive to normal metabolic bone processes, such as osteoporosis, but serum ICTP may be a marker of bone degradation in pathological conditions (eg, bone metastasis, rheumatoid arthritis).

**SALIVARY STUDIES ON CTX:**

**Pelligrini *et al***[71] in **2006** correlated between CTX and bone alkaline phosphatase in serum and saliva of rats. He concluded that salivary and serum CTX and b-AP correlated in both, normal conditions and in states of increased bone remodelling; the potential use of salivary markers for bone remodelling showed promising results.

**Pelligrini *et al***[72] *in* **2008** conducted study on osteopenic rats to determine the correlation between serum and salivary markers on bone turnover. He concluded saliva may be one of the best candidate markers to determine the activity and severity of periodontal disease.

**Pelligrini *et al***[73] in **2012** determined the salivary bone turnover marker in healthy and pre- and post menopausal women on daily and circadian rhythm. His results concluded that as in serum and urinary samples, salivary CTX exhibits daily and a slight seasonal rhythmicity. Whole non-stimulated saliva is a useful and promising tool to test changes in bone metabolism contributing to diagnose and to monitor the therapy of several metabolic bone diseases.

**Al Sabbagh M et al** in **2012**[2] examined the leves of salivary biomarkers associated with biological aspects of bone remodeling in subjects with chronic periodontitis. The markers analysed in the study are macrophage inflammatory protein 1 – α (MIP - 1α), osteoprotegerin, C-terminal pyridinoline crosslinks of type I collagen (ICTP) and β – C terminal type I collagen telopeptide (βCTX). Amongst the markers anlysed, he concluded that MIP1 – α levels were significantly increased in chronic periodontitis subjects as compared to healthy controls. βCTX levels were at or below the detection limit (0.80μg/ml) in all samples. Hence βCTX was neither compared nor correlated with the clinical parameters.

**Gursoy *et al*** in **2012**[42] studied salivary degradation products of type I collagen and matrix metalloproteinases to detect potential markers of periodontitis with high sensitivity and specificity. He demonstrated that salivary CTX differed in patients with and without periodontitis. He concluded that MMP8 is a strong biomarker candidate for detecting alveolar bone destruction.

# MATERIALS AND METHODS

STUDY DESIGN AND SUBJECT SELECTION:

The study was approved by the Institutional Ethical Committee. A total of 45 subjects were included in the study, from the out-patient ward of the Department of Periodontics, Tamil Nadu Government Dental College and Hospital, Chennai – 600 003. Subjects were chosen based on the following inclusion and exclusion criteria.

INCLUSION CRITERIA:

1. Subjects willing for voluntary participation and signing the informed consent
2. Systemically healthy subjects
3. Age: 30 – 40 years
4. Gender: Both males and females
5. Patients with generalized chronic periodontitis with minimum of 20 teeth

EXCLUSION CRITERIA:

1. History of any systemic illness
2. History of systemic antibiotics or anti-inflammatory drugs during past 6 months
3. Pregnancy, lactation and menopause[62,69]
4. Patients who smoke or use tobacco in any form
5. 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)

CONTROL GROUP:

20 subjects exhibiting the following criteria[4,5] were included:

- Healthy controls with minimum of 20 teeth
- < 20% sites with bleeding on probing
- No sites with probing depth more than 3 mm
- No evidence of radiographic bone loss
- Good oral hygiene status (Plaque Index score less than 1)

STUDY GROUP

25 subjects exhibiting the following criteria[4,5] were included:

- Chronic periodontitis with minimum of 20 teeth
- Presence of bleeding on probing
- >30% sites with Probing depth and Clinical Attachment Level (CAL) ≥5mm
- Radiographic evidence of bone loss
- Poor oral hygiene status ( plaque index score of 2.0 to 3.0)

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

**STUDY PROTOCOL**

1. Medical History and Informed Consent
2. Complete Periodontal Examination using clinical parameters namely Gingival Bleeding Index, Plaque Index, Probing Pocket Depth (PPD) and Clinical Attachment Level (CAL)
3. Orthopantamogram (OPG) for radiographic evaluation of generalized chronic periodontitis (Group II)
4. Bitewing radiographs (posterior) for Group II subjects.
5. Collection of saliva samples.
6. Oral hygiene instructions were given for group II subjects.
7. Phase I therapy performed for Group II patients
8. Re-evaluation of Group II patients using clinical parameters namely, Gingival Bleeding Index, Probing Pocket Depth and Clinical Attachment Level.
9. Collection of saliva samples in Group II patients 1 month after Phase I therapy
10. Estimation of CTX in saliva samples by ELISA method.

**CLINICAL PARAMETERS:**

**GINGIVAL BLEEDING INDEX (Ainamo and Bay 1975)**[1]

Teeth examined - All teeth except third molars

Surfaces examined - 6 sites per tooth (Mesiobuccal, Midbuccal, Distobuccal, Mesiolingual,, Midlingual and Distolingual)

The presence or absence of bleeding is determined by gentle probing of the gingival crevice with a periodontal probe.

CRITERIA FOR SCORING

Positive score (+) - Presence of bleeding within 10 seconds

Negative score (-) - Absence of bleeding

$$\% \text{ of bleeding sites} = \frac{\text{Total number of positive score}}{\text{Total number of surfaces of all teeth}} \times 100$$

**PLAQUE INDEX (Silness and Loe 1964)**[81]

Teeth examined – All teeth

Surfaces examined – 4 sites per tooth (disto-facial, facial, mesio-facial, lingual /palatal).

Criteria for Scoring:

Score 0 – No plaque

Score 1 – Film of plaque adhering to the tooth surface seen only by running a probe along the tooth surface

Score 2 – Moderate accumulation of soft deposits within the gingival pocket; can be seen by naked eye

Score 3 – Abundance of soft matter within the gingival pocket / margin / adjacent tooth

Calculation:

Plaque index for the tooth = total score from 4 areas/4

Plaque index for the individual = Total Plaque indices for all teeth / No. of teeth examined.

Interpretation: Score 0 – Excellent oral hygiene

0.1 to 0.9 – Good oral hygiene

1.0 to 1.9 – Fair oral hygiene

2.0 to 3.0 - Poor oral hygiene

**PROBING POCKET DEPTH (PPD in mm) (Grant 1965)[41] (Carranza 10th edn)[12]**

Probing Pocket Depth was measured from the gingival margin to the base of the pocket using William's Periodontal Probe. The probe was walked within the gingival sulcus along the circumference of the tooth. Six measurements were made per tooth (Mesiobuccal, Midbuccal, Distobuccal, Mesiolingual, Midlingual, Distolingual).

Calculation:

$$\text{PPD per tooth} = \frac{\text{Sum of all scores per tooth}}{6}$$

$$\text{Mean PPD per person} = \frac{\text{Sum of each tooth score}}{\text{Total number of teeth examined}}$$

**CLINICAL ATTACHMENT LEVEL (CAL in mm) (Carranza 10th edn)**[12]

- 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 – total of six sites per tooth (Mesiobuccal, Midbuccal, Distobuccal, Mesiolingual, Midlingual, and Distolingual).

- If 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 were added to the PPD.

**Calculation:**

$$\text{CAL per tooth} = \frac{\text{Sum of all scores per tooth}}{6}$$

$$\text{Mean CAL per person} = \frac{\text{Sum of each tooth score}}{\text{Total number of teeth examined}}$$

Subjects were selected randomly, with no discrimination in age and sex between both the groups. Study protocol was explained and written informed consent (Annexure 2, 3) was obtained from all subjects. Thorough medical and dental history was obtained (Annexure 4). 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 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.

**ARMAMENTARIUM**

CLINICAL EXAMINATION

Mouth Mirror

Williams Periodontal Probe

Explorer

Tweezer

Cotton balls

Kidney tray

Disposable Head Cap and Face mask

Surgical Gloves

**COLLECTION OF SALIVA SAMPLE**

Vaccutainer tubes

Sterile Cotton

**SAMPLE STORAGE**

-20°C Freezer

**FOR PHASE I THERAPY**

Mouth Mirror

Explorer

Scalers and Curette

Kidney Tray with cotton Rolls

Disposable facemask and headcap

Surgical Gloves

Disposable syringe with 23 gauge needle

Local Anesthetic solution

Aspirating Needle

0.9% Normal Saline

**ELISA PROCEDURE**

Centrifuge Machine

Plastic rack

Autoclaved plastic pipette tips

Micropipette

ELISA washer and reader

**SALIVA SAMPLE COLLECTION**

Unstimulated whole saliva samples were obtained from Group I (control group) at baseline and from Group II (study group) subjects before and after Phase I therapy. The subjects were advised to report in the morning following an overnight fast[61]. Samples were collected prior to clinical examination or any periodontal intervention. 2ml of saliva was collected in a vaccutainer immediately after single mouth rinse with water and stored in -20°C freezer.

**ELISA PROCEDURE**

Before testing, the collected samples were thawed to room temperature and centrifuged. The supernatant was used for the test. In this study, ELISA kit for Cross linked C-Telopeptide of Type I collagen (CTX 1), manufactured by USCN Life Science Inc., China was used.

**MATERIALS PROVIDED IN THE KIT**

Pre-coated, ready to use 96-well strip plate

Standard (Lyophilised)

Detection Reagent A (green)

Detection Reagent B (red)

TMB Substrate

Wash Buffer (30 x concentrate)

Plate sealer for 96 wells

Standard diluent

Assay Diluent A (2 x concentrate)

Assay Diluent B (2 x concentrate)

Stop solution

**REAGENT PREPARATION**

1. All kit components were brought to room temperature (18-25°C) before use.

2. Standard - The Standard was reconstituted with 0.5 mL of Standard Diluent, kept for 10 minutes at room temperature. The concentration of the standard in the stock solution is 10000pg/mL.

3. 5 tubes containing 0.6mL Standard Diluent were prepared and a triple dilution series was done.

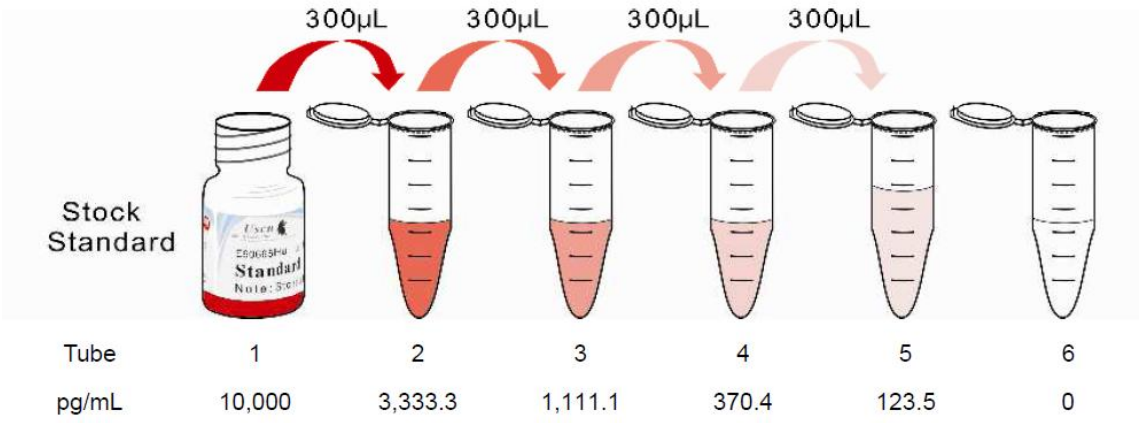



**Figure 3: Triple dilution series for Standard diluents**

4. Each tube was mixed thoroughly before the next transfer. 5 points of diluted standard such as 10,000pg/ml, 3,333pg/ml, 1,111pg/ml, 370.4pg/ml, 123.5pg/ml was set and the last EP tubes with Standard Diluent was considered blank as 0pg/mL.

5. Assay Diluent A and Assay Diluent B - 6mL of Assay Diluent A or B Concentrate(2×) was diluted with 6mL of deionized or distilled water to prepare 12 mL of Assay Diluent A or B.

6. Detection Reagent A and Detection Reagent B -The working concentration was diluted with working Assay Diluent A or B, respectively (1:100).

7. Wash Solution - 20mL of Wash Solution concentrate (30×) was diluted with 580mL of deionized or distilled water to prepare 600 mL of Wash Solution (1×).

8. TMB substrate - The needed dosage of the solution was aspirated with sterilized tips.

ASSAY PROCEDURE

1. The wells for diluted standard, blank and sample were determined- 5 wells for standard, 1 well for blank.
2. 50µL each of dilutions of standard, blank and samples were added into the appropriate wells.
3. 50µL of Detection Reagent A working solution was added to each well. The plate was sealed with plate sealer and incubated at 37°C.
4. The solution was aspirated and washed with 350µL of 1× Wash Solution using an autowasher. The remaining liquid from all wells was removed completely by snapping the plate onto absorbent paper. The process was repeated 3 times.
5. 100µL of Detection Reagent B working solution was added to each well. The plate was covered with plate sealer and incubated for 30 minutes at 37°C
6. The aspiration/wash process was repeated for five times.

7. 90µL of Substrate Solution was added to each well. Covered with plate sealer and incubated for 15 - 25 minutes at 37°C. The liquid turned blue after the addition of Substrate Solution.
8. 50µL of Stop Solution was added to each well. The liquid turned yellow by the addition of Stop solution.
9. The plate was placed on the microplate reader and measured at 450nm immediately.

CALCULATION OF RESULTS

This assay employs the competitive inhibition enzyme immunoassay technique, so there is an inverse correlation between CTX I concentration in the sample and the assay signal intensity. Average was calculated for the duplicate readings for each standard, control and samples. In order to make the calculation easier, standard curve was drawn with optical density value on the Y-axis and concentration of the standard on the X-axis.

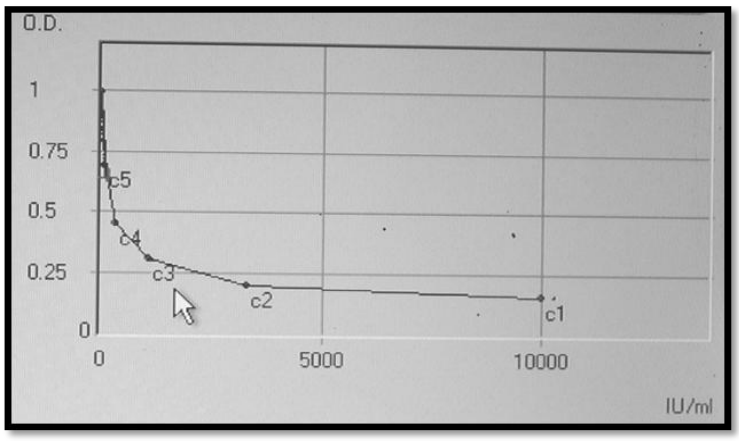
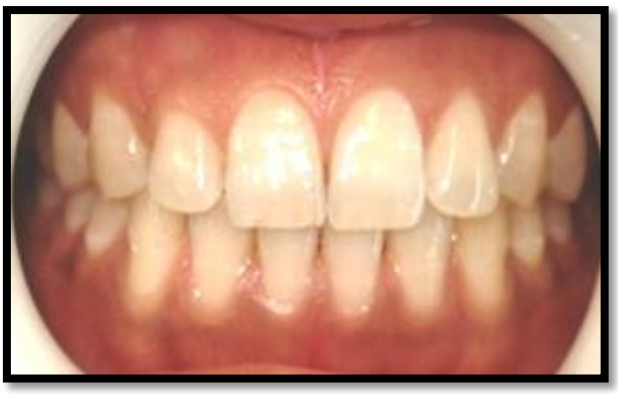
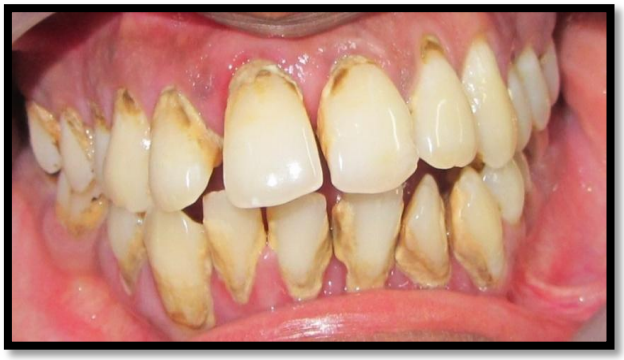


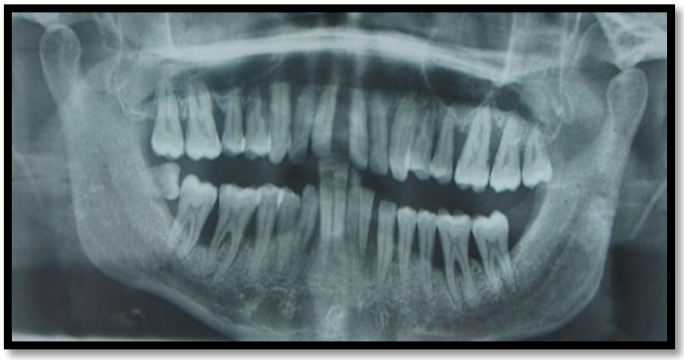

**Figure 4: Standard Curve for CTX**



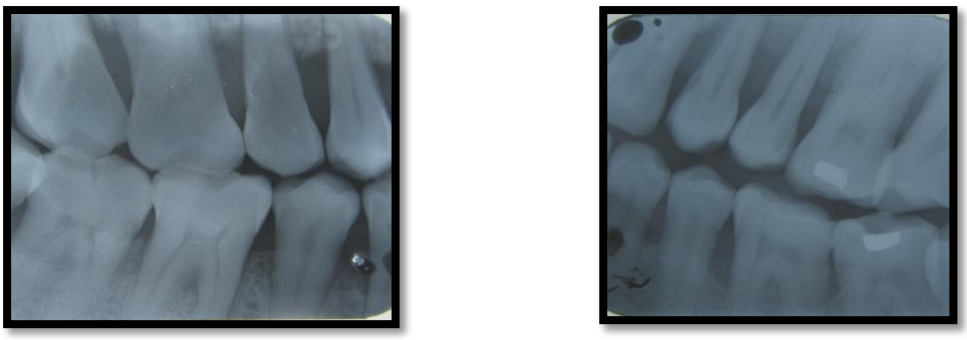
**Photograph 1: Control group**



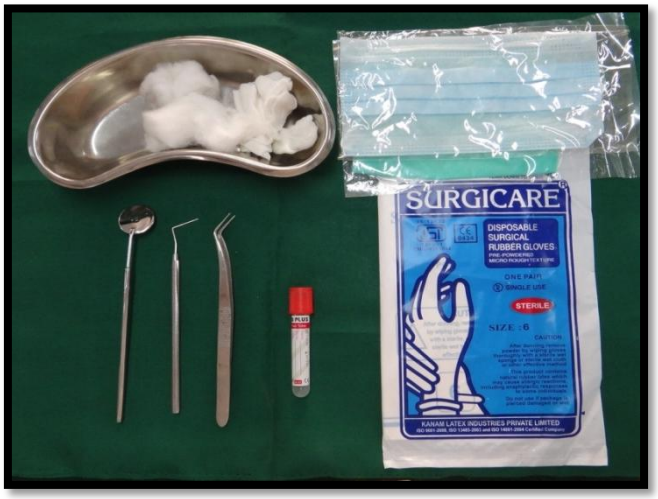
**Photograph 2: Generalised Chronic Periodontitis (study group)**



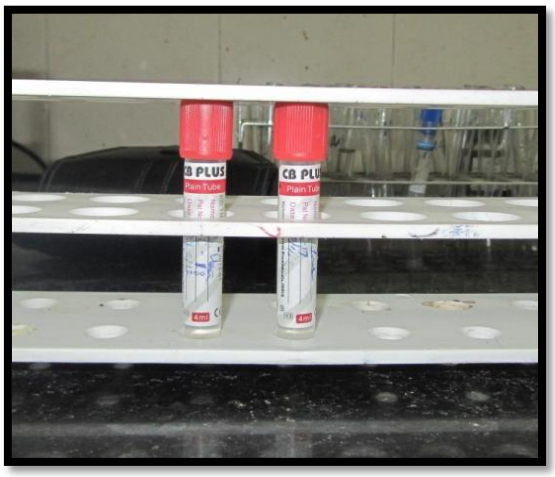
**Photograph 3: Orthopantamogram of study group**



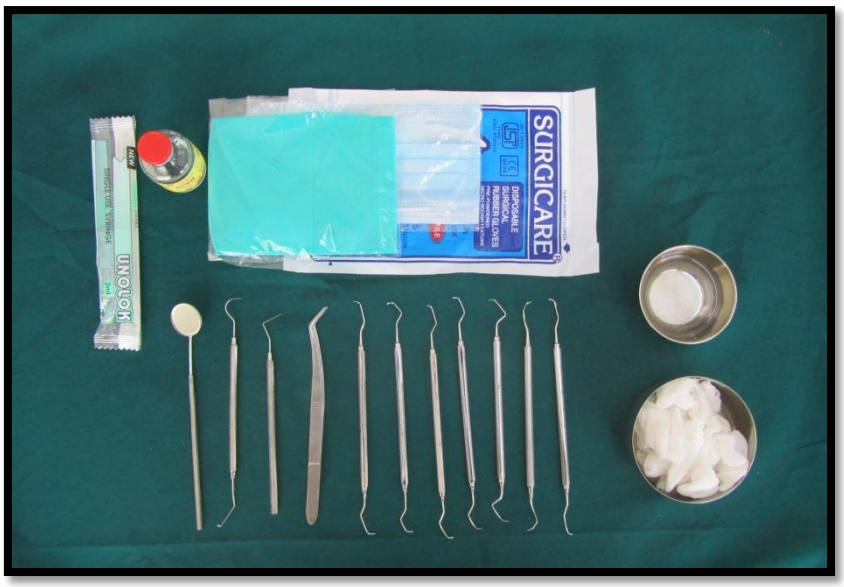
**Photograph 4: Posterior horizontal bitewing radiograph of study group**



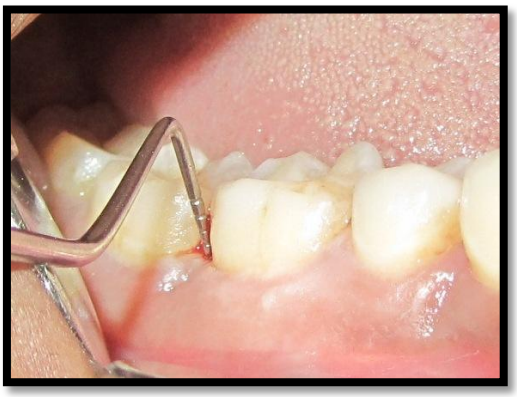

**Photograph 5: Armamentarium for periodontal examination**



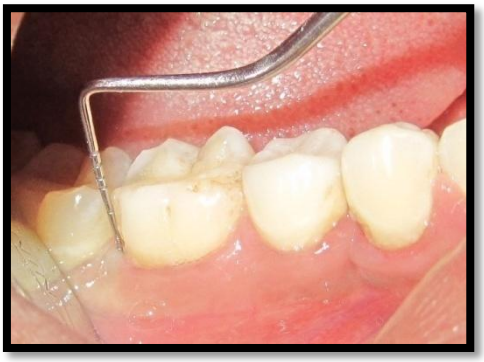
**Photograph 6: Collected saliva samples in vaccutainer**




**Photograph 7: Armamentarium for Phase I therapy**



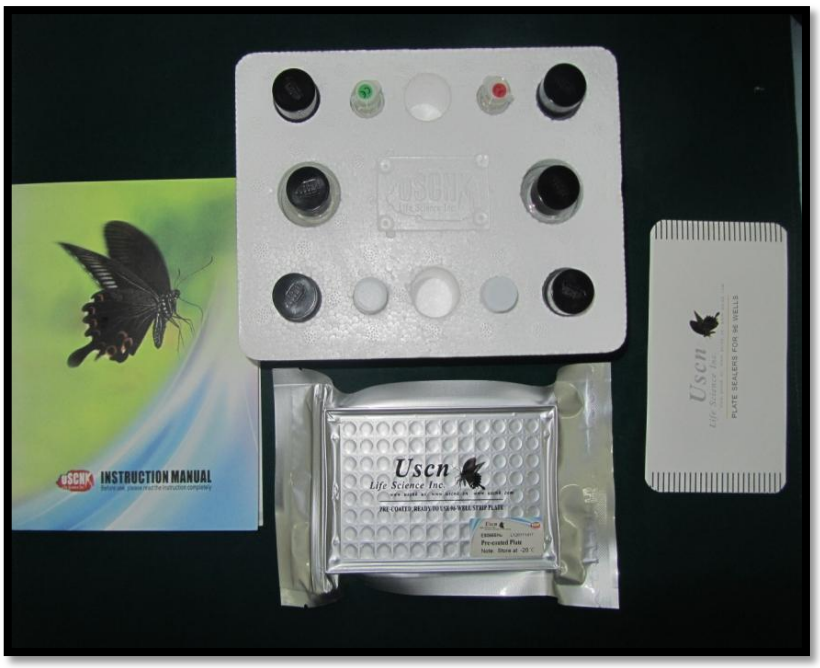
**Photograph 8: Probing depth measured using Williams periodontal probe before phase I therapy**



**Photograph 9: Probing depth measured using Williams periodontal probe after phase I therapy**

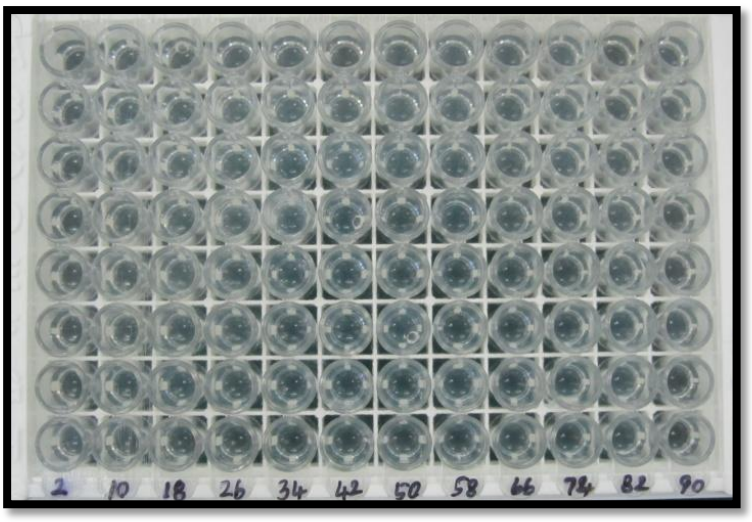
**Photograph 10: Centrifuge Machine**

**Photograph 11: Deep Freezer and samples stored at -20°C**





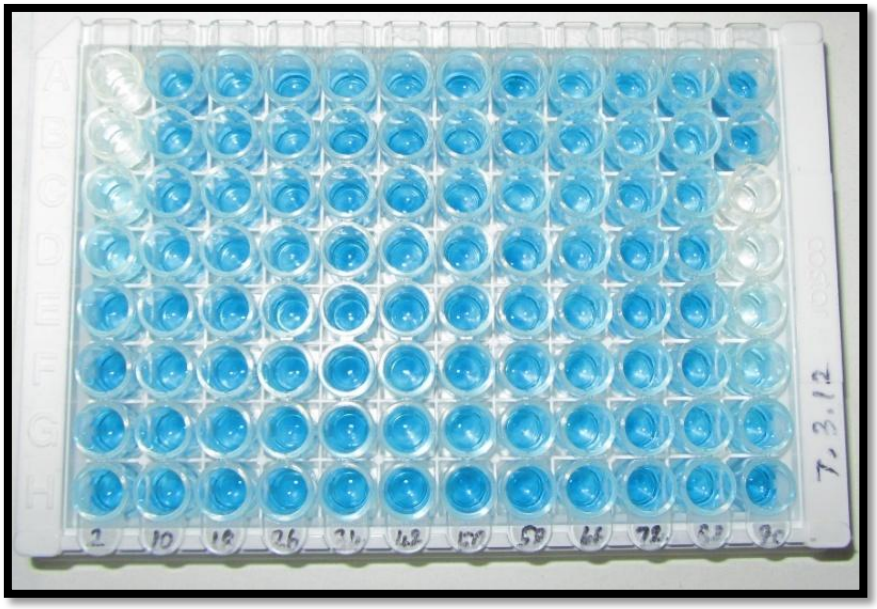

**Photograph 12 : ELISA kit contents**



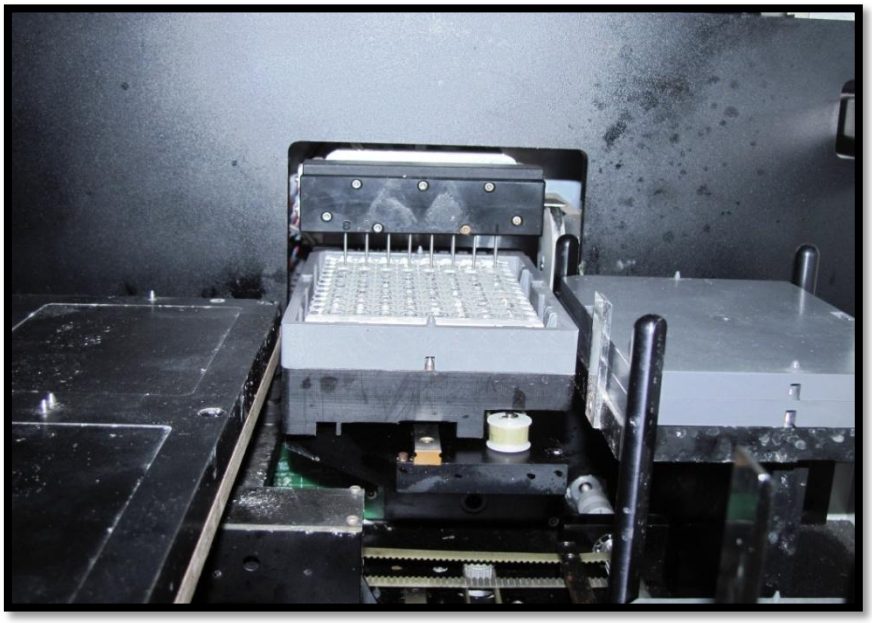

**Photograph 13: 96 microplate of ELISA kit**

**Photograph 14: Micropipettes**

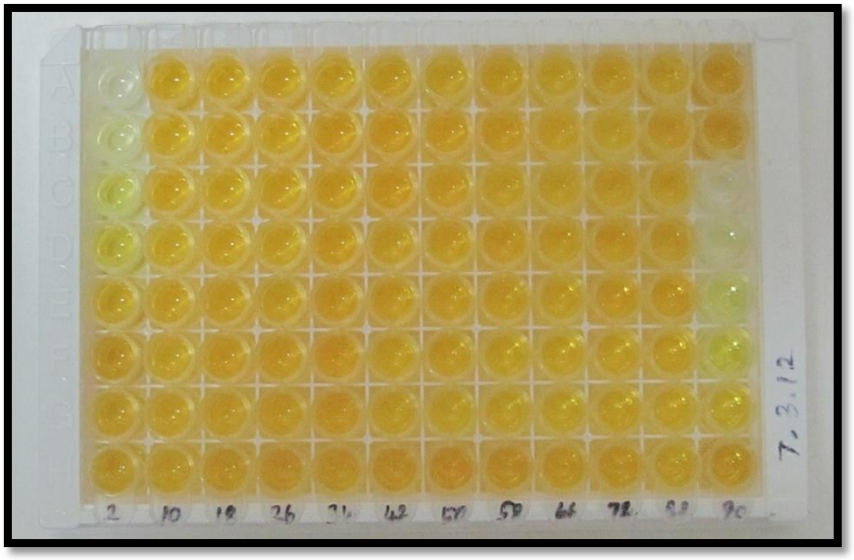

**Photograph 15: ELISA reader and washer**



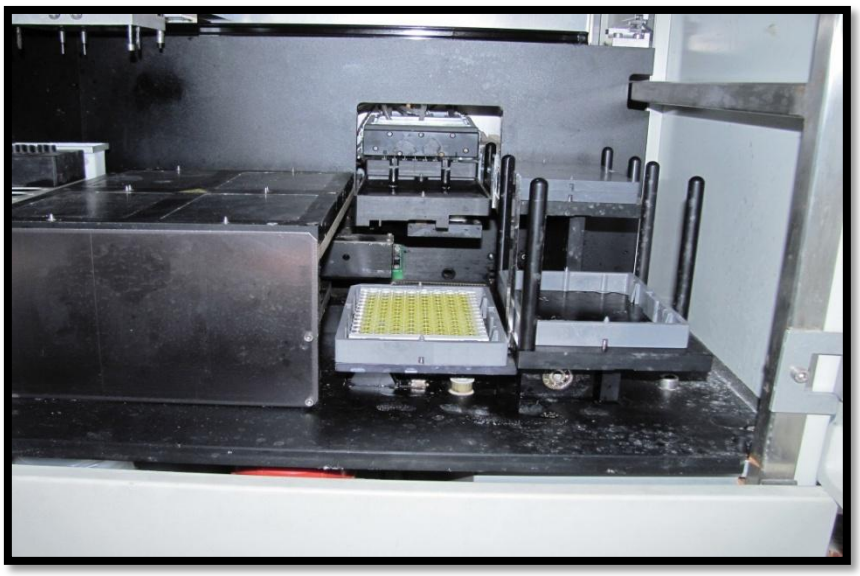
**Photograph 16: 96 well microplate after adding substrate solution**



**Photograph 17: 96 well microplate on autowasher**




**Photograph 18: 96 well microplate with conjugate solution**



**Photograph 19: 96 well microplate on ELISA reader**

## 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 co-efficient** was used to analyse 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**.**

## STATISTICAL FORMULAE USED FOR DATA ANALYSIS

### Student's independent t-test

The independent t-test was used to compare the statistical significance of a possible difference between the means of two groups on some independent variable and the two groups were independent of one another.

The formula for the independent t-test was

$$t = \frac{X_1 - X_2}{\sqrt{\left(\frac{SS_1 + SS_2}{n_1 + n_2 - 2}\right)\left(\frac{1}{n_1} + \frac{1}{n_2}\right)}},$$

Where,

$X_1$ is the mean for group 1,

$X_2$ is the mean for group 2,

$SS_1$ is the sum of squares for group 1,

$SS_2$ is the sum of squares for group 2,

$\mathbf{n_1}$ is the number of subjects in group 1, and

$\mathbf{n_2}$ is the number of subjects in group 2.

The t-value found was the difference between the two means divided by their sum of squares and taking the degrees of freedom into consideration.

$$SS_1 = \sum X_1^2 - \frac{\left(\sum X_1\right)^2}{n_1} \quad \text{and} \quad SS_2 = \sum X_2^2 - \frac{\left(\sum X_2\right)^2}{n_2}$$

The degree of freedom for the independent t-test used was:

$$df = n_1 + n_2 - 2$$

**Paired t - test:**

A paired t-test is used to compare two population means where you have two samples in which observations in one sample can be paired with observations in the other sample.

$$t = \frac{\Sigma d}{\sqrt{\frac{n\left(\Sigma d^2\right) - \left(\Sigma d\right)^2}{n-1}}}$$

The top of the formula is the sum of the differences (i.e. the sum of *d*). The bottom of the formula reads as:

The square root of the following: n times the sum of the squared differences minus the sum of the differences squared, all over n-1.

- The sum of the squared differences: $\sum d^2$ means take each difference in turn, square it, and add up all those squared numbers.
- The sum of the differences squared: $(\sum d)^2$ means add up all the differences and square the result.

**PEARSON'S CHI-SQUARE TEST**

Chi-square is a statistical test commonly used to compare observed data with data we would expect to obtain according to a specific hypothesis.

The formula used was

$$\chi^2 = \sum \frac{(\text{Observed Value} - \text{Expected Value})^2}{(\text{Expected Value})}$$

**P – VALUE**

The p-value measures consistency between the results actually obtained in the trial and the pure chance explanation for those results. In statistical hypothesis

testing, the ***p*-value** is the probability of obtaining a test statistic at least as extreme as the one that was actually observed, assuming that the null hypothesis is true.

| | |
|---|---|
| **0.05 < P < 0.10** | Weak evidence against the null hypothesis in favor of the alternative |
| **0.01 < P < 0.05** | Moderate evidence against the null hypothesis in favor of the alternative. |
| **P < 0.01** | Strong evidence against the null hypothesis in favor of the alternative. |
| **P < 0.001** | Very strong evidence against the null hypothesis in favor of the alternative |

In this study differences between the two populations were considered significant when $p < 0.05$.

## PEARSON'S CORRELATION CO-EFFICIENT

Pearson's correlation coefficient is the method of measuring the correlation.

**Degree of correlation:**

1. **Perfect:** If the value is near ± 1, then it is said to be a perfect correlation.
2. **High degree:** If the value lies between ± 0.75 and ± 1, then it is said to be a high degree of correlation.
3. **Moderate degree:** If the value lies between ± 0.25 and ± 0.75, then it is said to be moderate degree of correlation.
4. **Low degree:** When the value lies between 0 and ± 0.25, then it is said to be a low degree of correlation.
5. **No correlation:** When the value is zero

## RESULTS

The present study is an interventional study with a total of 45 subjects. The subjects were divided into 2 groups:

Group I – control group (n=20)

Group II – study group (n=25)

Samples were collected from Group I and II at baseline and from Group II after phase I therapy. Hence Group II was further categorized into

Group II A – before phase I therapy

Group II B – after phase I therapy

The statistical analysis was performed and results were tabulated.

***Table 1 and 2*** shows the master chart of the Group I and Group II with the clinical parameters and salivary CTX level.

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***Table 3 and Figure 5*** shows the comparison of age between Group I and Group II. The mean age in the Group I was 36.05 years and 35.08 years in the Group II respectively. There was no statistically significant difference in the distribution of age in both the groups.

***Table 4 and Figure 6*** 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% Group II. There was no statistically significant difference in distribution of the gender between the groups.

**Plaque Index (PI)**

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**). 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) ***(Table 5, 11, 12 and Figure 7, 14, 17)***

**Gingival Bleeding Index (GBI)**

The gingival bleeding index was significantly increased in the Group IIA (96.22±2.54) as compared to the control group (15.57 ± 6.06) (***Table 5 and Figure 8)*** with p value < 0.01**, which reduced to 21.79 ± 7.25 following phase I periodontal therapy with a p <0.01** ***(Table 7 and Figure 8).*** The gingival bleeding index showed positive correlation with salivary CTX in group I, IIA and IIB but the correlation was not significant. (p>0.05) ***(Table 11, 12, 13 and Figure 15, 18, 21)***

***Probing Pocket Depth (PPD)***

The PPD was significantly increased in the study group (6.27 ± 0.93mm) as compared to control group (2.84 ± 0.53mm) with p value <0.01** ***(Table 5 and Figure 9)***. The probing depth reduced considerably following treatment to 3.07 ± 0.68 mm (p value <0.01**) ***(Table 7 and Figure 9),*** but not to the level of the control group (p value <0.01**) ***(Table9 and Figure 9).*** Linear correlation was observed between probing depth and salivary CTX levels in all the groups but the correlation was not significant***.(Table 11, 12, 13 and Figure 16, 19, 22)***

**Clinical Attachment Level (CAL)**

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** ***(Table 7 and Figure 10).*** Since the loss in the clinical attachment level in control group was absent the comparisons with that group was highly significant. Positive correlation was observed between CAL and salivary CTX but the correlation was not significant.***(Table 11, 12, 13 and figure 20, 23)***

**Salivary CTX level**

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 high statistical significant difference in salivary CTX levels between the study and the control group**.** ***(Table 6 and Figure 1)***

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**.** ***(Table 8 and Figure 12)***

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. ***(Table 10 and Figure 13)***

## TABLE 1 – MASTER CHART – GROUP I (CONTROL GROUP)

| S.No | AGE (yrs) | SEX | PI | GBI (%) | PPD (mm) | CAL (mm) | CTX (pg/ml) |
|---|---|---|---|---|---|---|---|
| C1 | 31 | M | 0.27 | 21.5 | 2.9 | 0 | 30.1 |
| C2 | 33 | M | 0.14 | 10.34 | 2.3 | 0 | 41.7 |
| C3 | 32 | F | 0.36 | 14 | 2.14 | 0 | 39.4 |
| C4 | 35 | M | 0.52 | 17.5 | 3.02 | 0 | 41.2 |
| C5 | 37 | F | 0.87 | 8.12 | 2.68 | 0 | 38.4 |
| C6 | 32 | F | 0.11 | 20.17 | 2.44 | 0 | 33.9 |
| C7 | 36 | M | 0.09 | 6.98 | 2.33 | 0 | 42.2 |
| C8 | 35 | F | 0.02 | 13.72 | 2.11 | 0 | 41.4 |
| C9 | 31 | F | 0.33 | 21.67 | 2.24 | 0 | 29.5 |
| C10 | 30 | M | 0.24 | 11 | 3.12 | 0 | 31.1 |
| C11 | 31 | F | 0.07 | 24.33 | 3.77 | 0 | 37.6 |
| C12 | 34 | F | 0.04 | 17.34 | 3.03 | 0 | 45.9 |
| C13 | 37 | M | 0.23 | 7.02 | 2.87 | 0 | 44.3 |
| C14 | 38 | M | 0.17 | 13.72 | 3.11 | 0 | 47.8 |
| C15 | 31 | M | 0.31 | 5.2 | 3.86 | 0 | 32.5 |
| C16 | 31 | F | 0.42 | 16.79 | 3.13 | 0 | 34.8 |
| C17 | 32 | F | 0.67 | 24.22 | 2.17 | 0 | 36.1 |
| C18 | 32 | M | 0.25 | 21.33 | 2.91 | 0 | 40.6 |
| C19 | 37 | F | 0.06 | 14.21 | 3.57 | 0 | 48.3 |
| C20 | 39 | F | 0.15 | 22.15 | 3.24 | 0 | 28.1 |

## TABLE 2 – MASTER CHART – GROUP II (STUDY GROUP)

| S.No | AGE (yrs) | SEX | PI | GBI-1 (%) | PPD-1 (mm) | CAL-1 (mm) | CTX-1 (pg/ml) | GBI-2 (%) | PPD-2 (mm) | CAL-2 (mm) | CTX-2 (pp/ml) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| S1 | 37 | M | 2.63 | 100 | 5.79 | 6.12 | 172.8 | 12.1 | 2.59 | 3.31 | 94.3 |
| S2 | 37 | F | 2.89 | 94.72 | 6.11 | 6.74 | 137.5 | 15.1 | 4.1 | 4.56 | 56.5 |
| S3 | 43 | M | 2.5 | 100 | 7.67 | 7.17 | 143.2 | 28.05 | 4.67 | 5.12 | 96.3 |
| S4 | 32 | M | 2.57 | 95.13 | 6.96 | 7.54 | 119.8 | 31.22 | 3.12 | 4.31 | 44.6 |
| S5 | 36 | F | 2.54 | 100 | 5.98 | 6.32 | 147.1 | 18 | 3.07 | 4.23 | 92.2 |
| S6 | 33 | F | 2.6 | 96.23 | 6.23 | 6.94 | 151.3 | 10.74 | 2.47 | 4.12 | 101.3 |
| S7 | 32 | M | 2.37 | 89.98 | 6.4 | 7.14 | 142.2 | 34.3 | 3.51 | 4.77 | 80.4 |
| S8 | 33 | M | 2.46 | 94.12 | 5.37 | 6.77 | 167.1 | 19.27 | 2.23 | 3.78 | 95.6 |
| S9 | 37 | M | 2.74 | 97.36 | 5.1 | 6.24 | 165.4 | 23.44 | 2.27 | 3.68 | 98.2 |
| S10 | 36 | F | 2.41 | 96.11 | 7.94 | 8.1 | 130.7 | 27.12 | 3.54 | 4.18 | 80.8 |
| S11 | 39 | F | 2.68 | 97.13 | 5.56 | 6.32 | 167.2 | 35.14 | 3.29 | 3.66 | 105.6 |
| S12 | 40 | F | 2.51 | 95.04 | 6.29 | 6.85 | 154.3 | 11.91 | 3.35 | 5.07 | 90.6 |
| S13 | 39 | F | 2.77 | 93.9 | 4.34 | 5.97 | 161.9 | 24.12 | 2.12 | 4.11 | 96.3 |
| S14 | 40 | M | 2.34 | 100 | 5.92 | 6.33 | 139.9 | 26.43 | 2.35 | 4.32 | 83.3 |
| S15 | 41 | M | 2.43 | 95.84 | 5.65 | 6.92 | 155.2 | 29 | 2.97 | 3.78 | 58.3 |
| S16 | 33 | M | 2.39 | 98.11 | 6.77 | 6.84 | 175.5 | 23.43 | 3.24 | 4.23 | 51.7 |
| S17 | 42 | F | 2.67 | 94.34 | 7.81 | 8.23 | 172.7 | 20.97 | 2.54 | 3.64 | 49.2 |
| S18 | 42 | F | 2.53 | 91.86 | 5.75 | 6.51 | 151.9 | 15.24 | 3.14 | 3.91 | 100.5 |
| S19 | 38 | M | 2.44 | 97.14 | 6.91 | 7.29 | 125.9 | 17.89 | 4.27 | 5.34 | 95.9 |
| S20 | 40 | M | 2.56 | 93.54 | 7.88 | 8.15 | 127.4 | 27.34 | 3.1 | 4.71 | 71.1 |
| S21 | 42 | M | 2.72 | 97.36 | 7.21 | 7.97 | 166.1 | 28.12 | 2.77 | 3.62 | 54.9 |
| S22 | 34 | F | 2.58 | 96.02 | 5.12 | 6.54 | 155.8 | 23.14 | 3.63 | 4.73 | 94.3 |
| S23 | 36 | M | 2.9 | 98.12 | 6.05 | 6.98 | 145.6 | 12 | 2.35 | 3.78 | 56.5 |
| S24 | 34 | F | 2.58 | 95.48 | 5.92 | 6.41 | 178.2 | 14.91 | 2.42 | 4.11 | 104 |
| S25 | 37 | F | 2.72 | 97.9 | 6.16 | 6.97 | 169.8 | 15.83 | 3.78 | 4.57 | 70.3 |

**TABLE 3 – COMPARISON OF AGE BETWEEN GROUP I AND GROUP II**

| AGE (in years) | Group I | | Group II | | P value |
|---|---|---|---|---|---|
| | Mean | S.D | Mean | S.D | |
| | 36.05 | 2.038 | 35.08 | 3.000 | 0.752$ |

$ – not significant

**TABLE 4 – COMPARISON OF GENDER BETWEEN GROUP I AND II**

| | | | Group | | Total | P value |
|---|---|---|---|---|---|---|
| | | | I | II | | |
| **Sex** | **Male** | Count | 11 | 13 | 24 | 0.841$ |
| | | % within sex | 45.8 | 54.2 | 100 | |
| | | % within group | 55 | 52 | 53.3 | |
| | **Female** | Count | 9 | 12 | 21 | |
| | | % within sex | 42.9 | 57.1 | 100 | |
| | | % within group | 45 | 48 | 46.7 | |
| Total | | Count | 20 | 25 | 45 | |
| | | % within sex | 44.4 | 55.6 | 100 | |
| | | % within group | 100 | 100 | 100 | |

$ – not significant

**TABLE 5 - COMPARISON OF CLINICAL PARAMETERS BETWEEN 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 6 - COMPARISON OF SALIVARY CTX BETWEENGROUP I AND GROUP II A**

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

** < 0.01 – highly significant *0.05-0.01 – significant $ >0.05 – not significant

**TABLE 7 – COMPARISON OF CLINICAL PARAMETERS BETWEEN GROUP II A AND GROUP II B**

| | Group | Mean | S.D | P value |
|---|---|---|---|---|
| **GBI (%)** | II A | 96.22 | 2.54 | |
| | II B | 21.79 | 7.25 | 0.000** |
| **PPD (mm)** | II A | 6.27 | 0.93 | |
| | II B | 3.07 | 0.68 | 0.000** |
| **CAL (mm)** | II A | 6.93 | 0.64 | |
| | II B | 4.22 | 0.52 | 0.000** |

** < 0.001 – highly significant *0.05-0.01 – significant $ >0.05 – not significant

**TABLE 8 – COMPARISON OF SALIVARY CTX BETWEEN GROUP II A AND GROUP II B**

| | Group II A | | Group II B | | P value |
|---|---|---|---|---|---|
| **CTX (pg/ml)** | Mean | S.D | Mean | S.D | |
| | 152.98 | 6.22 | 80.90 | 19.90 | 0.000** |

** < 0.001 – highly significant *0.05-0.01 – significant $ >0.05 – not significant

**TABLE 9 – COMPARISON OF CLINICAL PARAMETERS BETWEEN GROUP I AND GROUP II B**

| | Group | Mean | S.D | P value |
|---|---|---|---|---|
| **GBI (%)** | I | 15.57 | 6.06 | 0.000** |
| | II B | 21.79 | 7.25 | |
| **PPD (mm)** | I | 2.84 | 0.53 | 0.000** |
| | II B | 3.07 | 0.68 | |
| **CAL (mm)** | I | 0.00 | 0.00 | 0.000** |
| | II B | 4.22 | 0.52 | |

** < 0.001 – highly significant *0.05-0.01 – significant $ >0.05 – not significant

**TABLE 10 – COMPARISON OF SALIVARY CTX BETWEEN GROUP I AND GROUP II B**

| | Group I | | Group II B | | P value |
|---|---|---|---|---|---|
| **CTX (pg/ml)** | Mean | S.D | Mean | S.D | |
| | 38.39 | 6.22 | 80.90 | 19.90 | 0.000** |

** < 0.001 – highly significant *0.05-0.01 – significant $ >0.05 – not significant

**TABLE 11 - CORRELATION BETWEEN CLINICAL PARAMETERS AND SALIVARY CTX LEVEL IN GROUP I**

| CLINICAL PARAMETER | | CTX |
|---|---|---|
| **PI** | Pearson Correlation | 0.254 |
| | Sig. (2-tailed) | 0.279$ |
| **GBI** | Pearson Correlation | 0.198 |
| | Sig. (2-tailed) | 0.402$ |
| **PPD** | Pearson Correlation | 0.32 |
| | Sig. (2-tailed) | 0.168$ |
| **CAL** | Pearson Correlation | 0.274 |
| | Sig. (2-tailed) | 0.242$ |

* significant ($p<0.05$) $ - not significant

**TABLE 12 – CORRELATION BETWEEN CLINICAL PARAMETERS AND SALIVARY CTX LEVEL IN GROUP II A (study group before Phase I therapy)**

| CLINICAL PARAMETER | | CTX-1 |
|---|---|---|
| **PI** | Pearson Correlation | 0.236 |
| | Sig. (2-tailed) | 0.256$ |
| **GBI -1** | Pearson Correlation | 0.365 |
| | Sig. (2-tailed) | 0.072$ |
| **PPD-1** | Pearson Correlation | 0.347 |
| | Sig. (2-tailed) | 0.089$ |
| **CAL-1** | Pearson Correlation | 0.156 |
| | Sig. (2-tailed) | 0.456$ |

* significant ($p<0.05$) $ - not significant

**TABLE 13 – CORRELATION BETWEEN CLINICAL PARAMETERS AND SALIVARY CTX LEVEL IN GROUP II B (study group after Phase I therapy)**

| CLINICAL PARAMETER | | CTX-1 |
|---|---|---|
| **GBI -2** | Pearson Correlation | 0.361 |
| | Sig. (2-tailed) | 0.076$ |
| **PPD-2** | Pearson Correlation | 0.293 |
| | Sig. (2-tailed) | 0.155$ |
| **CAL-2** | Pearson Correlation | 0.214 |
| | Sig. (2-tailed) | 0.304$ |

* significant ($p<0.05$) $ - not significant

**Figure 5: Comparison of age between control (group I) and study (group II) group**

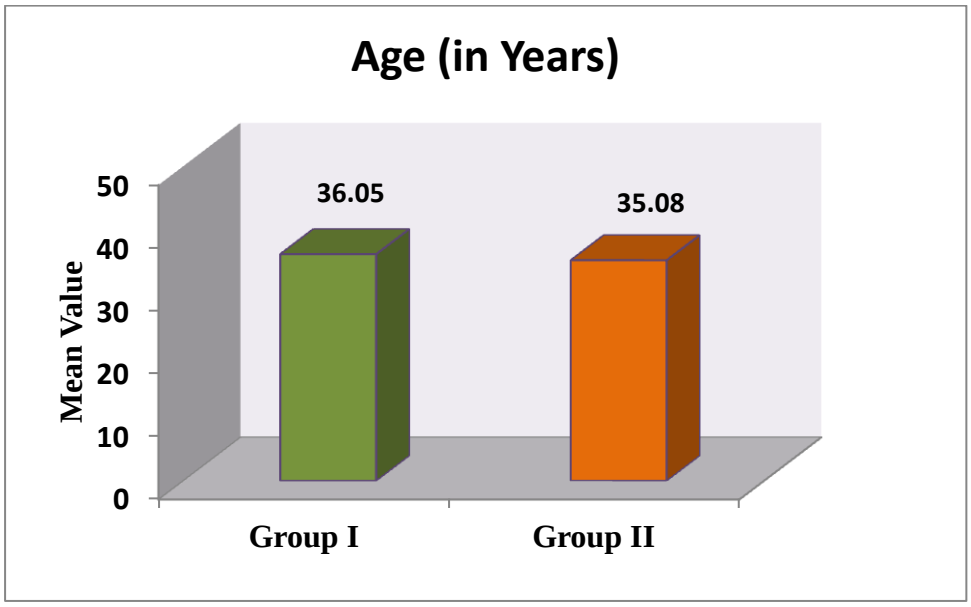



**Figure 6: Comparison of gender between group I and group II**

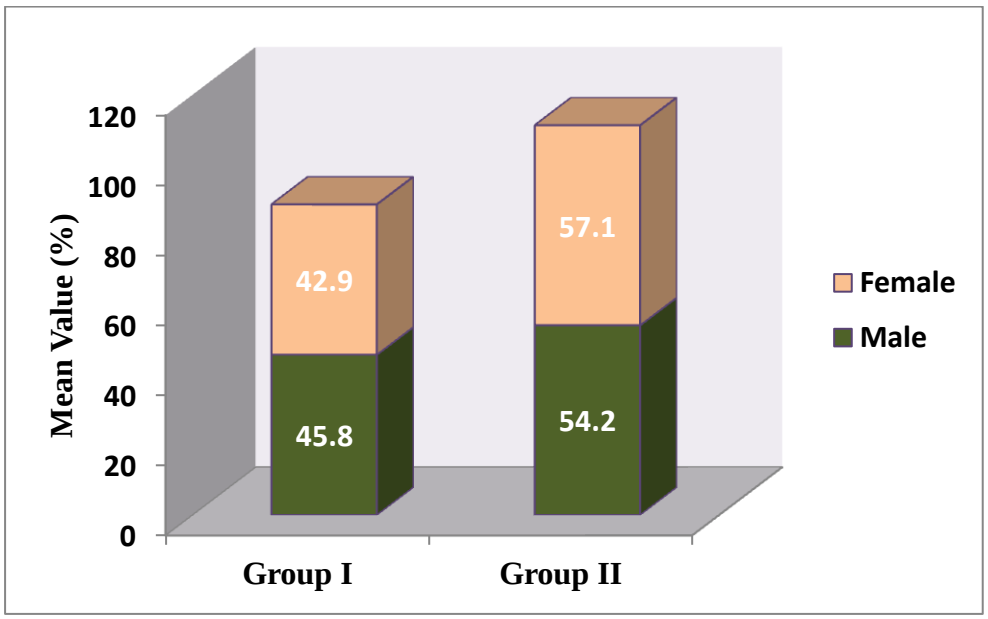


**Figure 7: Comparison of mean plaque index between group I (control) and group IIA (study group before phase I therapy)**

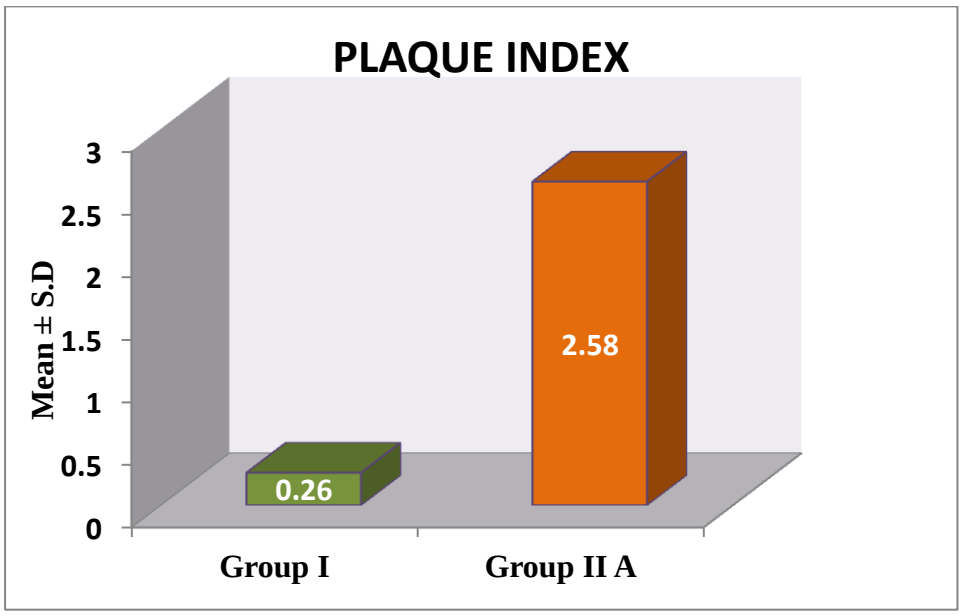



**Figure 8: Comparison of Gingival Bleeding Index between group I, group IIA and group IIB (study group after phase I therapy)**

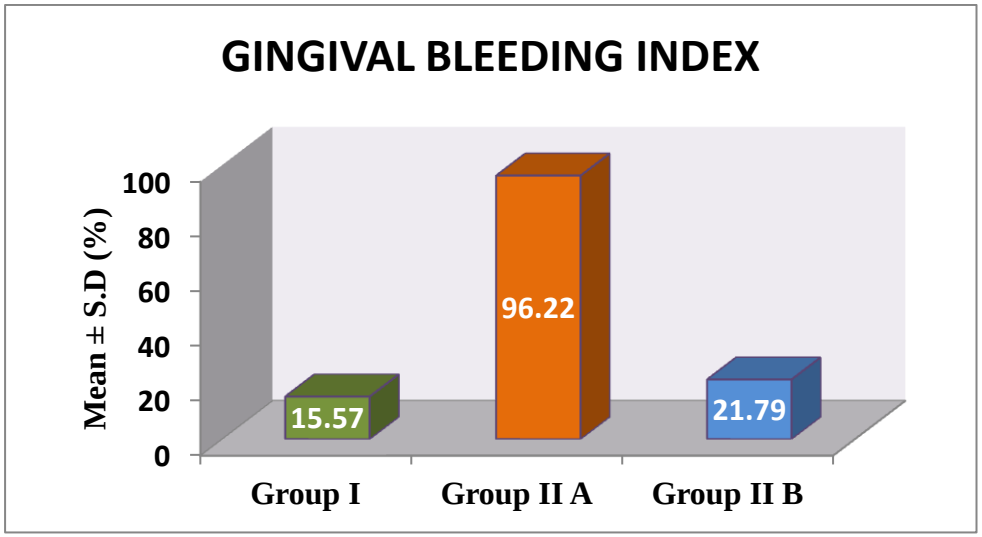


**Figure 9: Comparison of probing pocket depth between group I , group IIA and group IIB**

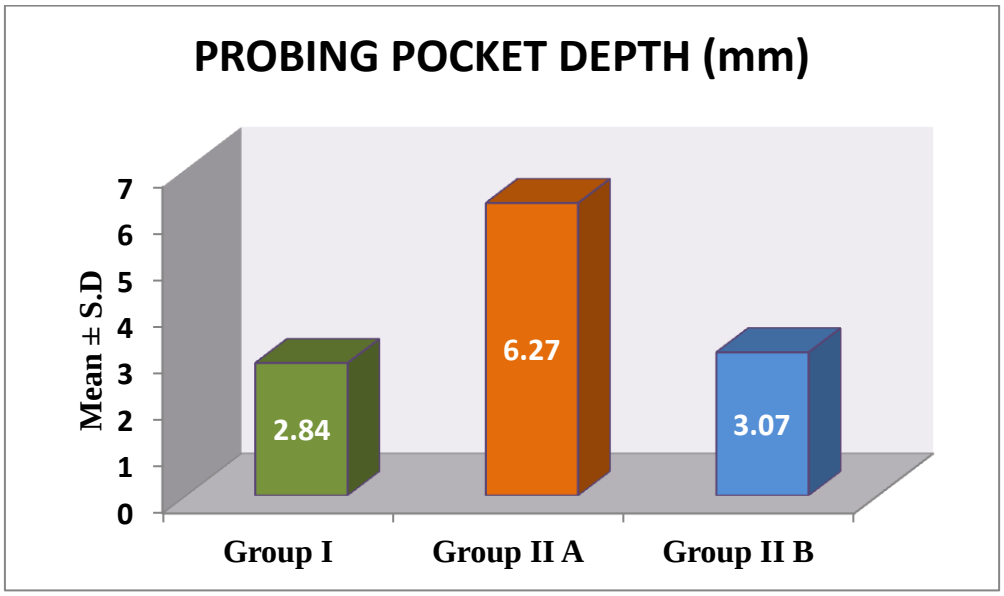



**Figure 10: Comparison of clinical attachment level between group I, group IIA and group IIB**

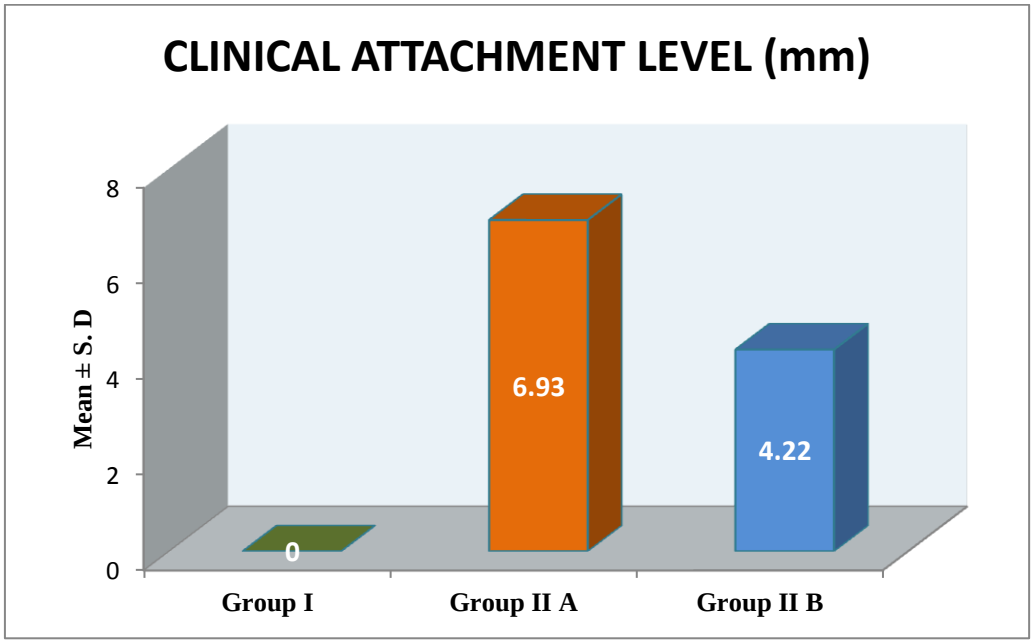


**Figure 11: Comparison of Mean salivary CTX between group I and group IIA**

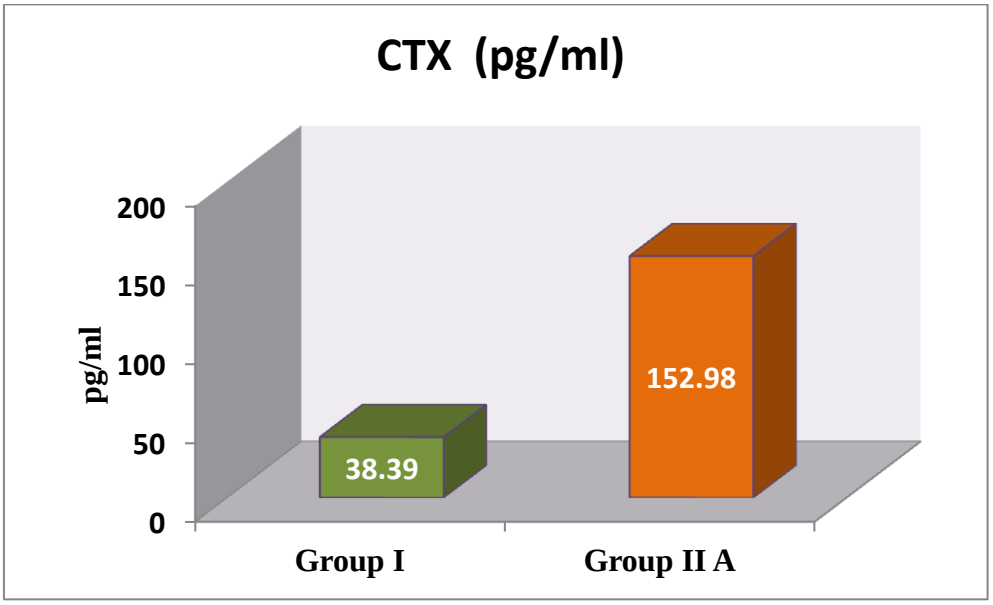



**Figure 12: Comparison of Mean salivary CTX between group IIA and group IIB**

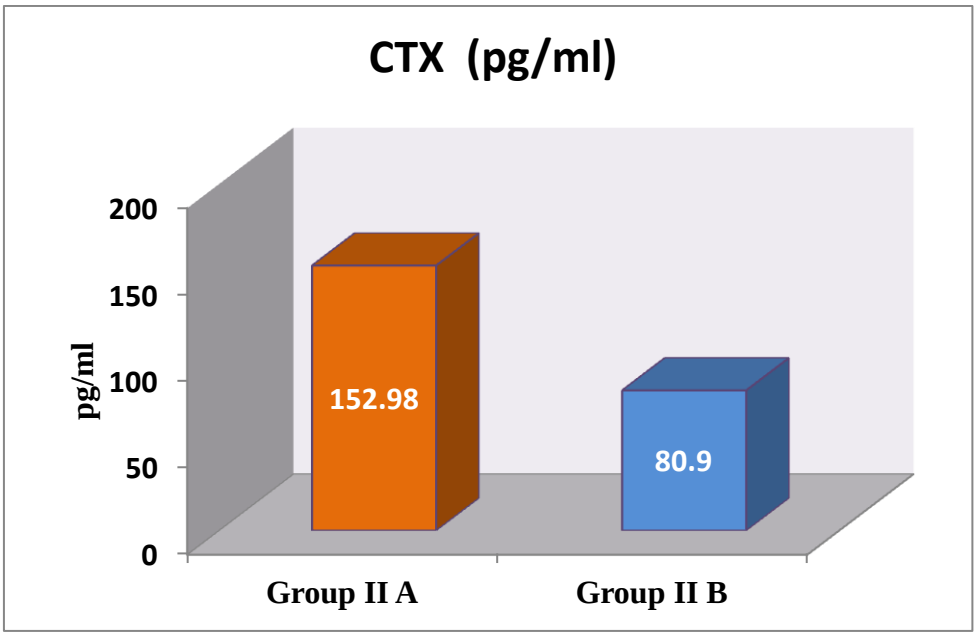


**Figure 13: Comparison of Mean salivary CTX between group I and group IIB**

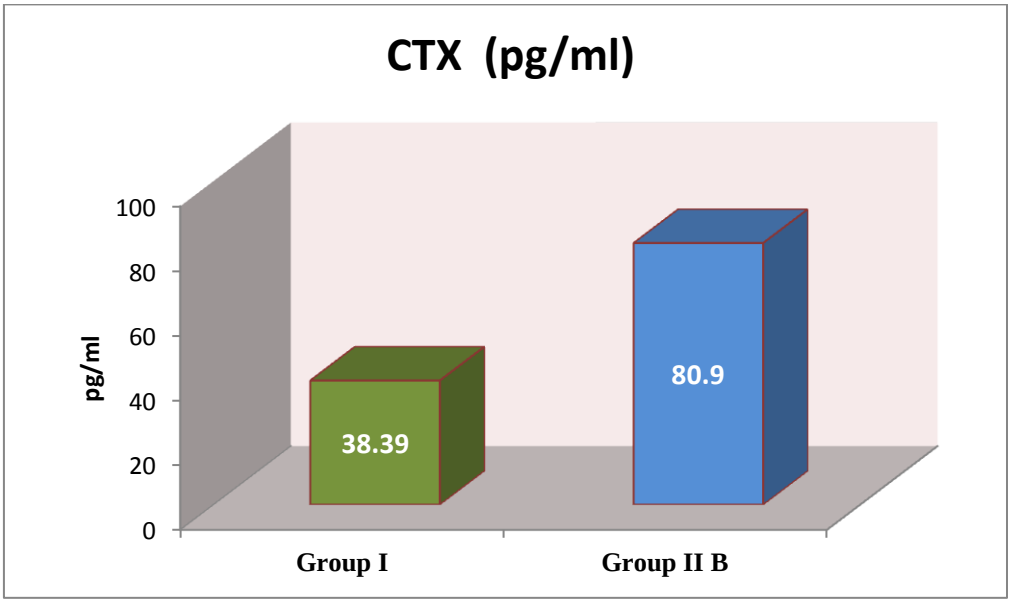



**Figure 14: Correlation of Plaque Index and salivary CTX level in control group**

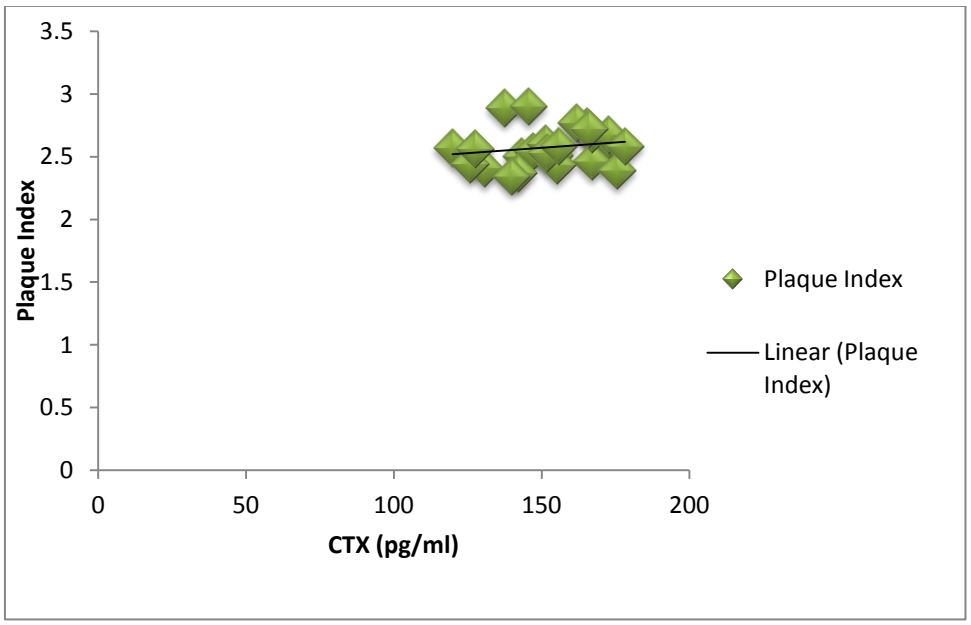


**Figure 15: Correlation of Gingival bleeding index (GBI) and salivary CTX level in control group**

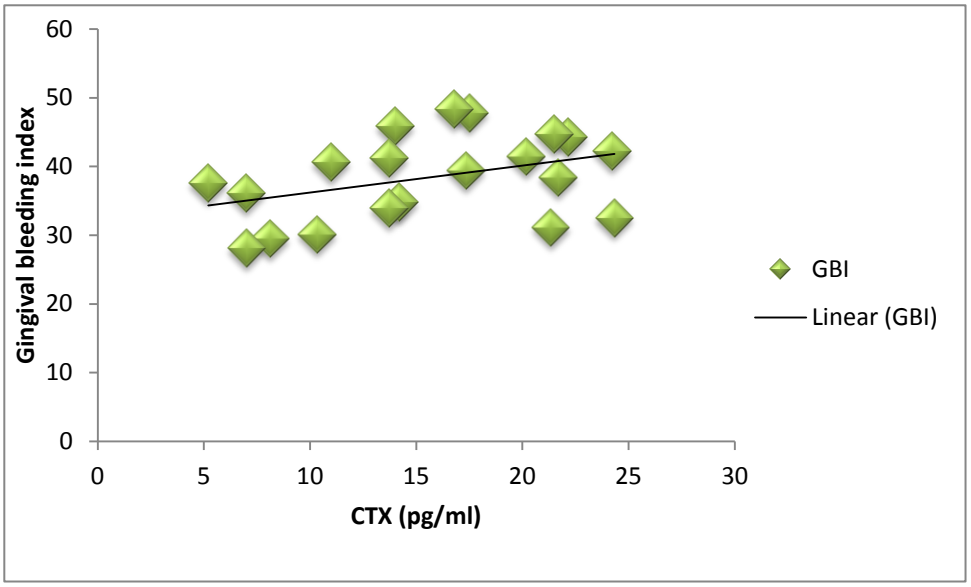



**Figure 16: Correlation of Probing Pocket Depth(PPD) and salivary CTX level in control group**

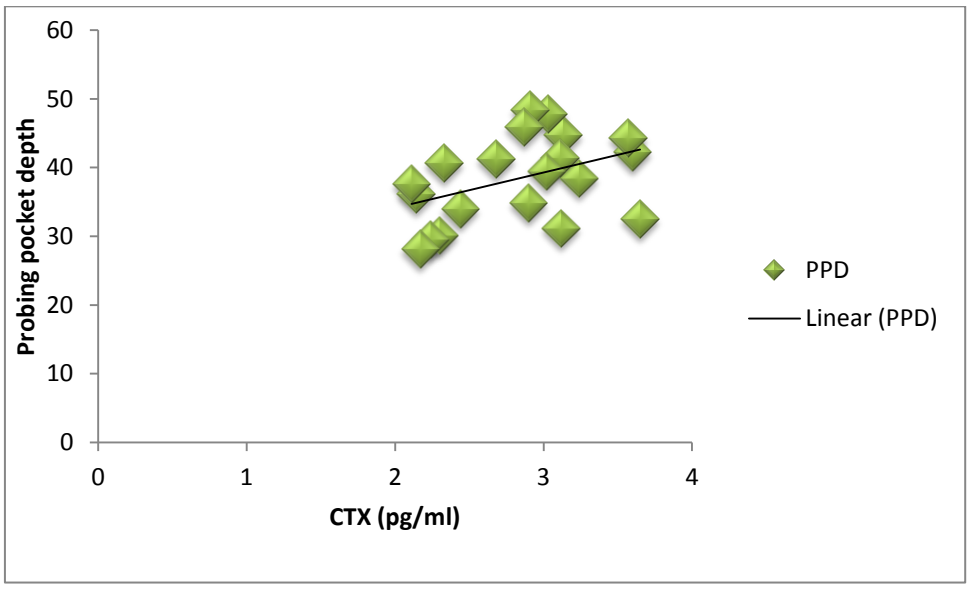


**Figure 17: Correlation of Plaque Index and salivary CTX level in study group (group IIA)**

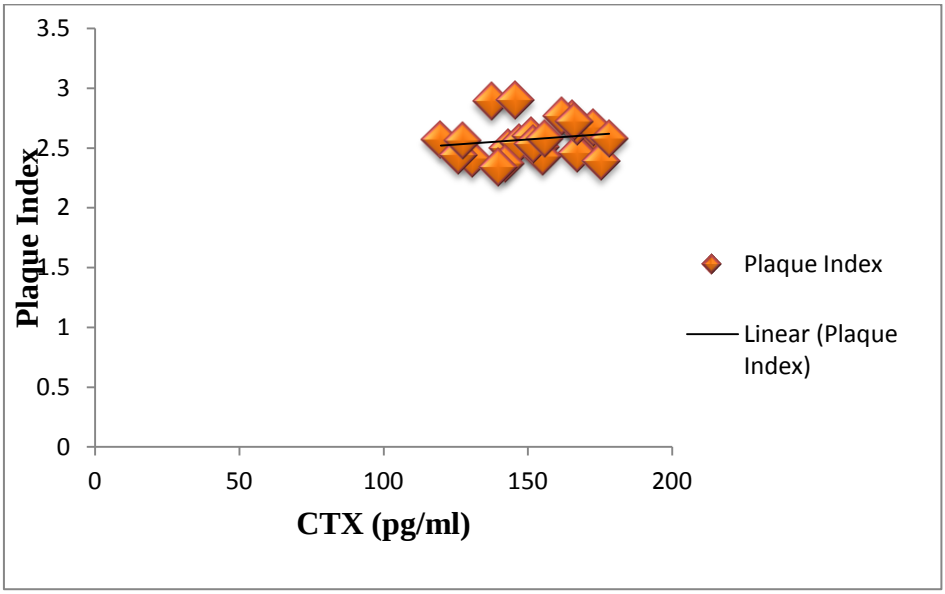



**Figure 18: Correlation of Gingival bleeding index and salivary CTX level in study group (group IIA)**

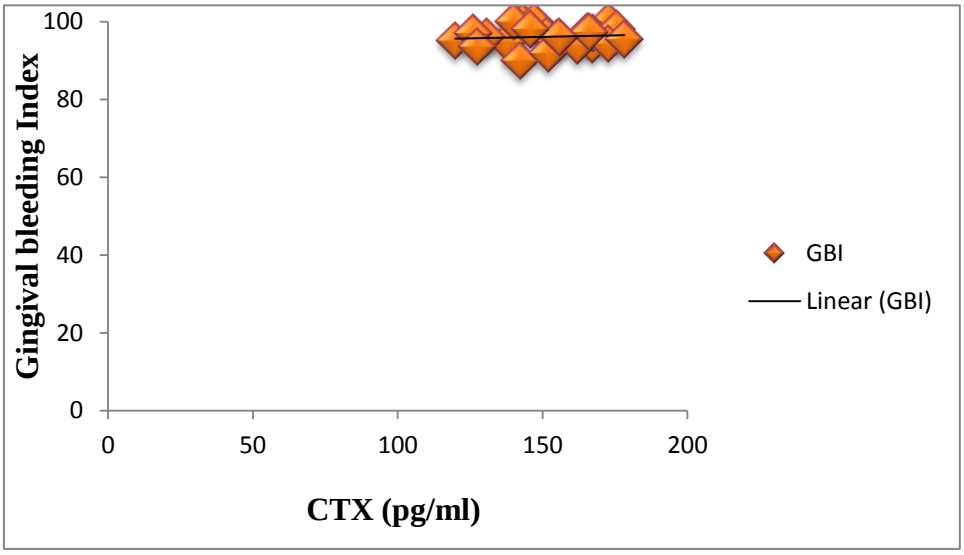


**Figure 19: Correlation of Probing pocket depth and salivary CTX level in study group (group IIA)**

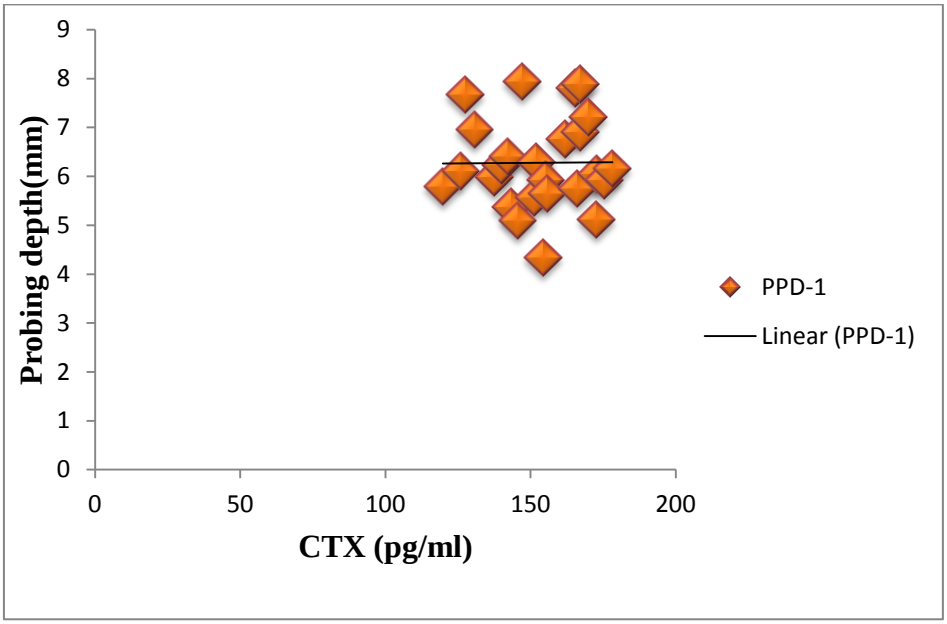



**Figure 20: Correlation of Clinical Attachment Level (CAL) and salivary CTX level in study group (group IIA)**

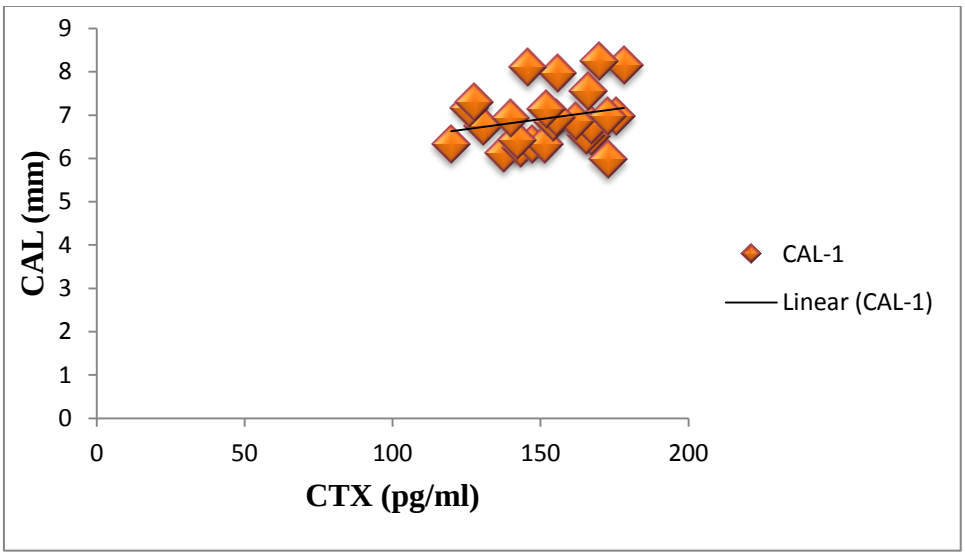


**Figure 21: Correlation of Gingival Bleeding Index (GBI) and salivary CTX level in study group IIB**

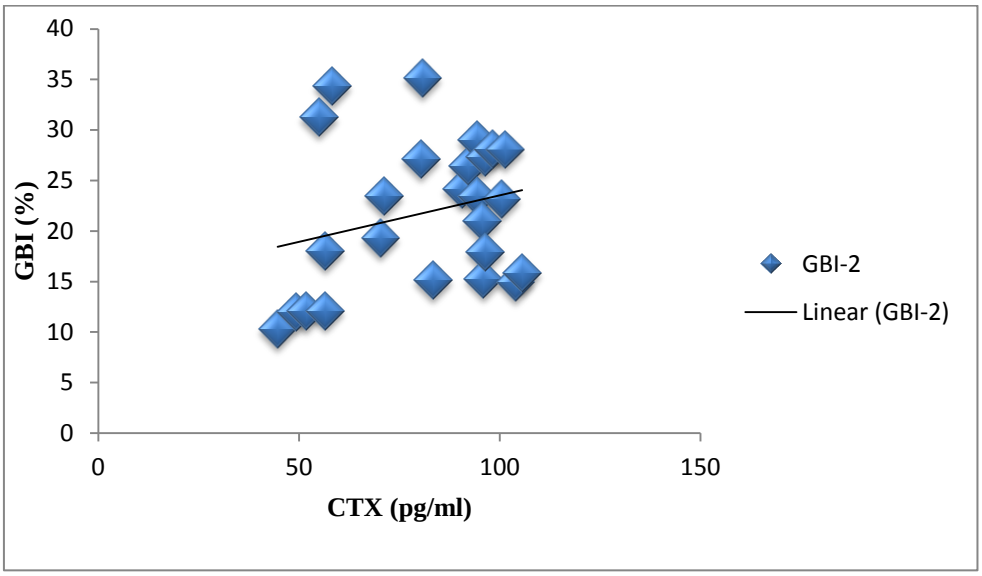



**Figure 22: Correlation of Probing Pocket Depth (PPD) and salivary CTX level in study group IIB**

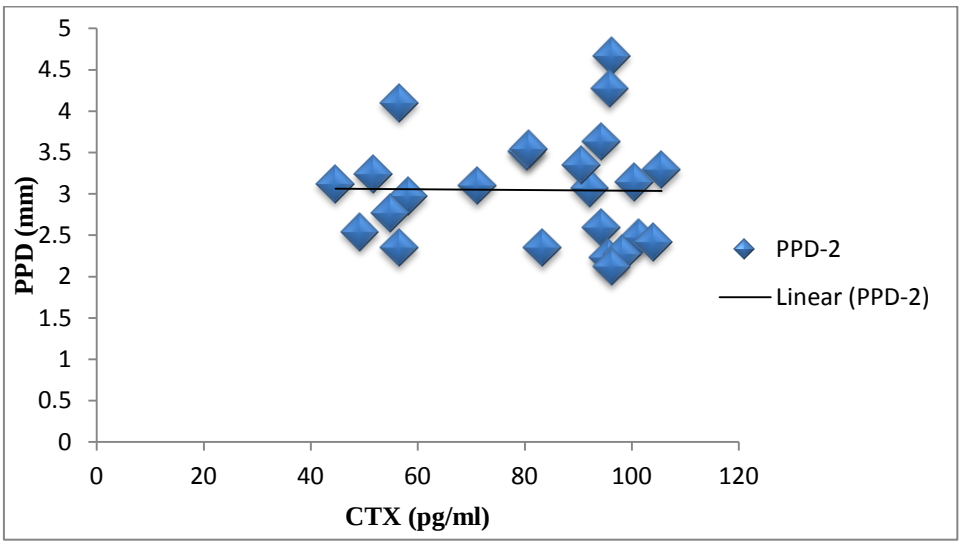
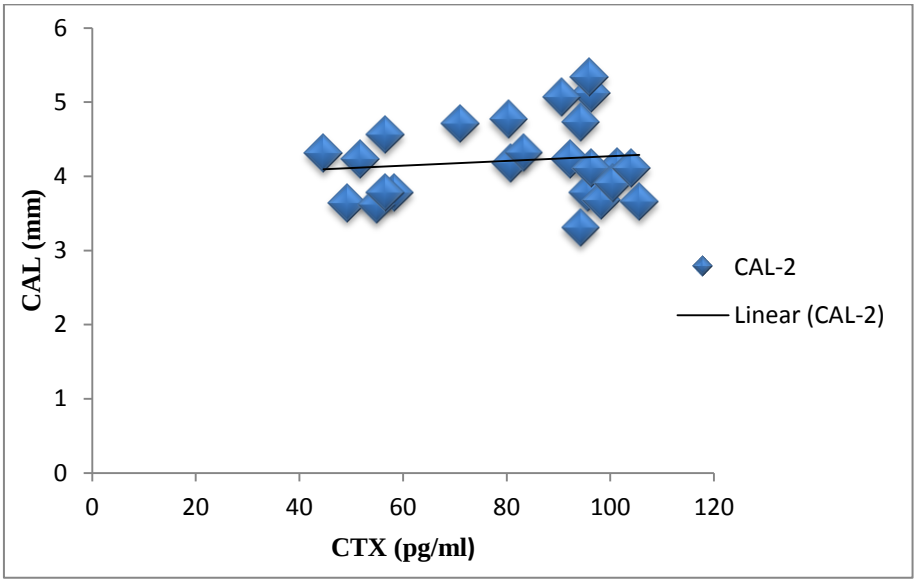


**Figure 23: Correlation of Clinical Attachment Level (CAL) and salivary CTX level in study group IIB**



## 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.[82] 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.[86] Moreover they are not reliable for identifying individuals with progressing disease activity.[26]

Biomarkers of disease activity facilitate earlier detection of disease and helps in monitoring therapy outcomes.[86]Recently, salivary markers have been investigated for 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 analyse the normal and pathologic processes in bone.[33]

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 as a good tool for assessing metabolic bone diseases like osteoporosis, rheumatoid arthritis and bone metastases.[86,39]

Amongst the diagnostic biomarkers for periodontal diseases, Giannobile et al reported that ICTP is a good predictor of future alveolar bone and attachment loss. Also ICTP levels correlated with clinical parameters, periodontal pathogens and demonstrates significant reductions after periodontal therapy.[39, 42]

But Garnero et al reported that type I collagen breakdown products ICTP and CTX, reveal two distinct enzymatic pathways and are detected in different pathological conditions. The cathepsin K related pathway releases large amounts of CTX but destroys the ICTP epitope. The other, MMP related pathway generates ICTP fragments.[37]

CTX is reported as an efficient marker of bone resorption in metabolic bone diseases characterized by increased osteoclastic activity like osteoporosis, Pagets disease etc. On the other hand, ICTP is a sensitive marker to detect osteolysis related to bone metastasis.[37, 42]

There are only few studies about CTX in relation to periodontal diseases. Miller et al analysed 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 whole saliva. Hence saliva can be used to assess the disease activity and to monitor periodontal therapy.[18]

Pellegrini G et al conducted a study in rats to correlate the serum and salivary markers of bone turnover and concluded that salivary CTX may be one of the best candidates to detect the activity and severity of periodontal disease.[71]

Recently Gursoy et al reported that salivary CTX levels differed in subjects with and without chronic periodontitis.[43]

Hence, the present study was conducted in subjects with generalized chronic periodontitis (n=25) and healthy subjects (n=20) to detect and compare salivary CTX levels before and after Phase I therapy. To our knowledge this is the first study in which salivary CTX has been evaluated in chronic periodontitis patients before and after periodontal therapy.

Thorough medical history was obtained from the subjects before including in the study, as variations in the marker level have been reported in liver diseases, renal diseases, diseases related to bone, etc.[62, 69]

Bone markers exhibit substantial short-term and long-term fluctuations related to age of the individual, health status, time of day, phase of the menstrual cycle, season of the year, diet and exercise. These biological factors produce intra and inter-individual variations in markers.[62, 69]

After 40 yrs of age, for every remodeling cycle there is a net loss of bone because the amount of bone resorption is more when compared to bone formation.[62,69] Hence in this study subjects of age group 30 – 40 yrs were recruited. The mean age calculated was 36.05 and 35.08 in the control and study group which was well within the inclusion criteria.

Bone marker concentrations exhibit considerable variations during puberty, menstruation, pregnancy and menopause due to the effect of various hormone levels. Hence female subjects in their menstruation, pregnancy and menopause period were excluded from the study.[62, 69]

Although GCF is site specific, 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[18]. In the present study, unstimulated whole saliva was collected from both the groups at baseline and in Group II after phase I therapy.

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 marker in serum and salivary samples are reported to be stable at -20$^0$C[61]. Therefore the collected samples were stored at -20$^0$C.

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 – 44.7pg/ml). On the contrary, in the study by Al Sabbagh et al[2], salivary CTX levels were below the detection limit (0.80µg/ml) in all the samples.

In another study by Gursoy et al CTX was detected only in 63% and 58% of subjects with and without generalized chronic periodontitis respectively.[43] Moreover, the minimum detection limit of the kit used by Gursoy et al[43] is 0.02ng/ml while the kit used in the present study had a higher minimum detection limit (44.7 pg/ml).

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 highly significant difference ($p<0.01$). This signifies that 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 has reached the saliva via GCF and has been detected by ELISA.

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. 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.

On comparing the mean values of the clinical parameters, a highly significant difference was shown before and after phase I therapy in study group. Also

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.

On evaluation of salivary CTX levels in 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 host response to periodontal treatment which varies from patient to patient. 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 surgical phase of periodontal therapy could have resulted in levels closer to control group. Hence further studies can be conducted to evaluate salivary CTX levels after the surgical phase of periodontal therapy.

In the present study significantly elevated salivary CTX levels were observed in patients with chronic periodontitis as compared to healthy controls and their levels reduced in study group after therapy. This suggests a close association between salivary CTX and periodontal disease. Thus, salivary CTX might be potentially useful in distinguishing health from disease and monitoring periodontal disease activity.

## SUMMARY AND CONCLUSION

In this study, bone resorption marker, carboxyterminal telopeptide of type I collagen (CTX) levels in unstimulated whole saliva of periodontally healthy subjects (n=20) and generalized chronic periodontitis subjects (n=25) were analysed at baseline using Enzyme linked Immuosorbent assay (ELISA). Group IIA underwent Phase I therapy and were analysed for salivary CTX after therapy using the same technique.

The collected data was analysed and showed that,

- CTX was detected in 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 the groups but the correlation was not significant.
- Significant reduction in salivary CTX levels was seen after phase I therapy in chronic periodontitis group but did not reduce to the level of healthy controls.

The limitations of the study was the small sample size (n=45) and short time period between observations in the study group. Within the limits of the study, it can be concluded that salivary CTX might be potentially useful in distinguishing health from disease and monitoring periodontal disease activity. Future longitudinal studies with larger sample size are needed to validate salivary CTX as a marker for periodontal disease.

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## Annexure - 1

## INFORMATION SHEET

- We are conducting a study on effect of phase I therapy on Salivary Carboxyterminal telopeptide levels in chronic periodontitis patients.
- The identity of the patients participating in the research will be kept confidential throughout the study. In the event of any publication or presentation resulting from the research, no personally identifiable information will be shared.
- Taking part in the study is voluntary. You are free to decide whether to participate in the study or to withdraw at any time; your decision will not result in any loss of benefits to which you are otherwise entitled.
- The results of the special study may be intimated to you at the end of the study period or during the study if anything is found abnormal which may aid in the management or treatment.

Name of the patient Signature / Thumb impression

Name of the investigator Signature Date

# Annexure – 2

## INFORMED CONSENT FORM

**STUDY TITLE:**

## EFFECT OF PHASE I THERAPY ON SALIVARY CARBOXYTERMINAL TELOPEPTIDE OF TYPE I COLLAGEN IN CHRONIC PERIODONTITIS PATIENTS

Name: O.P.No:

Address: Code No:

Age / Sex:

Tel. no:

I, _____age____years

exercising my free power of choice, hereby give my consent to be included as a participant in the study "**Effect of phase I therapy on salivary carboxyterminal telopeptide of type I collagen in chronic periodontitis patients "**

I agree to the following:

- I have been informed to my satisfaction about the purpose of the study and study procedures including investigations to monitor and safeguard my body function.
- I agree to co-operate fully and to inform my doctor immediately if I suffer any unusual symptom.
- I have informed the doctor about all medications I have taken in the recent past and those I am currently taking.
- I hereby give permission to use my medical records for research purpose. I am told that the investigating doctor and institution will keep my identity confidential.

Name of the patient Signature / Thumb impression

Name of the investigator Signature
Date

# Annexure - 3

# TAMIL CONSENT FORM

ஆராய்ச்சி ஒப்புதல் படிவம்

ஆராய்ச்சி தலைப்பு:-

ஈறு நோயாளிகளுக்கு, முதல் கட்ட ஈறு நோய் சிகிச்சைக்கு முன் மற்றும் பின் உமிழ் நீரில் கார்பாக்ஸி டெர்மினல் டிலோபெப்டைடு அளவு ஒப்பீடு.

பெயர் : .....
முகவரி : .....
.....
.....

புற நோயாளியின் எண். : .....
ஆராய்ச்சி சேர்க்கை எண் : .....
வயது
பாலினம் ☐ ஆண் ☐ பெண்

நான் ..... வயது ..... என்னுடைய சுயநினைவுடன் மற்றும் முழு சுதந்திரத்துடன், இந்த மருத்துவ ஆராய்ச்சியில் என்னை சேர்த்துக் கொள்ள சம்மதிக்கிறேன்.

எனக்கு விளக்கப்பட்ட விஷயங்களுக்கு நான் எனது சம்மதத்தை தருகிறேன்.

- இந்த ஆராய்ச்சியின் நோக்கம், மருத்துவ முறைகள், பரிசோதனை முறைகள் எனக்கு திருப்த்தியுறும் வகையில் விளக்கப்பட்டன.
- பரிசோதனை செய்வதற்காக என் உமிழ் நீர் எடுக்கப்பட வேண்டியுள்ளதாக அறிகிறேன்.
- நான் எடுத்து வருகின்ற மற்றும் முன் உட்கொண்ட மருந்துகள் பற்றிய விவரங்களை ஆராய்ச்சியாளரிடம் அறிவிக்க சம்மதம்.
- என் உடல்நலம் பாதிக்கப்பட்டாலோ அல்லது எதிர்பாராத வழக்கத்திற்கு மாறான நோய்குறி தென்பட்டாலோ உடனே அதை மருத்துவரிடம் தெரிவிப்பேன் என உறுதியளிக்கிறேன்.
- எனக்கும் மற்றும் மருந்து ஆராய்ச்சியாளருக்கும் இந்த ஆராய்ச்சியிலிருந்து எந்த ஒரு நிலையிலும் விலகுவதற்கோ அல்லது விலக்குவதற்கோ முழு உரிமை இருப்பதாக அறிகிறேன்.
- என்னுடைய மருத்துவக் குறிப்பேடுகளை இந்த ஆராய்ச்சியில் பயன்படுத்திக் கொள்ள சம்மதிக்கிறேன். ஆராய்ச்சி மையமும், ஆராய்ச்சியாளரும் என்னுடைய பெயர் மற்றும் சில விவரங்களை இரகசியமாக வைப்பதாக அறிகிறேன்.

| | | |
|---|---|---|
| ..... | ..... | ..... |
| நோயாளின் பெயர் | கையெழுத்து | தேதி |
| ..... | ..... | ..... |
| ஆராய்ச்சியாளரின் பெயர் | கையெழுத்து | தேதி |

## Annexure 4

# EFFECT OF PHASE I THERAPY ON SALIVARY CARBOXYTERMINAL TELOPEPTIDE LEVELS IN CHRONIC PERIODONTITIS PATIENTS

## PROFORMA

Name : Age / Gender:

O.P. No : Code No:

Occupation : Income :

Address and Contact No.:

**Chief Complaints**

**Duration:**

**Medical history**

**Dental history**

**Periodontal Examination**

**PLAQUE INDEX – SILNESS & LOE (1964)**

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | | | | | |
| **18** | **17** | **16** | **15** | **14** | **13** | **12** | **11** | **21** | **22** | **23** | **24** | **25** | **26** | **27** | **28** |
| **48** | **47** | **46** | **45** | **44** | **43** | **42** | **41** | **31** | **32** | **33** | **34** | **35** | **36** | **37** | **38** |
| | | | | | | | | | | | | | | | |

**Score:**

## GINGIVAL BLEEDING INDEX – AINAMO & BAY (1975)

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **18** | **17** | **16** | **15** | **14** | **13** | **12** | **11** | **21** | **22** | **23** | **24** | **25** | **26** | **27** | **28** |
| **48** | **47** | **46** | **45** | **44** | **43** | **42** | **41** | **31** | **32** | **33** | **34** | **35** | **36** | **37** | **38** |
| | | | | | | | | | | | | | | | |

**Score:**

## PROBING DEPTH (PD) & CLINICAL ATTACHMENT LEVEL (CAL) (in mm)

**MAXILLARY:**

**Palatal**

| **CAL** | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **PPD** | | | | | | | | | | | | | | | | |
| | **18** | **17** | **16** | **15** | **14** | **13** | **12** | **11** | **21** | **22** | **23** | **24** | **25** | **26** | **27** | **28** |
| **PPD** | | | | | | | | | | | | | | | | |
| **CAL** | | | | | | | | | | | | | | | | |

**Buccal**

**MANDIBULAR:**

**Lingual**

| **CAL** | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **PPD** | | | | | | | | | | | | | | | | |
| | **48** | **47** | **46** | **45** | **44** | **43** | **42** | **41** | **31** | **32** | **33** | **34** | **35** | **36** | **37** | **38** |
| **PPD** | | | | | | | | | | | | | | | | |
| **CAL** | | | | | | | | | | | | | | | | |

**Buccal**

***DIAGNOSIS:***

***INVESTIGATIONS:***

***OPG***

***BITEWING RADIOGRAPHS***

***SALIVARY CTX LEVEL***

***<u>TREATMENT</u>***

***<u>AFTER PHASE I THERAPY (after 1 month)</u>***

**GINGIVAL BLEEDING INDEX – AINAMO & BAY (1975)**

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **18** | **17** | **16** | **15** | **14** | **13** | **12** | **11** | **21** | **22** | **23** | **24** | **25** | **26** | **27** | **28** |
| **48** | **47** | **46** | **45** | **44** | **43** | **42** | **41** | **31** | **32** | **33** | **34** | **35** | **36** | **37** | **38** |
| | | | | | | | | | | | | | | | |

**Score:**

**PROBING DEPTH (PD) & CLINICAL ATTACHMENT LEVEL (CAL) (in mm)**

**MAXILLARY:**

**Palatal**

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **CAL** | | | | | | | | | | | | | | | | |
| **PPD** | | | | | | | | | | | | | | | | |
| | **18** | **17** | **16** | **15** | **14** | **13** | **12** | **11** | **21** | **22** | **23** | **24** | **25** | **26** | **27** | **28** |
| **PPD** | | | | | | | | | | | | | | | | |
| **CAL** | | | | | | | | | | | | | | | | |

**Buccal**

**MANDIBULAR:**

**Lingual**

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **CAL** | | | | | | | | | | | | | | | | |
| **PPD** | | | | | | | | | | | | | | | | |
| | **48** | **47** | **46** | **45** | **44** | **43** | **42** | **41** | **31** | **32** | **33** | **34** | **35** | **36** | **37** | **38** |
| **PPD** | | | | | | | | | | | | | | | | |
| **CAL** | | | | | | | | | | | | | | | | |

**Buccal**

***INVESTIGATION:***

***SALIVARY CTX LEVEL:***

***INFERENCE:***

**Signature of the P.G student** **Signature of Guide**

**Date**