"ESTIMATION OF SALIVARY MATRIX METALLOPROTEINASES- 8 (MMP-8) AND ITS CORRELATION TO PERIODONTAL INFLAMED SURFACE AREA (PISA) BEFORE AND AFTER NON-SURGICAL PERIODONTAL THERAPY"

BY

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Dissertation

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BELAGAVI, KARNATAKA

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This is to certify that the dissertation **"Estimation of salivary Matrix Metalloproteinases- 8 (MMP-8) and its correlation to Periodontal Inflamed Surface Area (PISA) before and after non-surgical periodontal therapy"** *is a bonafide research work done by* ***REG NO. IK0222004.***

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I, ***REG NO. IK0222004***, a postgraduate student in Department of Periodontics, have completed my research work on the topic **"Estimation of salivary Matrix Metalloproteinases- 8 (MMP-8) and its correlation to Periodontal Inflamed Surface Area (PISA) before and after non-surgical periodontal therapy"** in the year 2022-2025.

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Date:18-04-2025

Place: Belagavi

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LIST OF ABBREVIATIONS

IL	**Interleukin**
TNF	**Tumor necrosis factor**
MMP	**Matrix metalloproteinases**
GCF	**Gingival crevicular fluid**
SRP	**Scaling and root planing**
PPD	**Pocket probing depth**
CAL	**Clinical attachment loss**
PISA	**Periodontal inflamed surface area**
BOP	**Bleeding on probing**
LGM	**Lower gingival margin**
mm^2	**Square millimeter**
pg/ml	**picograms per milliliter**
ALSA	**Attachment loss surface area**
PI	**Plaque Index**
GI	**Gingival index**
RSA	**Recession surface area**
PESA	**Periodontal epithelial surface area**
NSPT	**Non-surgical periodontal therapy**
T2DM	**Type II Diabetes Mellitus**

ELISA	**Enzyme linked immunoassay**
CEJ	**Cementoenamel junction**
BOP	**Bleeding on probing**
AGE	**Advanced Glycation end products**
RAGE	**Receptor of Advanced Glycation end products**
IFMA	**Immunofluorometric assay**
TIMP	**Tissue Inhibitor of Metalloproteinases**
ICTP	**Pyridinoline cross-linked carboxyterminal telopeptide of type I collagen**
SD	**Standard Deviation**
MPO	**Myeloperoxidase**
CDC-AAP	**Centers for Disease Control and Prevention and American Academy of Periodontology**

ABSTRACT

INTRODUCTION

Matrix metalloproteinase-8 (MMP-8) is a key enzyme involved in collagen degradation and is considered a promising indicator of periodontal tissue destruction. Given its involvement in periodontal inflammation, MMP-8 holds promise as a biomarker for identifying individuals at risk of developing periodontitis. MMP-8 is detectable in oral fluids such as saliva and gingival crevicular fluid. The available studies used conventional periodontal parameters which inadequately measured the inflammatory burden posed by the periodontal disease. A new tool Periodontal Inflamed Surface Area (PISA) was developed which provides a quantitative measure of inflammation by calculating the surface area of bleeding pocket epithelium using CAL, recession and bleeding on probing. No existing studies have explored the relationship between MMP-8 and PISA levels pre and post non-surgical periodontal therapy.

AIM

Estimation of salivary MMP-8 and its correlation to PISA before and after non-surgical periodontal therapy

MATERIALS AND METHODS

A total of 42 participants were enrolled and categorized into two groups: Group A - periodontal health and Group B- severe periodontitis (Stage III and IV, Grade A). After a comprehensive periodontal examination, PISA scores were recorded and baseline saliva samples were collected. All patients underwent thorough Phase I periodontal therapy. After 3 months, participants were recalled for re-evaluation and

PISA scores were reassessed and saliva samples were recollected. These values were then compared to the baseline measurements. The saliva samples were collected from all the subjects and MMP-8 values were evaluated using ELISA. Statistical analysis was done using dependent t test, independent t test and Karl Pearson's correlation coefficient ($p<0.05$).

RESULTS

The mean salivary MMP-8 levels at baseline in periodontal health was 479.81 ± 140.41 pg/ml and at 3 months was 237.57 ± 100.52 pg/ml where as in periodontitis group MMP-8 levels were 2332.14 ± 163.39 pg/ml and 617.95 ± 195.97 pg/ml at baseline and 3 months respectively. The mean PISA scores at baseline for healthy group was 393.86 ± 107.43 mm^2 and 3 months post therapy was 243.80 ± 76.51mm^2 whereas in periodontitis group at baseline and 3 months the values were 1779.33 ± 530.32 mm^2 and 798.00 ± 120.78 mm^2 respectively. The correlation between MMP-8 levels and PISA scores showed a positive association in periodontitis patients.

CONCLUSION

The findings of this study showed that MMP-8 levels were higher in periodontitis group and post NSPT there was reduction in MMP-8. Similarly, PISA scores were higher in periodontitis group and reduction was post NSPT. A positive correlation was seen in MMP-8 and PISA values in periodontitis group. This association indicates that MMP-8 could potentially serve as a valuable diagnostic measure for assessing periodontal disease progression which can be measured by PISA.

KEYWORDS: Biomarkers, non-surgical periodontal therapy, PISA**,** periodontitis, salivary MMP-8

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INTRODUCTION

The intricate interactions between pathogenic microorganisms and the host's immune system result in periodontitis that eventually results in tooth loss and the destruction of periodontium. The development of bacterial plaque on the surfaces of tooth is predominantly linked to the pathophysiology of periodontal disease, which triggers both direct and indirect mechanisms of tissue destruction. Direct mechanisms involve bacterial-derived lytic enzymes and cytotoxins while indirect mechanisms are mediated by host inflammatory cytokines that exacerbate tissue damage.[1] Biomarkers are the indicator of normal biological and pathological processes and therapeutic interventions. They are important for the early detection of periodontitis, assessment of disease severity and prediction of future risk for the progression of gingivitis into irreversible periodontitis. The biomarkers commonly associated with periodontal disease include interleukins, IL1β, IL6, tumour necrosis factor-α (TNF-α), MMP and so on.[2]

Many biomarkers have been discovered to date which are commonly found inside bodily fluids like saliva, gingival crevicular fluid (GCF) and blood for studying diseases of the periodontium.[2] Selecting saliva as a point of care has many benefits including ease of sample collection, time efficiency and is cost-effective. Saliva also contains biomarkers that reflect components derived from both GCF and dental plaque making it a viable alternative for periodontal diagnostics. Unstimulated saliva has advantage over stimulated saliva as the latter alters the pH which increases the water content resulting in the dilution of target analytes. Therefore, the use of unstimulated saliva not only ensures greater analyte concentration but also offers a practical solution for large-scale population screening and non-invasive disease

monitoring. Salivary biomarkers also serve as an early detection method for identifying host immune responses and inflammatory processes that typically occurs before clinical or radiographic signs of periodontal tissue damage emerges. [3]

Some of these biomarkers are implicated in collagen breakdown, while others are associated with bone remodelling processes. Since collagen makes up a large portion of the periodontal tissue, the activity of MMPs in periodontal diseases has garnered significant attention. MMPs are zinc-containing endopeptidases and are calcium-dependent, that has a role in pathological processes including cancer, degenerative neurological disorders, cardiovascular abnormalities, arthritis and inflammation and in physiological events like angiogenesis, apoptosis and immune response. [4] In 1986, MMPs were discovered in human leukocytes for the first time. They are a group of endopeptidase enzymes that share structural similarities but differ genetically. MMP is one of the main enzymes in the remodeling of the extracellular matrix. MMPs also acts intracellularly by activating receptors on the cell surface, adhesion molecules and growth factors. This family presently consists of 28 members, 25 of which are found in humans. They are divided into six sub-families according to their structure and function.[4,5]

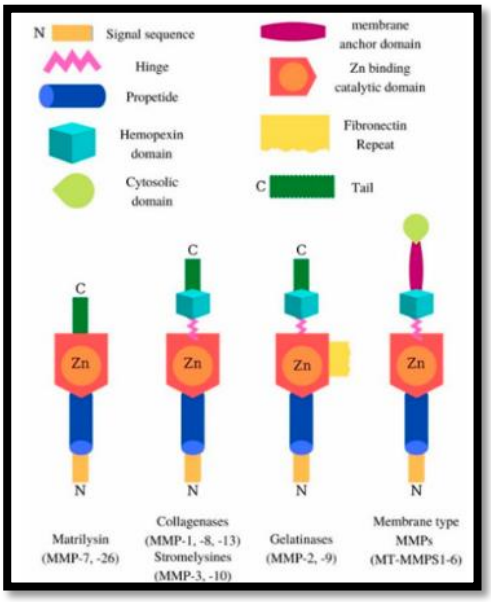
Structure of MMP

Members of the MMP family share a conserved structural framework (Figure 1), which consists of 200 amino acid hemopexin domain that is essential for the breakdown of collagen, a pro-peptide region of 80 amino acids containing an N-terminal signaling peptide, 170 amino acids of catalytic domain that incorporates a zinc ion that is necessary for enzymatic activity and a linking region that joins the hemopexin and catalytic domains.[4,5]

Figure 1- Classification of MMP

Subgroup	MMP member	Nomenclature
Collagenases	MMP-1 MMP-8 MMP-13	Collagenase 1 Collagenase 2 Collagenase 3
Gelatinases	MMP-2 MMP-9	Gelatinase A Gelatinase B
Stromelysins	MMP-3 MMP-10 MMP-11	Stromelysin 1 Stromelysin 2 Stromelysin 3
Matrilysins	MMP-7 MMP-26	Matrilysin 1 Matrilysin 2
Membrane matrix metalloproteinases	MMP-14 MMP-15 MMP-16 MMP-24	MT1-MMP MT2-MMP MT3-MMP MT5-MMP
Others	MMP-12 MMP-19 MMP-20 MMP-21 MMP-23 MMP-27 MMP-28	RASI-I Enamelysin Epilysin

Figure 2: Structure of MMP



One of the main characteristics of periodontal disease is the breakdown of ECM proteins by MMPs. MMP-8 is accounted for over 90% of collagenolytic activity, frequently referred to as collagenase 2 and is regarded as a promising biomarker for periodontitis in oral fluids. This enzyme is one of several collagenases frequently detected in periodontal disorders along with MMP-2, MMP-13 and MMP-9. It can be detected in oral fluids including saliva and GCF and is a major agent of irreversible tissue deterioration in severe periodontitis.[5, 6]

MMP-8 which is abundant in oral fluids during inflammation originates from neutrophils which are the primary defence cells. Once MMP-8 is released from inflammatory cells in an inert state, it is activated at the location of inflammation by a variety of host and microbe-derived stimuli. Numerous investigations have shown that, when compared to healthy controls, sites affected by periodontal inflammation had higher concentrations of MMP-8 in GCF and saliva.[7,8,9] According to research conducted by Fatemi et al.[10], MMP-8 values were almost 3.6 times greater in periodontitis patients than in healthy people.

Despite advancements in therapeutic modalities over the past decades, scaling and root planing (SRP) continues to be regarded as the most effective method for treating periodontitis. A substantial body of evidence from numerous clinical trials consistently demonstrates the efficiency of SRP in the treatment of periodontitis, highlighting its fundamental role in periodontal therapy. Various studies have reported reduced MMP-8 levels after carrying out less-invasive interventions such as ultrasonic scaling.[11,12,13] According to a study by Kinane et al.[14], individuals with periodontitis who had undergone non-surgical periodontal therapy (NSPT) reported less values in GCF MMP-8, three months after treatment.

Previously conducted studies that measured MMP-8 values in periodontitis patients have classified or defined periodontitis using non-continuous variables which includes CAL and PPD.[7,8,9,10] The available studies used conventional periodontal parameters which inadequately measured the inflammatory burden caused by the periodontal disease.

A new tool was introduced by Nesse et al.[15] in 2008 is a novel quantitative measure which is known as Periodontal Inflamed Surface Area (PISA). It provides a more

precise estimation of the inflammatory burden by offering more thorough assessment of the level of periodontal disease. The surface area (in square millimeters) of a bleeding pocket epithelium can be calculated by CAL, BOP and recession. The critical sign of inflammation is BOP and it is a useful parameter for identifying active inflammation. PISA values can be utilized to differentiate between individuals with active inflammation and those with non-inflamed dentition. They quantitatively describe the amount of periodontal inflammatory burden caused by periodontitis. [15]

At present there are no studies correlating MMP-8 levels in periodontitis and health to PISA which determines the total inflammatory condition of an individual. Also, there is limited literature available associating the salivary MMP-8 before and after NSPT. Therefore, the current study was done with an aim to estimate salivary MMP-8 levels and correlate it with the PISA before and after NSPT in periodontitis and healthy periodontium.

AIMS AND OBJECTIVES

AIM OF THE STUDY:

Estimation of salivary MMP-8 and its correlation to PISA before and after NSPT

OBJECTIVES:

1. To estimate salivary MMP-8 before and after NSPT in individuals with healthy periodontium and periodontitis
2. To assess PISA before and after NSPT in individuals with healthy periodontium and periodontitis
3. To correlate salivary MMP-8 to PISA before and after NSPT in individuals with healthy periodontium and periodontitis

REVIEW OF LITERATURE

Thomas et al.[16] (2024) measured salivary active MMP-8, advanced glycation end products (AGE), AGE receptors and soluble RAGE in three groups which included individuals with periodontal inflammation and uncontrolled diabetes mellitus (T2DM), patients having periodontal disease, controlled T2DM and periodontal healthy individuals. They collected salivary specimens and recorded PD and CAL. The findings indicated that individuals with uncontrolled T2DM and severe periodontitis exhibited significantly elevated levels of salivary AGE, RAGE and aMMP-8 in comparison to systemically healthy individuals.

Gul et al.[17](2022) conducted a systematic review where baseline MMP-8 levels in GCF were evaluated which can serve as a predictor for the results of NSPT in patients with periodontitis after three months. A total of 5 studies were included which demonstrated substantial variability in baseline GCF MMP-8 levels. The results showed that after three months of NSPT, there was an average PPD reduction of 1.20 mm for pockets that were initially 4 to 6 mm and 2.30 mm for pockets that were larger than 6 mm.

Miller et al.[18] (2021) assessed whether salivary biomarkers could discriminate between periodontally healthy individuals with diabetes and those with both diabetes and periodontitis. Salivary specimens were collected and periodontal parameters were recorded for 92 participants which were categorized into three groups which included 29 participants with T2DM and chronic periodontitis, 32 with T2DM without chronic periodontitis and 31 non-periodontitis controls. Unstimulated saliva was collected and standard periodontal measurements were recorded. Immunoassays quantified the biomarkers and salivary IL-1 β, MMP-8 and resistin were positively related with

periodontitis. MMP-8 showed strong correlation with PD exceeding 5 mm. These results highlight salivary MMP-8 as a promising marker for identifying periodontitis in patients with T2DM.

Ramenzoni et al.[19] (2021) enrolled twenty subjects, allocating ten in periodontitis test group and ten in periodontally healthy group, to identify the source of MMP-8 and lactoferrin and relate their concentrations to clinical findings. Stimulated and unstimulated saliva together with GCF, were collected and examined for MMP-8 and lactoferrin by ELISA. All periodontitis cases showed markedly higher levels of both biomarkers in periodontal pockets. A strong positive association was seen in increasing probing pocket depth and elevated MMP-8 and lactoferrin, indicating that periodontal pockets are the principal reservoir of these molecules.

Hernandez et al.[7] (2021) did a clinico-biochemical trial to compare diagnostic accuracy of an active MMP-8 (aMMP-8) immunoassay with a total MMP-8 (tMMP-8) ELISA for site-specific, real-time grading of periodontitis severity. GCF was collected from 91 volunteers, encompassing 30 healthy sites, 42 sites with mild periodontitis and 59 sites with severe periodontitis. Receiver operating characteristic analysis demonstrated that aMMP-8 was superior in distinguishing healthy from diseased sites, while tMMP-8 differentiated mild from severe periodontitis. These findings indicate that aMMP-8 and tMMP-8 assays can serve as useful adjuncts for diagnosing and staging periodontitis.

Chaparro et al.[20] (2021) investigated the association between the risk of gestational diabetes mellitus (GDM) and the extent of MMP-8 and 9 in GCF. 314 pregnant women with gestational ages ranging from 11 to 14 weeks were involved in this research. Detailed periodontal and obstetric inspections were done as well as GCF

concentrations of MMP-8 and 9 were evaluated using ELISA. GDM was diagnosed in 14% of the participants and women classified with stage III or IV periodontitis showed increased levels of biomarkers than those with stage I disease. This suggests that higher GCF level of biomarkers are linked to more severe periodontitis and an increased risk of GDM in early pregnancy.

Kim et al.[21] (2021) examined the changes in salivary inflammatory cytokines following NSPT by conducting a systematic review of 11 studies. Biomarkers that consistently showed raised concentrations in periodontal disease were MMP-8, IL-6,4,1 and TIMP-2. Following NSPT combined with oral-hygiene instruction, most studies reported decrease in the above salivary biomarkers. Pooled standardized mean differences for MMP-8 and IL-1 were 35.90 and 1.04, respectively; however, the overall differences between healthy controls and periodontitis patients did not show statistical significance. The authors concluded that salivary cytokine profiling may aid in monitoring periodontal therapy outcomes even though post-treatment shifts in IL-1 and MMP-8 were not significant.

Seinost et al.[22] (2020) conducted a study investigating the effect of NSPT in both periodontitis and peripheral artery disorder. In this randomized controlled trial, 90 patients were enrolled and were split into three groups in which 30 received systemic antibiotics and non-surgical periodontal therapy. Another 30 received the same periodontal therapy without antibiotics and the remaining 30 did not receive any periodontal treatment. The outcomes measured were the reduction in vascular inflammation and changes in the PISA three months post-treatment. Substantial enhancement in periodontal health was observed within the treatment groups following a three-month intervention period which highlights the efficacy of NSPT. However, no changes were noted in vascular inflammation in patients.

Fatemi et al.[10] (2020) enrolled 42 participants, allocating them to healthy control group and to generalised moderate-to-severe periodontitis case group. All subjects had their saliva and GCF samples collected and ELISA was used to measure the MMP 8 levels. Both salivary as well as GCF levels of MMP-8 were substantially elevated in the case group, supporting the notion that elevated MMP-8 is associated with severity of periodontitis and may serve as a useful biomarker for the disease.

Zhu et al.[23] (2019) explored the link between MMP-8, β-catenin and disease severity in 21 generalised chronic periodontitis patients and 21 healthy controls. GCF was analysed for MMP-8 concentrations and mRNA expression of beta-catenin was determined. Chronic periodontitis patients showed markedly higher levels of both β-catenin and MMP-8 than controls indicating that these molecules are linked to greater disease severity and may contribute to periodontal disease progression.

Mauramo et al.[24] (2018) carried out a cross-sectional study of 258 Swiss adults to explore the association between salivary and GCF MMP-8 levels and periodontitis. Saliva and GCF samples were taken following comprehensive dental and periodontal examination and IFMA was used to quantify MMP 8. Increased MMP 8 was substantially related with periodontitis, indicating that MMP 8 may be useful in detecting periodontitis suggesting that MMP-8 could help identify the disease in this population.

Zhang et al.[25] (2018) performed a systematic review to assess salivary MMP-8 as a diagnostic marker for periodontitis. 10 eligible studies, comprising 485 cases of periodontitis and 37 healthy controls were analysed. All studies reported increased MMP-8 levels in case group supporting the notion that MMP-8 can be used as a non-invasive indicator of periodontitis.

Rangbulla et al.[26] (2017) evaluated salivary MMP-8, IgA and IL -1β in fifty adults with moderate to severe periodontitis. Unstimulated saliva was collected at baseline and again 12 weeks after oral prophylaxis and routine periodontal indices were recorded at both visits. Post-treatment analysis showed significant reductions in salivary MMP-8, IgA and IL -1β indicating that periodontal therapy lowers inflammatory and immune markers in saliva.

Gupta et al.[27](2015) executed a cross-sectional study with 40 subjects, equally divided into chronic periodontal disease cases and periodontal health. Unstimulated saliva was taken from each participant and MMP-8 concentrations were quantified and compared with CAL, PPD, PI and GI. Elevated salivary MMP-8 levels correlated positively with greater periodontal measurements reinforcing its value as a non-invasive biomarker of periodontal status.

Kraft et al.[28] (2012) evaluated clinical periodontal parameters and active aMMP-8 seen in GCF of 9 individuals diagnosed with generalised chronic periodontitis. GI, PI, PD as well as BOP were recorded and GCF aMMP-8 concentrations varied between 3.2 ng/mL to 23.7 ng/mL. Deeper pockets were consistently associated with higher aMMP-8 levels indicating a direct link between enzymatic activity and disease severity.

Marcaccini et al.[29] (2010) analyzed biomarkers associated within GCF in chronic periodontitis sites and healthy control subjects at initial visit and subsequent 3-month period following NSPT. Levels of TIMP-1 & 2, myeloperoxidase (MPO), MMP-8 & 9 were determined by ELISA. Periodontitis sites initially showed higher concentrations of all the measured biomarkers. Three months after NSPT, MMP-8, TIMP-2 and MPO were significantly reduced, whereas TIMP-1 and MMP-9 remained

largely unchanged, demonstrating that NSPT selectively lowers the key inflammatory mediators.

Gursoy et al.[30] (2010) analysed saliva from 165 periodontitis patients (PD $\geq$ 4 mm) and 81 periodontally healthy controls to assess MMP-8 by ELISA and IFMA was used to assess MMP-14, TIMP-1 and ICTP. IFMA proved to be more sensitive than ELISA for detecting salivary MMP-8. The group with periodontitis displayed significantly elevated levels of biomarkers, confirming these salivary marker's diagnostic value for distinguishing advanced disease from health.

Marcaccini et al.[31] (2009) quantified plasma MMP-8, MMP-3, MMP-9 along with other biomarkers in 28 individuals with chronic periodontitis and 22 healthy subjects both before and three months after NSPT. Initial concentrations of biomarkers were noticeably higher in the periodontitis group. Following therapy, MMP-8 & 9 decreased by 35 % and 39 % respectively, but MMP-3 remained unchanged. These findings highlight the link between circulating MMP activity to periodontitis and showed that NSPT can partially reverse this proteolytic burden.

Kinane et al.[14] (2003) evaluated the influence of SRP on GCF MMP-8 concentrations followed by a 3 month follow up. 20 periodontitis patients were enrolled and clinical parameters together with GCF samples were collected from 4 sites per patient at baseline, immediately after SRP and after the maintenance period of 3 months. MMP-8 levels were quantified by IFMA. MMP-8 levels significantly dropped as a result of SRP and it reduced additionally by 50% post 3 months of SRP.

MATERIALS AND METHODS

SOURCE OF DATA:

The study was done in the Outpatient Department of Periodontics, KAHER's KLE V.K. Institute of Dental Sciences, Belagavi. The laboratory processes were conducted at Dr. Prabhakar Kore Basic Science Research Centre (BSRC), KLE Academy of Higher Education and Research, Belagavi. An ethical clearance was issued from the Ethical Committee, KAHER's KLE V.K. Institute of Dental Sciences, Belagavi before conducting the study.

CRITERIA FOR GROUP SELECTION:

A thorough clinical examination was done using William's graduated probe. The sample population was divided into 2 groups based on proposal elements for inclusion in the classification of periodontitis according to 2017 World Workshop.[32]

Group A (Control): Subjects with healthy periodontium

-Pocket probing depth (PPD) ≤ 3mm

-Bleeding on probing (BOP) < 10%

Group B (Case): Subjects with Periodontitis (Stage III and Stage IV, Grade A)

- Clinical attachment loss (CAL) ≥5 mm

- Probing pocket depth (PPD) ≥6 mm

Before starting, the study was described to the patients in a language they understood and signed written consent was acquired from them. All participants were examined for their periodontal condition. After recording the demographic and clinical data, collection of saliva sample was carried out.

INCLUSION CRITERIA

- Subjects in the age group of 35-55 years.
- Subjects having at least 20 teeth.
- Patients in whom history of any kind of systemic diseases was not present.
- Patients who have not taken periodontal therapy during the previous 6 months.

EXCLUSION CRITERIA

- Subjects with history of smoking and tobacco chewing
- Patients on any kind of medications
- Nursing or pregnant females

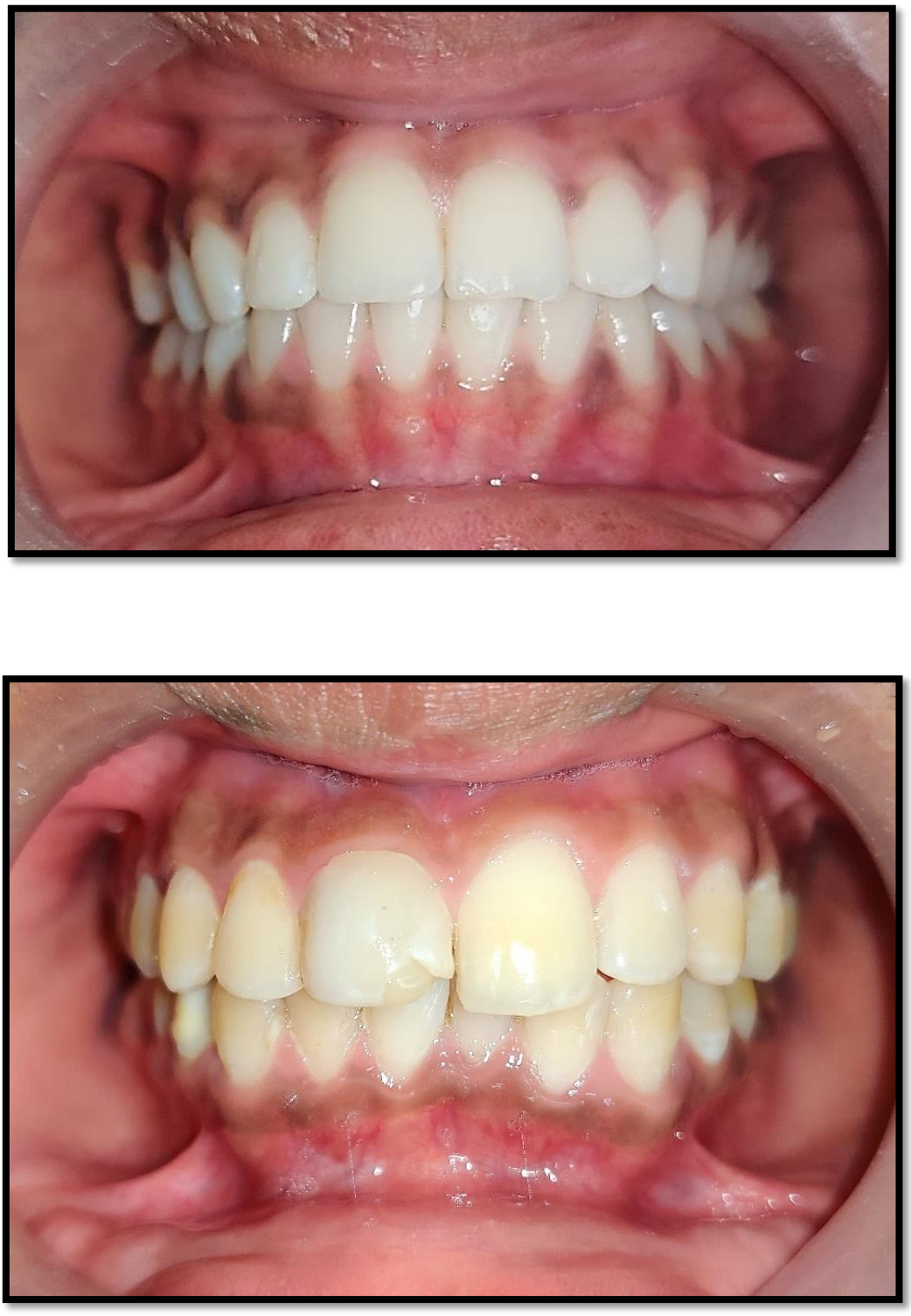


Figure 3: Periodontal health

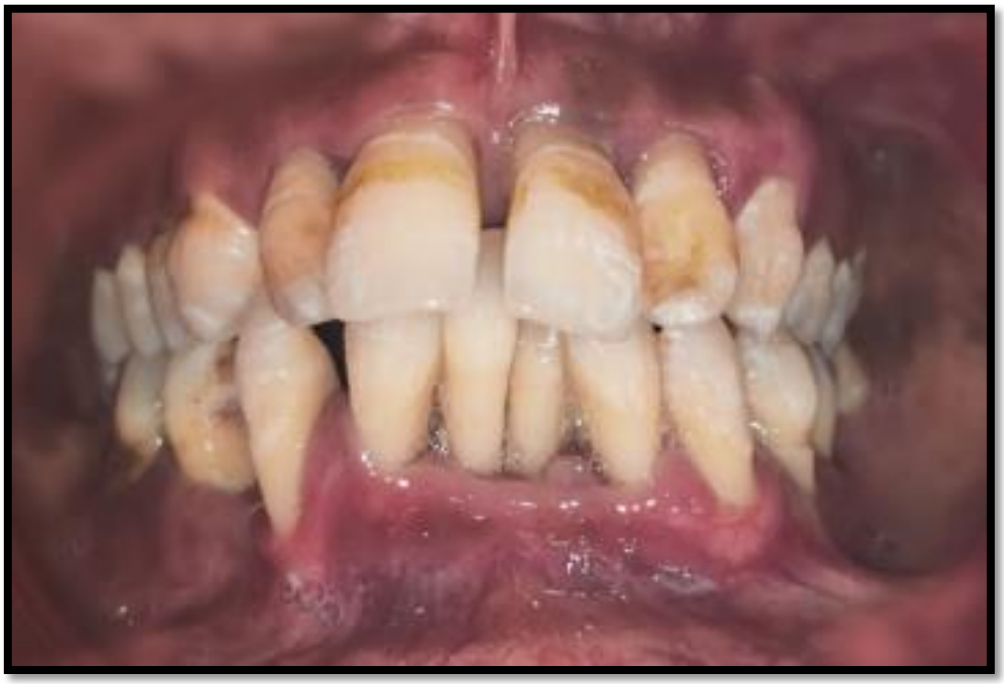


Figure 4: Periodontitis (Stage IV Grade A)

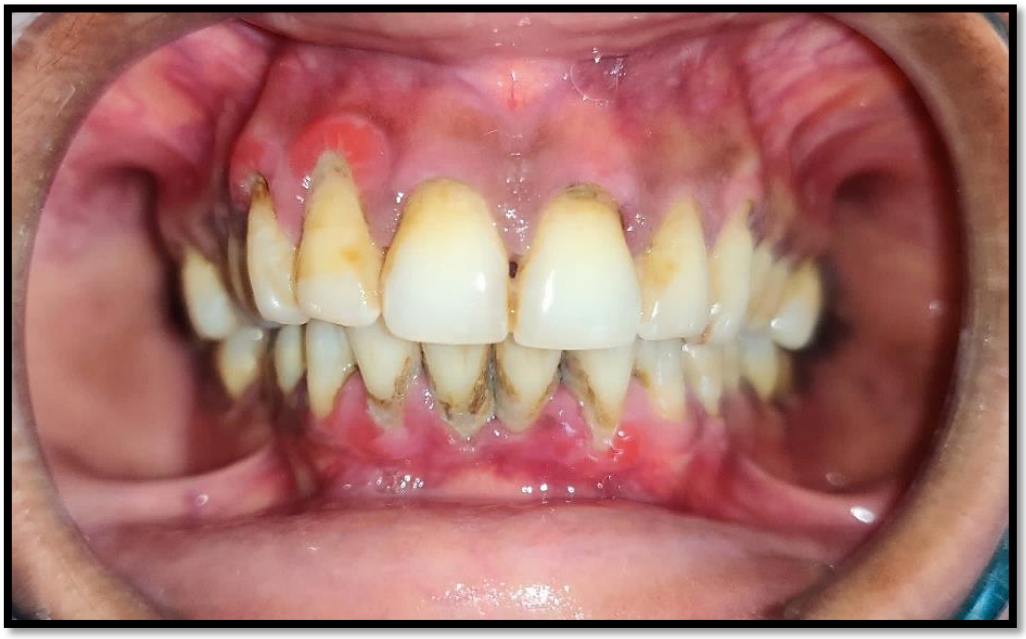


Figure 5: Periodontitis (Stage III Grade A)

CLINICAL ARMAMENTARIUM:

1. Mouth mirror
2. Explorer
3. Straight probes
4. Tweezers
5. Kidney tray
6. Cotton roll and gauze
7. William's graduated periodontal probe
8. Mouth mask
9. Disposable latex gloves
10. Distilled water for rinsing
11. 15 ml falcon tube
12. Ice for transportation to the laboratory

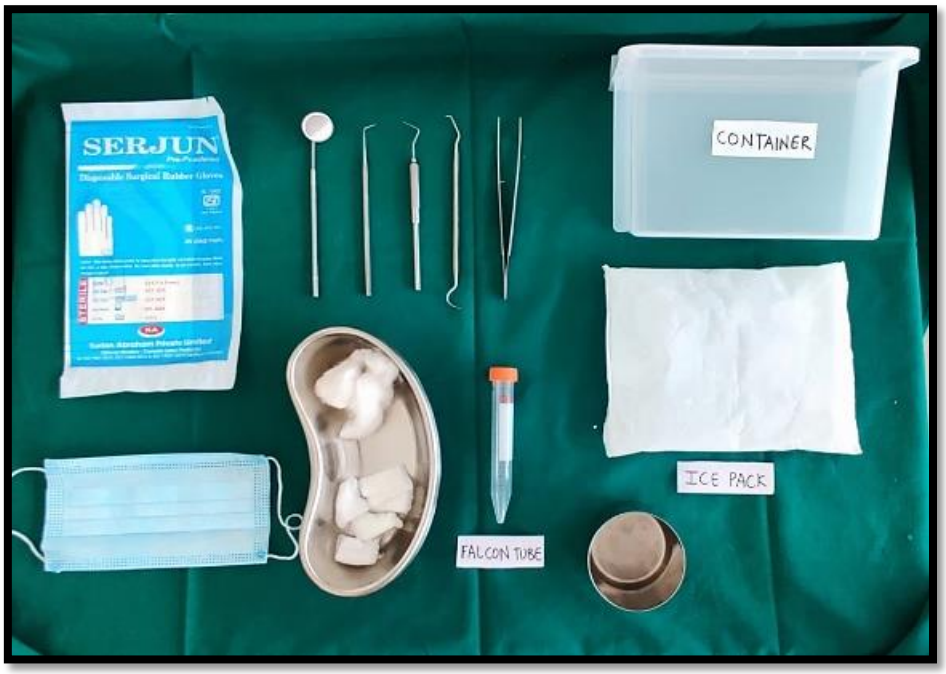


Figure 6: Clinical Armamentarium

PARAMETRES RECORDED:

Following parameters were recorded:

1. Clinical attachment loss
2. Recession
3. BOP
4. PISA

Clinical Attachment Loss (CAL) [33]:

CAL was seen from the CEJ to the base of pocket using a Williams graduated periodontal probe.

A. If the gingival margin was coronally placed (above) to the CEJ, the attachment level was calculated by reducing the length between the margin of gingiva and the CEJ from the PPD.
B. If it was at the level of the CEJ, the attachment level was considered the same as the PPD.
C. The attachment level was calculated by adding the length between the margin of gingiva and the CEJ to PPD if the margin of gingiva was apical or below to the CEJ.

Measurements were done on 6 sites of a particular tooth (Mesio-facial, facial, disto-facial, Mesio-lingual, lingual, disto-lingual).

Recession[33]:

Recession was determined by measuring from CEJ to lower gingival margin (LGM) using a William's graduated periodontal probe. 6 sites of a specific tooth (lingual, disto-lingual, mesio-lingual, facial, disto-facial, and mesio-facial) were measured.

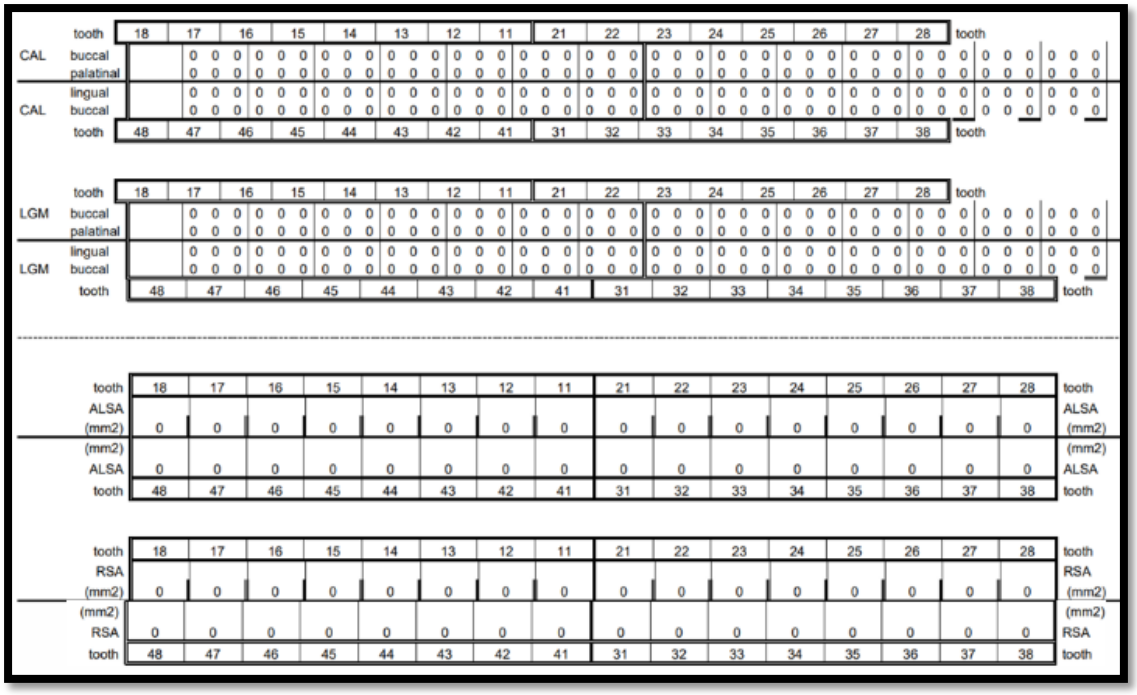
Bleeding on probing[33]:

BOP was determined by inserting a probe between the sulcus and tooth and sites of bleeding were recorded. Six sites of each tooth were recorded.

Estimation of Periodontal Inflamed Surface Area (PISA)[15]:

A Microsoft Excel spreadsheet which is accessible at www.parsprototo.info was used to calculate PISA.

CAL and Recession at 6 sites per tooth were entered and their mean values were calculated by the computer. These linear values were converted to ALSA and RSA per tooth. RSA was subtracted from ALSA to obtain PESA for each tooth. The number of sites affected by BOP was multiplied by PESA to obtain PISA of a particular tooth. Total PISA for an individual was calculated as the sum of all individual PISA values.



ESTIMATION OF SALIVARY MMP-8 USING ELISA KIT (SHANGHAI COON KOON BIOTECH CO., LTD) [34]

LABORATORY ARMAMENTARIUM:

1. -20°C Refrigerator for storage of samples
2. ELISA microplate reader (absorbance at 450 nm)
3. Micropipette
4. Centrifuge machine
5. Laminar air flow
6. Eppendorf tubes of 2 ml
7. 37 ℃ incubator
8. Precision pipettes to deliver 2 ml to 1 ml volumes.
9. 100 ml and 1 litre graduated cylinders.
10. Absorbent paper
11. Precision pipettes and disposable tip
12. Log graph paper standard or sample dilutions

ELISA KIT CONTENTS:

1. 96-well Strip Microplate (Pre-coated)- 1
2. Standard A: 0ng/mL
3. Standard B: 1.25ng/mL
4. Standard C: 2.5ng/mL
5. Standard D: 5ng/mL
6. Standard E: 10ng/mL
7. Standard F: 20ng/mL
8. Diluent: 6.0ml
9. HRP-Conjugate reagent: 10.0ml
10. Wash solution (20X): 25ml
11. Chromogen Sol A: 6.0ml
12. Chromogen Sol B: 6.0ml
13. Stop Sol: 6.0ml
14. Closure plate membrane: 2
15. User manual: 1
16. Sealed bag: 1

Figure 7: -20° C Refrigerator

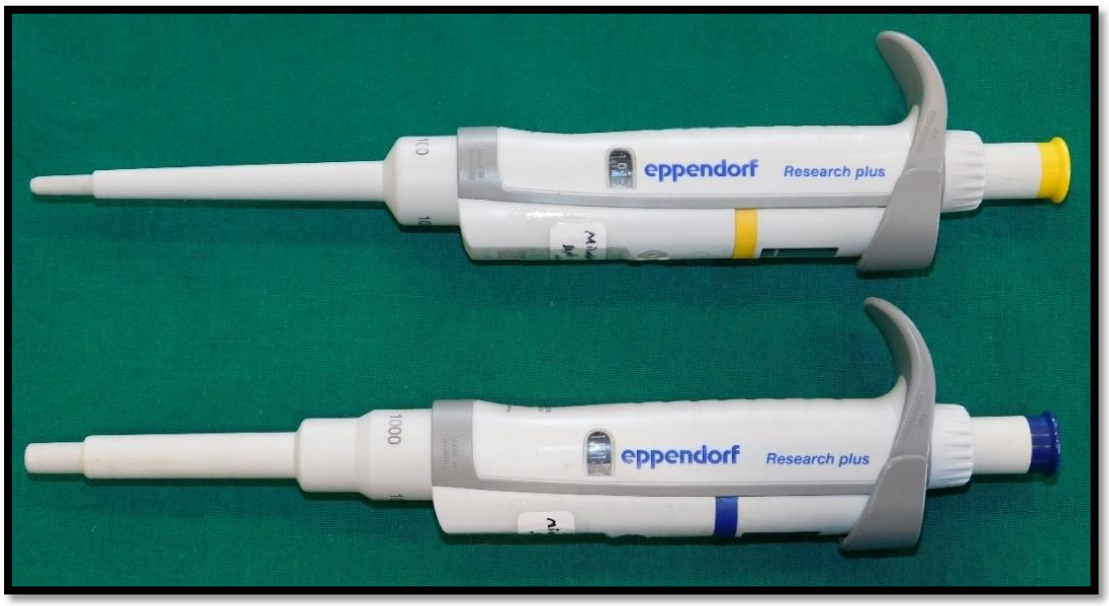


Figure 8: Micropipette

Figure 9: Centrifuge machine

Figure 10: Vortex machine

Figure 11: ELISA microplate reader

Figure 12: ELISA kit contents

PROCEDURE

Collection of Saliva Sample:

The study was described to all patients in the language they understood. Using the spitting technique as outlined by Navazesh[35], unstimulated whole saliva was obtained. Patients were advised not to consume any liquid or solid food substances 1 hour prior to saliva collection. At first patients rinsed their mouth with water. This was followed by expectoration of whole saliva into 15 ml Falcon tube. A final volume of 3 to 5 ml whole saliva was obtained for each patient. Each saliva sample was immediately placed on icepacks for transportation to the laboratory. Until the analysis, the salivary samples were refrigerated at -20° C. Initially, samples were centrifuged for 10 minutes at 1100g in 4°C and then estimation of MMP-8 was done by ELISA kit.

Figure 13: Collection of saliva

ASSAY PROCEDURE:

Estimation of Salivary MMP-8 was done using ELISA kit (Shanghai Coon Koon Biotech Co., Ltd).[34]

1. The standard was introduced into the designated Standard wells and sample wells were tested accordingly. In particular, the well was filled with 50 microliters of standard, while the blank well remained empty.
2. 40 microliters of sample diluent were introduced to the testing sample well after a 10-microliter sample had been deposited inside.
3. Both the Standard and testing sample wells received HRP-conjugate reagent (100µl), and the plate was then sealed with a membrane. After that, the plate was gently stirred and incubated for 1 hr.

4. For later use, the washing solution (20X) was diluted with deionized water to make the washing solution.
5. In the manual washing method, the sealing film was delicately removed, the liquid was drained, and each well was dried. Next, the wells were filled with washing solution, left for 1 minute, drained again, and this process was repeated 5 times before pat drying the plate. Alternatively, for automatic washing, 350µL of wash solution was injected into each well, soaked for 1 minute, and the plate was washed 5 times.
6. Chromogen sol- A (50µL) and sol-B one after the other were added to develop the color. After shaking the plate, it was again incubated for 15 minutes without being exposed to light.
7. Stop Solution (50µL) was introduced to terminate reaction which resulted in sudden color change from blue to yellow. If any wells showed a green color or if the color change was uneven, gentle tapping ensured thorough mixing.
8. The Optical density (OD) was measured for each well separately at 450 nm wavelength during the test, with the blank well serving as the zero point. Within fifteen minutes of the stop solution being added the measurement was taken. The linear regression equation for the standard curve using the standards and their respective OD values were done.

Calculation of results

1. The sample quantity was measured with a standard curve.
2. This curve was made by adding the average O.D. values for every 6 standard concentrations at 450 nm on Y-axis.
3. Subsequently, O.D was adjusted through subtracting the value of zero standard. Generation on graph paper was done using the standard curve.
4. A horizontal line intersected the standard curve, and the O.D. value was placed on the Y axis to verify the quantity in each sample. The comparable concentration was then read by making a vertical line from the crossing point to the X-axis.

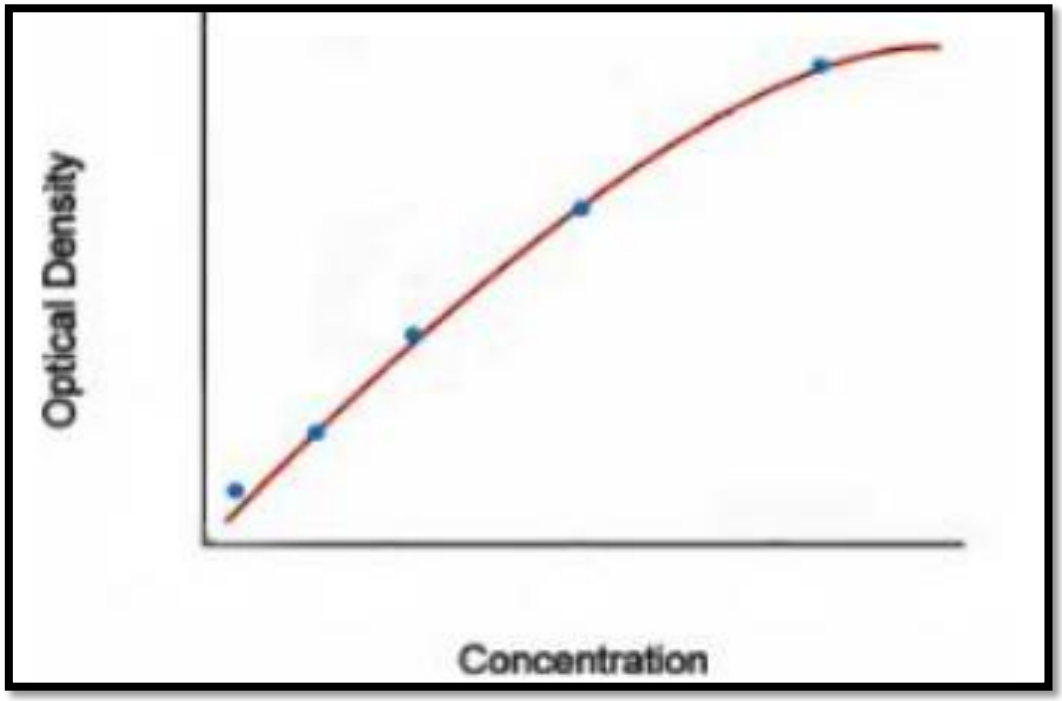


Figure 14: OD value vs Concentration

SAMPLE SIZE ESTIMATION:

At

95 % confidence interval

$$n = \frac{(Z_{1-\alpha/2} + Z_{1-\beta})^2 (SD_1^2 + SD_2^2)}{(x_1 - x_2)^2}$$

n = 19 per group

With 10% attrition, n= 21 per group

The total estimated sample size was 42

SD_1: 143.89

SD_2: 202.10

$Z_{1-\alpha/2}$: 1.96

$Z_{1-\beta}$: 1.64

x_1: 190.91

x_2: 348.76

n: sample size number

α error = 5%

β error = 80%

STATISTICAL ANALYSIS:

- The data was inserted into Excel sheet and SPSS version 20.0 was used to estimate mean and SD for each parameter within each of the two groups.
- Distribution of age and gender was assessed in both groups. Percentage distribution of gender was done for both groups.
- The test done to determine the normality of data was Shapiro-Wilk test after which parametric tests were applied.
- To compare the groups at baseline and after three months, an independent t-test was used.
- Dependent t-test was done for comparison of pre and post NSPT within the groups.
- Association between PISA values with MMP-8 was done using Karl Pearson's Correlation coefficient.
- A value was deemed statistically significant if it was less than or equal to 0.05.

RESULTS

Table 1: Comparison of Group A and Group B with gender

Gender	Group A	%	Group B	%	Total	%
Male	9	42.86	12	57.14	21	50.00
Female	12	57.14	9	42.86	21	50.00
Total	21	100.00	21	100.00	42	100.00

Observation-

1. The frequency percentages of male in Group A were 42.86%, while percentages of female were 57.14%.
2. The frequency percentages of male and female in Group B were 57.14% and 42.86%, respectively.

Table 2: Comparison of Group A and Group B with mean age by independent t test

Group	n	Mean	SD	SE	t-value	P-value
Group A	21	41.43	5.77	1.26		
Group B	21	44.38	7.57	1.65	1.4214	0.1630

Observation-

1. Group A and Group B had mean ages of 41.43 ± 5.77 and 44.38 ± 7.57, respectively, which were not statistically significant (p=0.1630).

Table 3: Normality of baseline and 3 months scores of PISA and MMP-8 in Group A and Group B by Shapiro-Wilk test

Parameters	Times	Groups	Shapiro-Wilk	df	p-value
PISA (mm2)	Baseline	Group A	0.9370	21	0.1870
		Group B	0.9740	21	0.1120
	At 3 months	Group A	0.9720	21	0.7670
		Group B	0.9750	21	0.8340
	Difference	Group A	0.9410	21	0.2270
		Group B	0.9780	21	0.1130
MMP-8 (pg/ml)	Baseline	Group A	0.9770	21	0.8800
		Group B	0.9150	21	0.0700
	At 3 months	Group A	0.9130	21	0.0640
		Group B	0.9340	21	0.1690
	Difference	Group A	0.9660	21	0.6440
		Group B	0.9400	21	0.2190

Observation-

1. The baseline and 3 months score of PISA and MMP-8 values of both groups followed a normal distribution. Thus, the parametric tests were applied.

Table 4: Comparison of Group A and Group B with MMP-8 (pg/ml) scores at baseline and 3 months treatment time points by independent t test

Time points	Group A		Group B		Effect size	t-value	p-value
	Mean	SD	Mean	SD			
Baseline	479.81	140.41	2332.14	163.39	12.19	39.4021	0.0001*
3 months	237.57	100.52	617.95	195.97	2.57	7.9146	0.0001*
Difference	242.24	178.50	1714.19	101.10	10.53	32.8817	0.0001*

*p<0.05

Observation-

1. Group A and Group B had statistically significant mean baseline MMP-8 values of 479.81 ± 140.41 and 2332.14 ± 163.39, respectively. (p=0.0001)
2. Group A and Group B had statistically significant mean MMP-8 values of 237.57 ± 100.52 and 617.95 ± 195.97, respectively at 3 months. (p=0.0001)
3. Group A and Group B had statistically significant mean difference of MMP-8 values at baseline and after 3 months 242.24 ± 178.50 and 1714.19 ± 101.10 respectively. (p=0.0001)

Table 5: Comparison of baseline and 3 months treatment time points with MMP-8 (pg/ml) scores in Group A and Group B by dependent t test

Groups	Time points	Mean	SD	Mean Diff.	SD Diff.	% of effect	t-value	p-value
Group A	Baseline	479.81	140.41					
	3 months	237.57	100.52	242.24	178.50	50.49	6.2190	0.0001*
Group B	Baseline	2332.14	163.39					
	3 months	617.95	195.97	1714.19	101.10	73.50	77.6996	0.0001*

*p<0.05

Observation-

1. The mean MMP-8 values in Group A at baseline was 479.81 ± 140.41 and at 3 months was 237.57 ± 100.52 which showed reduction of 50.49% and was statistically significant. (p=0.0001)
2. Mean MMP-8 values in Group B at baseline was 2332.14 ± 163.39 and at 3 months was 617.95 ± 195.97 which showed reduction of 73.50% and was statistically significant. (p=0.0001)

Table 6: Comparison of Group A and Group B with PISA (mm^2) scores at baseline and 3 months treatment time points by independent t test

Time points	Group A		Group B		Effect size	t-value	p-value
	Mean	SD	Mean	SD			
Baseline	393.86	107.43	1776.33	530.32	4.34	11.7083	0.0001*
3 months	243.80	76.51	798.00	120.78	5.62	17.7623	0.0001*
Difference	150.06	76.33	978.34	456.01	3.11	8.2094	0.0001*

*p<0.05

Observation-

1. Group A and Group B had statistically significant mean baseline PISA values of 393.86 ± 107.43 and 1776.33 ± 530.32, respectively. (p=0.0001)
2. After three months, the statistically significant mean PISA values for Group A were 243.80± 76.51 and Group B were 798.00± 120.78. (p=0.0001)
3. The statistically significant difference between mean PISA values of Group A and Group B at baseline and three months was 150.06 ± 76.33 and 978.34 ± 456.01, respectively. (p=0.0001)

Table 7: Comparison of baseline and 3 months treatment time points with PISA (mm^2) scores in Group A and Group B by dependent t test

Groups	Time points	Mean	SD	Mean Diff.	SD Diff.	% of effect	t-value	p-value
Group A	Baseline	393.86	107.43	150.06	76.33	38.10	9.0089	0.0001*
	3 months	243.80	76.51					
Group B	Baseline	1776.33	530.32	978.34	456.01	55.08	9.8316	0.0001*
	3 months	798.00	120.78					

*p<0.05

Observation-

1. The mean PISA values in Group A at baseline was 393.86 ± 107.43 and at 3 months was 243.80 ± 76.51 which showed reduction of 38.10% and was statistically significant. (p=0.0001)
2. The mean PISA values in Group B at baseline was 1779.33 ± 530.32 and at 3 months was 798.00 ± 120.78 which showed reduction of 55.08% and was statistically significant. (p=0.0001)

Table 8: Correlation between PISA (mm^2) and MMP-8 (pg/ml) in two groups by Karl Pearson's correlation coefficient

Group	PISA (mm2)	Summery	MMP-8 (pg/ml)		
			Baseline	At 3 months	Difference
Group A	Baseline	r-value	-0.1730	0.3394	-0.3272
		p-value	0.4530	0.1320	0.1480
	3 months	r-value	-0.1643	0.4122	-0.3613
		p-value	0.4770	0.0630	0.1080
	Difference	r-value	-0.0788	0.0645	-0.0983
		p-value	0.7340	0.7810	0.6720
Group B	Baseline	r-value	0.7347	0.5985	0.0274
		p-value	0.0001*	0.0040*	0.9060
	3 months	r-value	0.6563	0.6299	-0.1604
		p-value	0.0010*	0.0020*	0.4870
	Difference	r-value	0.6807	0.5292	0.0743
		p-value	0.0010*	0.0140*	0.7490

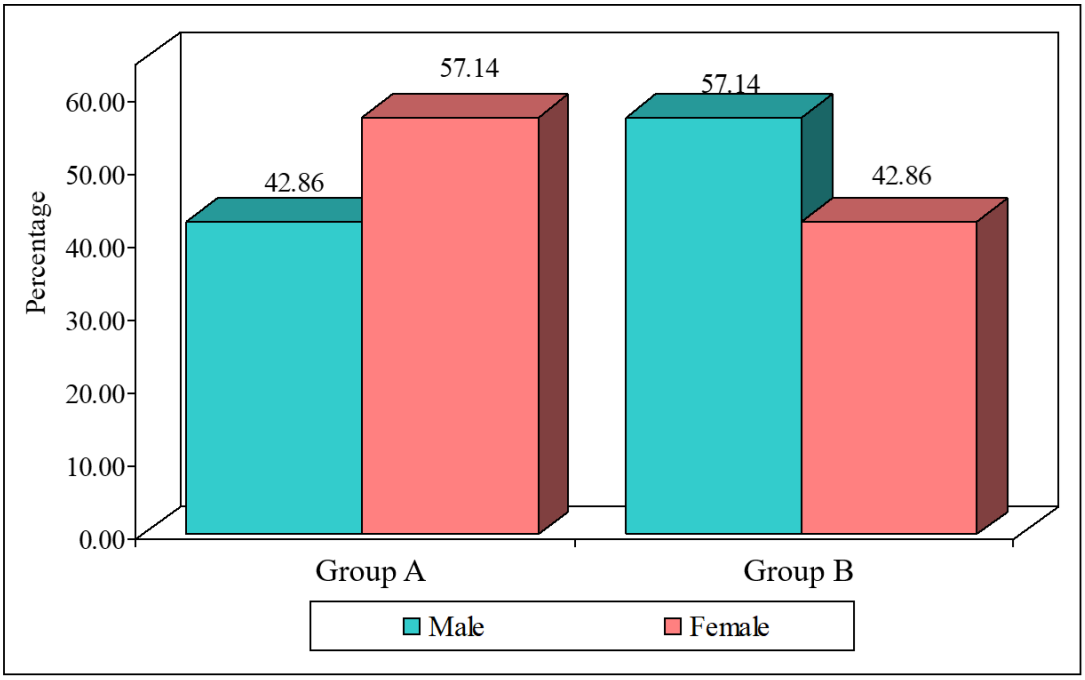
*$p<0.05$

Observation-

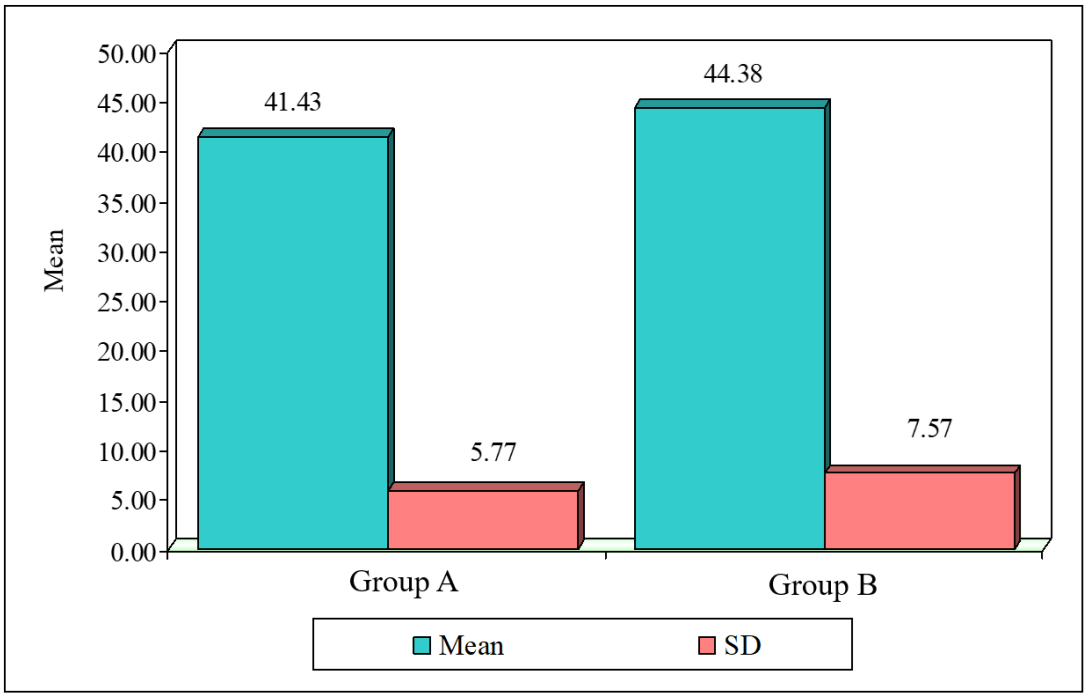
1. MMP-8 levels and mean PISA scores at baseline and three months showed no correlation in Group A.
2. MMP-8 levels and mean PISA scores at baseline and three months had a positive correlation in Group B and were statistically significant.

GRAPHS

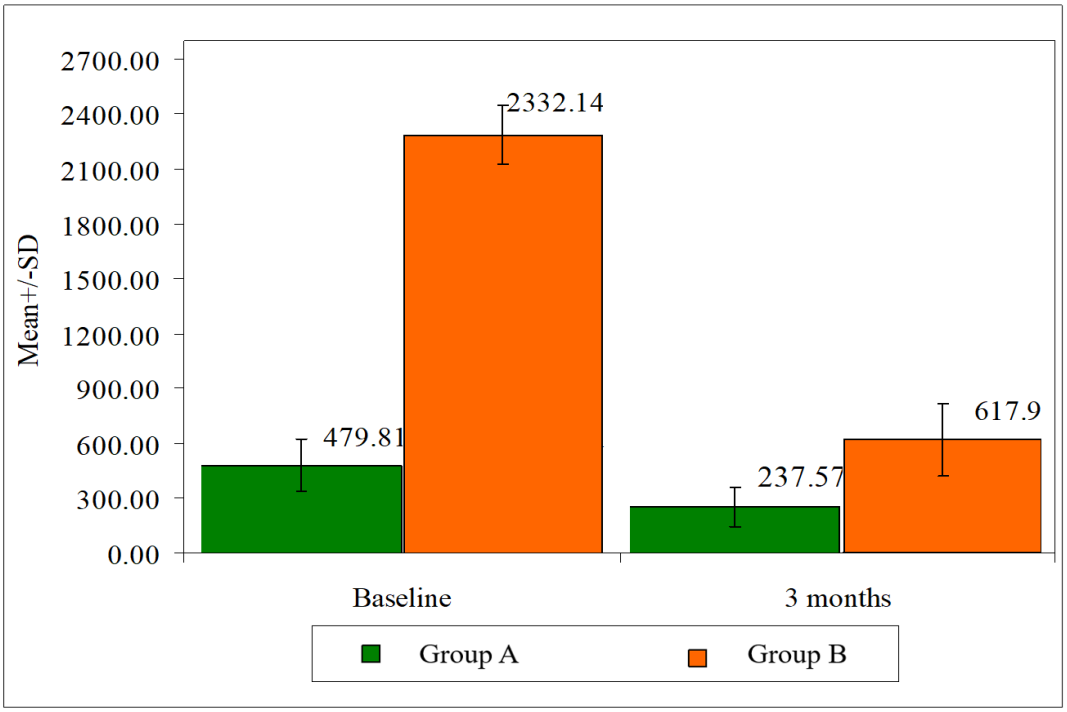
Graph 1: Comparison of Group A and Group B with gender



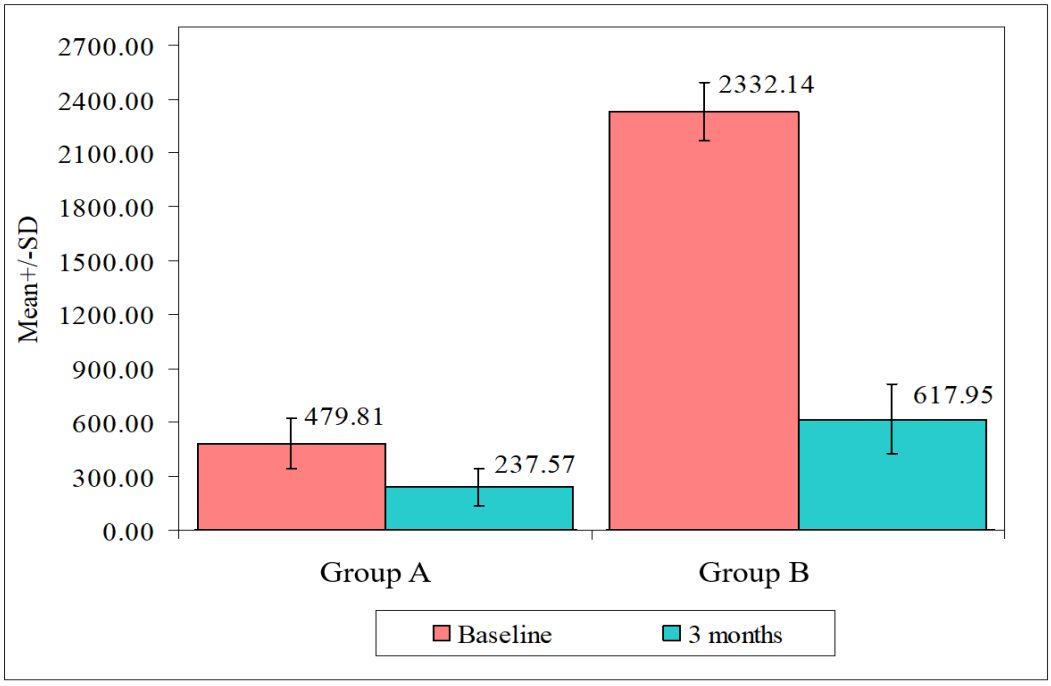
Graph 2: Comparison of Group A and Group B with mean age



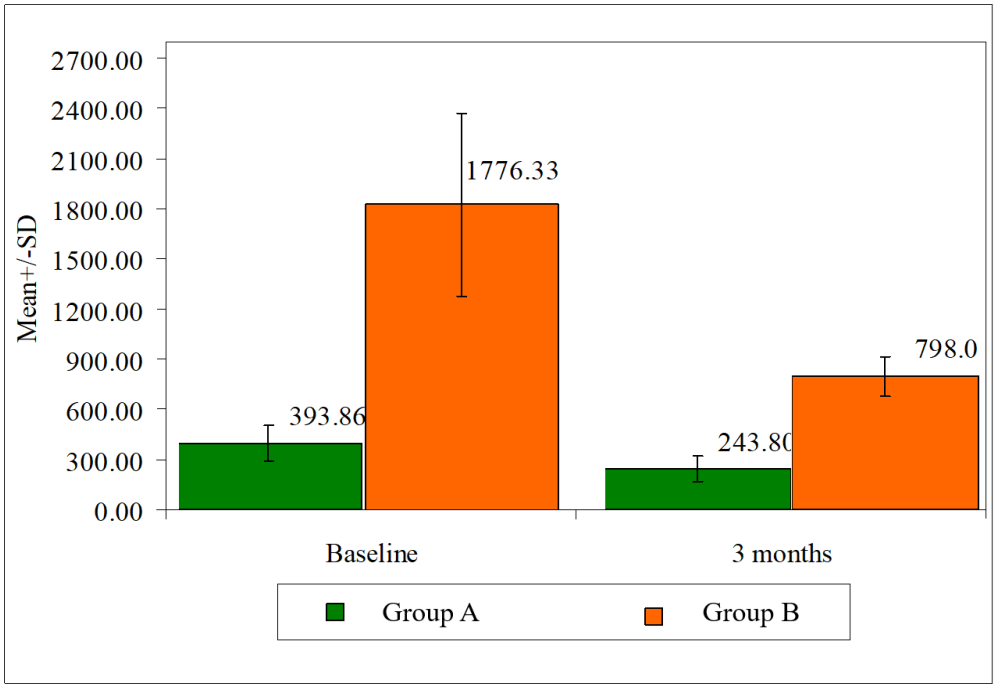
Graph 3: Comparison between Group A and Group B with MMP-8 (pg/ml) scores at baseline and 3 months treatment time points



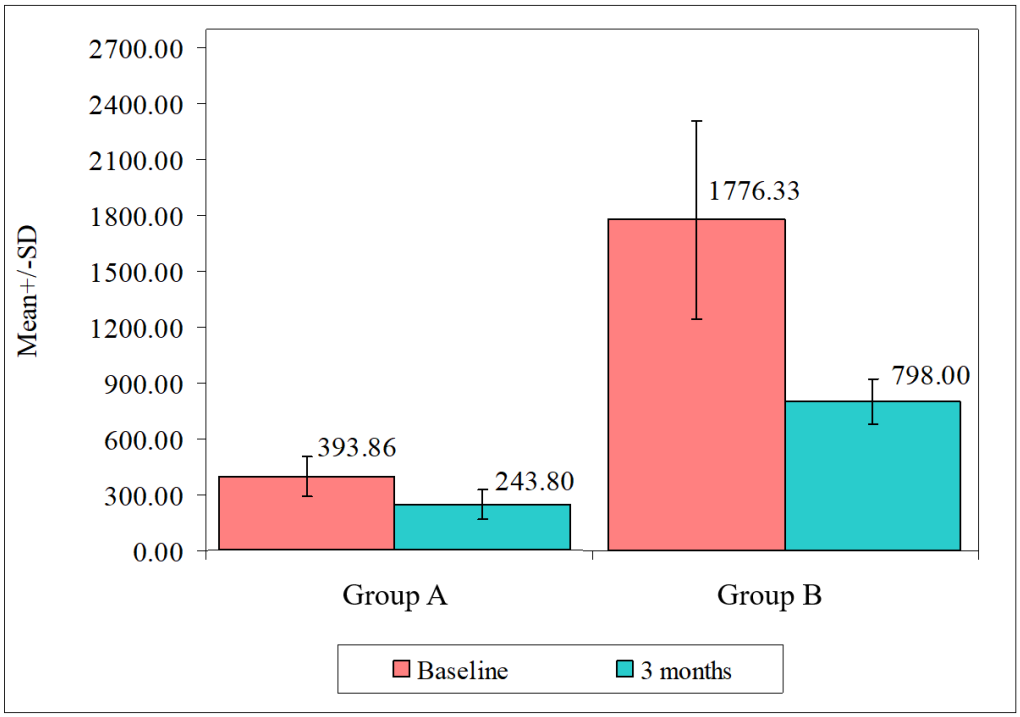
Graph 4: Comparison of MMP-8 (pg/ml) scores with Group A and Group B at baseline and 3 months



Graph 5: Comparison between Group A and Group B with PISA (mm^2) scores at baseline and 3 months treatment time points



Graph 6: Comparison of PISA (mm^2) scores with Group A and Group B at baseline and 3 months



DISCUSSION

Periodontal tissue destruction results from a complex interplay between microbial components derived from immune response of the host and dental plaque. Endotoxins released by pathogenic bacteria are known to trigger an inflammatory cascade by activating immune effector mechanisms. This activation leads to the release of biologically active mediators which increases vascular permeability and promotes neutrophil chemotaxis. These early inflammatory changes often result in edema and enhanced gingival permeability allowing deeper penetration of bacterial antigens and greater stimulation of the immune system. Neutrophils in their defence against bacterial invasion releases protein degrading enzymes such as MMP-8 along with MMP-9 and 3 during phagocytosis contributing to the degradation of periodontal tissues. With persistent microbial challenge the inflammatory condition becomes chronic leading to progressive tissue breakdown. [2,3]

The current interventional study was undertaken to assess and correlate MMP-8 and PISA levels in individuals with healthy periodontium and periodontitis from baseline to 3 months after NSPT. The study enrolled 42 participants who were systemically healthy and categorized into two groups according to 2017 World Workshop classification.[32] Group A consisted of individuals of healthy periodontium and Group B included patients with periodontitis (Stage III and IV, Grade A). PISA values and salivary MMP-8 values were recorded at baseline after which NSPT was carried out. Patients were recalled after 3 months of NSPT for follow up and recording of parameters to be tested.

The demographic variables with respect to gender is depicted in (Table1, Graph 1) showing equal distribution of males and females for the total sample size tested for

the two groups. The mean age in healthy group was 41.43 ± 5.77 and in periodontitis was 44.38 ± 7.57 which showed no statistical significance (p=0.1630). (Table 2, Graph 2)

The mean salivary MMP-8 levels at baseline in periodontal health was 479.81 ± 140.41 pg/ml and at 3 months was 237.57 ± 100.52 pg/ml where as in periodontitis group MMP-8 levels were 2332.14 ± 163.39 pg/ml and 617.95 ± 195.97 pg/ml at baseline and 3 months respectively in the present study. On intergroup evaluation, significant difference was noted in the values. ($p<0.05$) (Table 4, Graph 3) According to intragroup analysis, MMP-8 levels significantly decreased in both groups from baseline to 3 months but greater reduction (73.5%) was seen in periodontitis group in comparison to the healthy group (50.49%) as depicted in (Table 5).

Increased BOP and PPD were linked to raised values of MMP-8 in saliva and GCF according to a number of cross-sectional studies which compared the MMP-8 levels in patients showing signs of clinical periodontal inflammation to controls. [7, 8, 19, 24, 26] A study done by Gupta et al.[27] demonstrated that MMP-8 in saliva among periodontitis patients were 348.76 ± 202.1 ng/ml and in healthy individuals were 190.91 ± 143.89 ng/ml which was statistically significant in periodontitis cases. The outcomes of this study are in congruence with the above results where healthy group had lower levels of MMP-8 as compared to periodontitis. Salivary MMP-8 concentrations along with TIMP-1 and ICTP were reduced in health as compared to periodontitis patients as reported by Gursoy et al[30] and similar observation was seen in the present study. In another research by Fatemi et al.[10] salivary and GCF MMP-8 levels were assessed in patients with generalized moderate to severe chronic periodontitis and in health. The periodontitis group exhibited MMP-8 concentrations approximately 3.6 times more than those of healthy individuals. The increased MMP-

8 levels observed during active periodontal disease are likely to result from increased infiltration of inflammatory cells. When exposed to bacterial lipopolysaccharides, these cells are known to produce proinflammatory cytokines including TNF-α and IL-6. An imbalance between lytic enzymes and natural inhibitors results from this inflammatory reaction, which accelerates the development of periodontitis and degrades connective tissue.

In research done by Rangbulla et al.[26] salivary MMP-8 was assessed before and after oral prophylaxis in periodontitis patients and healthy group. The results inferred that significant decrease in salivary MMP-8 level was seen in moderate to severe periodontitis after 12 weeks of oral prophylaxis which are in alignment to the results of our study. Comparable to the findings of this investigation, Kinane et al.[14] showed that MMP-8 in GCF decreased by more than 50% following NSPT over a three-month maintenance period. Similar observations were seen in a study done by Marcaccini et al.[29] demonstrating that post-therapy there was a reduction in local inflammatory factors such as MMP-8 along with MMP-9, MPO and TIMP-2 at 3 months. This highlights the positive impact of NSPT which is demonstrated by a significant reduction in MMP-8 levels indicating effective control of inflammation and further periodontal tissue degradation.

The mean PISA scores at baseline for healthy group was 393.86 ± 107.43 mm^2 and 3 months post therapy was 243.80 ± 76.51mm^2 whereas in periodontitis group at baseline and 3 months the values were 1779.33 ± 530.32 mm^2 and 798.00 ± 120.78 mm^2 respectively. The results of the intergroup analysis revealed statistical significance with p-value below 0.05. (Table 6, Graph 5). According to CDC-AAP classification,[36] the PISA values ≥130.33 mm^2 indicated chronic periodontitis and values between 934.71 mm^2 and 3,274.96 mm^2 indicated severe periodontitis. In our

study, periodontitis group showed PISA values between 1200 mm^2 to 2649 mm^2 which falls under the above-mentioned range.

On intragroup analysis, a significant lowering in PISA values was seen in the two groups from baseline to 3 months with periodontitis group showing greater reduction (55%) as compared to the healthy group (38%) (Table 7). This outcome suggests that NSPT is particularly effective in reducing the inflammatory burden in individuals with active periodontal disease. Our results align with those reported by Seinost et al.[22], who demonstrated a decrease in PISA values in patients receiving NSPT in combination with systemic antibiotics compared to those who did not undergo NSPT. This supports the notion that periodontal therapy especially when targeted towards individuals with higher baseline inflammation can substantially lower the ulcerated and inflamed periodontal surface area thereby reducing the risk of systemic inflammatory dissemination.

As far as we are known, this is the first study to investigate the correlation between MMP-8 and PISA levels from baseline to 3 months following NSPT. A positive association was observed between MMP-8 and PISA values in the periodontitis group from baseline to 3 months follow up. (Table 8) This could be due to the reason that MMP- 8 has been well documented as an oral biomarker for periodontitis [7, 8, 19, 24, 26] PISA and MMP-8 and 9 values seemed to be associated in a study done by Shimizu et al.[37], while a negative association was noted with TIMP-2. The positive correlation suggests that elevated PISA values imply an increase in inflammatory burden, which is correlated with increased production of MMP-8, hence accelerating the progression of periodontal destruction. The decrease in MMP-8 and PISA values indicated that therapeutic intervention effectively reduces the inflammatory burden and the associated periodontal tissue degradation.

However, in the healthy group no correlation was noted between MMP-8 and PISA values. This finding can be attributed to the minimal inflammatory burden seen in periodontal health thereby leading to minimal degradation of ECM and less ulcerated epithelium which could have reflected in the negative correlation of statistical changes for MMP-8 and PISA values at baseline and 3 months post NSPT.

LIMITATIONS AND SCOPE FOR FUTURE RESEARCH

The study has the following limitations-

1. The study was limited by a small sample size
2. Gingivitis group was not included in the study.
3. As periodontitis is a complex and multifactorial disease there is a need to study a panel of inflammatory mediators and correlate with PISA which can reflect different stages of disease.

More research is necessary in future to check if the expression levels of MMP-8 and PISA are affected by the Grade classification which includes smoking and diabetes or other systemic diseases. Also, longitudinal studies with larger sample size are recommended where gingivitis group can be taken in consideration.

SUMMARY AND CONCLUSION

The present study enrolled a total of 42 participants who were categorized into periodontal health and severe periodontitis (Stage III and IV Grade A). After detailed periodontal examination PISA scores were recorded and the patients were subjected for collection of baseline saliva samples. The samples were transferred to Dr. Prabhakar Kore Basic Science Research Centre (BSRC), KLE Academy of Higher Education and Research, Belagavi for estimation of MMP-8 which was done using an ELISA kit (Shanghai Coon Koon Biotech Co., Ltd). Following this NSPT was performed on all patients. After 3 months, subjects were recalled for re-evaluation and PISA scores were recorded and saliva was collected to assess salivary MMP-8 levels. These values were compared with the baseline values.

The findings of this study demonstrated that MMP-8 levels were greater in periodontitis group and post NSPT there was reduction in MMP-8. Similarly, PISA scores were greater in periodontitis group and reduction was seen post NSPT. A positive correlation was noted in MMP-8 and PISA values in periodontitis group. This association indicates that MMP-8 and PISA can serve as diagnostic measure to indicate the inflammatory process associated with periodontitis.

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ANNEXURES

ANNEXURES- I: ETHICAL CLEARANCE CERTIFICATE

Research and Ethics Committee
KLE VK INSTITUTE OF DENTAL SCIENCES
A Constituent Unit of KLE Academy of Higher Education & Research
Accredited 'A' Grade by NAAC Placed in Category 'A' by MHRD (GoI)
Nehru Nagar, Belagavi - 590 010, Karnataka State
☎: 0831-2470362 Web: http://www.kledental-bgm.edu.in
FAX: 0831-2470640 E-mail:principal@kledental-bgm.edu.in

Sl. No. : 1640

CERTIFICATE

This is to Certify that the synopsis titled

Estimation of Salivary Matrix Metalloproteinase-8 (MMP-8) and its correlation to Periodontal Inflamed Surface area (PISA) before and after Non Surgical Periodontal Therapy *Submitted by*

Dr. REG NO. IK0222004 *P. G. Student /*

Staff, Guided by _____ *from Department of*

Periodontics *has been critically evaluated by committee members and granted ethical clearance to conduct the above mentioned study*

Date : 8/4/21

Member Secretary
Research and Ethical Committee
KLEVK Institute of Dental Sciences
Belagavi

Chairman
Research and Ethical Committee
KLEVK Institute of Dental Sciences
Belagavi

MEMBER SECRETARY
Research & Ethical Committee
KLEVK Institute of Dental Sciences
BELAGAVI

Chairman
Research and Ethical Committee
KLE VK Institute of Dental Sciences
Belgaum

ANNEXURE- II: BIOSTATISTICIAN CERTIFICATE

KAHER's K L E
VISHWANATH KATTI
INSTITUTE OF DENTAL SCIENCES
(Constituent College of K.L.E. University, Belgaum)
J.N.M.C. Campus, Nehru Nagar, Belgaum-590 010, Karnataka, India
☎: 0831-2470362
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Biostatistics Clearance Certificate

This is to certify that Biostatistics aspect of the Dissertation/Research work of **REG NO. IK0222004** **Graduate Student**, under the guidance of **Professor and Head, Department of Periodontics**, entitled **"Estimation of salivary matrix metalloproteinases- 8 (MMP-8) and its correlation to periodontal inflamed surface area (PISA) before and after non-surgical periodontal therapy."** has been done under my guidance and completed satisfactorily.

Place: Belagavi
Date: 20|3|2025

Name & Signature of Biostatistician

Dr. S. B. Javali. Ph.D.
Professor in Statistics
Department of Community Medicine
USM KLE International Medical Programmes
BELAGAVI-590010

ANNEXURE- III- PLAGIARISM REPORT

Scientific Correspondence and Review Committee

KLE VK Institute of Dental Sciences
A Constituent Unit of KLE Academy of Higher Education and Research
(Deemed-to-be-University u/s 3 of the UGC Act, 1956)
Nehru Nagar, Belagavi - 590 010, Karnataka State
Accredited 'A+' Grade by NAAC (3rd Cycle) Placed in Category 'A' by MHRD (GoI)
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Date : 18/04/2025 Serial No. : 419

PLAGIARISM CHECK REPORT

Name of the Applicant : 1 **REG NO. IK0222004**
UG / PG / Ph.D / Staff : PG
Batch & Year : 2022-2025
Department : Department of Periodontics

The soft copy of Research Work / Manuscript by . **REG NO. IK0222004** .. entitled
" Estimation of salivary MMP-8 (Matrix metalloproteinases) and its corelation to PISA (Periodontal Inflamed Surface Area) before & after non-surgical Periodontal therapy "
under the guidance of ....has been submitted for Anti-Plagiarism check to the Scientific Correspondence & Review Committee of KLE VK Institute of Dental Sciences using "Turn-it-in" software.

The scan has been carried out and the scanned output reveals a Similarity Index of .....6.....%, which is ✓**within / not within** the acceptable limits of 10% as per the UGC guidelines.

18/4/2025

Member Secretary
Scientific Correspondence and Review Committee
KLEVK Institute of Dental Sciences
KAHER-Belagavi

Chairman
Scientific Correspondence and Review Committee
KLEVK Institute of Dental Sciences
KAHER - Belagavi

ANNEXURE-IV- CONSENT FORM

CONSENT FORM

DEPARTMENT OF PERIODONTICS

KLE V.K. INSTITUTE OF DENTAL SCIENCES

BELAGAVI.

ESTIMATION OF SALIVARY MMP-8 LEVEL AND ITS CORRELATION TO PERIODONTAL INFLAMED SURFACE AREA (PISA) BEFORE AND AFTER NON-SURGICAL PERIODONTAL THERAPY

Principal Investigator: **REG NO. IK0222004**

I _____, aged _____ years have been informed about my involvement in the study.
I agree to give my personal details like Name, Age, Gender, Residential Address, past and Present dental history and any other details if required for the study to the best of my knowledge.
I will co-operate with the dentist.
I will follow the instructions given by the dentist during study.
I permit the dentist to utilize the information given by me and the results obtained from this study for presentation and publication without disclosing my identity.
I have been informed that saliva sample will be collected, which will be used for the study, and non-surgical intervention such as scaling and root planing will be performed. I permit the dentist to perform the same.
If by chance any complications arise during the above said procedure, I permit the dentist to take necessary actions to prevent the same.
In my full consciousness and presence of mind, after understanding all the procedures and related complications if any, in my vernacular language, I am willing and give my consent to participate in this study.

Date:

Name of the Patient:

Signature:

Address & Ph. No:

Name of witness/guardian:

Signature:

DEPARTMENT OF PERIODONTICS
KLE V.K. INSTITUTE OF DENTAL SCIENCES
BELAGAVI.

ESTIMATION OF SALIVARY MMP-8 LEVEL AND ITS CORRELATION TO PERIODONTAL INFLAMED SURFACE AREA (PISA) BEFORE AND AFTER NON-SURGICAL PERIODONTAL THERAPY

Principal Investigator: **REG NO. IK0222004**

मी, _____ , वय _____ वर्षे, मला ह्या अभ्यासाबद्दल पूर्ण कल्पना देण्यात आली आहे.

मी माझी वैयक्तिक माहिती ज । सध्याची दंत उपचाराची माहिती व अन्य तपशील देण्यास सहमत आहे.

मी दंत चिकित्सकांना त्यांच्या अभ्यासासाठी पूर्ण सहकार्य करेन.

दंतचिकित्सकांचा अभ्यास चालू असताना, मी त्यांनी दिलेल्या सर्व सूचनांचे पालन करेन.

दंत चिकित्सकांच्या अभ्यासदरम्यान त्यांनी प्राप्त केलेली माझी सर्व माहिती व अभ्यासाचे परिणाम माझी ओळख लपवून कुठल्याही प्रकाशनात सादर करायला माझी परवानगी आहे.

मला कळविण्यात आले आहे की माझ्या लाळेचा नमुना घेतला जाईल, जो अभ्यासासाठी वापरण्यात येईल आणि स्केलिंग व रूट प्लेनिंग प्रक्रिया केली जाईल. मी दंतवैद्याला हे करण्याची परवानगी देते/देतो.

वर दिलेल्या प्रक्रियेत, जर कधी चुकून काही झालच तर मी दंत चिकित्सकांना योग्य तो उपाय करण्याची परवानगी देत आहे.

मी पूर्ण शुद्धीत व माझ्या मनाच्या जागृत अवस्थेत, सर्व प्रक्रिया व त्यांचे क्वचित होऊ शकणारे दुष्परिणाम समजून, माझ्या मातृभाषेत ह्या अभ्यासात सहभागीहोण्यास संमती देतो/देते.

तारीख :-

पत्ता व दूरध्वनी क्रमांक :-

स्वाक्षरी :-

पत्ता आणि फोन नंबर :-

साक्षीदार/पालकाचे नाव :-

स्वाक्षरी :-

DEPARTMENT OF PERIODONTICS

KLE V.K. INSTITUTE OF DENTAL SCIENCES

BELAGAVI.

ESTIMATION OF [illegible] AND ITS CORRELATION TO PERIODONTAL INFLAMED SURFACE AREA (PISA) BEFORE AND AFTER NON-SURGICAL PERIODONTAL THERAPY

Principal Investigator: **REG NO. IK0222004**

ನಾನು _____ ವಯಸ್ಸಿನ _____ ವರ್ಷಗಳ ಅಧ್ಯಯನದಲ್ಲಿ ನನ್ನ ತೊಡಗಿರುವ ಬಗ್ಗೆ ಮಾಹಿತಿ ಮಾಡಲಾಗಿದೆ.

ನಾನು ಹೆಸರು, ವಯಸ್ಸು, ಲಿಂಗ, ವಸತಿ ವಿಳಾಸ, ಹಿಂದಿನ ಮತ್ತು ಪೆರಿಯೊಂಟ್ ಹಲ್ಲಿನ ಇತಿಹಾಸ ಮತ್ತು ನನ್ನ ಜ್ಞಾನದ ಅತ್ಯುತ್ತಮ ಅಧ್ಯಯನಕ್ಕೆ ಬೇಕಾಗುವ ಯಾವುದೇ ಇತರ ವಿವರಗಳು ಹಾಗೆ ನನ್ನ ವೈಯಕ್ತಿಕ ವಿವರಗಳನ್ನು ನೀಡಲು ಒಪ್ಪುತ್ತೀರಿ.

ದಂತವೈದ್ಯ ನಾನು ಕಾಣಿಸುತ್ತದೆ ಸಹಕಾರ.

ನಾನು ಅಧ್ಯಯನದ ಸಮಯದಲ್ಲಿ ದಂತವೈದ್ಯ ನೀಡಿದ ಸೂಚನೆಗಳನ್ನು ಅನುಸರಿಸಿ.

ನಾನು ನನ್ನ ಗುರುತನ್ನು ಬಹಿರಂಗಪಡಿಸದೇ ನೀಡಿದ ಮಾಹಿತಿ ಮತ್ತು ಪ್ರಸ್ತುತಿ ಮತ್ತು ಪ್ರಕಟಣೆಗೆ ಈ ಅಧ್ಯಯನದಿಂದ ಪಡೆದ ಫಲಿತಾಂಶಗಳನ್ನು ಬಳಸಿಕೊಳ್ಳಲು ದಂತವೈದ್ಯ ಅನುಮತಿ.

ಲಾಲಾರಸದ ಮಾದರಿಯನ್ನು ಸಂಗ್ರಹಿಸಲಾಗುವುದು ಎಂದು ನನಗೆ ತಿಳಿಸಲಾಗಿದೆ, ಅದನ್ನು ಅಧ್ಯಯನಕ್ಕಾಗಿ ಬಳಸಲಾಗುತ್ತದೆ ಮತ್ತು ಸ್ಕೇಲಿಂಗ್ ಮತ್ತು ರೂಟ್ ಪ್ಲಾನಿಂಗ್‌ನಂತಹ ಶಸ್ತ್ರಚಿಕಿತ್ಸಕವಲ್ಲದ ಹಸ್ತಕ್ಷೇಪವನ್ನು ನಡೆಸಲಾಗುತ್ತದೆ. ನಾನು ದಂತವೈದ್ಯರಿಗೆ ಅದೇ ರೀತಿ ಮಾಡಲು ಅನುಮತಿ ನೀಡುತ್ತೇನೆ.

ನಾನು ಯಾವುದೇ ಸಂಸ್ಥೆ ಪ್ರಾಯೋಜಿಸುತ್ತಿದೆ ಮಾಡಲಾಗುತ್ತಿದೆ ಸಹ, ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಸಹಕಾರವನ್ನು ಯಾವುದೇ ಆದಾಯ ಮಾತನ್ನಾಡುವುದಿಲ್ಲ. ನನ್ನ ಸ್ವಂತ ಇಚ್ಛೆಯ ಮತ್ತು ಇಚ್ಛೆ ಭಾಗವಹಿಸುವ ನಾನು.

ಯಾವುದೇ ಕಾರಣಕ್ಕೆ ನಾನು ಅಪರಿಚಿತ ಕಾರಣಗಳಿಗಾಗಿ, ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ಸಾಧ್ಯವಾಗುವುದಿಲ್ಲ ಏಕೆ ವೇಳೆ, ನಾನು ಸಮಯ ಯಾವುದೇ ಹಂತದಲ್ಲಿ ಅಧ್ಯಯನದಿಂದ ಹಿಂದಕ್ಕೆ.

ನನ್ನ ಪೂರ್ಣ ಪ್ರಜ್ಞೆ ಮತ್ತು ಮನಸ್ಸಿನ ಉಪಸ್ಥಿತಿಯಲ್ಲಿ, ನನ್ನ ಸ್ಥಳೀಯ ಭಾಷೆಯಲ್ಲಿ ಎಲ್ಲಾ ಕಾರ್ಯವಿಧಾನಗಳು ಮತ್ತು ಸಂಬಂಧಿತ ತೊಡಕುಗಳು ಇದ್ದಲ್ಲಿ, ಅದನ್ನು ಅರ್ಥಮಾಡಿಕೊಂಡ ನಂತರ, ನಾನು ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ಸಿದ್ಧನಿದ್ದೇನೆ ಮತ್ತು ನನ್ನ ಸಮ್ಮತಿಯನ್ನು ನೀಡುತ್ತೇನೆ

ದಿನಾಂಕ:

ದಂತವೈದ್ಯ ಹೆಸರು: ಸಹಿ:

ರೋಗಿಯ ಹೆಸರು: ಸಹಿ:

ವಿಳಾಸ ಮತ್ತು ದೂರವಾಣಿ ಸಂಖ್ಯೆ.:

ಸಾಕ್ಷಿ / ಪೋಷಕರ ಹೆಸರು: ಸಹಿ

ANNEXURE-V- PROFORMA

DEPARTMENT OF PERIODONTICS

KAHER'S KLE's V.K. INSTITUTE OF DENTAL SCIENCES

BELAGAVI.

ESTIMATION OF SALIVARY MMP-8 LEVEL AND ITS CORRELATION TO PERIODONTAL INFLAMED SURFACE AREA (PISA) BEFORE AND AFTER NON-SURGICAL PERIODONTAL THERAPY

Case No: OPD No:

Name:

Age: Sex: Occupation:

Address:

Chief Complaint:

Medical History:

Dental history:

Clinical Examination :

Periodontal inflamed surface area (PISA) calculation (at baseline)

	tooth	18	17	16	15	14	13	12	11	21	22	23	24	25	26	27	28	tooth
CAL	buccal		0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	
	palatinal		0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	
	lingual		0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	
CAL	buccal		0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0
	tooth	48	47	46	45	44	43	42	41	31	32	33	34	35	36	37	38	tooth

	tooth	18	17	16	15	14	13	12	11	21	22	23	24	25	26	27	28	tooth
LGM	buccal		0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	
	palatinal		0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	
	lingual		0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	
LGM	buccal		0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0
	tooth	48	47	46	45	44	43	42	41	31	32	33	34	35	36	37	38	tooth

tooth	18	17	16	15	14	13	12	11	21	22	23	24	25	26	27	28	tooth
ALSA (mm2)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	ALSA (mm2)
(mm2) ALSA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	(mm2) ALSA
tooth	48	47	46	45	44	43	42	41	31	32	33	34	35	36	37	38	tooth

tooth	18	17	16	15	14	13	12	11	21	22	23	24	25	26	27	28	tooth
RSA (mm2)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	RSA (mm2)
(mm2) RSA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	(mm2) RSA
tooth	48	47	46	45	44	43	42	41	31	32	33	34	35	36	37	38	tooth

tooth	PESA	nr of sites with BOP	PISA (mm2)
18	0		0
17	0		0
16	0		0
15	0		0
14	0		0
13	0		0
12	0		0
11	0		0
21	0		0
22	0		0
23	0		0
24	0		0
25	0		0
26	0		0
27	0		0
28	0		0

tooth	PESA	nr of sites with BOP	PISA (mm2)
38	0		0
37	0		0
36	0		0
35	0		0
34	0		0
33	0		0
32	0		0
31	0		0
41	0		0
42	0		0
43	0		0
44	0		0
45	0		0
46	0		0
47	0		0
48	0		0

Total **Periodontal Epithelial Surface Area**
0

Total **Periodontal Inflamed Surface Area** (
0

CAL = Clinical Attachment Level relative to CEJ
LGM = Location of Gingival Margin relative to CEJ
ALSA = Attachment Loss
Surface Area
RSA = Recession Surface Area
PESA = Periodontal Epithelial Surface Area
PISA = Periodontal Inflamed Surface Area

	tooth	18	17	16	15	14	13	12	11	21	22	23	24	25	26	27	28	tooth
CAL	buccal		0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	
	palatinal		0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	
	lingual		0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	
CAL	buccal		0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0
	tooth	48	47	46	45	44	43	42	41	31	32	33	34	35	36	37	38	tooth

	tooth	18	17	16	15	14	13	12	11	21	22	23	24	25	26	27	28	tooth
LGM	buccal		0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	
	palatinal		0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	
	lingual		0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	
LGM	buccal		0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0
	tooth	48	47	46	45	44	43	42	41	31	32	33	34	35	36	37	38	tooth

tooth	18	17	16	15	14	13	12	11	21	22	23	24	25	26	27	28	tooth
ALSA (mm2)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	ALSA (mm2)
(mm2) ALSA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	(mm2) ALSA
tooth	48	47	46	45	44	43	42	41	31	32	33	34	35	36	37	38	tooth

tooth	18	17	16	15	14	13	12	11	21	22	23	24	25	26	27	28	tooth
RSA (mm2)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	RSA (mm2)
(mm2) RSA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	(mm2) RSA
tooth	48	47	46	45	44	43	42	41	31	32	33	34	35	36	37	38	tooth

Periodontal inflamed surface area (PISA) calculation (3 months post therapy)-

tooth	PESA	nr of sites with BOP	PISA (mm2)
18	0		0
17	0		0
16	0		0
15	0		0
14	0		0
13	0		0
12	0		0
11	0		0
21	0		0
22	0		0
23	0		0
24	0		0
25	0		0
26	0		0
27	0		0
28	0		0

tooth	PESA	nr of sites with BOP	PISA (mm2)
38	0		0
37	0		0
36	0		0
35	0		0
34	0		0
33	0		0
32	0		0
31	0		0
41	0		0
42	0		0
43	0		0
44	0		0
45	0		0
46	0		0
47	0		0
48	0		0

Total **Periodontal Epithelial Surface Area**
0

Total **Periodontal Inflamed Surface Area** (
0

CAL = Clinical Attachment Level relative to CEJ
LGM = Location of Gingival Margin relative to CEJ
ALSA = Attachment Loss
Surface Area
RSA = Recession Surface Area
PESA = Periodontal Epithelial Surface Area
PISA = Periodontal Inflamed Surface Area

Estimation of MMP-8 levels (at baseline)-

Biochemical Parameter	Reading of Salivary MMP-8 (ng/ml)
Saliva Sample	

Estimation of MMP-8 levels (3 months post therapy)-

Biochemical Parameter	Reading of Salivary MMP-8 (ng/ml)
Saliva Sample	

ANNEXURE VI- MASTER CHART

Group – A (Healthy)

S.No.	Gender	Age	Baseline		3 months	
			PISA (mm^2)	MMP-8 level (pg/ml)	PISA (mm^2)	MMP-8 level (pg/ml)
1	F	35	371.9	767.0	220.7	156.0
2	F	36	339.8	665.0	198.0	166.0
3	F	35	473.0	432.0	321.2	378.0
4	M	46	487.0	221.0	278.2	155.0
5	F	37	502.6	426.0	302.6	320.0
6	M	36	412.0	356.0	261.5	115.0
7	F	41	511.6	436.0	217.0	191.0
8	F	45	524.6	576.0	292.0	262.0
9	F	46	204.0	445.0	96.5	150.0
10	M	38	478.0	443.0	345.2	191.0
11	F	40	342.0	682.0	206.0	125.0
12	M	44	289.0	346.0	178.2	255.0
13	M	54	310.0	568.0	245.6	432.0
14	F	50	236.6	332.0	123.4	113.0
15	F	46	410.5	467.0	378.7	245.0
16	F	35	406.0	232.0	313.5	353.0
17	F	36	546.5	478.0	282.3	238.0
18	M	47	209.0	587.0	115.0	158.0
19	M	37	496.0	488.0	189.4	402.0
20	M	42	289.0	579.0	246.6	220.0
21	M	42	432.0	550.0	308.3	364.0

Group – B (Periodontitis Stage III and IV Grade A)

S.No.	Gender	Age	Baseline		3 months	
			PISA (mm^2)	MMP-8 level (pg/ml)	PISA (mm^2)	MMP-8 level (pg/ml)
1	F	55	1200.9	2440.0	745.2	748.0
2	F	53	1696.7	2432.0	868.3	859.0
3	M	55	1765.5	2501.0	823.4	915.0
4	M	52	1906.6	2179.0	926.4	586.0
5	F	36	1494.7	2238.0	767.6	481.0
6	F	35	2158.0	2513.0	946.5	747.0
7	F	45	2649.5	2556.0	879.0	745.0
8	F	37	1209.7	2279.0	783.7	581.0
9	M	51	1493.0	2303.0	654.2	615.0
10	M	42	2387.1	2482.0	824.2	671.0
11	F	35	1366.8	2208.0	887.6	467.0
12	M	39	2567.6	2513.0	1024.4	718.0
13	M	52	1188.3	2051.0	578.0	63.0
14	F	48	1455.8	2258.0	646.5	498.0
15	M	44	1695.2	2398.0	903.4	695.0
16	M	40	1534.2	2265.0	686.2	492.0
17	F	36	1592.2	2302.0	702.6	601.0
18	M	36	1263.8	2053.0	724.0	348.0
19	M	37	2588.7	2442.0	848.0	744.0
20	M	54	2786.2	2510.0	915.7	886.0
21	M	50	1302.5	2052.0	623.0	517.0