COMPARATIVE EVALUATION OF 1% METFORMIN GEL PLUS PLATELET RICH FIBRIN AND PLATELET RICH FIBRIN ALONE IN TREATMENT OF CLASS II FURCATION DEFECTS: A CLINICO-RADIOGRAPHIC STUDY

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LIST OF ABBREVIATIONS

Table No.	Short Form	Full Form
1.	AMPK	AMP-activated protein kinase
2.	ANOVA	Analysis of variance
3.	ALG	Sodium alginate
4.	ALN	Alendronate
5.	ATF3	Activating transcription factor-3
6.	AV	Aloe vera
7.	BOP	Bleeding upon probing
8.	BD	Bone density
9.	BG	Bioactive Glass
10.	BL-H	Bone loss in the horizontal direction –
11.	BMPCs	Bone marrow progenitor cells
12.	BMPs	Bone morphogenetic protein
13.	β-TCP	β-tricalcium phosphate
14.	BL-V	Bone loss in the vertical direction
15.	BW	Bone width
16	CAL	Clinical attachment level
17.	CBCT	cone beam computed tomography
18.	CEJ	Cementoenamel Junction
19.	CMC	Carboxy methyl cellulose sodium
20.	CV-MSCs	Human chorionic villous mesenchymal stem cells
21.	CT	Computed tomography
22.	cPRP	Concentrated platelet-rich plasma
23.	DICOM	Digital Imaging and Communications in Medicine
24.	DDR%	Defect depth reduction
25.	DFDBA	Demineralized Freeze-dried bone allograft
26.	DM	Diabetes mellitus
27.	ECs	Endothelial cells
28.	ELISA	Enzyme linked immunosorbent assay
29.	FOV	Field of volume
30.	FDBA	Freeze dried bone allograft
31.	FGM	Free gingival margin
32.	FI	Furcation involvement
33.	FW	Furcation width

Table No.	Short Form	Full Form
34.	GI	Gingival Index
35.	GML	Gingival marginal level
36.	GR	Gingival recession
37.	GTR	Guided tissue regeneration barriers
38.	HA	Hydroxyapatite
39.	HBF	Horizontal bone fill
40.	HCAL	Horizontal clinical attachment level
41.	H-DOF	Horizontal depth of furcation
42.	HDD	Horizontal defect depth
43.	HPD	Horizontal probing depth
44.	IGF-I	Insulin growth factor-I
45.	IL-1β	Interleukin-1β
46.	IL-6 and IL-8	Interleukin-6 and Interleukin-8
47.	IOPA	Intraoral periapical radiograph
48.	IBDs	Intrabony defects
49.	eNOS	endothelial nitric oxide synthase
50.	LPS	Lipopolysaccharide
51.	LDL	Low density lipoprotein
52.	LDD	Local drug delivery
53.	Ms	Macrophages
54.	MF	Metformin
55.	mSBI	Modified sulcular bleeding index
56.	NSAID’s	Non-steriodal anti-inflammatory drugs
57.	NF-B	Nuclear factor-kappa B
58.	OFD	Open flap debridement
59.	OPG	Osteoprotegrin
60.	OVX	Ovariectomized
61.	PDGF	Platelet derived growth factor
62.	PKC	Protein kinase C
63.	PB	Probing depth
64.	PPD	Probing pocket depth
65.	PDGF-BB	Platelet derived growth factor BB
66.	PPP	Platelet-poor plasma
67.	PI	Plaque Index
68.	PRP	Platelet rich plasma
69.	PRF	Platelet rich fibrin

Table No.	Short Form	Full Form
70.	qRT-PCR	Quantitative Real-time polymerase chain reaction
71.	RANKL	Receptor activator of nuclear factor kappa beta ligand
72.	RBC	Red blood cell
73.	r-HDF	Horizontal depth of furcation
74.	RHAL	Relative horizontal clinical attachment level
75.	ROI	Region of interest
76.	RSV	Rosuvastatin
77.	RVAL	Relative vertical clinical attachment level
78.	RVG	Radiovisiography
79.	r-VHF	Relative vertical height of furcation
80.	RT-PCR	Real-time polymerase chain reaction
81.	SRP	Scaling and root planing
82.	SMCs	Smooth muscle cells
83.	TGFb-1	Transforming growth factor b-1
84.	TRAP	Tartrate-resistant acid phosphatase
85.	TSA	Thiolated sodium alginate
86.	TNF-α	Tumor necrosis factor α
	UNC-15	University of North Carolina-15
87.	VCAL	Vertical clinical attachment level
88.	VDD	Vertical defect depth
89.	VEGF	Vascular endothelial growth factor
90.	V-HOF	Vertical height of furcation
91.	VPD	Vertical probing depth
92.	WHI	Wound healing index
93.	2D	Two dimensional
94.	3D	Three dimensional

INTRODUCTION

The complex interplay between microbial challenge, host response and other modifying factors triggers the immunoinflammatory reaction, which leads to gingival connective tissue and alveolar bone damage culminating into periodontitis.[1] The mechanisms to understand the pathogenesis of periodontal diseases was intensified in 1960's and 1970's which demonstrated microbial specificity of subgingival plaque at the sites with periodontitis. Later, the conceptual models of pathogenesis also explained the role of genetic and environmental factors along with exacerbated inflammatory host immune response and dysbiotic plaque biofilm in leading destructive events of periodontal disease.[2,3]

Risk factors increases the susceptibility to inflammatory response which explains the variations in destruction patterns and severity of clinical outcomes. The primary features of this inflammatory condition include attachment loss and alveolar

bone resorption.[4] When this destruction progresses apically it involves the furcation area of multirooted tooth further leading to pathologic resorption of bone within the furcae.[5]

"Furcation involvement may be defined as the incursion of the bifurcation and trifurcation of multirooted teeth by periodontal disease. Furcation involvement is one of the most compelling challenges faced during the management of the periodontal disease, owing to its complex anatomic morphology, which is extremely strenuous and frequently inappropriate for adequate debridement. Molars are more often lost than any other tooth type since it responds less favourably to the conventional therapy.[7]Its intricate anatomy, primarily the location and width of the furcation entrance, root concavities, cervical enamel projections, root trunk length and variable morphology with ridges in the inter-radicular root surface area frequently render the defect impossible to instrument effectively. This unique morphology of the root complex in the furcal region of multirooted teeth favours the advancement of destructive periodontitis lesions in the furcation, thus resulting in chronic inflammation. These anatomical peculiarities and irregularities not only dictate the modifications in treatment approaches but also affects the maintenance. Various contributing and predisposing factors precipitates further attachment loss in the furcation area thus reducing the efficacy of periodontal therapy. Vital determinants of successful periodontal therapy of furcation lesions are the extent and degree of furcation involvement which can be determined by evaluating vertical bone loss, horizontal bone loss or both.[8] Various classifications of furcation involvement have been proposed based on these assessment parameters. Following are few widely used

classification systems which have been devised to measure and record the severity of furcation involvement.

1. Glickman (1953)[9]

Grade I: Pocket formation into the furcation but intact interradicular bone.

Grade II: Loss of inter radicular bone and pocket formation but not extending through to the opposite side.

Grade III: Through-and-through lesion.

Grade IV: Through-and-through lesion with gingival recession, leading to a clearly visible furcation area.

2. Hamp et al. (1975)[10]

Degree 0 - Furcation defect is not accessible with a periodontal probe.

Degree I - The horizontal loss of periodontal tissue support is up to 3mm.

Degree II - The horizontal loss of support exceeds 3 mm, but is not more than 6mm.

Degree III - Consists of through and through horizontal destruction of the periodontal tissue support in furcation area.

3. Tarnow & Fletcher (1984)[11]

Sub-divided each grade of horizontal involvement into three subgroups, based on the degree of vertical involvement

Subclass A - 0–3 mm

Subclass B - 4–6 mm

Subclass C - >7 mm

Most of the classifications considered the severity of alveolar bone destruction but **Pajnigara et al. in 2018**[12] proposed a classification system which related the severity of alveolar bone destruction horizontally as well as vertically in the furcation along with the corresponding gingival position to enable the clinicians to address minute details during the treatment. The authors classified the furcation defects into four Grades (Grade I, Grade II, Grade III, Grade IV). Depending on the position of marginal gingiva these four grades were further sub-classified as a,b,c and depending on pattern of bone loss Grade II, III and IV were sub-classified as type 1 and type 2.

The goal of treating furcation defects is to clean the furcation and facilitate the oral hygiene by establishing an anatomy that enables proper plaque control, thus preventing further attachment loss. Different strategies have been used to deal with the furcation involvement. Various treatment approaches such as scaling and root planing, open flap debridement, bone replacement grafts, root resection, hemisection and guided tissue regeneration barriers (GTR) were proposed to deal with furcation involvement. Literature has demonstrated the efficacy of bone grafts and barrier membranes in furcation involved molars and a less favourable response to conventional therapy.[8,13] Despite a promise of high predictability of success for the use of bone grafts in furcation defects, the need for additional surgical intervention and expense of procurement for autografts, the greater expenses, availability of bone grafts, variability in repair and regeneration predictability has presented certain limitations with its use.[14]

Regenerative attempts by employing various biomimetic agents such as, bone morphogenetic protein (BMPs), platelet derived growth factor (PDGF), enamel matrix derivatives (EMDs), platelet rich Plasma (PRP) has even given better results in furcation resolution. The Platelet concentrates plays a very important role in periodontal regeneration. Platelet Rich Fibrin (PRF), a second-generation of platelet concentrate is a powerful healing biomaterial with inherent regenerative capacity and can be used in treatment of periodontal osseous defects. A protocol was suggested by **Choukroun et al**. in France, according to which PRF was produced without the addition of any anticoagulant to the collected blood sample. The basic difference between PRP and PRF is that PRF does not require any additives, such as anticoagulants, thrombin, or any other gelling agent while preparation. The slow polymerization of this smart blood derivative leads to formation of autologous fibrin matrix enmeshed with leukocytes, platelets, cytokines, glycan chains and structural glycoproteins. PRF has shown its beneficial effect on bone regeneration by stimulating proliferation of osteoblasts. It has also demonstrated specific cell type action by providing a scaffold for breeding human periosteal cells and suppressing oral epithelial cell downgrowth, which is valuable for periodontal regeneration. Gingival connective tissue is stimulated by PRF and it also offers the root surface with the fibronectin and vitronectin which are mandatory for cell migration. Current evidences have shown the efficacy of PRF in facilitating cell events that are favourable for periodontal regeneration leading to mineralized tissue formation in intrabony as well as mandibular grade II furcation defects.[15.16]

Periodontitis a multifactorial disease and its progression is a result of complex interaction between pathogens and the host response. The advancement in

understanding the pathogenesis of periodontal disease thus introduced the concept of host response modulation as a treatment option. Host modulation with chemotherapeutics or drugs presented a exciting new adjunctive therapeutic option for management of periodontal disease and regeneration of its structures. Considering the limitations with the use of bone grafts and in a view of modulating the host responses for periodontal repair and regeneration, various pharmacological bone regenerative agents such as Melatonin, Atorvastatin, Alendronate etc were tried for promoting periodontal regeneration.[17] Amongst these agents, Metformin (MF) an oral hypoglycaemic drug has shown a stimulating effect on osteoblastic lineages, leading to osteoblast differentiation and bone formation. It is a biguanide, derived from perennial herb "Galegaofficinalis" that is used to treat diabetes mellitus (DM) type 2. This potent insulin sensitizing drug decreases hepatic glucose output, increases peripheral glucose uptake and improves insulin secretion. A two-way relationship has been found to exist between periodontitis and DM. DM affects the periodontal health and the periodontal disease in turn affects glycaemic control. An increase in the activity of alkaline phosphatase in MC3T3E1 type of osteoblasts and collagen fabrication (type1), in both UMR106 and MC3T3E1 types of cells has been observed with the use of MF.[18] Its favourable effect on alveolar bone by promoting osteogenic differentiation of osteoblast-like cells and human chorionic villous mesenchymal stem cells (CV-MSCs) has been confirmed in vitro and in vivo. While no effect of MF has been recorded on osteoclasts and adipocytes.[19] MF also has shown anti-inflammatory effect and inhibitory effect on IL-1β induced activation of proinflammatory phosphokinases Akt p38 and Erk.[20] It is available at different concentrations (0.5%, 1% and 1.5%) but 1% concentration has been verified to be more efficacious

clinically as well as radiographically.[21] Therefore, it is novel to explore this antidiabetic agent for the purpose of periodontal tissue regeneration.

Various diagnostic tools have been developed to detect and evaluate the extent of disease in the furcation area. Traditionally furcation involvement (FI) is assessed by utilizing both clinical and radiographic examination. This assessment includes evaluation of horizontal probing depth of furcation entrance based on horizontal bone loss, vertical probing pocket depth (PPD), clinical attachment level (CAL) clinically and detection of radiolucency at the site of furcation by using intraoral periapical radiograph (IOPA). The 2D imaging modality (IOPA or bitewing radiographs) might not be accurate for the assessment of furcation involvement since it has got low sensitivity and high specificity for detection of furcation defects. This conventional radiographic method gives only 2D information of the 3D structures. Also, the inherent shortcomings of 2D projections such as superimposition of anatomical structures and distortion of images due to angulation problems further obscures the bony changes in the defect. Computed tomography (CT) replaced 2D imaging and 3D imaging became possible but with a high radiation exposure to the patient. Along with this surgical re-entry and histologic evaluation were also the methods opted to precisely detect the extent and type of bone destruction as well as to determine the amount of bone gain after regenerative therapy. However, certain limitations such as, lesser time for the surgeon to evaluate the exact morphology of the defect during surgery and the ethical constraints led to the advent of cone beam computed tomography (CBCT) which assess the images three-dimensionally with higher accuracy, higher resolution, and low cost as compared to CT. [22]

CBCT imaging modality uses a cone shaped X-ray beam cantered on a 2D X-ray sensor rotating 180-369 degrees to scan a 360-degree rotation about the patient's head, scanning a defined anatomical volume. A series of 360 exposures or projections, one for each degree of rotation, captures planed data in a single scan which provides the raw digital data for reconstruction of the exposed volume by computer algorithm. It provides 3D volumetric images with multiplanar reconstruction in axial, coronal, and sagittal planes without magnification. Voxel is a unit of graphic information which defines three-dimensional space and hence in CBCT 3D imaging voxel is used instead of pixel for volumetric assessment. DICOM (Digital Imaging and Communications in Medicine) file format enables ease of its usage with other third-party imaging software. The 3D volume evaluated on CBCT as region of interest (ROI). The smaller the ROI the better the resolution of the images. The resolution is indirectly related to field of volume (FOV). So, when FOV decreases, X- ray scattering decreases thus producing an image of higher resolution. The radiation dose of CBCT is about 6-15 times lesser than conventional CT and depends on the voltage and amplifier settings. The lower mA and/or collimation reduces the amount of radiation but affects the quality of the captured image. The CBCT machine used in our study was the Orthophos® XG 3D/ Ceph manufactured by Sirona Dental Systems GmbH, Germany.[23]

Rapid scan time comparable to panoramic radiograph, less radiation as compared to conventional CT and full mouth IOPA, more image accuracy with better resolution ranging from 0.4 mm to as low as 0.076 mm, lesser cost, multiplanar reformation of images along with unique 3D images demonstrating features are

advantages of CBCT over intraoral, panoramic, and cephalometric imaging modalities.

Previously, very few clinical studies have been performed to evaluate the efficacy of 1% MF gel and PRF in combination in the treatment of Class II furcation defects. Very encouraging results have been recorded in terms of periodontal regeneration with the use of 1% MF gel in treatment of Class II furcation defects. Also, CBCT being one of the latest methods of evaluation of periodontal regeneration very few studies have used CBCT for evaluation of regeneration. So, the present study was planned to compare and evaluate the effectiveness of 1% MF gel with PRF and PRF alone in the treatment of Class II furcation defects clinically and radiographically by CBCT.

AIM AND OBJECTIVES

The study was aimed to evaluate and compare 1% Metformin gel (MF) plus Platelet Rich Fibrin (PRF) and Platelet Rich Fibrin (PRF) alone in the treatment of Grade II furcation defects, clinically and by CBCT.

Also, attached to this aim were certain objectives:

1. To evaluate the efficacy of 1% MF Gel in combination with PRF in the treatment of Grade II furcation defects.

2. To evaluate the efficacy of PRF alone in the treatment of Grade II furcation defects.

3. To clinically evaluate and compare the clinical attachment level (CAL) and probing pocket depth (PPD) in Grade II furcation defects treated with 1% MF gel with PRF and PRF alone.

4. To evaluate and compare by CBCT, the amount of bone fill leading to reduction in bone defect height in Grade II furcation defects treated with 1% MF gel with PRF and PRF alone, 6 months postoperatively.

5. To evaluate and compare by CBCT, the amount of bone fill leading to reduction in bone defect width in Grade II furcation defects treated with 1% MF gel with PRF and PRF alone, 6 months postoperatively.

6. To evaluate and compare by CBCT, the amount of bone fill leading to reduction in bone defect depth in Grade II furcation defects treated with 1% MF gel with PRF and PRF alone, 6 months postoperatively.

7. To evaluate and compare by CBCT, the amount of bone fill leading to reduction in bone defect volume in Grade II furcation defects treated with 1% MF gel with PRF and PRF alone, 6 months postoperatively.

REVIEW OF LITERATURE

The definitive goal of periodontal treatment is the regeneration of tissues damaged as result of periodontal tissues. Various regenerative techniques such as root conditioning, often combined with coronally advanced flaps, bone grafts, organic or synthetic barrier membranes etc have been tried with the aim of closing Grade II furcation defects. Research has given sufficient evidence to support these treatment strategies. The surrogate therapeutic end points were gains in vertical and horizontal attachment, reductions in probing depth, bone fill or changes in density radiographically. However, assessment of regeneration also presents a challenge. Radiographs provide abundant information about the periodontium but it gives a two-dimensional representation of three-dimensional structures and their limitations are well known. In addition, the exact morphology of these periodontal defects cannot be determined by using conventional radiography. To overcome the inherent difficulties

of 2D projection radiographs, 3D image analysis by CBCT has been introduced and used in this study.

Considering the paramount literature available and for the ease of understanding, the review of literature has been segregated into four parts

1. Review of studies on Metformin gel.
2. Review of studies on Platelet Rich Fibrin.
3. Review of studies on the combination therapy (1%MF gel +PRF).
4. Review of studies on the methods of analysis of regeneration.

1. Review of studies on Metformin gel

Isoda K et al. (2005)[20] examined the inhibitory pro-inflammatory responses of Metformin in human vascular smooth muscle cells (SMCs), macrophages (Ms), and endothelial cells (ECs). Investigations demonstrated dose dependent action of metformin on inhibiting IL-1 β –induced release of the pro-inflammatory cytokines IL-6 and IL-8 and nuclear translocation of nuclear factor-kappa B (NF-B) in SMCs. Also, a suppressed action of IL-1β–induced activation of the pro-inflammatory phosphokinases Akt, p38, and Erk in SMCs and decreased phosphorylation of Akt and protein kinase C (PKC) in ECs under hyperglycaemic conditions were reported in this study. The data thus suggested a novel anti-inflammatory action of Metformin by inhibiting NF-KB through blockade of the PI3K–Akt pathway.

Cortizo AM et al. (2006)[18] investigated the efficacy of Metformin on the growth and differentiation of osteoblasts in culture. UMR106 rat osteosarcoma cells and MC3T3E1 mouse calvaria-derived cells were used to prepare a cell culture. A

dose-dependent increase in cell proliferation of two osteoblast-like cells (UMR106 and MC3T3E1) was found when administered with Metformin (25–500μM) for 24 hrs. An increase type-I collagen production in both cell lines and stimulated alkaline phosphatase activity was observed in specifically MC3T3E1 osteoblasts. Thus, in this study an activation/redistribution of ERK-1/2 and induction of e/ iNOS mediated, direct osteogenic effect was observed in osteoblast culture when administered with Metformin.

Bak E. J et al. (2010)[24] analysed the effect of Metformin on alveolar bone loss in ligature- induced periodontitis and osteoblast, osteoclast, and adipocyte differentiation. After inducing periodontitis by a ligature around mandibular first molars in rats, they were divided into two groups. First group received a vehicle and the second group received Metformin. The alveolar bone volume between the molars was assessed using microcomputed tomography while the effect of metformin on osteoblast, osteoclast was assessed using MC3T3-E1, cocultures of mouse bone marrow cells and calvaria-derived osteoblasts and adipocyte differentiation was evaluated using 3T3-L1/C3H10T1/2 cells. The differentiation of all the above-mentioned cell types was estimated by the degree of mineralization, the formation of tartrate-resistant acid phosphatase–positive multinucleated cells, and the accumulation of triglycerides, respectively. Results showed a twofold increase of mineralization of MC3T3-E1 cells with regards to osteoblasts, while little effect was reported with respect to the differentiation of osteoclasts and adipocytes. A significant reduction of alveolar bone was observed in the Metformin treated rats as compared to vehicle treated rats. The authors thus found a beneficial effect of Metformin on alveolar bone in periodontitis, induced by osteoblast differentiation.

Gao Y et al. (2010)[25] investigated the action of metformin on bone mass in ovariectomized (OVX) rats. Three months after either a sham surgery or bilateral ovariectomy, 32 female Sprague–Dawley rats were randomly assigned into four groups: (1) Sham group; (2) OVX group; (3) OVX+ metformin (50 mg/kg/day) group; and (4) OVX + metformin (100 mg/kg/day) group. Tibiae was harvested for dual energy X-ray absorptiometry, microcomputed tomography (micro-CT) and histology analysis after 2 months from all the groups. While the bone marrow cells from tibiae were collected for measurement of the mRNAs expressions for three osteoblast genes and estrogen receptors alpha by using real-time RT-PCR. An impaired bone density and quality induced by bilateral ovariectomy was found to be significantly improved in the groups that received metformin (both 50 and 100 mg/kg/day) and this action was observed to be partly mediated by regulating bone marrow cells development through induction of mechanisms regulating osteoblast markers core binding factor a1 and LDL receptor-related protein 5. These findings thus provided an evidence of Metformin induced direct inhibitory effect on bone loss in OVX rats, in addition to its well-documented osteogenic potency in vitro.

Kanazawa I et al. (2008)[26] investigated the effects of metformin on the differentiation and mineralization of osteoblastic MC3T3-E1 cells as well as intracellular signal transduction. MC3T3-E1 cells, a clonal osteoblastic cell line was isolated from calvariae of late-stage mouse embryo and were cultured in a-MEM containing 10% FBS, 1% penicillin–streptomycin, and 10 mM b-glycerophosphate for 2 weeks after reaching confluence. It was followed by Real-time PCR quantification of gene expression, assay of alkaline phosphatase activity and mineralisation along with immunoblotting to quantify the signal intensity of AMPK. Metformin

significantly showed increased collagen-I and osteocalcin mRNA expression, stimulated alkaline phosphatase activity, and enhanced cell mineralization. A significantly activated AMPK in dose- and time dependent manners, and induced endothelial nitric oxide synthase (eNOS) and bone morphogenetic protein-2 (BMP-2) expressions was also noted in this study. The findings thus suggested a beneficial role of Metformin on differentiation and mineralisation of osteoblast and also emphasised its use in diabetes as well as osteoporosis owing to its bone promoting action.

Liu L et al. (2012)[27] investigated the effects of systemically administered metformin on alveolar bone resorption and on the ratio of receptor activator of nuclear factor kappa B ligand/osteoprotegerin (RANKL/OPG) in rats subjected to experimental periapical lesions. The pulp chambers of 40 male rats were exposed to induce periapical lesion. The test group received daily intramuscular injections of metformin at 40 mg/kg doses, whereas the control group received only the saline vehicle. The injections were started 1 day before the periapical lesion induction and then continued throughout the experiment. Two to four weeks later the rats were killed and their mandibles were prepared for histologic analysis, enzyme histochemistry, immunohistochemistry, and immunofluorescence. The results showed a decrease in the number of RANKL positive and tartrate-resistant acid phosphatase (TRAP)–positive cells in the metformin-treated groups on day 14, whereas an increase in the number of OPG positive cells on day 28. A significant decrease in periapical bone loss area in the metformin-treated group was observed as compared to control group on day 28. This study thus suggested Metformin's potential role in inhibiting the periapical lesions by possibly lowering the

RANKL/OPG ratio, subsequently reducing the number of osteoclasts and bone resorption areas.

Pradeep A R et al. (2013)[21] explored the efficacy of 0.5%, 1%, and 1.5% Metformin (MF) gel as a local drug delivery system in adjunct to scaling and root planing (SRP) for treatment of intrabony defects (IBDs) in patients with chronic periodontitis. Total 41 patients aged 30 to 50 year diagnosed with chronic periodontitis were with 0.5%, 1%, or 1.5% MF gel or placebo gel. In the test site SRP was done followed by the placement of 0.5%, 1%, and 1.5% MF gel while in the control same SRP was followed by placement of placebo gel. The outcome parameters; PI, mSBI, PD, and CAL were recorded at baseline, 3 and 6, months whereas, IBD depth was recorded on standardized radiographs by using image analysis software at baseline and 6 months. MF group showed a greater mean PD reduction and mean CAL gain than the placebo group at both 3 and 6 months. Additionally, the test group showed a significantly greater reduction of IBD depth as compared to the placebo group, with greatest reduction in 1% MF treated sites. It was concluded that the local delivery of different concentrations of MF gel resulted in significantly greater improvement in PD, CAL, IBD depth as compared to placebo gel when used as an adjunct to SRP.

Rao N S et al. (2013)[28] conducted a study to investigate the effectiveness of 1 % MF, indigenously prepared biodegradable controlled-release gel as, as an adjunct to scaling and root planing (SRP) in treatment of vertical intrabony defects in smoker patients affected with generalized chronic periodontitis. Fifty male smokers, aged 30 to 50 years who were diagnosed with generalized chronic periodontitis were equally divided into two groups. In the test group SRP was performed followed by the

placement of 1% MF gel while in the control group SRP was performed followed by the placement of placebo gel in the periodontal pockets. 10 µl prepared MF gel was prepared by adding weighed amount of MF in 10 ml of distilled water mixture. Clinical parameters; plaque index (PI), modified sulcus bleeding index (mSBI), probing depth (PD), and clinical attachment level (CAL) were recorded at baseline, 3 months and 6 months. The depth of IBD was evaluated at baseline and 6 months by using computer aided software programme. 45 patients out of 50 completed the study. These patients showed an improvement in PI score from baseline. No significant difference was noted for mSBI in both groups at baseline but a significant decrease was observed in test group (1% MF) as compared to placebo group at 3 and 6 months. Intergroup comparison of PD and CAL showed no difference at baseline. However, the PD reduction and CAL gain was significant in the test group than control group at 3 and 6 months. Moreover, a significant greater mean percentage of bone fill was seen in MF group (26.17%±6.66 %) as compared to the placebo sites (3.75%± 8.06 %) at 6 months of time interval. It was concluded that the local delivery of 1% MF gel into the periodontal pocket of smokers stimulated a significant increase in PD reduction, CAL gain, and enhanced bone fill as compared to Placebo treated sites when used as an adjunct to SRP.

Pradeep A R et al. (2016)[29] investigated the effectiveness of 1% Metformin gel as an adjunct to scaling and root planing in the treatment of intrabony defects in patients with chronic periodontitis. Total 65 individuals were equally divided into two groups. The test group was treated with 1% MF gel with SRP while the control group was treated with SRP plus placebo gel. Plaque index, modified sulcus bleeding index, probing depth (PD), and clinical attachment level (CAL) were calculated at baseline,

3 and 6 months while at the end of 6 months by using computer-aided software Intrabony defect depth (IBD) was assessed. A significantly greater mean PD reduction, CAL gain, and IBD depth reduction were found in the MF group as compared to placebo group at all visits. Significantly greater reduction in defect depth was observed in the MF group (26.8 ± 5.52%) than in the placebo sites (4.79 ± 2.30%). Thus, 1% MF gel was found to be effective in significantly improving clinical and radiographic parameters in intrabony defects in patients with chronic periodontitis.

Kassem A A et al. (2017)[30] developed and evaluated the effect of Thiolated-alginate based mucoadhesive multiple layer film loaded with small dose of MF for intrapocket application in chronic periodontitis patients. This multiple layer film was established by double casting and compression method. Either 6% carboxy methyl cellulose sodium (CMC) or sodium alginate (ALG) constituted the inner drug (0.6%) loaded layer. to enhance mucoadhesion and achieve controlled drug release thiolated sodium alginate (TSA; 2 or 4%) constituted the outer drug free layer of the film. Total 20 subjects were selected to evaluate the optimized formulation of MF drug. In the control group 10 interproximal sites were treated with SRP alone while in the test group SRP was done followed by the application of 0.6% Metformin multiple layer film (TL4.CMC). Bleeding upon probing (BOP), probing pocket depth (PPD) and clinical attachment level (CAL) clinical parameters were recorded at baseline and 6 months post therapy. Based on water uptake and in vitro drug release, CMC based film with 4% TSA as an outer layer showed enhanced mucoadhesion and controlled drug release of 83.73% over 12 hrs. Scanning electron micrography showed the effective fabrication of the triple layer film in which connective lines between the

layers were be observed. On FTIR (infrared spectra) examination the possibility of hydrogen bonding between the –NH groups of metformin and –OH groups of CMC and DSC thermograms was suggested and also revealed the presence of MF mainly in the amorphous form. An improvement in all the clinical parameters was observed in the test site as compared to control site six months post treatment. The study thus suggested the effective role of local application of the mucoadhesive multiple layer films loaded with metformin hydrochloride in the management of moderate chronic periodontitis.

Kotry G et al. (2016)[31] assessed the effect of a muco-adhesive, multiple layer film of MF as an intra-pocket application in non-surgical management of moderate to severe chronic periodontitis patients. Of total 20 selected patients 10 sites were treated with SRP with placebo gel while remaining 10 sites were treated with SRP plus Metformin film. Clinical parameters recorded were bleeding on probing (BOP), Clinical attachment level (CAL) and probing depth (PB). These parameters were recorded at baseline, 3 and 6 months while the radiographic parameters; Intrabony defect depth (IBD) and bone density (BD) were recorded at baseline and 6 months post-treatment. A significant reduction in mean probing depth and CAL gain were observed at the test site as compared to placebo treated site. A statistically significant reduction in IBD depth and increase in BD were recorded at MF treated site. This study thus suggested the beneficial role of local application of films loaded with MF in non –surgical management of cases of moderate to severe chronic periodontitis.

Pradeep A R et al. (2017)[32] evaluated the efficacious role of MF 1 % gel when used as an adjunct to scaling and root planing (SRP) while treating omoderate and severe chronic periodontitis subjects. Seventy subjects were divided in tow

groups where in group I (test group) the subjects were treated with SRP plus 1% MF gel and group II (control group) was treated with SRP plus placebo gel. Each group was divided into two subgroups with an initial pocket depth of ≥5 mm and ≥7 mm. Clinical parameters included were plaque index (PI), modified sulcus bleeding index (mSBI), probing depth (PD) and clinical attachment level (CAL). These parameters were recorded at baseline, 3, 6 and 9 months. Radiographic parameters valuation of intrabony defect (IBD) and percentage defect depth reduction (DDR%) was done at baseline, 6 and 9months' time interval by using computer aided software programme. A greater mean probing depth reduction and mean clinical attachment level gain was documented in MF group than placebo group at all visits. Clinical parameters (PD, CAL) in the subgroup of initial pocket depth ≥5mm and ≥7mm exhibited significant improvement in 1% MF group as compared to placebo group. Similarly, MF group showed a significant greater mean percentage of defect depth reduction at 6 months and 9 months in both ≥5mm and ≥7mm of initial pocket depth. A significant reduction of PPD, CAL gain, IBD and DDR% reduction at the locally delivered MF treated sites highlighted its valuable usage in treating the in chronic periodontitis subjects in both the subgroups as compared to placebo.

Grace S U et al. (2017)[33] assessed the effectiveness of Metformin gel as an adjunct to scaling and root planning in the treatment of periodontitis. This was a parallel design study which comprised of total 16 individual with chronic periodontitis. These patients were divided into two groups: In group I 8 patients underwent SRP plus local drug delivery Metformin application while 8 patients in group II underwent SRP with local drug delivery of placebo gel. Gingival index, probing depth, clinical attachment loss was recorded clinically at baseline and 1

month. Results showed a significant difference in all the parameters at one month as compared to baseline for both the groups but no statistically significant difference was observed between both the test and control groups. Smaller sample size and short-term follow-ups were the limitations of this study. Considering these limitations this study demonstrated efficacy of Metformin in treating periodontal tissues but the effect was found to be non-significant when compared to placebo gel.

Kang W et al. (2017)[34] assessed the action of Metformin on P.gingivalis Lipopolysaccharide-influenced inflammatory response in human gingival fibroblasts (HGFs). Dose-dependent effects of metformin on LPS-influenced HGFs were evaluated. Cell-counting assay was used to govern the effects of Metformin as well as LPS on the viability of HGFs. The levels of interleukin (IL) -1β, IL-6 and tumor necrosis factor (TNF)-α in different treated cells were detected by using Enzyme linked immunosorbent assay (ELISA) and quantitative Real-time polymerase chain reaction (qRT-PCR). Activating transcription factor-3 (ATF3) siRNA transfection was used to determine the mechanism of action of Metformin while the transfection efficiency was observed by fluorescence microscope. The qRT-PCR and Western blot determined the effects of ATF3. No toxicity to HGFs was observed by 5 μg/ml P. gingivalis LPS and 0.1, 0.5, 1 mM Metformin. Also, the inhibitory effect of metformin was observed on LPS-influenced IL-1β, IL-6 and TNF-α production in a dose dependent manner. It was found that Metformin and LPS could synergistically facilitate ATF3 expression which, on the other hand has found to abolish the inhibitory effects of metformin on LPS-influenced inflammatory cytokines production in HGFs. The results thus confirmed the inhibitory effect of metformin on LPS-

enhanced IL-6, IL-1β and TNF-α production in HGFs via increasing ATF3 expression.

Mushtaq I et al. (2018)[35] evaluated the efficacy of 1% metformin gel as an adjunct to SRP in the treatment of chronic periodontitis patients. Total 30 patients selected for the study were divided into two groups. In the firs control group SRP was done while in the second test group SRP was done followed by Local drug delivery of 1% MF gel. Probing depth (PD), modified sulcus bleeding index (mSBI) and clinical attachment level (CAL) were recorded at baseline, 1 and 3 months of time interval. The intragroup comparison showed a significant reduction of mean PD and CAL gain from baseline to 3 months with a more significant reduction in test group as compared to control group. The percentage change of mSBI was also found to be more significant in the site treated with MF gel. This study the favoured the use of Metformin as LDD in treating chronic periodontitis.

Pankaj D et al. (2018)[36] explored and compared the clinical efficacy of locally delivered 1.2% Rosuvastatin (RSV) and 1% Metformin (MF) gel when used as an adjunct to scaling and root planning (SRP) in the treatment of intrabony defects in chronic periodontitis patients. Total 90 subjects were randomly divided into three groups. Group I patients were treated with SRP plus placebo gel, Group II with SRP plus 1% RSV gel and group III with SRP plus 1% MF gel. Clinical parameters such as modified sulcus bleeding index (mSBI), plaque index (PI), pocket probing depth (PD) and clinical attachment level (CAL) were documented at baseline, 6 and 12 months and the radiological parameter of bone defect fill was assessed at 6 and 12 months. Without using any active ingredients RSV, MF gel and the placebo gel were prepared. All the gels, which were prepared, were similar in colour and consistency.

All parameters showed improvement in all groups, however a significant reduction of PD, CAL gain and bone defect fill was observed in group II and III as compared to group I. The results of this study thus showed that both drugs are effective in treating chronic periodontitis and can be used in LDD treatment mode, however RSV showed comparatively better improvement in calculated parameters than MF.

Kurian I G et al. (2018)[37] investigated the effectiveness of locally delivered 1% metformin (MtF) and Aloe vera (AV) gel as an adjunct to scaling and root planning (SRP) in the treatment of intrabony defects in chronic periodontitis patients. Total 90 volunteers were assigned into three groups where group I underwent SRP plus placebo gel treatment, Group II was treated with SRP plus 1% MF gel while group III was treated with SRP plus AV gel. Clinical parameters such as gingival index (GI), bleeding on probing (BoP), pocket probing depth (PPD), and clinical attachment level (CAL), were documented at baseline, and 6 and 12 months. The radiological parameter of bone defect fill was evaluated at 6 and 12 months. All gels were prepared in same colour and consistency following the documented procedure. GI, BoP, PPD, and CAL showed improvement in all the treated groups; nevertheless, the mean PPD reduction, CAL gain, and percentage of bone fill was found to be greater in the MtF treated and AV treated groups than the placebo treated group at all visits. The authors thus concluded that the local delivery of 1% MtF and AV gel stimulates a significant PPD reduction, CAL gain, and improved bone fill and regeneration when compared with placebo gel. Results were found to be significantly improved with the use of 1% MtF gel than AV gel.

2. Review of studies on Platelet Rich Fibrin

Dohan et al. (2006)[38] described the conceptual and technical evolution from fibrin glues to platelet concentrates. This study analysed retrospectively the fibrin technologies and the biochemical properties of 3 generations of surgical additives, respectively fibrin adhesives, concentrated platelet-rich plasma (cPRP) and PRF was done in this study. The 3- dimensional fibrin architecture was found to be deeply dependent on artificial clinical polymerization processes, such as massive bovine thrombin addition. A fibrin network similar to the natural one was seemed to be generated due slow polymerization during PRF preparation. This network was observed to lead, to a more efficient cell migration and proliferation and thus cicatrisation.

Dohan et al. (2006)[39] investigated the platelet-associated features of PRF & carried out a comparative study by undertaking to quantification of PDGF-BB, TGFb-1, and IGF-I within PPP (platelet-poor plasma) supernatant and PRF clot exudate serum. These evaluations discovered that slow fibrin polymerization during PRF processing leads to the intrinsic incorporation of platelet cytokines and glycanic chains in the fibrin meshes which, unlike the other platelet concentrates and thus aids the progressive release of cytokines during fibrin matrix remodeling.

Dohan et al. (2006)[40] examined the immune features of PRF. It was found that during PRF processing, leucocytes also secrete cytokines in reaction to the hemostatic and inflammatory phenomena artificially induced in the centrifuged tube. So, the authors started to quantify 5 significant cell mediators within platelet poor plasma supernatant and PRF clot exudate serum: 3 pro-inflammatory cytokines (IL-

1β, IL-6, and TNF-α), an anti-inflammatory cytokine such as IL-4, and a key growth promoter of angiogenesis such as VEGF. This data was correlated with that obtained in plasma (non-activated blood) and in sera (activated blood). These initial analyses therefore revealed that PRF could be an immune regulation node with inflammation retro control abilities and eventually thus explained the reduction of postoperative infections when PRF was used as surgical additive.

Choukroun et al. (2006)[41] explored the previously evaluated biology of PRF with the first established clinical results and determined the potential fields of application for this biomaterial. Platelet-rich fibrin (PRF) is a second generation of platelet concentrates, with simplified processing method and without biochemical blood handling. The reasoning is structured around 4 fundamental events of cicatrization, which includes angiogenesis, immune control, circulating stem cells trapping, and wound-covering epithelialization. This initial research therefore makes it probable to plan several impending applications of PRF in plastic and bone surgery, provided that the real effects are evaluated both impartially and rigorously.

Choukroun et al. (2006)[42] attempted to assess the potential of PRF in combination with freeze-dried bone allograft (FDBA) for augmenting the pneumatized sinus floor. Nine sinus floor augmentations were accomplished; in 6 sites, PRF was added to FDBA particles (test group), and in 3 sites FDBA without PRF was used (control group). After 4 months in the test group and 8 months in the control group bone specimens were harvested from the respected augmented region during the implant insertion procedure. The histologic maturation of the test group after 4 months was observed to be identical to that of the control group which was for a period of 8 months. Moreover, the newly formed bone was found to be equivalent in

quantity between the 2 protocols. Sinus floor augmentation with the combination of FDBA and PRF thus led to a reduction of healing time prior to implant placement. From a histologic point of view, the authors found that the healing time could be reduced to 4 months, and also emphasised the need to carry out large-scale studies to validate these results.

Sharma A and Pradeep AR et al. (2011)[43] evaluated the efficacy of autologous PRF in comparison with OFD in the treatment of mandibular degree II furcation defects. By using a split-mouth design, 18 patients with 36 mandibular degree II furcation defects were randomly allotted in two groups. Test group was treated with autologous PRF and OFD and the control group with OFD alone. Clinically plaque index, sulcus bleeding index, probing depth, relative vertical and horizontal clinical attachment level, gingival marginal level, and radiographically bone defect was recorded at baseline and 9 months postoperatively. Statistically significant improvement was observed for all clinical and radiographic parameters at the sites treated with PRF and OFD compared to those with OFD alone thus implying a significant role of autologous PRF as a regenerative material in the treatment of furcation defects.

Chang et al. (2011)[44] evaluated the clinical and radiographic changes of a patient with periodontal intrabony defects when treated with PRF. The left mandibular first molar and left maxillary second molar with intrabony defects were filled with PRF as the sole grafting material in a 38-yearold female patient. The primary outcomes evaluated at baseline and 6 months post-operatively were probing depth, attachment level, and radiographic bone density. The results thus displayed that the application of PRF as the sole grafting material in intrabony defects exhibited pocket

reduction and gain in clinical attachment after 3 months and 6 months. Thus by using National institute of health program, the 6 months postoperative radiographic density of images for left mandibular first molar and left maxillary second molar showed an increase of 1.6 and 1.3 fold compared with each preoperative radiography, respectively.

Bajaj P et al. (2013)[45] analysed the clinical and radiographical effectiveness of autologous platelet-rich fibrin (PRF) and autologous platelet rich plasma (PRP) in the treatment of mandibular degree II furcation defects in subjects with chronic periodontitis. Of total seventy-two mandibular degree II furcation defects 24 defects were treated with autologous PRF with OFD; 25 defects were treated with autologous PRP with OFD and 23 defects were treated with OFD alone. Clinical and radiological parameters such as probing depth, relative vertical clinical attachment level and horizontal clinical attachment level along with gingival marginal level were recorded at baseline and 9 months postoperatively. All the clinical and radiographical parameters showed statistically significant improvement at the test sites as compared to control sites. Relative vertical clinical attachment level gain was found to be greater in PRF (2.87 ± 0.85 mm) and PRP (2.71 ± 1.04 mm) sites as compared to control site (1.37 ± 0.58 mm), and statistically significant greater relative horizontal clinical attachment level gain was valuated at both PRF and PRP treated sites than in the control group. The use of autologous PRF as well as PRP were found effective in the treatment of furcation defects with uneventful healing of sites.

Gupta S et al. (2014)[46] analysed and compared the efficacious role of platelet-rich fibrin (PRF) and enamel matrix derivative (EMD; Emdogain®) in the treatment of periodontal intrabony defects in patients with chronic periodontitis, six months

post-surgery. Forty-four intrabony defects in 30 patients were randomly allocated into two treatment groups. In the control group EMD (n = 22) was used while in the test group PRF (n = 22) was used. Measurement of the defects was done by using clinical and cone beam computed tomography at baseline and 6 months. Clinical parameters such as probing depth, clinical attachment level, radiographic parameters such as intrabony defect depth and defect angle, were recorded at baseline and 6 months post-operatively. In Intragroup analysis change was evaluated by using the Wilcoxon signed rank test while intergroup comparisons were made by using the Mann-Whitney U test. Postsurgical measurements revealed that there was an equal reduction in probing depth for both the groups but a greater statistically non-significant attachment gain was found for the Emdogain® group when compared to the platelet-rich fibrin group. The Emdogain® group presented with significantly greater percentage defect resolution (43.07% ± 12.21) than did the platelet-rich fibrin group (32.41% ± 14.61). The changes in defect width and defect angle post-operatively were significant in both groups, but upon intergroup comparison they were found to be statistically non-significantly different. Both Emdogain® as well as platelet-rich fibrin were found to be effective in the regeneration of intrabony defects with Emdogain® being slightly superior in terms of percentage defect resolution.

Pradeep AR et al. (2016)[46] evaluated the potency of combination of Rosuvastatin (RSV) 1.2mg in situ gel with 1:1 mixture of autologous platelet rich fibrin and Hydroxyapatite (HA) bone graft over autologous PRF and HA bone graft placed after open flap debridement (OFD) in the surgical treatment of mandibular degree II furcation defects. Total 105 mandibular furcation defects were treated with either OFD + Placebo gel (Group 1) or PRF+HA with OFD (Group 2) or RSV 1.2mg

gel + PRF + HA with OFD (Group 3). PD, and RVAL and RHAL, intrabony defect depth and %defect fill were recorded at baseline and at 9 months postoperatively. A greater mean PD reduction was observed in Group 2 (3.68 ± 1.07 mm) and Group 3 (4.62 ± 1.03mm) than Group 1 (2.11 ± 1.25 mm) while greater mean RVAL and RHAL gain was also found in Group 2 (3.31 ± 0.52 and 2.97 ± 0.56 mm) and Group 3 (4.17 ± 0.70 and 4.05 ± 0.76 mm) as compared to Group 1 (1.82 ± 0.78 and 1.62 ± 0.64 mm respectively). Furthermore, significantly higher percentage of mean bone fill was found in the Group 2 (54.69 ± 1.93 %) and Group 3 (61.94 ± 3.54%) as compared to Group 1 (10.09 ± 4.28 %). Treatment of furcation defects with RSV 1.2mg in situ gel combined with autologous PRF and porus-HA bone graft, resulted in significant improvements of clinical and radiographic parameters when compared with OFD alone. This study implied the synergistic effect of the combination of RSV with PRF and HA thus explaining their role as a regenerative material in the treatment of furcation defects.

Biswas S et al. (2016)[48] investigated the efficacious role of a second-generation bioactive glass putty biomaterial over platelet rich fibrin in treating Grade II furcation defects. Fifteen systemically healthy patients comprising of 10 males and 5 females with an age range of 20–50, with 20 mandibular molar class II furcation defects according to Glickman's classification were treated in this study. These 20 Grade II furcation defects were randomly allocated as follows: Group I, 10 furcation defects were treated using bioactive glass (NovaBone) bone graft putty material; Group II, 10 furcation defects were treated using platelet rich fibrin (PRF). Customized acrylic stents were fabricated on study casts were used as a fixed reference point for measurements. Clinical parameters such as gingival index, plaque

index, vertical probing depth (from gingival margin to base of the pocket), clinical attachment level (CEJ to the base of the pocket), and horizontal probing depth of furcation involvement (using stent) resulting improvement in gingival index (GI) and plaque index (PI) were calculated in both groups. An overall reduction in both vertical and horizontal probing depth was found in both test and control groups; however, the Putty group (Group I) showed consistently more vertical probing depth reduction than the PRF group (Group II) at the end of third month (P- value ¼ 0.0004), sixth month (P-value ¼ 0.00001), and ninth month (P-value ¼0.0004). It was concluded that bioactive glass is a osteo-stimulative biomaterial that yields superior clinical results, including increased pocket depth reduction of class II furcation defects as compared to an autologous platelet concentrate.

Siddiqui ZR et al. (2016)[49] investigated clinically and radiographically the efficacy of platelet-rich fibrin (PRF) versus β-tri-calcium phosphate (β-TCP) in the treatment of Grade II mandibular furcation defects. Forty-five Grade II furcation defect in mandibular molars which were allotted to OFD with PRF Group I, to OFD with β-TCP Group II, and to OFD alone Group III which were analysed for clinical parameters such as; (probing pocket depth [PPD], vertical clinical attachment level [VCAL], horizontal clinical attachment level [HCAL], gingival recession, relative vertical height of furcation [r-VHF], and relative horizontal depth of furcation [r-HDF]) and radiographical parameters such as; (horizontal depth of furcation [H-DOF], vertical height of furcation [V-HOF]) using cone-beam computed tomography (CBCT) at 6 months interval. Greater reduction in PPD and gain in VCAL and HCAL were found to be in Group II as compared to Group I. Also change in r-VHF and r-HDF was found to be greater in Group II as compared to Group I. A

higher mean percentage clinical vertical defect fill was found in Group II as compared to Group I (58.52% ± 11.68% vs. 53.24% ± 13.22%, respectively). CBCT measurements showed a nonsignificant difference in mean change for all parameters at 6 months between the two experimental groups. Mean change in V-HOF was higher in Group I as compared to Group II, but mean change in H-DOF and furcation width was more in Group II as compared to Group I. Thus, a statistically significant improvement was observed at 6 months follow-up from baseline values for both experimental and the control groups.

Kanoriya D et al. (2017)[50] assessed the effectiveness of PRF and 1% ALN gel combination in mandibular degree II furcation defects treatment in comparison to PRF and access therapy alone. Seventy-two mandibular molar furcation defects were treated with either access therapy alone (Group 1), access therapy with PRF (Group 2), and access therapy with PRF+1% ALN (Group 3). PI, mSBI, PD, RVAL and RHAL, IBD were recorded at baseline and 9 months post-operatively. Radiographically defect fill was assessed in percentage at baseline before surgery and 9 months post therapy. Group 3 exhibited greater PD reduction and RVAL and RHAL gain as compared to Group 1 and 2 post-operatively. Moreover, Group 3 sites displayed a significantly greater percentage radiographic defect fill (56.01±2.64%) as compared to Group 2 (49.43±3.70%) and Group 1 (10.25±3.66%) at 9 months. The treatment of furcation defects with autologous PRF combined with 1% ALN gel thus resulted in significant therapeutic outcomes when compared with PRF and access therapy alone.

Patel G K et al. (2017)[51] evaluated the adjunctive use of PRF in regenerative management of intrabony defects in comparison with open flap debridement. Twenty-

six bilateral defects (13 per group) in 13 patients were randomly allocated to two groups, which were treated with either PRF (Test group) or Open flap debridement alone (control group). Clinical parameters included in this study were probing depth (PD), clinical attachment level (CAL) and bone probing depth. Radiographic parameters assessed were reduction in defect depth and percentage of bone fill. All primary outcome parameters were evaluated at 6months, 9 months and 12 months. Secondary outcome assessment included wound healing using a wound healing index. The test group showed significant improvement in clinical parameters over control group at 6, 9, and 12 months. The test group showed a bone fill of 45.18±7.57 % which was statistically significant when compared to 21.6±9.3 % in control group. Wound healing index (WHI) also showed significant advantages for the PRF group over control group. A significant soft tissue healing and reduction in probing depth thus showed that PRF can be potentially used as an adjunctive to conventional open flap debridement in the treatment of intrabony defects.

Rani N et al. (2018)[52] evaluated the effectiveness of PRF with β-tricalcium phosphate (β-TCP) graft (R. T. R) over β-TCP allograft alone in the treatment of mandibular Grade II furcation defects. A total of 20 mandibular Grade II furcation defects sites were included in the study and treated with either β-TCP alone (Group I) or β-TCP with PRF membrane (Group II). The clinical parameters recorded were probing pocket depth (PPD), clinical attachment level (CAL), gingival recession (GR), horizontal defect depth (HDD), and vertical defect depth (VDD), at baseline and at 6 months re-entry. Intragroup analysis showed statistically significant results for all parameters from baseline to 6 months while the intergroup analysis was found to be statistically insignificant. In Control group, gain in CAL was 2.80 ± 1.40 and in

Test group it was 3.00 ± 1.44. Bone fill in Control group was VDD (3.50 ± 2.12) and HDD (3.70 ± 0.67), whereas Test group showed VDD (3.70 ± 1.57) and HDD (4.0 ± 0.88), respectively. PPD reduction was found to be higher in Group I (3.50 ± 2.27) than Group II (2.80 ± 1.93). Control group showed higher GR (0.70 ± 0.67) than Test group (0.40 ± 0.52) at re-entry. Although significant improvement was found in both groups, the combination of PRF with β-TCP allograft led to a more favourable improvement in the management of Grade II furcation defect except PPD.

Kaur J et al. (2018)[53] assessed and compared the regenerative potential of autologous PRF with and without amnion membrane in the treatment of Grade II furcation defects. This double-masked randomized, split-mouth design study included fifteen patients with thirty mandibular degree II furcation defects which were randomly allotted into Group I (PRF and amnion membrane) and Group II (PRF). Clinically parameters such as PI and GI-at defect site along with probing pocket depth, and relative attachment level and furcation defect depth were recorded at baseline, 3 months, and 6 months postoperatively. Radiographic parameters by using computer-assisted tomography (Dentascan) were evaluated at baseline and 6 months postoperatively. Statistical Analysis was performed by using Wilcoxon signed-rank test, and for comparison between the two groups/intergroup variations, Independent t-test and Mann–Whitney test was performed. All evaluated parameters showed statistically significant improvement at the sites treated with PRF and amnion membrane as compared to those treated with PRF alone. Bearing in mind the limitations of this study a greater pocket reduction, attachment level gain, and bone fill was found at the sites treated with PRF and amnion membrane as compared to sites treated with PRF alone.

Agrawal A et al. (2019)[54] explored the effectiveness and usefulness of PRF alone and with DFDBA in the treatment of mandibular degree II furcation defects in subjects with chronic periodontitis. A total of sixty mandibular molars were treated with either open flap debridement (OFD) alone, PRF and OFD combination or OFD + PRF + DFDBA combination. The clinical parameters such as vertical probing depth (VPD), vertical clinical attachment level (VCAL), gingival marginal level (GML), horizontal probing depth (HPD) and radiographic parameters such as vertical bone fill (VBF), horizontal bone fill (HBF) and furcation width (FW) were recorded at baseline and 9 months post-therapy. To measure the statistical significance between time period within each group for clinical and radiographic parameters a paired t-test was conducted. ANOVA and post-hoc Tukey's tests were used for intergroup comparison of soft and hard tissue parameters. All treatment groups showed significant ($p < 0:001$) improvement in soft and hard tissue parameters at 9 months, except GML in all three groups and HBF and FW in the OFD group as compared to baseline. The mean VBF change was found to be highest in the OFD + PRF + DFDBA group (1:90 ± 0:45) mm, followed by that in the PRF + OFD and OFD groups (1:60 ± 0:88 and 0:45 ± 0:51 mm, respectively). Both PRF + OFD and PRF + DFDBA + OFD combinations exhibited significant advantage for the management of mandibular degree II furcation defects. However, the PRF + DFDBA + OFD combination showed profound benefits over PRF + OFD combination in terms of VBF.

3. Review of studies on combination therapy (1% MF + PRF)

Pradeep et al. (2015)[55] assessed the efficacy of1%MF gel with or without PRF, PRF alone with open flap debridement (OFD), in the treatment of intrabony defects in chronic periodontitis (CP) patients. One hundred and twenty patients with

single defects were treated with either OFD alone (group I), OFD with PRF (group II), OFD with 1% MF gel (group III) or with OFD with PRF+1%MF (group IV). PI, mSBI, PD, relative attachment level (RAL) and gingival marginal level (GML) were recorded at baseline, before surgery and 9 months postoperatively. Radiographic intrabony-defect depth reduction was calculated in percentage at baseline and 9 months using computer aided software programme. Group II, III and IV showed significant PD reduction and RAL gain than group I. Mean PD reduction and mean RAL gain was found to be greater in group IV as compared to group II and III at 9 months. Furthermore, a significantly greater percentage radiographic defect depth reduction was observed in sites treated with PRF+1% MF (52.65% ± 0.031%) as compared to MF alone (48.69± 0.026 %), PRF alone (48 % ± 0.029%) and OFD (9.14±0.04%) at 9 months. Combined approach therapy of PRF+1% MF for intrabony defects treatment in CP patients showed better clinical parameters outcomes with greater defect depth reduction in comparison to PRF, 1%MF and access therapy alone.

Sharma P et al (2017)[56] evaluated the effectiveness of PRF and 1% MF gel combination in mandibular Grade II furcation defects treatment in comparison to PRF and access therapy alone. Thirty mandibular molar furcation defects were treated with either access therapy and PRF alone (Group 1) or access therapy with PRF+1% MF (Group 2). PI, mSBI, PD, relative vertical attachment level (RVAL), and relative horizontal attachment level (RHAL) were recorded at baseline and at 6 months post-operatively. Radiographically defect depth (IBD) was assessed at baseline and 6 months post therapy by using image J software. Test group showed greater PD reduction and RVAL and RHAL gain as compared to Control group post-operatively.

Not a very significant difference in RVAL was observed in both the groups at 6 months. Furcation defects treatment with autologous PRF combined with 1% MF gel thus resulted in significant therapeutic outcomes when compared with access therapy and PRF alone.

4. Review of studies using cone beam computed tomography (CBCT)as an investigative modality for analysis of periodontal osseous defects and regeneration.

Misch KA et al. (2006)[57] compared linear measurements of periodontal defects obtained via traditional methods to those obtained via CBCT images. Artificial osseous defects were created on premolar and molar of mandibles of dry skulls. Each investigation in this study utilised CBCT scan, periapical radiograph, and direct measurements using a periodontal probe which was then compared to defect measurements made by impressionmaterial for standard reference by an electronic caliper. Statistically no differences were seen between bone sounding, radiography, and CBCTfor linear measurements. Whereas a significant variance was observed on comparing isolated interproximal measurements using a probe versus the caliper ($p < 0.001$) but not for CBCT or other radiography. All included periodontal osseous defects were recognizable and measurable directly or with CBCT. In comparison, radiographs could not measure buccal and lingual defects. It was thus concluded that all three modalities are useful for identifying interproximal periodontal defects. Compared to radiographs, the 3D capability of CBCT offered a significant advantage of detecting and as well as quantifying the bony defects.

Walter C et al. (2009)[58] assessed the accuracy of CBCT in detecting furcation involvement (FI) in maxillary molars. Fourteen (14) patients with generalized advanced chronic periodontitis were successively recruited and treated non-surgically. Maxillary molars with the furcation involvement were considered for furcation surgery. Due to increased FI and/or increased probing pocket depths during re-evaluation, CBCT was performed to evaluate the degree of FI on CBCT images. Furcation surgery was performed in 25 maxillary molars. The data collected from CBCT images was compared with the intra-surgical FI assessments. The intra-surgical findings confirmed overall, 84% of the CBCT data. Total 14.7% (11 sites) data was underestimated (CBCT less than intra-surgical value) and in only 1.3% (one site) CBCT data was overestimated as compared with the intra-surgical analysis. Agreement for final evaluation between both assessment methods was found to be the highest in distopalatal furcation entrances, followed by buccal and mesiopalatal. So, it was concluded that CBCT images determine a high accuracy in evaluating the loss of periodontal tissue and classifying the degree of FI in maxillary molars.

Cimbaljevic MM et al. (2015)[59] compared the use of periodontal probing and CBCT images in the diagnosis of FI in patients with chronic generalized severe periodontitis. A total 15 patients with 174 furcation sites (38 maxillary and 30 mandibular molar teeth) were analysed for FI at three sites (buccal, mesiopalatal, and distopalatal) of maxillary molars, and at buccal and oral sites of mandibular molars. A dichotomous scale (present/absent) was used to assess FI both clinically (using Naber's periodontal probe) and radiographically on CBCT images. Overall, FI was more often detected by CBCT analysis than by clinical examination. The agreement between the evaluation methods was found to be 46.9%, with a stronger agreement in

maxillary sites than in mandibular sites (63.3% and 45.0% respectively). Clinically detected 24% FI sites was confirmed by means of CBCT evaluation. in 24%. Total 73% of strongest agreement was observed between two methods for FI detection in the distopalatal maxillary sites, whereas the smallest agreement (36.6%) was found in the buccal sites of the mandibular molars, in which 63.3% of FI were detected by using CBCT only and not clinically. The results of this study highlighted a greater accuracy of CBCT in assessing FI than clinical probing and also indicated its use as an adjunct tool in diagnosing FI in cases of surgical intervention.

Pajnigara N et al. (2017)[60] evaluated the dimensions of Grade II furcation defects clinically (pre-and post-surgery), intra-surgically, and by CBCT (pre and post-surgery). Total 200 grade II furcation defects in forty patients with a mean age of 38.05 ± 4.77 years, diagnosed with chronic periodontitis were analysed in the study. After the pre surgical baseline clinical and CBCT measurements, DFDBA was placed in the furcation defect and the flaps were sutured back. After six months, these defects were assessed by recoding post-surgery clinical and CBCT measurements (40 defects each). In both aspects (vertical and horizontal), pre-surgery clinical measurements (vertical 6.15 ± 1.71 mm and horizontal 3.05 ± 0.84 mm) and CBCT measurements (vertical 7.69 ± 1.67 mm and horizontal 4.62 ± 0.77 mm) underestimated intra-surgery measurements (vertical 8.025 ± 1.67 mm and horizontal 4.82 ± 0.67 mm) but the difference was not statistically significant (vertical $p = 1.000$, 95% CI; horizontal $p = 0.867$, 95% CI). Further, post-surgery clinical measurements (vertical 2.9 ± 0.74 mm and horizontal 1.52 ± 0.59 mm) underestimated CBCT measurements (vertical 3.67 ± 1.17 mm and horizontal 2.45 ± 0.48 mm). The difference between pre-surgery clinical and CBCT values ($p< 0.0001$, 95% CI) versus post-surgery clinical and

CBCT values ($p< 0.0001$, 95% CI) in both vertical and horizontal aspects was statistically significant. It was thus concluded that the use of CBCT appears to be considerate for accurate diagnosis and treatment planning of furcation defects in advanced periodontal diseases.

Padmanabhan S et al. (2017)[61] compared the diagnostic efficacy of cone-beam computed tomography (CBCT) as against direct intra-surgical measurements of furcation defects in mandibular molars. Total 14 patients with 25 mandibular molar furcation sites were analysed in this study. CBCT was performed to measure the deepest height, width, and depth of Grade II and Grade III furcation defects of mandibular molars. For the specified tooth the intra-surgical measurements of the FI were evaluated during periodontal flap surgery using UNC-15 periodontal probe which were then compared with CBCT measurements. Paired t-test and Bland–Altman plot were applied for statistical analysis. The CBCT as opposed to intra-surgical furcation measurements were 2.18 ± 0.86 mm versus 2.30 ± 0.89 mm for furcation height, 1.87 ± 0.52 mm versus 1.84 ± 0.49 mm for furcation width, and 3.81 ± 1.37 mm versus 4.05 ± 1.49 mm for furcation depth, respectively. Results showed no statistically significant difference between the measured parameters, indicating the two methods to be statistically similar. Thus, the accuracy of CBCT in assessing mandibular molar furcation defects was found to be comparable to that of direct surgical measurements further indicating the use of CBCT as an exceptional diagnostic tool in periodontal treatment planning.

Warda H et al. (2018)[62] determined the accuracy of cone beam computed tomography (CBCT) in the assessment of mandibular molar furcation defects over intra-surgical assessment. Total 19 mandibular molar furcation defects with degree II

or III involvement were included in the study. CBCT measurements of furcation involvement was recorded in three dimensions. These included the measurement in horizontal plane which was calculated by measuring the distance between the outer root surface and the inter-radicular bone (bone loss in the horizontal direction – 'BL-H'), the degree of bone loss in vertical direction (BL-V) which was calculated by measuring the distance from the furcation entrance to the base of the defect in the vertical direction and lastly the width of the furcation entrance (FW) which was measured by measuring the greatest mesio–distal distance in the furcation. Intra-surgical measurements also recorded furcation height (BL-V) and width (BW) by using Periodontal probe and defect depth (BL-H) by using Naber's probe. No statistically significant difference was observed between both the methods with respect to the measured parameters giving a p value of 0.101, 0.201 and 0.910 for FW, BL-V and BL-H respectively. The authors thus confirmed the use of CBCT for assessing mandibular furcation defect.

Zang W et al. (2018)[22] compare and correlate accuracy of molar furcation assessment via three different evaluation methods i.e., clinical detection, intraoral radiography and CBCT images. Total eighty-three patients having chronic periodontitis with an existing CBCT scans were included. Furcation involvement was assessed on maxillary and mandibular first molars. Furcation involvement on buccal and palatal/lingual sites were evaluated by using Periodontal charts (modified Glickman's classification), intraoral (periapical and/or bitewing) radiographs (recorded as presence or absence) and axial CBCT sections. The correlation of furcation assessment by the three methods was assessed by using Pearson analysis. Substantial correlation ($p < 0.05$) was found between clinical detection and intraoral

radiography, clinical detection and CBCT, as well as intraoral radiography and CBCT at all the measured sites (r values range between 0.230 to 0.644). While, CBCT exhibited higher correlation with clinical detection relative to intraoral radiography, especially at distal palatal side of maxillary first molar ($p < 0.05$). In addition, CBCT provided more accurate assessment of bone loss measurement up to 2 decimals in millimeters, whereas clinically all 3 classes FI were detected and the intraoral radiographs usually only detected the presence of furcation involvement in Glickman Class 2 and 3. This study thus validated the use of CBCT as a valuable tool in assessing molar furcation defects in addition to clinical detection and intra-oral radiographs.

Bodhare et al. (2018)[63] evaluated the efficacy of Bioactive Glass (BG) with and without autologous platelet-rich fibrin (PRF) in the treatment of intrabony defects in chronic periodontitis patients and assessed the changes clinically and radiographically using CBCT. Total 20 chronic periodontitis patients with a mean age: 35.9 years having at least one pair of bilateral intrabony defect were analysed in this study. 20 sites in Group I were treated with a combination of BG and autologous PRF whereas 20 sites in Group 2 were treated with BG alone. Clinical parameters such as, probing pocket depth (PPD), clinical attachment level (CAL) and gingival recession (GR) were evaluated at 3 and 6 months while the radiographical parameter such as, bone fill was evaluated at 6 months by using cone beam computed tomography (CBCT). Chief study outcomes were changes in PPD, CAL, GR and bone fill. The results showed a significant CAL gain in Group 1 (5.05 ± 1.09 mm) as compared to Group 2 (4.2 ± 1.70 mm) and a significantly greater bone fill in Group 1 at 6 months. Statistically significant reduction in PPD was found in Group 1 as well as

in Group 2. The authors found a significant effect of BG morsel in combination with PRF on clinical and radiographical parameters. CBCT evaluation of bone fill confirmed the enhanced periodontal regeneration with the use of BG morsel and PRF as compared to treatment with BG alone in periodontal intrabony defects.

Wanikar et al. (2018)[64] evaluate clinically and radiographically the efficacy of 1% ALN gel in combination with PRF over PRF alone in the treatment of grade II furcation defects. Total 40 bilateral furcation defects were randomly divided into PRF group (control group) and PRF+ALN group (test group). The reduction in Bone defect volume was the primary outcome which was evaluated at the end of 6 months by using CBCT while the secondary outcomes evaluated were changes in clinical parameters including Probing pocket depth (PPD) and Clinical attachment level (CAL) and Horizontal probing depth (HPD) at baseline, 3 and 6 months. The results showed a mean reduction in PPD (1.85 ± 0.59 mm), CAL (1.9 ± 0.64mm) and HPD (1.7 ± 0.73mm) for PRF group and 2.85 ± 0.88 mm, 3.05 ± 0.98mm and 2.3 ± 0.73mm respectively for PRF+ ALN group. CBCT evaluation at the end of 6 months showed a mean reduction in bone defect volume for both PRF (8.65 ± 3.84 mm3) and PRF+ ALN group (11.98 ± 4.13 mm3) with greater reduction in the test group as compared to control group. Better clinical and radiographic outcomes using CBCT in PRF+ALN treated sites suggests enhanced periodontal regeneration with the combination of PRF+ALN as compared to PRF alone treated sites.

Shah et al. (2019)[65] evaluated and compared DFDBA alone and in combination with CM in treatment of Grade II furcation defects, clinically and radiographically by CBCT. This study comprised of total 20 patients with a mean age of 40.85 ± 7.32 years (13 females and 7 males) affected by chronic periodontitis

having at least a pair of bilateral grade II furcation. The patients were randomly divided into two groups i.e Group I and II. In Group I after open flap debridement DFDBA was placed while in Group II DFDBA along with chorion membrane was placed at the site of Grade II furcation defect. Probing pocket depth (PPD) and Clinical attachment level (CAL), Gingival recession (GR) and Horizontal probing depth (HPD) at baseline, 3 and 6 months while the bone defect volume was evaluated at 6 months by using CBCT. Comparison of clinical parameters from baseline to 6 months yielded statistically significant reduction in PPD ($p < 0.001$), gain in CAL ($p < 0.001$), increase in GR ($p=0.0001$ and $p= 0.004$ for Group I and II respectively) and reduction in HPD in both groups ($p < 0.001$). A statistically significant decrease in bone defect volume in both the groups ($p < 0.001$) was also observed on CBCT analysis. A significant improvement in all clinical and radiographical parameters using CBCT in DFDBA+CM group indicated additional benefits of this combination therapy.

Shirke P et al. (2019)[66] analysed the efficacy of 1.2% ATV as an adjunct to scaling and root planing (SRP) in the treatment of intraosseous defects clinically and radiographically by CBCT. Total 20 CP patients, with a minimum of one pair of bilateral intraosseous, were randomly divided into Group 1 (SRP with subgingival delivery of a placebo gel) and Group 2 (SRP along with subgingival delivery of 1.2% ATV gel). The parameters evaluated clinically at baseline and 3- and 6-month intervals were plaque index (PI), modified sulcus bleeding index (mSBI), probing pocket depth (PPD) and clinical attachment level (CAL), while bone fill, the radiographic parameter was recorded at baseline and 6-month interval using cone-beam computed tomography (CBCT). A greater reduction in the mean PPD and gain

in CAL was demonstrated in Group 2 as compared to group 1 at 3- and 6-month intervals. Furthermore, a significantly greater bone fill was obtained in group 2 (1.70 ± 0.54 mm) as compared to group 1 (0.22 ± 0.43 mm) after six months. The authors thus confirmed the use of ATV with SRP as a non-invasive way to enhance periodontal regeneration in periodontal intraosseous defect.

MATERIALS AND METHOD

Periodontal attachment loss in the furcation area of multirooted teeth are not amenable to definitive and predictable treatment of conventional periodontal procedures. The most unique and challenging task is the reliable detection, assessment and measurement of furcation and the degree of its involvement. Use of three-dimensional imaging could facilitate treatment planning and periodontal regenerative therapy could provide an adequate prognosis of furcation defects.

The present study was a randomized clinical trial undertaken to evaluate 1% MF gel in conjunction with PRF and PRF alone in the treatment of Class II furcation defects. The evaluation was done clinically and by CBCT.

One of the most commonly used classifications for furcation involvement was the one given by Irvin Glickman in 1953.[9] With reference to this classification the

present study utilizes Grade II furcation defects to evaluated the regenerative potential of 1%MF with PRF versus PRF alone.

Clinical research reported remarkable gains in clinical attachment levels using PRF in human intraosseous lesions.

Metformin HCL is second generation biguanide which is the used in the treatment of diabetes mellitus. Previous studies have demonstrated Metformin to increases the bone formation by stimulating the differentiation of osteoblasts and also diminishing bone loss by restraining the differentiation of osteoclasts. Since there is paucity of literature available on its use in periodontal regeneration it was felt necessary to conduct clinical trial and evaluate the efficacy of this drug and its combination with autologous PRF in the treatment of Grade II furcation defect.

21 patients (11 females and 10 males) affected with Stage III periodontitis[67] comprising of both the sexes in age range of 33-58 years (mean age of 43.33 ± 7.65 years) were selected from those visiting the Department of Periodontology of our Institute. Each patient with a bilateral Grade II furcation defects in either maxillary or mandibular molars were included in this study. The study was initiated after the clearance from the Institutional Ethics Committee of our institute. A special proforma was designed so as to have systematic and methodological recording of observation and information. This included a detailed case history, clinical examination, radiographic evaluation, periodontal indices and written consent of the patient.

INCLUSION CRITERIA

1. Patients with Stage III periodontitis[67] as assessed by probing pocket depth (PPD) ≥ 6mm, interdental clinical attachment loss (CAL) ≥ 5mm and Horizontal Probing depth (HPD) > 2mm.

2. Patients with at least one pair of bilateral Grade II furcation defects in either maxillary or mandibular arches.

EXCLUSION CRITERIA

1. Patients with history of known systemic diseases, allergies or drug usage.

2. Patients who have undergone periodontal therapy in last 6 months.

3. Pregnant or lactating women.

4. Smokers, tobacco chewers and patients with poor oral hygiene index (PI>1.5) will be excluded from the study.

PRESURGICAL THERAPY

All the selected patients were subjected to presurgical hygiene therapy and received thorough scaling and root planing session, oral hygiene instructions, and any occlusal adjustment prior to surgery. Three weeks after the initial therapy the patients were re-evaluated to assess the plaque control and overall oral hygiene. Recording of clinical data of such as oral hygiene and gingival health, PI and GI and clinical parameters (PPD, CAL, HPD) was carried out by the same examiner in all the patient. These parameters were evaluated at baseline, 3 and 6 months post-therapy.

ALLOTMENT

The selected sites were randomly assigned to Group I that is Control group (PRF) and Group II that is Test group (1%MF + PRF) by a computer-generated random table number by the operator, who was the second examiner. Every patient was explained about the treatment procedure to be performed and its outcome and a written informed consent was obtained prior to beginning of the study.

A. PLAQUE INDEX (PI) (SILNESS AND LOE, 1964)[68]

Plaque was assessed on labial / buccal, lingual/ palatal, mesial and distal surfaces of these selected teeth.

The teeth selected as the index teeth were

16 - Maxillary Right First Molar

12 - Maxillary Right Lateral Incisor

24 - Maxillary Left First Premolar

36 - Maxillary Left First Molar

32 - Mandibular Left Lateral Incisor

44 - Maxillary Right First Premolar

Score	Criteria
0	No plaque.
1	A film of plaque adhering to the free gingival margin and adjacent area of the tooth which cannot be seen with the naked eye but only detected using probe or disclosing agent.

2	Moderate accumulation of deposits within the gingival pocket or the gingival margin and /or adjacent tooth surface which can be seen with the naked eye.
3	A band of plaque wider than 1mm but covering less than one third of the crown.
4	Abundance of soft matter within the gingival pocket and/or on the tooth and gingival margin.

Calculations

The total plaque score for the entire mouth per person was obtained by totalling all of the plaque scores and dividing by the number of surfaces examined.

PI = Total plaque score

Number of surfaces examined

Plaque Scores	Condition
0	Excellent
0.1-0.9	Good
1.0-1.9	Fair
2.0-3.0	Poor

B. GINGIVAL INDEX (GI) (LOE AND SILNESS, 1963)[69]

The severity of gingivitis was scored on mesial, distal, buccal and palatal/lingual surfaces of selected index teeth.

The teeth selected as the index teeth were

16 - Maxillary Right First Molar

12 - Maxillary Right Lateral Incisor

24 - Maxillary Left First Premolar

36 - Mandibular Left First Molar

32 - Mandibular Left Lateral Incisor

44 - Mandibular Right First Premolar

A UNC-15 periodontal probe was used to assess the bleeding potential of gingival margin according to following criteria.

Score	Criteria
0	Absence of gingival inflammation/normal gingiva.
1	Mild inflammation, slight change in colour, slight oedema, no bleeding on probing
2	Moderate inflammation, moderate glazing, redness, oedema, hypertrophy, bleeding on probing.
3	Severe inflammation, marked redness and hypertrophy, ulceration, tendency to spontaneous bleeding

Calculations

If the scores around each tooth are totalled and divided by four, the gingival index score for the tooth is obtained. To obtain the GI score for the individual the total index score for each of the teeth are totalled and divided by total number of surfaces examined.

$$\textbf{GI} = \frac{\textbf{Total GI scores}}{\textbf{Number of surfaces examined}}$$

The numerical scores of the GI may be associated with varying degrees of clinical gingivitis as follows:

Gingival Scores	Condition
0.1-1.0	Mild Gingivitis
1.1-2.0	Moderate Gingivitis
2.1-3.0	Severe Gingivitis

After the hygiene phase of therapy, soft tissue measurements were determined to the nearest millimeter mark by using UNC-15 graduated periodontal probe from the cementoenamel junction (CEJ) and free gingival margin (FGM) to base of periodontal pocket (BP) and Clinical attachment level (CAL) and Probing Pocket Depth (PPD) were recorded. While Horizontal probing depth (HPD) was measured using Naber's probe. Custom made occlusal acrylic stents were used to standardize the probe angulation and position. Occlusal stents were fabricated with cold cured acrylic resin on a cast model obtained from an alginate impression. The occlusal stents covered the occlusal surface of the tooth being treated and occlusal surfaces of at least one tooth in mesial and distal directions. Stents also extended apically on the buccal and lingual surfaces so as to cover the coronal third of the teeth. A groove (guide plane) was made on the stent in relation to each involved tooth to guide the UNC-15 periodontal probe while taking measurements. This technique provided a fixed reference point and fixed angulations for measurements at each site.

CBCT ANALYSIS

CBCT measurements were taken for each Group i.e. the Group I (PRF) and the Group II (1% MF + PRF) at baseline and at 6 months. The CBCT analysis included the measurement of bone defect height, bone defect width in sagittal view and the bone defect depth in the axial view which generated the total volume of the bone defect.

SURGICAL ARMAMENTARIUM

Instruments were arranged in a definite order on a sterilized drape placed on a surgical trolley. All the equipment's were autoclaved.

The surgical armamentarium consisted of –

- Mouth mirrors.
- UNC-15 periodontal probe (Hu-Friedy, USA).
- Straight probe.
- Naber's probe
- Explorer number 23 and number 17.
- Tweezer.
- Disposable gloves.
- Disposable face masks.
- Disposable syringe – 5ml and 2ml.
- Disposable test tubes 10 ml
- Centrifugation machine (R-4C, REMI, Mumbai, India)
- Local anaesthetic (2% Xylocaine HCl with adrenaline 1:200000).

- Bard parker handles.
- No. 11, 12, and 15 blades.
- Periosteal elevator (24G Hu-Friedy, USA).
- Gracey curettes.
- Scissors – Castroviejo and straight
- Tissue forceps.
- Needle holder.
- Mersilk suture material.
- Cotton swabs.
- Kidney tray with saline and irrigation syringe.
- Dappen dish.
- Coe – pak.
- Normal saline.
- Denatured spirit.
- Listerine mouthwash .

Materials Used in Periodontal Regeneration

PRF preparation

The PRF was prepared following the protocol developed by Choukroun et al. Just before surgery, intravenous blood (by venipuncture of the antecubital vein) was collected in two 10 mL sterile tubes without anticoagulant and immediately centrifuged in a centrifugation machine (R-4C, REMI, Mumbai, India) at (approximately 400 g) for 10 min. Blood centrifugation allowed the composition of a

structured fibrin clot in the middle of the tube, just between the red corpuscles at the bottom and acellular plasma [platelet-poor plasma (PPP)] at the top. Just after the removal of PPP, PRF was easily separated from the red corpuscle base [preserving a small red blood cell (RBC) layer] using a sterile tweezers and scissors and then transferred on to a sterile compress. A stable fibrin membrane was obtained by squeezing serum out of the PRF clot.

1% MF gel preparation

S. No	Component	1%
1	Metformin HCL	10 gm
2	Distilled water equivalent to	100 mL/
3	Methyl paraben	50 mg
4	Propyl paraben	25 mg
5	Carbopol 934P	1 gm
6	Triethanolamine	0.5 mL

Procedure for Gel Preparation

1) To prepare 1% Metformin gel at first methylparaben (0.1%) and propylparaben (0.05%) were dissolved in 100 mL of distilled water.

2) After that Sonication was done to allow proper mixing of preservatives and water.

3) It was followed by dispersion of 1% Carbopol 934P in the solution was kept for 24 hrs.

4) After a complete dispersion of Carbopol 1% of Metformin HCL was added to the solution.

5) And finally, Triethanolamine (1%) was added to form the gel.

SURGICAL PROCEDURE

After the completion of initial therapy and recording baseline values, the patients entered into the surgical phase of the regenerative therapy. The selected sites to be treated were anesthetized with local anaesthesia containing 2% Xylocaine HCl with adrenaline (1:200000). The surgical procedure was initiated after adequate anaesthesia.

Incision and reflection of flap

Full thickness mucoperiosteal flap was reflected after giving an intrasulcular incision extending at least one tooth mesial and distal to the treatment site. An attempt to preserve the interdental papilla was made wherever possible. After gaining access, the defect site was debrided by removing granulation tissue from the bony wall of the defects with the help of curettes. The root surfaces were scaled and planed using hand instruments. At the time of surgery baseline hard tissue measurements were recorded from the CEJ to the base of furcation defect. Horizontal probing defect depth was also measured.

Placement of 1% MF gel and PRF

For Test group, 10 mL prepared 1% MF gel (10mg/mL) was injected into the furcation defects using a syringe followed by placement of PRF. The reflected flap was repositioned over the PRF and secured with interrupted sutures (3-0 mersilk

suture) and periodontal dressing was placed. For Control group, the sites received the same treatment with the application of PRF but without application of the 1% MF gel.

POST-SURGICAL CARE

Post-surgical instructions were given and appropriate oral hygiene measures were explained to all the patients. Patients were placed on antibiotics (Amoxicillin + Clavulanic acid: 625 mg) two times a day for five days. Analgesics (Ketorolac tromethamine 10mg) were prescribed to control postsurgical discomfort. Sutures and Coe-pak were removed after seven days. Patients were instructed to use a Chlorhexidine mouth rinse (10 ml twice daily) for 15 days and refrain from chewing hard or sticky foods, forcefully brushing the treated sites, or using any interdental aids until the next appointment at 3 months. Adverse effects were recorded at recall visits, and supragingival deposits were removed.

POST SURGICAL EVALUATION

The patients were evaluated clinically at 3 and 6 months and by CBCT at 6 months intervals. Using UNC 15 graduated periodontal probe the measurements of CAL and PPD were taken while using Naber's probe HPD measurements were taken similar to the pre procedures.

CBCT MEASUREMENTS

All the sites in both Test and Control groups were subjected to CBCT assessment. The Orthophos® XG 3D manufactured by Sirona Dental Systems GmbH, Germany using 3Diagnosys 4.2 Imaging software was used for the CBCT assessment.

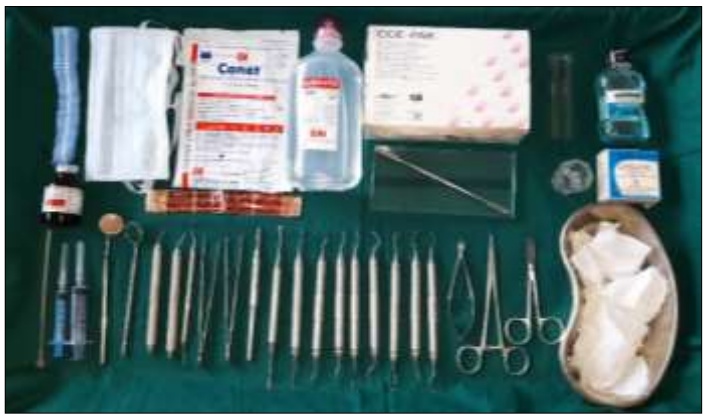
Patient was asked to remove all metal objects and wear a lead apron. The patient was asked to bite gently and naturally on the bite block without joining the incisors. The upper incisors centered with the bite block. The patient was adjusted using two positional laser beams-

- The mid-sagittal positioning laser beam
- The 3D FoV positioning laser beam

The digital readout was seen on the computer screen.

The CBCT analysis was done at baseline and at 6 months post-surgery to measure the defect height, depth and width, thus, giving the total volume of the bone defect.

COLOR PLATE I



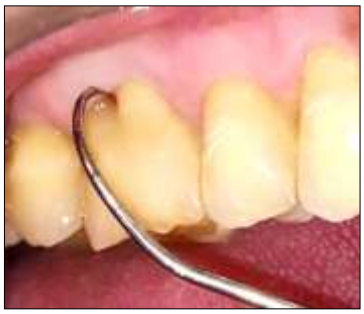
Surgical Armamentarium

Platelet Rich Fibrin (PRF)

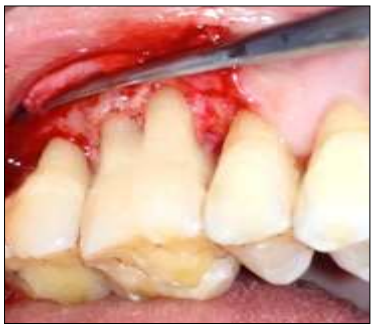
1% Metformin(1%MF) **Gel in Cannula**

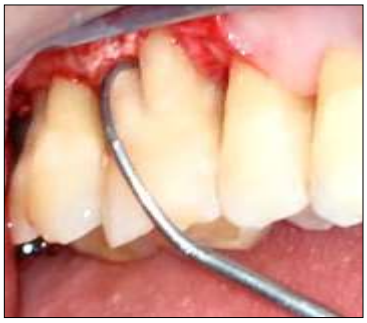
COLOR PLATE II

SURGICAL PROTOCOL FOR GROUP I (PRF)

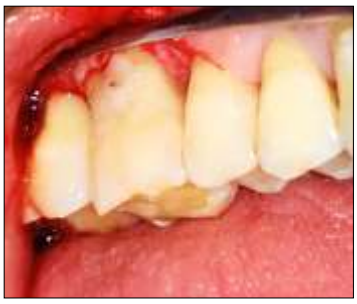



Pre-operative Photograph

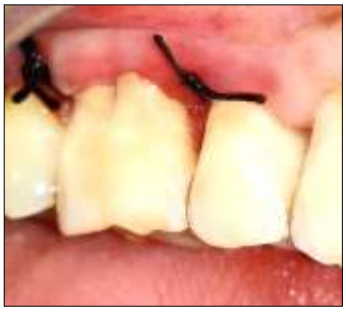
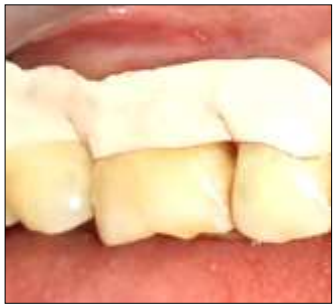




After Debridement **Vertical Probing Depth** **Horizontal Probing Depth**



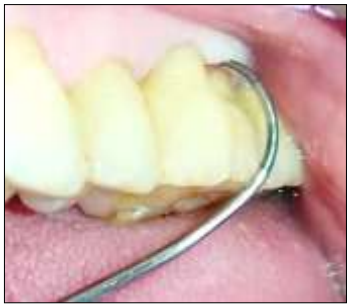
Placement of Platelet Rich Fibrin

Sutured flap **Periodontal Dressing**

COLOR PLATE III

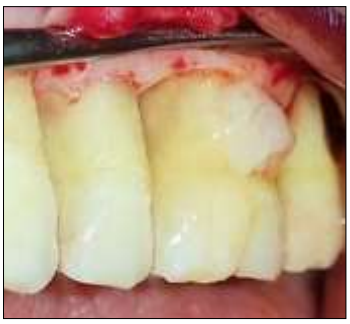
SURGICAL PROTOCOL FOR GROUP II (1%MF + PRF)

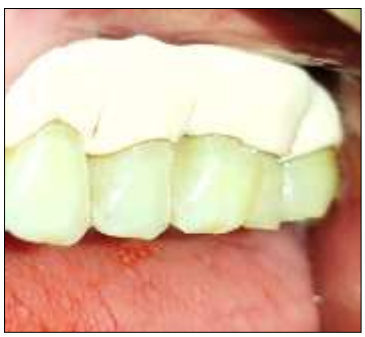
Pre-operative Photograph



After Debridement **Vertical Probing Depth** **Horizontal Probing Depth**

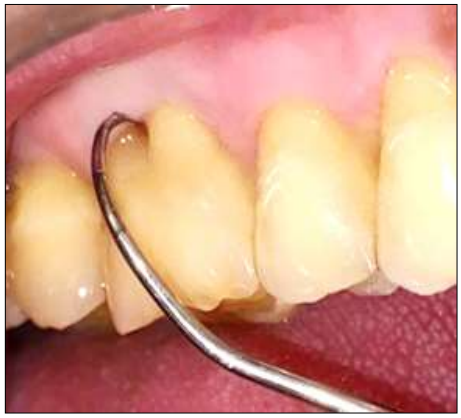
Placement of 1% Metformin gel + Platelet Rich Fibrin

Sutured flap **Periodontal Dressing**

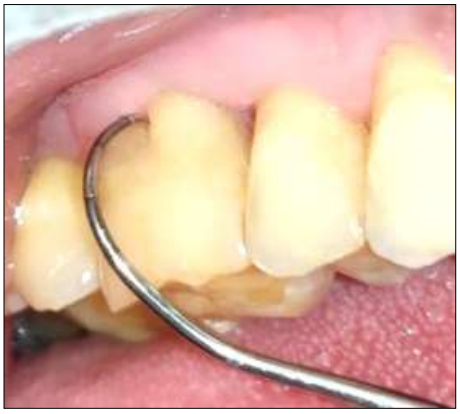
COLOR PLATE IV

RECALL: CLINICAL PARAMETERS FOR GROUP I (PRF)



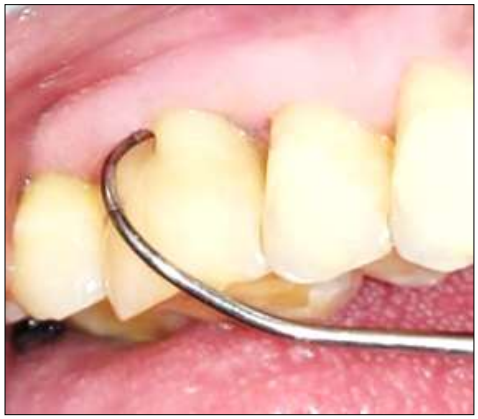
Baseline

Baseline



3 months

3 months

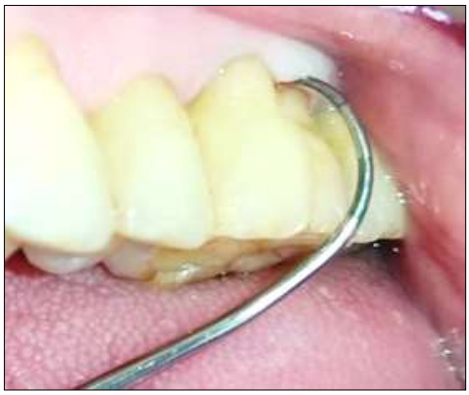


6 months

6 months

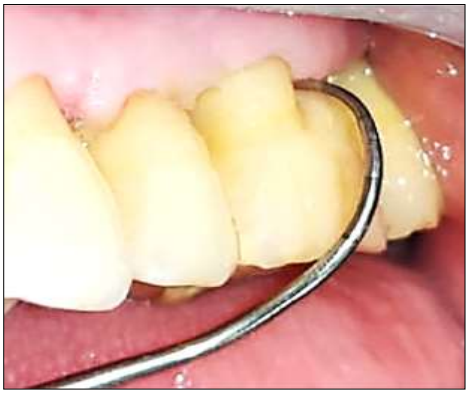
COLOR PLATE V

RECALL: CLINICAL PARAMETERS FOR GROUP II (1%MF + PRF)

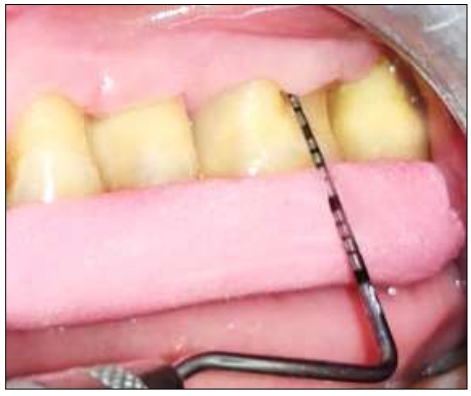


Baseline

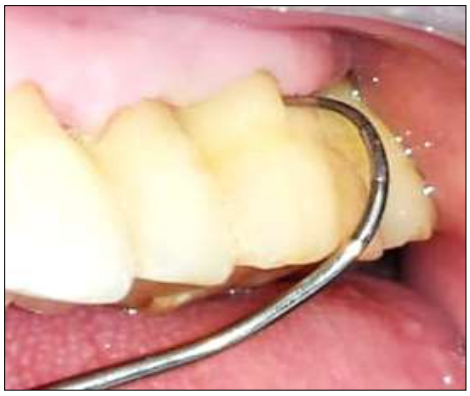
Baseline



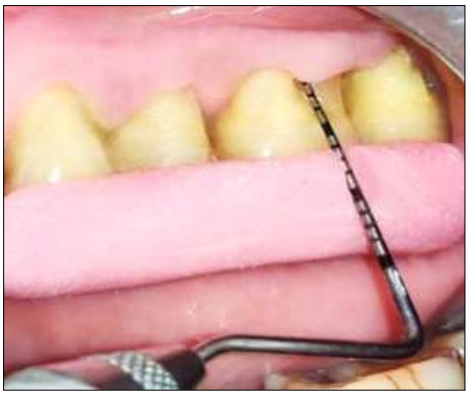
3 months



3 months



6 months

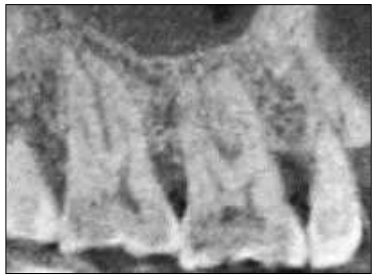


6 months

COLOR PLATE VI

RECALL: CBCT PARAMETERS

GROUP I (PRF)



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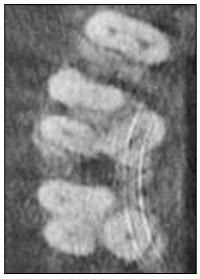

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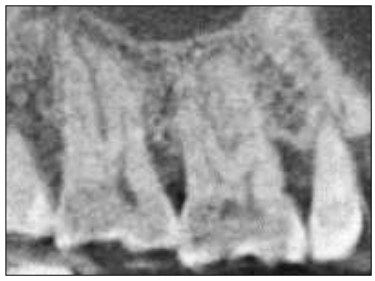
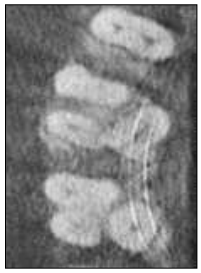
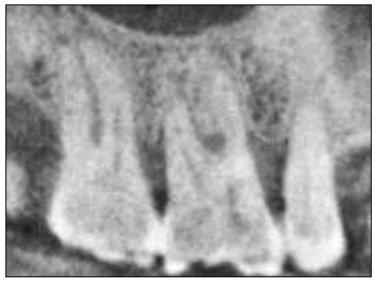


6 months

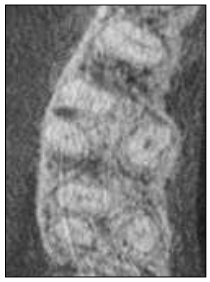


6 months

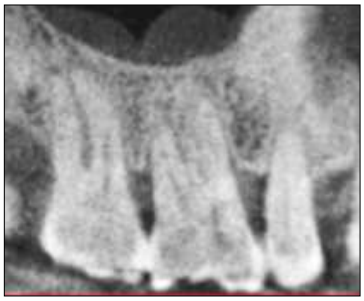
GROUP II (1%MF + PRF)



Baseline



Baseline



6 months

6 months

RESULTS

At baseline, the parameters assessed were PI, GI, PPD, CAL, Horizontal PD (HPD); height, width and depth of the defect by CBCT. PI, GI, PPD, CAL, HPD was measured at all recall visits (3 months and 6 months). The CBCT analysis for defect height, width and depth was done at recall interval of 6 months.

STATISTICAL ANALYSIS

The data was analyzed using the STATA Version 20.0. The p-value was taken as significant when less than 0.05. The tests applied were

1. Continuous variables (Age, PI, GI, PPD, CAL, Horizontal PD, Defect height, Defect width, Defect depth, Volume of defect) were presented as Mean ± SD.

2. PI, GI, PPD, CAL and Horizontal PD were compared at different time point in each group by performing repeated measures of Analysis of Variance

(ANOVA). Pairwise comparisons were made by Tukey's multiple comparison tests.

3. Mean changes in these study parameters were compared between Group I and Group II by independent t-test.

4. Defect height, defect width, defect depth were compared before and after 6 months by performing paired t- test for normalized data. Mean change in defect height, width and depth between 2 groups were compared by independent t-test.

5. Volume of defect was compared before and after 6 months by Wilcoxon Signed Rank test for non-normalized data. Changes in volume of defect between 2 groups were compared by performing Wilcoxon Rank Sum Test (Mann-Whitney test).

21 patients (11 females and 10 males) affected with Stage III periodontitis comprising of both the sexes in age range of 33-58 years (mean age of 43.33 ± 7.65 years) presenting with 21 bilateral grade II furcation defects were included in the present study. The selected sites were randomly included into Group I (PRF) and Group II (1% MF + PRF).

During the course of study, wound healing was uneventful in both the groups, without any signs of infections or complications. There was no untoward local and systemic reaction, indicating the biocompatibility of the Metformin as well as Platelet rich fibrin.

Clinical indices findings at baseline, 3 months and 6 months:

In general, patients showed good oral hygiene through-out the complete duration of the study. Baseline full mouth plaque score was 3.17 ± 0.37, while at 3 months, it decreased to 2.50 ± 0.36, while at 6 months, the mean PI was 1.93 ± 0.33. The difference in PI scores when compared with baseline measurements versus 3 months, showed statistically significant decrease in plaque score ($p<0.0001$). At 6 months post-surgical PI measurements showed statistically significant decrease ($p<0.0001$) when compared to baseline, also, the reduction of plaque score from 3 months to 6 months was statistically significant ($p<0.0001$). **(Table 1) (Graph 1)**

The mean GI dropped from 2.06 ± 0.46 at baseline to 1.52 ± 0.33 at 3 months and to 1.17 ± 0.19 at 6 months. GI scores when compared with baseline to 3 months showed statistically significant decrease ($p<0.0001$) and also when compared at 6 months, the difference was statistically significant ($p<0.0001$). The mean decrease in GI from 3 months to 6 months was also statistically significant ($p<0.0001$) **(Table 1) (Graph 1)**

Soft tissue clinical parameters at baseline, 3 months and 6 months: Probing pocket depth (PPD)

In Group I, the mean PPD at baseline was 6.00 ± 0.77 mm and that at 3 months was 4.10 ± 0.83 mm and in Group II, the mean PPD at baseline was 6.14 ± 0.73 mm and that at 3 months was 3.86 ± 0.73 mm (Table 2) (Graph 2, 3). At baseline there was no significant difference between Group I and Group II ($p=0.5447$). At 3 months, the mean PPD reduction was 1.90 ± 0.60 mm for Groups I and 2.28 ± 0.64 mm for Group II. There was a statistically significant reduction in PPD for Group I

and Group II at 3 months ($p<0.0001$) but the difference at 3 months was not statistically significant between the two groups ($p=0.2860$) **(Table 2) (Graph 2, 3,4).**

In the Group I, the mean PPD at baseline was 6.00 ± 0.77 mm and that at 6 months was 2.90 ± 0.77 mm and in Group II the mean PPD at baseline was 6.14 ± 0.73 mm and that at 6 months was 2.43 ± 0.51 mm **(Table 3) (Graph 2, 3).** There was statistically significant reduction at 6 months compared to 3 months in both the groups. However, the reduction in PPD at 3-6 months between both the groups was not statistically significant. **(Table 4, 5) (Graph 6)** At 6 months, the mean PPD reduction was 3.10 ± 0.89 mm for Group I and 3.71 ± 0.78 mm for Group II. There was statistically significant reduction in PPD for Group I and Group II at 6 months when compared to baseline ($p<0.0001$) and in Group II when compared to Group I ($p=0.0215$). **(Table 3, 5) (Graph 5).**

Clinical attachment level (CAL)

In Group I, the mean CAL at baseline was 5.95 ± 0.74 mm and that at 3 months was 4.57 ± 0.75 mm. The mean CAL at baseline in Group II was 6.38 ± 1.07 mm and that at 3 months was 4.24 ± 0.70 mm. **(Table 2) (Graph 2, 3)** At baseline there was no significant difference between Group I and Group II ($p=0.1074$). A mean CAL gain of 1.38 ± 0.58 mm was observed in Group I and Group II exhibited a mean CAL gain of 2.14 ± 0.79 mm. Both groups exhibited a statistically significant increase in CAL at the end of 3 months ($p<0.0001$). **(Table 2) (Graph 2, 3)** but the difference was not statistically significant between the groups at 3 months ($p=0.1098$). **(Table 5) (Graph 4)**

In Group I, the mean CAL at baseline was 5.95 ± 0.74 mm and that at 6 months was 3.43 ± 0.87 mm. Group II showed a mean baseline CAL of 6.38 ± 1.07 mm and at 6 months, it was 3.14 ± 0.65 mm. **(Table 3) (Graph 2, 3)** There was statistically significant gain in CAL at 6 months compared to 3 months in both the groups. **(Table 4, Graph 2, 3)** However, the gain in CAL at 3-6 months between both the groups was not statistically significant. **(Table 5) (Graph 6)** The mean CAL gain at 6 months in Group I was 2.52 ± 0.87 mm and in Group II was 3.24 ± 0.94 mm. There was statistically significant CAL gain for Group I and Group II at 6 months when compared to baseline ($p<0.0001$). (**Table 3) (Graph 2, 3)** There was a statistically significant more CAL gain at 6 months in Group II when compared to Group I ($p=0.0149$). **(Table 5) (Graph 5)**

Horizontal probing depth (HPD)

In Group I, the mean HPD at baseline was 4.90 ± 0.77 mm and that at 3 months was 3.81 ± 0.81 mm and in Group II, the mean HPD at baseline was 5.29 ± 0.90 mm and that at 3 months was 3.76 ± 0.89 mm. **(Table 2) (Graph 2, 3)** At baseline, there was no statistically significant difference between the two groups ($p= 0.1338$). At 3 months, the mean HPD reduction was 1.09 ± 0.43 mm for Group I and 1.53 ± 0.67mm for Group II. There was a statistically significant reduction in HPD for Group I as well as Group II at 3 months compared to baseline ($p<0.0001$). **(Table 2) (Graph 2, 3)** However, the reduction in HPD was not statistically significant between Group II and Group I ($p=0.8528$). **(Table 5) (Graph 4)**

In the Group I, the mean HPD at baseline was 4.90 ± 0.77 mm and that at 6 months was 3.10 ± 0.89 mm and in Group II the mean HPD at baseline was 5.29 ± 0.90 mm and that at 6 months was 2.67 ± 0.66 mm. **(Table 3) (Graph 2, 3)** There was

statistically significant reduction in horizontal PD at 6 months as compared to 3 months in both the groups. **(Table 4) (Graph 2, 3)** Also, the reduction in HPD at 3-6 months between both the groups was statistically significant (p=0.0587) **(Table 5) (Graph 6).** At 6 months, the mean HPD reduction was 1.81 ± 0.81 mm for Group I and 2.62 ± 0.74 mm for Group II. There was statistically significant reduction in horizontal PD for Group I and Group II at 6 months when compared to baseline (p<0.0001). **(Table 3) (Graph 2,3)** Also, the reduction in horizontal PD was statistically greater in Group II as compared to Group I (p=0.0016). **(Table 5) (Graph 5)**

Radiographic parameters at baseline and 6 months

CBCT analysis of Bone defect height

The mean bone defect height at baseline for Group I was 2.78 ± 0.46 mm and for Group II it was 2.97 ± 0.46 mm. At 6 months, the mean bone defect height for Group I was 2.15 ± 0.39 mm, showing a mean reduction of 0.63 ± 0.15 mm of bone defect height. The mean bone defect height at 6 months for Group II was 1.87 ± 0.37 mm thus exhibiting a reduction of 1.10 ± 0.41 mm of bone defect height. There was a significant reduction of bone defect height in both the groups (p<0.0001) at 6 months. The higher reduction of bone defect height was statistically significant for Group II when compared to Group I (p<0.0001). **(Table 6, 7) (Graph 7, 8, 9)**

CBCT analysis of Bone defect width

The mean bone defect width at baseline for Group I was 2.26 ± 0.49 mm and for Group II it was 2.34 ± 0.35 mm. At 6 months, the mean bone defect width for Group I was 1.82 ± 0.32 mm, showing a mean reduction of 0.44 ± 0.44 mm of bone

defect width. The mean bone defect width at 6 months for Group II was 1.55 ± 0.37 mm thus exhibiting a reduction of 0.79 ± 0.30 mm of bone defect width. There was a significant reduction of bone defect width in both the groups ($p<0.0001$) at 6 months. The higher reduction of bone defect width was statistically significant for Group II when compared to Group I ($p=0.0059$). **(Table 6, 7) (Graph 7, 8, 9)**

CBCT analysis of Bone defect depth

The mean bone defect depth at baseline for Group I was 2.54 ± 0.57 mm and for Group II it was 2.42 ± 0.35 mm. At 6 months, the mean bone defect depth for Group I was 1.82 ± 0.41 mm, showing a mean reduction of 0.72 ± 0.35 mm of bone defect depth. The mean bone defect depth at 6 months for Group II was 1.48 ± 0.38 mm thus exhibiting a reduction of 0.94 ± 0.34 mm of bone defect depth. There was a significant reduction of bone defect depth in both the groups ($p<0.0001$) at 6 months. The higher reduction of bone defect depth was statistically significant for Group II when compared to Group I ($p=0.049$). **(Table 6, 7) (Graph 7, 8, 9)**

CBCT analysis of Bone defect volume

The mean bone defect volume at baseline for Group I was 16.22 ± 6.31 mm^3 and for Group II it was 16.92 ± 5.33 mm^3. At 6 months, the mean bone defect volume for Group I was 7.20 ± 2.81 mm^3 showing a mean reduction of 9.02 ± 4.21 mm^3 of bone defect volume. The mean bone defect volume at 6 months for Group II was 4.47 ± 2.31 mm^3 thus exhibiting a reduction of 12.45 ± 3.88 mm^3 of bone defect volume. There was a significant reduction of bone defect volume in both the groups ($p<0.0001$) at 6 months. The higher reduction of bone defect volume was statistically significant for Group II when compared to Group I ($p=0.0099$). **(Table 6, 7) (Graph 7, 8, 9)**

DISCUSSION

Periodontitis is a chronic inflammatory disease resulting in progressive loss of bone and supporting periodontal structures. When this chronic inflammation invades into the bifurcations and trifurcations of multirooted teeth it leads to furcation involvement. Periodontal furcation involvement (FI) represents a challenge to the dentist in the treatment of periodontally compromised molar and also confers molars with FI at a high risk of tooth loss. The complex anatomic morphology makes it difficult to debride this area properly during periodontal instrumentation as well as during routine home-care practices. The main goal of treating furcation involvement is to eliminate microbial plaque from the exposed surfaces of the root complex and establishment of an anatomy facilitating proper self-performed plaque control. Different treatment modalities have been advocated for the treatment of FI, including nonsurgical maintenance, resective and regenerative treatment, according to site factors and degree of involvement of individual affected molars.[70]

The primary objective of periodontal therapy is the regeneration of new bone, cementum, and periodontal ligament which has been demonstrated by numerous therapeutic grafting modalities and barrier membranes. Even though bone grafts have been considered as a viable option for treating furcation defects there are some major limitations with its use. The donor site morbidity, volume of acquired bone, the unpredictable replacement rate of autografts, the risk of disease transmission and greater expenses with the use of allografts and xenografts has possibly restrained their use as bone regenerative material in treating the periodontal osseous defects.[14]

The introduction of biomimetic agents such as enamel matrix derivatives, platelet rich plasma (PRP), platelet derived growth factor, platelet rich fibrin (PRF), and bone morphogenetic proteins have shown to enhance the periodontal regeneration in grade II furcation defects. Platelet concentrates also plays an important role in maturation of soft tissue and regeneration of bone. The first generation incorporates the platelet rich plasma (PRP), while the second generation involves the platelet rich fibrin (PRF). The second generation platelet concentrate was developed by **Choukroun et al**. in France. It is defined as an autologous healing biomaterial, incorporating in a matrix of autologous fibrin most leukocytes, platelets and growth factors harvested from a simple blood sample. It is rich in leukocyte cytokines such as IL-1, 4, 6 and growth factors such as TGF- 1, PDGF-AB, and VEGF .[71] PRF not only differs from its predecessor by its lack of anticoagulant use during preparation, but also it's easier fabrication as it requires only one centrifugation cycle with less time, as opposed to two centrifugation cycles with PRP.[41] There are added advantages of PRF over PRP which further supports its use in regenerative therapy. PRF contains a fibrin network that facilitates blood clot formation and tissue repair.[72] Secondly, its

slow release of growth factors as compared to PRP enhances the process of regeneration over a more extended period of time. Moreover, PRF contains leukocytes and macrophages, known cell types which are implicated in immunity and host defense and are hypothesized to further act as a bacterial resistant matrix to fight against bacterial pathogens.[73]

The periodontal micro-organisms and destructive host response are involved in the initiation and progression of periodontal disease and activates the innate immune system, pro-inflammatory molecules and cytokines like interleukin-1 and tumor necrosis factor-α. This is followed by the activation of cascade of events that leads to osteoclastogenesis and subsequent bone loss via the receptor activator of nuclear factor-kappa B (RANK)–RANK ligand RANKL)–osteoprotegerin (OPG) axis.[74] RANKL induces osteoclast differentiation and activation, whereas OPG counteracts this process by acting as a decoy receptor for RANKL.[75] Thus with a aim to regulate the osteoblastic and osteoclastic activity and stabilize or even regenerate the periodontium various pharmacological agents were studied to modulate the rate of bone formation and resorption.

Of all the host modulating pharmacological agents, Metformin HCl (1, 1-dimethylbiguanide) an antihyperglycemic agents is one of the most commonly used therapeutic agent which modulates the disease process by stimulating osteoblasts and reducing alveolar bone loss. In diabetic patients it acts by inhibiting gluconeogenesis, which in turn leads to a decline in hepatic glucose production and decreases peripheral insulin resistance. MF also regulates carbohydrates and lipid metabolism via insulin signalling and activating AMP-activated protein kinase (AMPK). Metformin is shown to inhibit advanced glycation end products induced cytosolic and

mitochondrial reactive oxygen species in endothelial and smooth muscle cells. MF down-regulates the production of receptor activator of nuclear factor kappa B ligand (RANKL) and up-regulates the production of osteoprotegerin (OPG) from osteoblasts This decreased RANKL/OPG ratio in turn declines the osteoclast activity, thus inducing bone formation and inhibiting bone resorption. [27] A dose-dependent increase of cell proliferation and osteoblastic differentiation was observed when two osteoblast-like cells (UMR106 and MC3T3E1) were treated with metformin (25–500 mM) for 24 hours. MF also has shown a stimulating effect in the ex vivo osteogenic potential of bone marrow progenitor cells BMPCs thus resulting in increase of bone formation and remodelling, via enhancing BMPC osteoblastic alkaline phosphatase, type I collagen, and osteocalcin.[76] Various animal studies have provided an evidence of the direct stimulatory effect of Metformin on osteoblast differentiation in addition to an inhibitory effect on osteoclasts.[18,25,27] **Pradeep et al.** investigated the efficiency of 0.5%, 1% and 1.5% concentrations of Metformin gel as local drug delivery in adjunct to scaling and root planing for the treatment of intrabony defects and found a greatest reduction of IBD depth especially in the sites treated with 1% MF gel. Thus, it was hypothesized that 1% MF gel provides clinical benefits at a lowest concentration.[21]

Studies in the literature have suggested the effectiveness of 1% Metformin gel in combination with PRF for periodontal regeneration.[55,56] However, there is paucity in literature evaluating the effectiveness of the use of 1% MF gel and PRF in the treatment of Grade II furcation defects. Hence, through this study it was aimed to evaluate additional benefits if any of 1% Metformin gel with PRF in achieving better healing and resolution of the Grade II furcation defects.

The precise assessment of furcation defects is of utmost importance before establishing a prognosis and treatment strategy. The furcation defect is assessed by using a combination of probing and radiographs. The most commonly used imaging modalities are bitewing radiographs, Intraoral periapical radiographs and panoramic radiographs. However, these radiographs give 2D representation of the 3D structures and also the incorrect positioning of radiographic films, superimposition of anatomic structures and reduced sensitivity adds the limitations to the use of these imaging modalities. To overcome these inherent difficulties computed tomography (CT), 3D imaging modality was introduced. Recently it has been replaced by a more promising relevant imaging modality i.e. cone beam computed tomography (CBCT). It generates 3D volumetric images at a very low effective radiation dose as compared to CT. Many studies in literature have encouraged the use of CBCT for assessing pre and post surgical dimensions of furcation defects.[22,60,62]

Thus, the present study was aimed to evaluate the efficacy of 1% MF gel with PRF over PRF alone in the treatment of human Class II furcation defects, clinically and by CBCT.

A total of 42 Grade II defects were selected in 21 patients with Stage III periodontitis in the age group of 33 to 58 years of either sex who met the inclusion criteria were recruited and randomly assigned to each treatment sequence. All the selected patients had probing pocket depth (PPD) ≥ 6mm, interdental clinical attachment loss (CAL) ≥ 5mm and Horizontal probing depth (HPD) > 2mm with a radiographic evidence of furcation defects in either of the molar region of the mouth bilaterally. A split-mouth design was employed as it allows the comparison of

outcome between the treatments. It also greatly facilitates the interpretation of trials by minimizing the effects of inter-patient variability.

At baseline, no significant differences in any of the investigated parameters were observed between Group I and Group II indicating that the randomization process was effective. One defect was treated with PRF alone and other with 1% MF gel + PRF. The clinical and radiographic parameters in the study were evaluated until 6 months as the dimensional alteration of the periodontal tissues after periodontal therapy occurs within the first 6 months.

During the course of study, uneventful wound healing was observed in both Group I and Group II without any signs of infection and complications. There was no untoward local or systemic reaction and undesirable immune response in test group as well as control group, indicating the biocompatibility of PRF as well as 1% MF gel. No study in the literature has yet reported any adverse reactions of Metformin when used either in a topical form, systemic form or as an adjunct to surgical or nonsurgical therapy. [28,29,32,,55,56] The uneventful healing in patients in the present study is in agreement with various previous studies. **Sharma A and Pradeep et al**. **(2011)**[43] evaluated the effect of autologous platelet rich fibrin in the treatment of mandibular degree II furcation defects, **Throat M et al. (2011)**[74] evaluated the effect of autologous platelet rich fibrin in the treatment of intrabony defects and **Kanoriya et al. (2017)**[50] evaluated the efficacy of combination therapy of PRF+1% ALN in mandibular degree II furcation defect. All of these authors found the biocompatibility and greater wound healing properties of PRF. Thus, these studies validate the use of PRF and 1% MF gel not only individually but also in combination to treat the periodontal osseous defects.

An association has been found with the composition of and quantity of dental plaque and changes in gingival health with the quality and frequency of oral hygiene measures. For the long term stability of clinical outcomes plaque control is essential. The decrease in PI was statistically significant at the end of 3 months and 6 months compared to baseline. The statistical decrease in plaque index scores in our study are in accordance with the finding of **Pradeep et al., (2015)**[55], **Sharma P et al. (2017)**[56], who evaluated the efficacy 1%MF+PRF combination in intrabony and grade II furcation defect respectively. A statistically significant GI reduction in our study was found to be similar to the study by **Biswas S et al. (2016)**[48] and **Kurian I G et al. (2018)**[37]. **Biswas et al**. compared PRF with bioactive glass in grade II furcation defect and found reduction in GI and PI in both the study groups. **Kuria G et al.** compared 1% MF gel with Aloe vera gel as an adjunct to SRP in Intrabony defects and found reduction in GI in both the study groups.

Remarkably, numerous studies approved on the lack effect of metformin use on plaque index and bleeding index.[21,28.36] Irrespective of intrabony defects receiving metformin or placebo gel, the bleeding and plaque indices in these studies was found to be relatively the same. This might be attributed to the potential effect of metformin on osteoblast differentiation and bone formation[24] rather than stimulating the gingival tissue.[18,26] The decrease in PI, GI in our study is thus assumed to be the result of repeated oral hygiene instructions given to the patients. These results showed good oral hygiene level and a clinically healthy gingival condition throughout the duration of study and also emphasized the significance of long-term reinforcement of oral hygiene instructions for maintenance of improved oral hygiene.

The identification and accurate assessment of periodontal pocket is very important for the evaluation of disease severity, disease progression and therapeutic efforts.[77] Custom stents with vertical grooves serves as fixed reference guides to calculate the depth of probe penetration. It has been found to be a more promising method to guide the accurate probe placement.[78,79] In the present study, the vertical clinical periodontal probing was performed by using a UNC-15 probe and customized acrylic stents with guiding vertical grooves for reproducible probing sites.

In Group I, the mean PPD at baseline was 6.00 ± 0.77mm and that at 3 months was 4.10± 0.83mm. In Group II, the mean PPD at baseline was 6.14 ± 0.73mm and that at 3 months was 3.86 ± 0.73mm. At 3 months, the mean PPD reduction for Group I was 1.90 ± 0.60 mm and for Group II was 2.28 ± 0.64mm. There was a statistically significant difference in PPD in both the groups at 3 months compared to baseline but not between the two groups.

In Group I, the mean PPD at baseline was 6.00 ± 0.77mm and that at 6 months was 2.90± 0.77mm. In Group II, the mean PPD at baseline was 6.14 ± 0.73mm and that at 6 months was 2.43 ± 0.51mm. At 6 months, the mean PPD reduction for Group I was 3.10 ± 0.89mm and for Group II was 3.71 ± 0.78mm and this difference between the groups was statistically significant. Also, there was statistically significant difference in PPD in both the groups at 6 months compared to baseline. These results were similar to those reported by **Pradeep et al. (2015)**[55] and **Sharma et al. (2017)**[56], who evaluated the combination of PRF+1%MF gel in intrabony defects and grade II furcation defects respectively and found a greater PD reduction in the PRF+1%MF gel treated sites as compared to sites treated with PRF alone.

The sites with a probing pocket depth of ≥6mm was selected to treat with 1%MF+PRF in this study and the sites treated with this combination showed a greater PD reduction as compared to the control (PRF) sites. **Pradeep AR et al. (2017)** also found a similar reduction of PD at 6 and 9 months in the subjects with both initial pocket depth of ≥5mm and ≥7mm subgroups treated with local drug delivery of 1%MF gel as compared to placebo group.[32]

Other studies which resulted in significant PPD reduction with PRF and MF as separate regenerative materials are those by **Pradeep et al. (2013)**[21], who compared MF gel with a placebo gel in chronic periodontitis patients, **Rao et al. (2013)**[28] who evaluated efficacy of MF gel +SRP over SRP alone in chronic periodontitis patients, **Kurian G et al. (2018)**[37] and **Pankaj D et al.(2018)**[36] who compared MF gel with aloe-vera gel and Rosuvastatin gel respectively in intrabony defects and also by **Kassem et al. (2017)**[30] and **Kotry G et al. (2016)**[31] who assessed the effect of muco-adhesive multiple layer films of MF as an intrapocket application in chronic periodontitis patients.

Sharma et al. (2011)[43] found a significant reduction in PPD in test group (PRF) as compared to control group (OFD) in mandibular degree II furcation defects at 9 months. **Kanoriya et al. (2017)**[50] and **Wanikar et al. (2018)**[64] evaluated the potential of ALN in combination with PRF in the treatment of mandibular molar furcation defects as compared to PRF alone and found a significant improvement in probing depth reduction at 9 and 6 months respectively in the both study groups with a higher reduction in the test groups.

The most commonly used clinical outcome variable in regenerative studies is the clinical attachment level (CAL). In Group I the mean CAL at baseline was 5.95 ± 0.74mm and that at 3 months was 4.57 ± 0.75 mm. In Group II, the mean CAL at baseline was 6.38± 1.07mm and that at 3 months was 4.24 ± 0.70mm. At 3months, the mean CAL gain for Group I was 1.38 ± 0.58mm and for Group II was 2.14 ± 0.79 and this difference between the groups was not statistically significant. In Group I, the mean CAL at baseline was 5.95 ± 0.74mm and that at 6 months was 3.43 ± 0.87mm. In Group II, the mean CAL at baseline was 6.38 ± 1.07mm and that at 6 months was 3.14 ± 0.65mm. At 6 months, the mean CAL gain for Group I was 2.52 ± 0.87 mm and for Group II was 3.24 ± 0.94 mm and this difference between the groups was statistically significant.

Our results go in accordance with the study conducted by **Pradeep et al. (2015)**[55] who reported a RVAL gain of 4.90mm in 1%MF+PRF treated intrabony defects as compared to 4.03mm of RVAL gain in PRF treated sites at 9 months. **Pradeep et al. in 2013**[21] when used MF as LDD in chronic periodontitis patients found a significant CAL gain of 3.83 mm in the 1% MF treated sites as compared to 1.33 mm in SRP treated sites at 6 months. Significant CAL gain at 1% MF treated sites was observed by **Rao et al. in 2013**[28], **Pradeep et al. in 2015**[29] ,**2017**[32] and **Kurian I G et al. in 2018**[37] who all assessed the effect of 1% MF gel as an adjunct to SRP in chronic periodontitis patients.

Sharma P et al. (2017)[56] showed a significant improvement of RVAL in mandibular grade II furcation defects from baseline to 6 months in both test (1%MF+PRF) and the control (PRF) groups. However, intergroup comparisons yielded nonsignificant result which was attributed by the author to the varied

anatomic factors influencing the outcomes of regenerative therapy in furcation defects.

The result similar to our study was reported by **Wanikar et al. (2018)**[64] who reported a significant CAL gain of 3.05 mm in mandibular furcation defects treated with 1% ALN gel + PRF as compared to 1.90 mm CAL gain in PRF treated sites at 6 months. **Sharma A et al**.[43] noted a significant CAL gain in PRF treated mandibular degree II furcation defects as compared to OFD while **Kanoriya et al**.[50] reported significant CAL gain in both PRF+ALN and PRF alone treated mandibular furcation defects. **Kanoriya et al**. ascribed the insignificant difference between the groups to a small sample size.

It is a known fact that in majority of the clinical trials wherein regenerative therapies have been utilized for the treatment of grade II furcation defects, a reduction in PPD as well as gain in CAL postoperatively has been noticed. In our clinical trial, we have substantiated the above findings. Also, the PPD reduction and CAL gain observed in our clinical trial with combination therapy (1%MF+ PRF) was better as compared to the monotherapy (PRF). This could be due to the action of Metformin induced stimulatory effect on osteoblasts and an inhibitory effect on osteoclasts along with the synergistic effect of PRF and MF on preventing bone loss and promoting osteoblast genesis.

The horizontal progression of periodontal attachment loss is one of the definite features of furcation lesions. It implies lateral extension of pocket towards the interior of the furcation which influences the clinical outcome of successful regenerative therapy in furcation defects.[8] The HPD of the furcation defect can be determined by

measuring the horizontal extent of probe penetration into the furcation.[80] The horizontal probing depth (HPD) was measured by using Naber's probe.[8]

In Group I, the mean horizontal PD (HPD) was 4.90 ± 0.81mm, 3.81 ± 0.81mm and 3.10 ± 0.89mm at baseline, 3 month and 6 months respectively. In Group II, the mean HPD was 5.29 ± 0.90mm, 3.76 ±0.89mm and 2.67 ± 0.66mm at baseline, 3 month and 6 months respectively. At 3 months, the mean HPD reduction for Group I was 1.09 ± 0.43mm and for Group II was 1.52 ± 0.67mm. At 6 months, the mean HPD reduction for Group I was 1.81 ± 0.81mm and for Group II was 2.62 ± 0.74mm and this difference between the groups was statistically significant. Also, there was statistically significant difference in HPD in both the groups at 3 and 6 months compared to baseline. These results were similar to those reported by **Wanikar et al. (2017)**[64] who found HPD reduction of 2.3 ± 0.73mm and 1.7 ± 0.73mm in test (PRF+1%ALN) and control (PRF) group respectively. **Sharma P et al. (2017)**[56] evaluated the efficacy of PRF+1%MF in grade II furcation defect and found a significant greater RHAL in the test group as compared to control group at 6 months. **Kanoriya et al. (2017)**[50] evaluated and compared the effectiveness of OFD; OFD and PRF; OFD, PRF and ALN in Grade II mandibular furcation treatment. The clinical efficacy of the treatment modalities was evaluated at 9 months postoperatively. The placement of ALN in the furcation defect with PRF resulted in a greater mean reduction of HPD when compared to regenerative therapy alone.

PRF enhances the proliferation and differentiation of cells such as osteoblasts, fibroblasts, and cementoblasts at the healing site, thus promoting tissue regeneration in addition to the repair. The process of tissue regeneration commences when the sustained release from the applied PRF's 3D fibrin matrix produces a local

concentration sufficient to form autologous PRF, which then acts as an effective sources of growth factors or grafting material. The sustained release of cytokines, growth factors, neovascularization augments the healing of surrounding tissue architecture.[38-42] Metformin increases collagen type I, osteocalcin mRNA expression, alkaline phosphatase activity and enhanced cell mineralization. It reduces the RANKL/OPG ration thus subsequently reducing osteoclasts and bone resorption areas. Metformin has shown to inhibit the IL1-β induced release of pro-inflammatory cytokines IL-6, IL-8 and nuclear translocation of nuclear factor-kappa B (NF-KB). Its anti-inflammatory action has been confirmed by its inhibitory action on Lipopolysaccharide enhanced IL-6, IL-1 β and TNF-α production in human gingival fibroblasts via increasing ATF3 expression. This anti-inflammatory action, osteogenic and antiresorptive effect of MF further arrests the further degradation of attachment apparatus. The resultant reduction in inflammation and soft tissue destruction at its site of action when applied locally as an adjunct to surgical therapy, thus leads to more reduction in gingival index score, probing pocket depth, more gain in attachment level and bone formation.[26,27,34]

Westfelt et al. (1985)[81] stated that most histological changes occurred in the first 6 months after surgery. Accordingly, a healing period of 6 months was considered for post-surgical evaluation of parameters. Therapeutic endpoint of successful regenerative therapy is determined traditionally by radiographic assessments in conjunction with clinical probing. The inability to detect initial alveolar bone changes, leading to variability in the perception of furcation involvement, distortions and variability in image quality due to processing errors, and overlapping of structures due to their 2-dimensional nature, has limited the reliability

of traditional radiographs in assessment of the periodontal osseous defects. Conventional CT solved this problem by providing axial slices throughout the object of interest but had major shortcomings, including high radiation dose, high cost and low resolution.[60] To address this issue cone beam computed tomography (CBCT) was introduced which revolutionized dental imaging from 2D to 3D images and expanded its role of from being a mere diagnostic tool to providing images for post-operative evaluation of defects. CBCT assess the images three-dimensionally with the added advantage of high accuracy, high resolution, and low cost as compared to CT.[60,61] So, in this study minute changes within the alveolar bone in terms of gauging the height, width and depth of a defect of grade II furcation defect was recorded by using CBCT, which no other tool could record with such a greater accuracy.

In the current clinical trial, the mean bone defect volume at baseline for Group I was 16.22 ± 6.31 mm 3 and that at 6 months was 7.20 ± 2.81 mm 3 showing a mean difference of 9.02 ± 4.21 mm3. Group II showed a mean bone defect volume of 16.92 ± 6.31 mm3 at baseline and 4.47 ± 2.31 mm3 at 6 months with mean reduction of 12.45± 3.88 mm3. Both groups showed statistically significant reduction in defect volume at 6 months when compared to baseline. Group II showed statistically significant defect volume reduction compared to Group I indicating better results for combination therapy over monotherapy. These results go in accordance with the study conducted by **Wanikar et al.** who evaluated the efficacy of 1% ALN+PRF over PRF alone in grade II furcation defect. The authors found statistically significant reduction in defect volume in the test site as against control site when evaluated by using CBCT.[64] **Pradeep AR et al. (2015)**[55] and **Sharma P et al. (2017)**[56] assessed effect of 1%MF+PRF in intrabony and furcation defect respectively. **Pradeep et al.**[55]

demonstrated a greater radiographic intrabony defect depth reduction in the test site (1%MF+PRF) at 9 months and **Sharma et al**.[56] reported a significant reduction of defect depth in furcation area in the test site (1%MF+PRF) at 6 months. These studies indicate a synergistic effect of these combination in periodontal osseous defect.

Darby I et al.[82] and **Walter C et al**.[83] stated that clinical probing over- and under-estimated FI relative to CBCT analysis. 84% of the CBCT data was correct when compared intra-surgically, 14.7% of the sites were underestimated while only 1.3% of the sites were overestimated. Probing angulation and force, soft tissue inflammation, and inter-radicular bone and root morphology, all contributed to variations of clinical detection. Although true periodontal regeneration can only be assessed by histologic evaluation, CBCT is considered as one of the most reliable imaging modalities to assess volumetric bone defect fill radiographically. Since the healing pattern in our clinical trial was similar to other trials which evaluated on bone fill via IOPA or RVG, it can be stated that the radiographic bone fill which was apparent in CBCT images was due to the regenerative process within the furcation defect.

Padmanabhan S et al. (2017)[61] and **Warda H et al. (2018)**[62] evaluated the efficacy of CBCT over intra-surgical measurements in furcation defects and confirmed the accuracy of CBCT for assessing the furcation defects. **Braun et al. (2014)**[84] emphasized CBCT as a better alternative to the conventional intraoral periapical radiographs when a higher exposure of radiation and precision of analysis is considered.

Pajnigara et al. (2017)[60] evaluate the dimensions of furcation defects clinically (pre- and post-surgery), intra-surgically, and by cone beam computed tomography (CBCT) (pre- and post-surgery) which were treated with DFDBA. The authors concluded the use of CBCT to be more prudent and accurate for diagnosis and treatment planning in advanced periodontal diseases. The accuracy in assessing the volumetric changes in intrabony defects by using CBCT was also emphasized in infrabony defect by **Bodhare G et al. (2018)**[63]. The authors evaluated Bioactive Glass (BG) with and without autologous PRF in intrabony defects clinically and radiographically, pre-operative and post-operatively by analysing CBCT images and reported an achievement of greater bone fill with BG+PRF than with PRF alone at 6 months. **Shah et al. in 2019**[65] on analysis of CBCT images at baseline and 6 months found a significant improvement in defect volume reduction in the furcation defects treated with combination therapy of DFDBA+CM as compared to defects treated with CM alone. **Shirke P et al. (2019)**[66] demonstrated the efficacy of 1.2% Atorvastatin as an adjunct to SRP in 1.2% Atorvastatin treated periodontal osseous defects clinically as well as radiographically by using CBCT.

The literature suggests that 1%MF and PRF when used alone and in combination can result in periodontal regeneration on a previously diseased contaminated root surface, but due to constraints we could not do histological analysis and thus cannot confirm that 1%MF with PRF results in true periodontal regeneration. Furthermore, no study to date has analysed the utility of CBCT in assessing the effect of the regeneration carried out by 1%MF with PRF and PRF alone in furcation defect. Nevertheless, if we correlate our study with the previous studies, then it will not be unreasonable to consider that the test group yielded healing was quite similar to that

of true periodontal regeneration as was assessed by clinical and radiographic parameters.

Like the previously mentioned studies CBCT analysis enabled us to assess the volumetric healing characteristics to a certain extent and also helped in analysing regeneration radiographically with relatively less invasion.

The following limitations were observed in the present study:

1. The sample size in the present study was limited to 21 bilateral furcation defects. A larger sample size would be desirable so as to substantiate the results.

2. Evaluation of radiographic parameter at 6 months may be speculated as too early for a final evaluation of bone fill in furcation defects.[8] Therefore, long term analysis is needed to determine the stability of the results and to improve the radiographic assessment of the results.

3. Ethical constraints as well as relative invasiveness restricted the assessment of bone filly by surgical re-entry as well as histologically.

CONCLUSION

The present split mouth, randomized, controlled clinical and CBCT study was undertaken to compare the effectiveness of 1%MF plus PRF and PRF alone in the treatment of grade II furcation defects. 21 systemically healthy subjects with 21 bilateral grade II furcation defects were selected for the study. Baseline measurements included PI, GI, PPD, HPD, CAL and bone defect height, depth and width by CBCT. At the time of surgery, defects were randomly assigned to either Group I i.e.control group (PRF) or Group II i.e. test group (1%MF + PRF). PI, GI, PPD, HPD, CAL were assessed at 3 and 6 months while the CBCT analysis was done at 6 months.

During the course of study, healing was uneventful in both Group I and Group II, without any signs of infection or complications. There was no untoward local and systemic reaction indicating the biocompatibility of the materials used. No clinical evidence of undesirable, immune response was detected and no evidence of tissue reaction was seen. The reductions in Plaque index and Gingival index indicated

satisfactory maintenance of oral hygiene by patients throughout the study period. PPD reduction and CAL gain in Group II was significantly greater than in Group I at 6 months. Between baseline and 3months as well as 3 and 6 months, there no significant difference between Groups was observed. The HPD reduction in Group II was significantly greater than in Group I at 6 months. The mean bone height, width and depth gain in Group II was statistically significantly greater than in Group I at 6 month evaluation. Also, there was statistically significantly gain in volume of defect in Group II as compared to Group I at 6 month evaluation.

From the analysis of results, following conclusions were drawn:

1. 1%MF + PRF resulted in reductions of probing pocket depth at 3 months and 6 months with a statistically greater reduction at 6 months compared to PRF alone.

2. 1%MF+ PRF showed clinical attachment level gain at 3 months and 6 months with a statistically greater CAL gain at 6 months compared to PRF alone.

3. 1%MF + PRF showed significantly better results in terms of gain of bone volume at 6 months, compared to PRF alone.

It is concluded within the limits of study, that the use of 1%MF + PRF was beneficial in achieving better results in terms of periodontal regeneration. Attempting to identify the most accurate method for evaluating hard tissue changes after periodontal therapy is an important task. To date, re-entry procedure appears to be the gold standard to assess the defect fill and, while no single method can produce similar information consistently. The images obtained by CBCT, combined with clinical

measurements, definitely increases our ability to determine the treatment outcome without the use of re-entry procedure.

It should be noted that the differences in healing patterns, microbial pathogens, study designs, patient population, measurement techniques and human defect variations make it difficult to compare clinical results. Also, different methods like clinical, histological and radiographic evaluations have been used in various studies for assessing the outcomes of treatments. This could be some of the reasons for variations observed amongst clinical trials. Further studies evaluating the efficacy of 1%MF and PRF as a regenerative treatment modality should be carried out.

SUMMARY

Periodontal disease is characterized by tissue inflammation and destruction of the tooth supporting structures that involves bone, cementum and periodontal ligament and eventually leads to the loss of affected teeth. The invasion of periodontitis in the furcation area leads to furcation involvement which are graded as Grade I, II, III, IV based on soft and hard tissue loss. The treatment of FI in molars represents an ominous problem due to its complex anatomy and poor access. In a view to gain optimum success, an open flap debridement should be supported with either an autogenous or allogenic bone graft with GTR or endogenous regenerative material or a combination of regenerative materials with biomimetic agents or pharmacological bone regenerative agents. Hence, the present study was conducted to evaluate clinically and radiographically the efficacy of 1% MF gel in combination with PRF and PRF alone in the treatment of grade II furcation defections. A total of 21 patients with bilaterally furcation defects were randomly divided into experimental

site (OFD and 1% MF with PRF) and control site (PRF alone). A written informed consent was obtained from all the patients after thorough clinical examination with parameters including PI, GI, PPD, HPD, CAL were recorded at baseline, 3 months and 6 months intervals. Radiographic evaluation using CBCT was done at baseline and 6 months intervals to evaluate the amount of change in the level of defect height, width and depth and hence volume was obtained. At the end of the study all the data of both the clinical and radiographic parameters were subjected to statistical analysis using the STATA ver 20.0 software and conclusions were drawn. Definite improvement was seen with regards to the clinical and radiographic findings at both the sites; however, experimental sites showed significantly better results thus promoting the regenerative combination therapy (1%MF + PRF) over monotherapy (PRF) in furcation defects.

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Table 1: Comparison of clinical indices among the study population

	Plaque Index	**Gingival Index**
Baseline	3.17 ± 0.37	2.06 ± 0.46
Month 3	2.50 ± 0.36	1.52 ± 0.33
Mean difference at month 3	0.67 ± 0.54	0.54 ± 0.37
p-value	< 0.0001 (HS)	< 0.0001 (HS)
Month 6	1.93 ± 0.33	1.17 ± 0.19
Mean difference at month 6	1.24 ± 0.56	0.89 ± 0.43
p-value	< 0.0001 (HS)	< 0.0001 (HS)
Mean difference between month 3 and month 6	0.57 ± 0.33	0.35 ± 0.26
p-value	< 0.0001 (HS)	< 0.0001 (HS)

SD: Standard deviation; HS: Highly significant; S: Significant

Table 2: Comparison of clinical parameters (in mm) in both the groups at 3 months

Parameter	**Group I**			**Group II**		
	Baseline	**3 months**	**p-value**	**Baseline**	**3 months**	**p-value**
PPD	6.00± 0.77	4.10± 0.83	< 0.0001 (HS)	6.14± 0.73	3.86± 0.73	< 0.0001 (HS)
CAL	5.95± 0.74	4.57± 0.75	< 0.0001 (HS)	6.38± 1.07	4.24± 0.70	< 0.0001 (HS)
HPD	4.90± 0.77	3.81± 0.81	< 0.0001 (HS)	5.29± 0.90	3.76± 0.89	< 0.0001 (HS)

SD: Standard deviation; HS: Highly significant

Table 3: Comparison of clinical parameters (in mm) in both the groups at 6 months

Parameter	Group I			Group II		
	Baseline	**6 months**	**p-value**	**Baseline**	**6 months**	**p-value**
PPD	6.0± 0.77	2.90± 0.77	< 0.0001 (HS)	6.14± 0.73	2.43± 0.51	< 0.0001 (HS)
CAL	5.95± 0.74	3.43± 0.87	< 0.0001 (HS)	6.38± 1.07	3.14± 0.65	< 0.0001 (HS)
HPD	4.90± 0.77	3.10± 0.89	< 0.0001 (HS)	5.29± 0.90	2.67± 0.66	< 0.0001 (HS)

SD: Standard deviation; HS: Highly significant

Table 4: Comparison of clinical parameters (in mm) in both the groups at 3-6 months

Parameter	Group I			Group II		
	3 months	**6 months**	**p-value**	**3 months**	**6 months**	**p-value**
PPD	4.09± 0.830	2.90± 0.77	< 0.0001 (HS)	3.85± 0.72	2.43± 0.51	< 0.0001 (HS)
CAL	4.57± 0.746	3.43± 0.87	< 0.0001 (HS)	4.23± 0.70	3.14± 0.65	< 0.0001 (HS)
HPD	3.80± 0.813	3.10± 0.89	< 0.0001 (HS)	3.76± 0.88	2.67± 0.66	< 0.0001 (HS)

SD: Standard deviation; HS: Highly significant

Table 5: Comparison of clinical parameters (in mm) between both the groups at different time intervals

Parameter	Baseline vs. 3 months		p-value	Baseline vs. 6 months		p-value*	3 months vs. 6 months		p-value
	Group I	Group II		Group I	Group II		Group I	Group II	
PPD reduction	1.90± 0.6	2.28± 0.64	0.28600 (NS)	3.10± 0.89	3.71± 0.78	0.0215 (S)	1.19± 0.6	1.43± 0.68	0.2351 (NS)
CAL gain	1.38± 0.58	2.14± 0.79	0.1098 (NS)	2.52± 0.87	3.24± 0.94	0.0149 (S)	1.14± 0.85	1.10± 0.44	0.8215 (NS)
HPD reduction	1.09± 0.43	1.53± 0.67	0.8528 (NS)	1.81± 0.81	2.62± 0.74	0.0016 (S)	0.71± 0.64	1.10± 0.62	0.0587 (S)

(HS: highly significant, S: significant, NS: non-significant)

Table 6: Comparison of radiographic parameters in both the groups at different time interval

CBCT parameters	Group I			Group II		
	Baseline	6 months	p-value	Baseline	6 months	p-value
Bone defect height (in mm)	2.78 ± 0.46	2.15 ± 0.39	< 0.0001 (HS)	2.97 ± 0.46	1.87 ± 0.37	< 0.0001 (HS)
Bone defect width (in mm)	2.26 ± 0.49	1.82 ± 0.32	< 0.0001 (HS)	2.34 ± 0.35	1.55 ± 0.37	< 0.0001 (HS)
Bone defect depth (in mm)	2.54 ± 0.57	1.82 ± 0.41	< 0.0001 (HS)	2.42 ± 0.35	1.48 ± 0.38	< 0.0001 (HS)
Bone defect volume (in mm3)	16.22 ± 6.31	7.20 ± 2.81	< 0.0001 (HS)	16.92 ± 5.33	4.47 ± 2.31	< 0.0001 (HS)

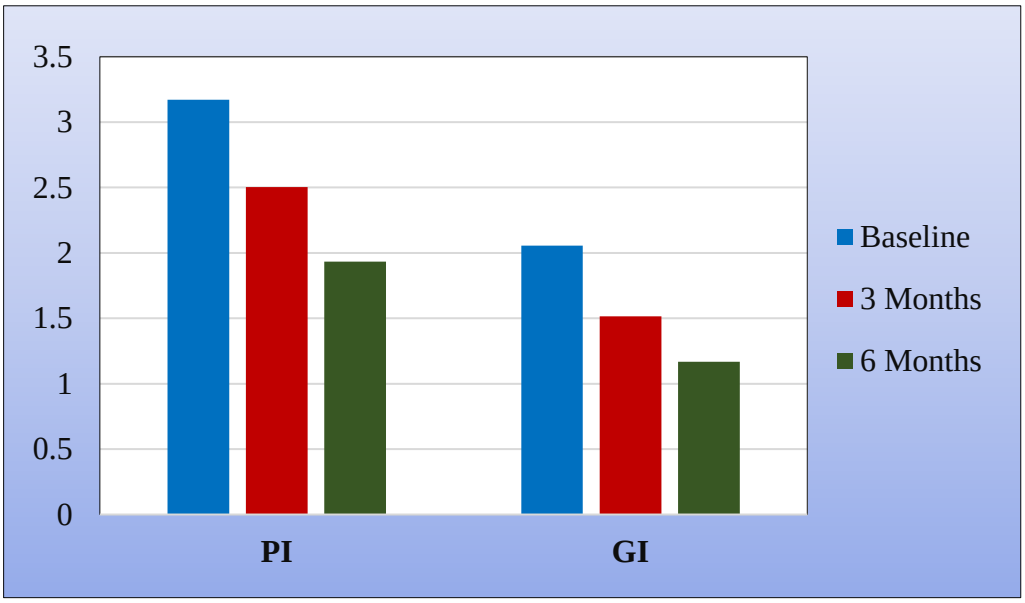
(HS: highly significant)

Table 7: Comparison of radiographic parameters between both the groups

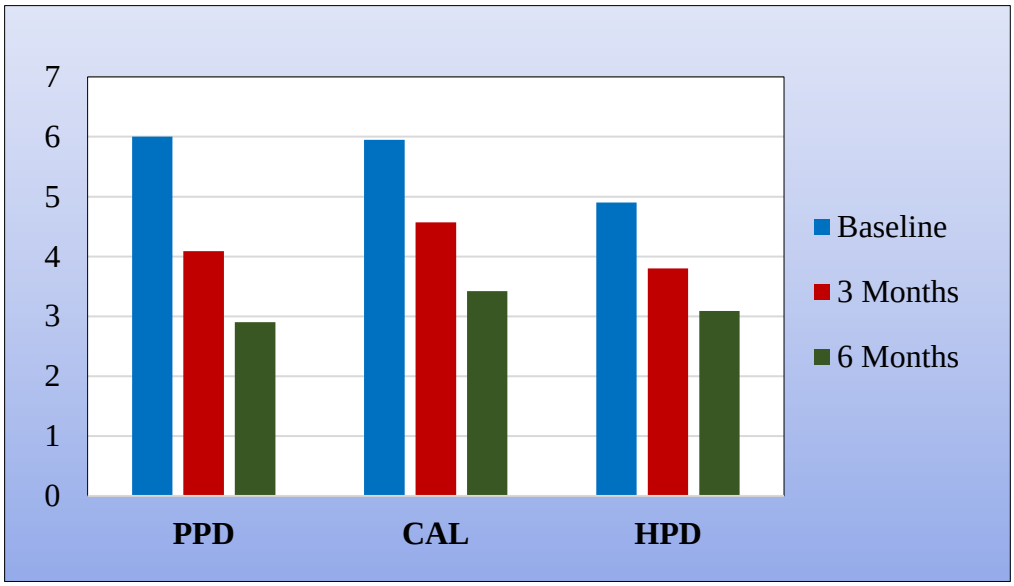
Reduction in	Baseline to 6 months		p-value
	Group I	Group II	
Bone defect height (in mm)	0.63 ± 0.15	1.10 ± 0.41	< 0.0001 (HS)
Bone defect width (in mm)	0.44 ± 0.44	0.79 ± 0.30	0.0059 (S)
Bone defect depth (in mm)	0.72 ± 0.35	0.94 ± 0.34	0.049 (S)
Bone defect volume (in mm3)	9.02 ± 4.21	12.45 ± 3.88	0.0099 (S)

(HS: highly significant, S: significant)

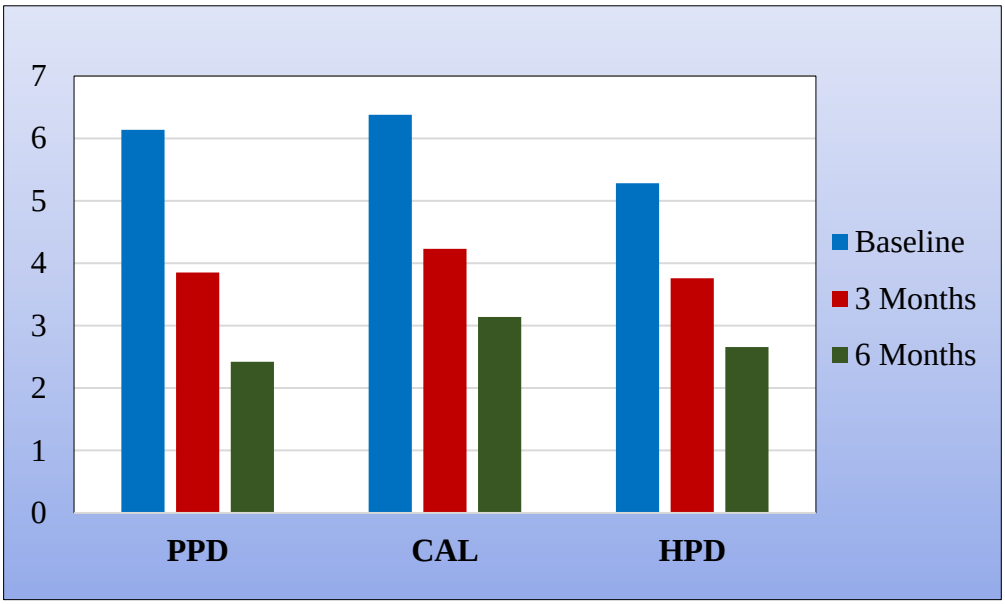
Graph 1: Comparison of clinical indices among the study population



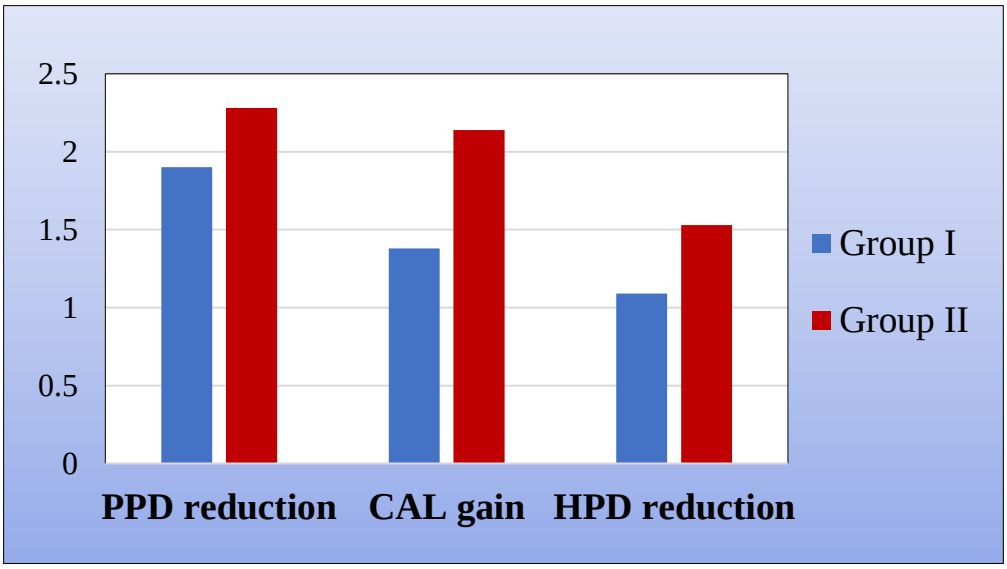
Graph 2: Comparison of clinical parameters at different time intervals in Group I



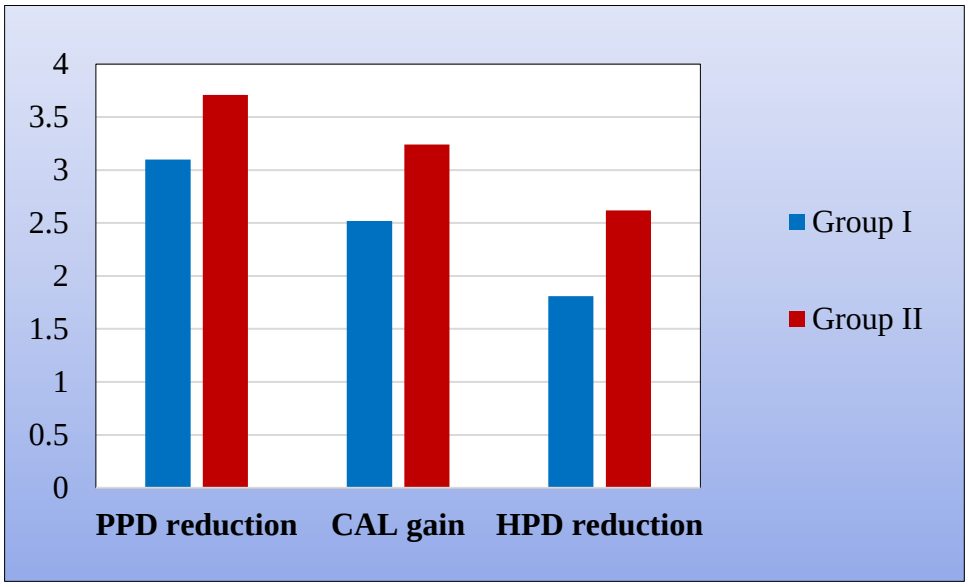
Graph 3: Comparison of clinical parameters at different time intervals in Group II



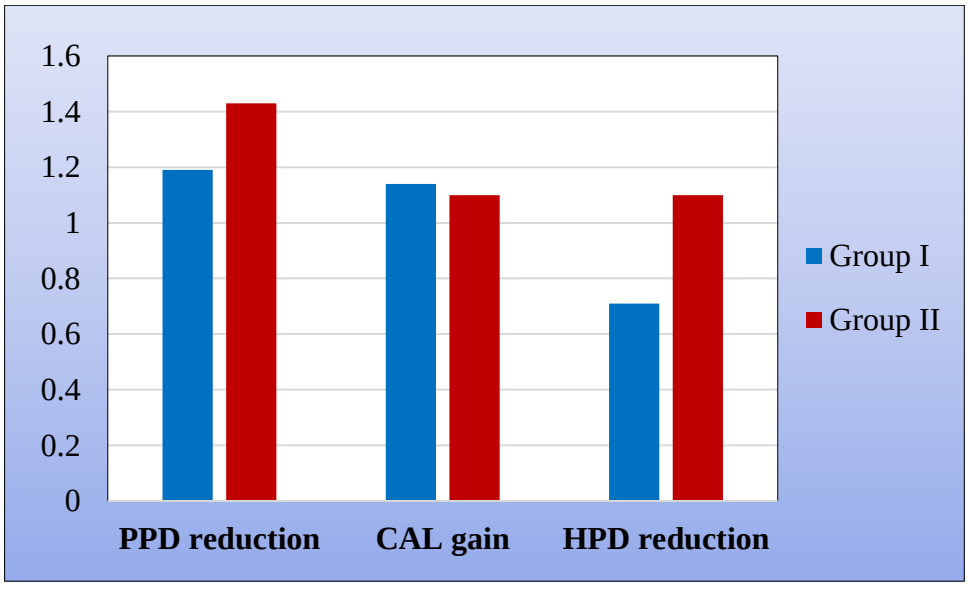
Graph 4: Comparison of clinical parameters between both the groups at 3 months



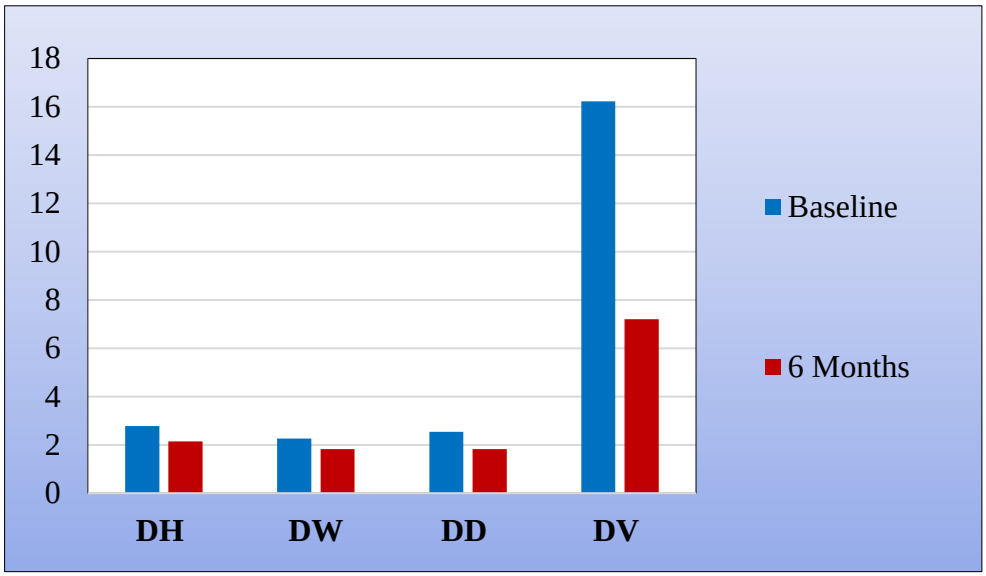
Graph 5: Comparison of clinical parameters between both the groups at 6 months



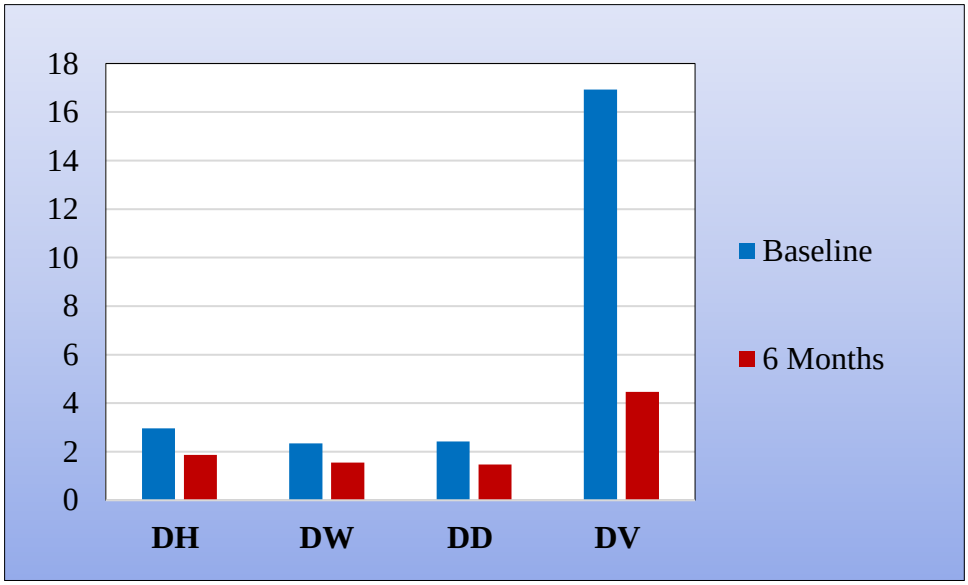
Graph 6: Comparison of clinical parameters between both the groups at 3-6 months



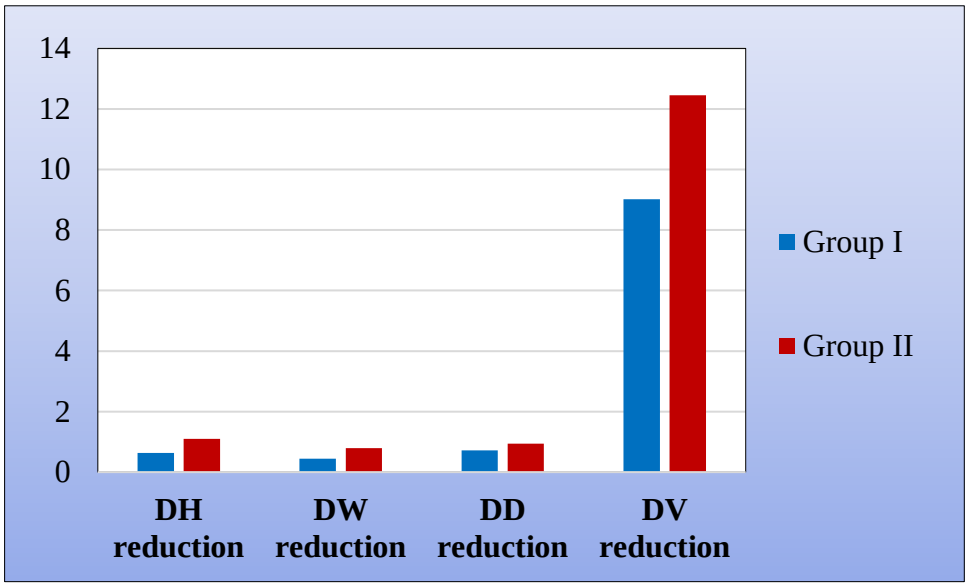
Graph 7: Comparison of radiographic parameters in Group I at different time interval



Graph 8: Comparison of radiographic parameters in Group II at different time interval



Graph 9: Comparison of radiographic parameters between both the groups at 6 months



MASTER CHART

PLAQUE INDEX

Sr. No	Baseline	3 months	6 months
1	2.9	2.2	1.3
2	3.09	3	2.13
3	2.24	2.13	2.1
4	3.06	2.51	2.2
5	3.5	2.67	2
6	2.93	2.4	2.23
7	3.06	2.65	2.4
8	3.86	2.55	2.1
9	3.14	2.78	1.81
10	3	2.89	1.6
11	3.15	2.87	2
12	3.25	2.87	1.8
13	2.89	2.5	2.1
14	3.18	2.45	2.1
15	3.7	1.43	1.12
16	3.76	2.13	1.43
17	2.9	2.56	1.9
18	3.1	2.87	2.3
19	3.66	2.6	2
20	3.15	2.34	2.11
21	3.1	2.2	1.9

GINGIVAL INDEX

Sr. No	Baseline	3 months	6 months
1	2.5	2.1	1.2
2	2.5	2	1.3
3	1.8	1.21	1.08
4	1.6	1.4	1.2
5	1.9	1.61	1.52
6	1.62	1.51	1.1
7	1.33	1.2	1.04
8	2.1	1.44	1
9	2.5	1.16	1.04
10	2.4	2	1.32
11	2.3	1.81	1.6
12	2.2	1.9	1.5
13	1.6	1.2	1
14	2.5	1.5	1.12
15	1.8	1.2	1
16	2.6	1.31	1.12
17	1.6	1.2	1
18	1.56	1.5	1
19	2	1.16	1.2
20	1.65	1.32	0.98
21	3.1	2.1	1.2

CLINICAL PARAMETERS

BASELINE

	Group I **PRF**			**Group II** **1%MF + PRF**		
	PPD (mm)	**CAL (mm)**	**HPD (mm)**	**PPD (mm)**	**CAL (mm)**	**HPD (mm)**
1	6	6	5	6	6	5
2	6	5	4	6	7	6
3	7	6	4	6	5	4
4	6	5	6	5	6	6
5	5	5	4	7	5	5
6	7	6	5	5	5	5
7	6	6	6	6	6	6
8	5	6	6	6	8	5
9	6	6	4	7	8	5
10	5	5	4	6	7	6
11	5	7	5	7	8	5
12	6	7	5	7	8	6
13	6	6	4	5	5	3
14	6	5	5	6	7	5
15	5	5	5	5	6	6
16	7	7	6	6	7	5
17	6	7	4	7	5	6
18	7	7	5	6	7	6
19	5	6	6	6	6	4
20	7	6	5	7	6	5
21	7	6	5	7	6	7

CLINICAL PARAMETERS

3 MONTHS RECALL

	Group I **PRF**			**Group II** **1%MF + PRF**		
	PPD (mm)	**CAL (mm)**	**HPD (mm)**	**PPD (mm)**	**CAL (mm)**	**HPD (mm)**
1	3	4	4	4	4	3
2	4	4	3	3	5	4
3	5	4	3	3	4	3
4	4	4	5	4	5	5
5	4	4	3	5	3	4
6	5	5	4	3	3	3
7	4	5	5	3	4	5
8	4	5	4	4	5	4
9	5	5	4	5	6	4
10	3	4	3	4	4	4
11	4	5	4	4	5	4
12	4	4	4	4	4	4
13	3	4	3	3	4	2
14	4	4	4	4	4	3
15	3	3	3	3	4	4
16	6	6	5	5	5	4
17	4	5	2	4	4	5
18	5	6	4	3	4	5
19	3	5	5	4	4	3
20	4	5	4	5	4	2
21	5	5	4	4	4	4

CLINICAL PARAMETERS

6 MONTHS RECALL

	Group I **PRF**			**Group II** **1%MF + PRF**		
	PPD (mm)	**CAL (mm)**	**HPD (mm)**	**PPD (mm)**	**CAL (mm)**	**HPD (mm)**
1	2	3	3	3	3	2
2	2	3	2	2	4	3
3	3	4	3	2	3	2
4	2	2	4	2	4	4
5	4	4	3	3	2	3
6	3	2	2	2	2	2
7	3	4	4	3	4	3
8	3	3	3	3	3	3
9	4	4	4	3	4	2
10	2	4	3	2	3	3
11	3	3	4	2	4	2
12	3	4	4	2	3	3
13	3	4	2	3	3	2
14	3	3	2	2	3	2
15	2	2	2	2	3	3
16	5	5	5	3	4	2
17	3	3	2	2	3	3
18	3	5	3	2	3	4
19	2	3	4	2	3	3
20	3	4	3	3	3	2
21	3	3	3	3	2	3

CBCT MEASUREMENTS OF DEFECT HEIGHT, DEFECT WIDTH, DEFECT DEPTH OF GROUP I

Group I (PRF)								
	BASELINE				6 MONTHS RECALL			
Sr. No	H (mm)	W (mm)	D (mm)	V (mm3)	H (mm)	W (mm)	D (mm)	V (mm3)
1	2.5	2.1	2.5	13.125	2	1.8	1.5	5.4
2	2.6	1.3	1.8	6.084	2.1	1.6	1.1	3.696
3	3	2	2	12	2.4	2.2	1.5	7.92
4	2.8	2.7	3.4	25.704	2	2.1	2.6	10.92
5	2.4	2.1	2.3	11.592	1.9	1.6	2	6.08
6	3.5	2.8	2.4	23.52	2.9	1.8	1.7	8.874
7	2.7	1.8	3.2	15.552	2.2	1.1	2.5	6.05
8	2.5	1.5	2.8	10.5	1.8	1.9	1.6	5.472
9	2.9	2	2.1	12.18	2.2	1.5	1.8	5.94
10	2.8	2	1.8	10.08	1.9	1.8	1.5	5.13
11	2.1	1.9	2.8	11.172	1.6	1.2	2.1	4.032
12	3.4	3.1	2.3	24.242	2.7	2.3	2	12.42
13	2.3	2.6	1.8	10.764	1.9	2.1	1.3	5.187
14	2.7	2.4	2.5	16.2	2	1.9	1.9	7.22
15	2.1	2.6	2.1	11.466	1.5	1.8	1.4	3.78
16	3.5	2.3	3	24.15	2.7	2.1	2.3	13.041
17	2.4	3	1.8	12.96	2	1.7	1.3	4.42
18	3.8	2	3	22.8	3	1.5	2.3	10.35
19	2.6	2.3	3.3	19.734	2	2.3	2.2	10.12
20	3.1	3	3	27.9	2.2	2	1.8	7.92
21	2.7	2	3.5	18.9	2.1	1.9	1.8	7.18

CBCT MEASUREMENTS OF DEFECT HEIGHT, DEFECT WIDTH, DEFECT DEPTH OF GROUP II

Group II (1%MF+PRF)								
	BASELINE				6 MONTHS RECALL			
Sr. No	**H (mm)**	**W (mm)**	**D (mm)**	**V (mm3)**	**H (mm)**	**W (mm)**	**D (mm)**	**V (mm3)**
1	3.1	1.8	1.9	10.602	1.8	1.2	1	2.16
2	3.4	2.1	2.4	17.136	2.1	1.5	1.6	5.04
3	2.5	2.1	2.4	10.5	2	1.1	1.5	3.3
4	2.8	1.9	2.6	13.832	2.2	1.2	1.4	3.69
5	2.2	2	2.3	10.12	1.4	0.8	1.3	1.456
6	2.4	2.9	2.2	15.312	1.3	2	1.2	3.12
7	2.6	2.3	2.7	16.164	1.8	1.6	1.5	4.32
8	3.1	2.4	2.6	19.344	2.1	1.4	1.3	3.822
9	3.2	2.6	2.1	17.472	2.4	1.4	1.4	4.704
10	3.7	2.5	3	27.75	1.9	2.1	2	7.98
11	3.8	3.1	2.3	27.094	2.1	2.1	1.8	7.938
12	3.4	2.4	3	24.48	1.8	1.9	2	6.84
13	2.5	2.1	2	10.5	1.6	1.8	1	2.88
14	3.4	1.8	2.3	14.076	1.4	1.2	1.1	1.848
15	3	2.4	2.6	18.72	1.6	1.8	2	5.76
16	3.4	2.4	2.2	17.952	2.8	2	1.9	10.64
17	2.9	2.4	2.9	20.184	1.8	1.4	2	5.04
18	3.1	2.7	2.6	21.762	2	1.2	1.9	4.56
19	2.9	2.6	1.9	14.326	2.1	1.7	1.1	3.927
20	2.4	2.6	2.9	18.096	1.4	1.9	0.9	2.394
21	2.5	2	2	10	1.6	1.3	1.2	2.496

CBCT MEASUREMENTS OF BONE DEFECT VOLUME

	Group I (PRF)		**Group II (1%MF+PRF)**	
Sr. No	**BASELINE (mm)**	**6 MONTHS (mm3)**	**BASELINE (mm)**	**6 MONTHS (mm3)**
1	13.125	5.4	10.602	2.16
2	6.084	3.696	17.136	5.04
3	12	7.92	10.5	3.3
4	25.704	10.92	13.832	3.69
5	11.592	6.08	10.12	1.456
6	23.52	8.874	15.312	3.12
7	15.552	6.05	16.164	4.32
8	10.5	5.472	19.344	3.822
9	12.18	5.94	17.472	4.704
10	10.08	5.13	27.75	7.98
11	11.172	4.032	27.094	7.938
12	24.242	12.42	24.48	6.84
13	10.764	5.187	10.5	2.88
14	16.2	7.22	14.076	1.848
15	11.466	3.78	18.72	5.76
16	24.15	13.041	17.952	10.64
17	12.96	4.42	20.184	5.04
18	22.8	10.35	21.762	4.56
19	19.734	10.12	14.326	3.927
20	27.9	7.92	18.096	2.394
21	18.9	7.18	10	2.496

CASE HISTORY PROFORMA

Comparative Evaluation of 1% Metformin gel plus Platelet Rich Fibrin and Platelet Rich Fibrin Alone in Treatment of Class II Furcation Defects: A Clinico-radiographic Study

NAME: **OPD NO:**

AGE/SEX: **DATE:**

ADDRESS: **PHONE NO:**

OCCUPATION:

CHIEF PERIODONTAL COMPLAINT:

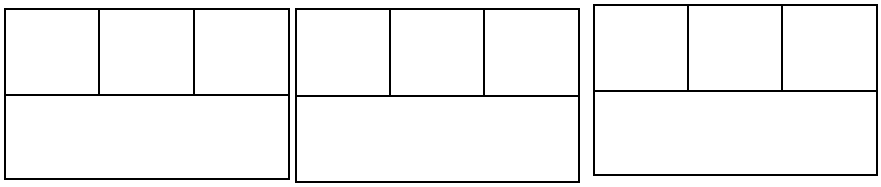
PAST DENTAL HISTORY:

PAST MEDICAL HISTORY:

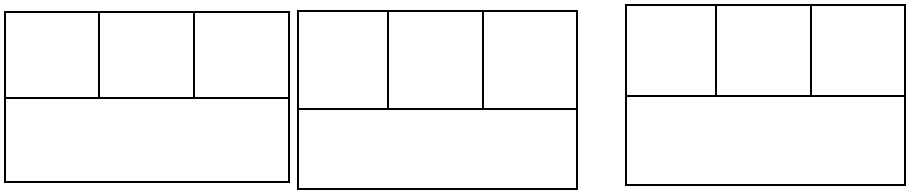
ORAL HYGIENE HABIT:

PLAQUE INDEX *(Sillness and Loe 1964)* Baseline

16 12 24



44 32 36



Total Score of all teeth

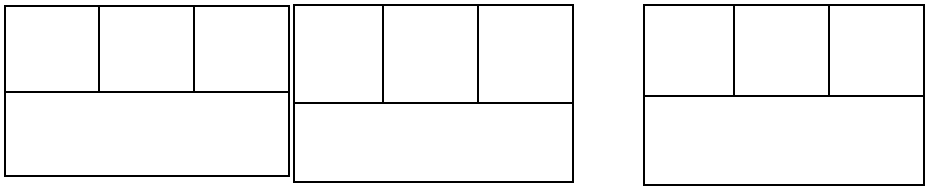
Total number of teeth examined

SCORE:

PLAQUE INDEX *(Sillness and Loe 1964)* 3 months

16 12 24

44 32 36



Total Score of all teeth

Total number of teeth examined

SCORE:

PLAQUE INDEX *(Sillness and Loe 1964)* 6 months

16 12 24

44 32 36

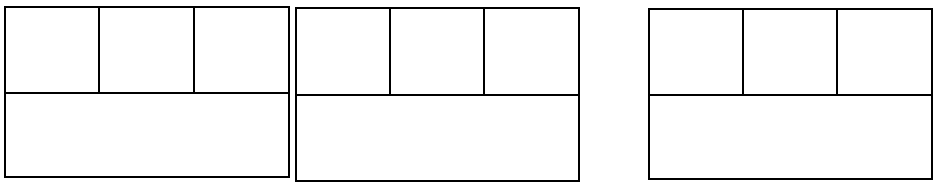
Total Score of all teeth

Total number of teeth examined

SCORE:

GINGIVAL INDEX (Loe and Sillness 1963) Baseline

16 12 24



44 32 36

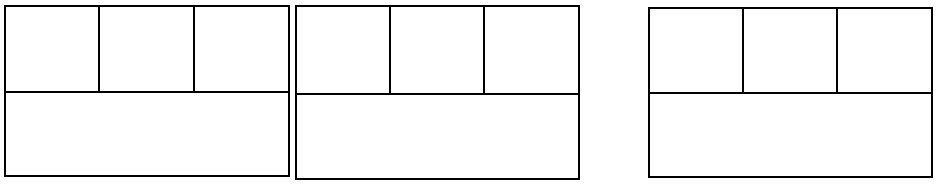
Total Score of all teeth

Total number of teeth examined

SCORE:

GINGIVAL INDEX (Loe and Sillness 1963) 3 Months

16 12 24



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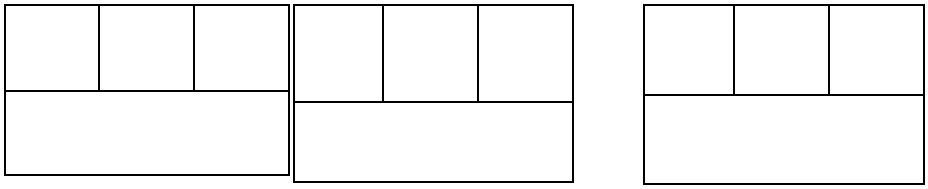


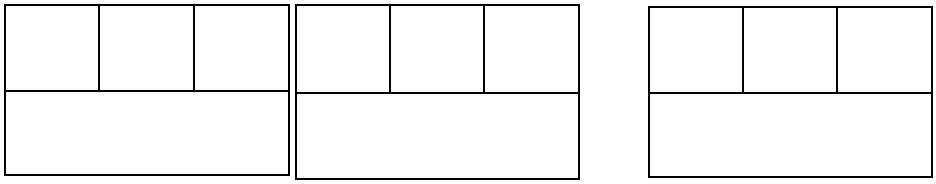
Total Score of all teeth

Total number of teeth examined

SCORE:

GINGIVAL INDEX (Loe and Sillness 1963) 6 Months

16 12 24



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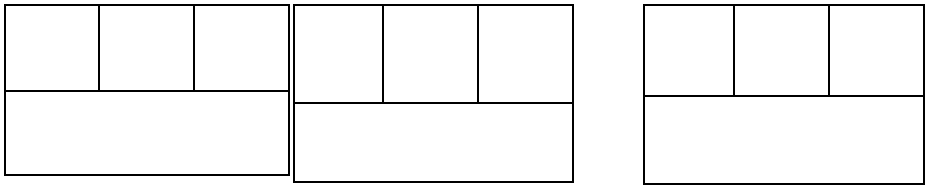
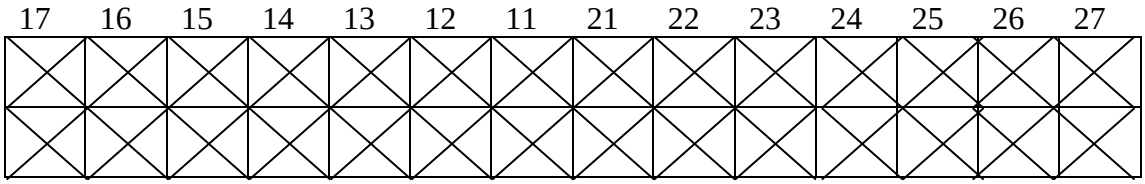


Total Score of all teeth

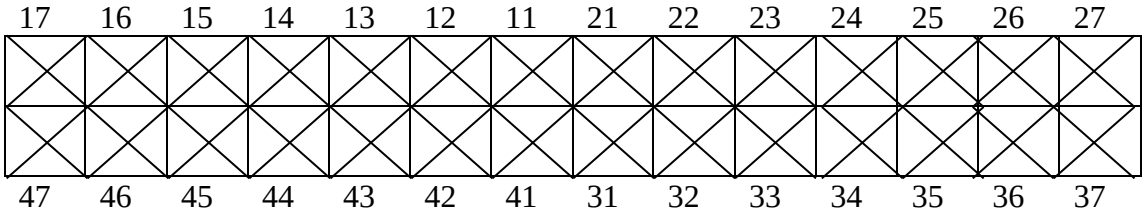
Total number of teeth examined

SCORE:

PROBING POCKET DEPTH (mm) (BASELINE):



PROBING POCKET DEPTH (mm) (3 MONTHS):



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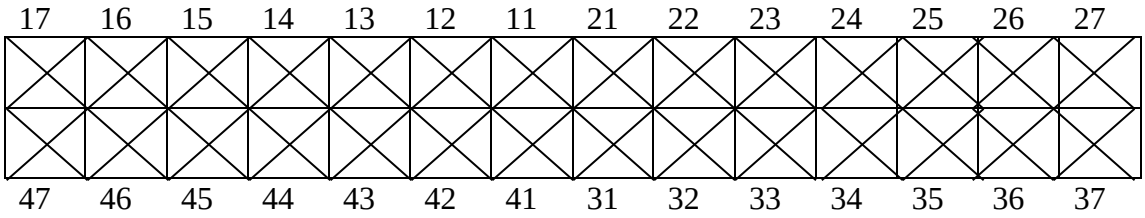
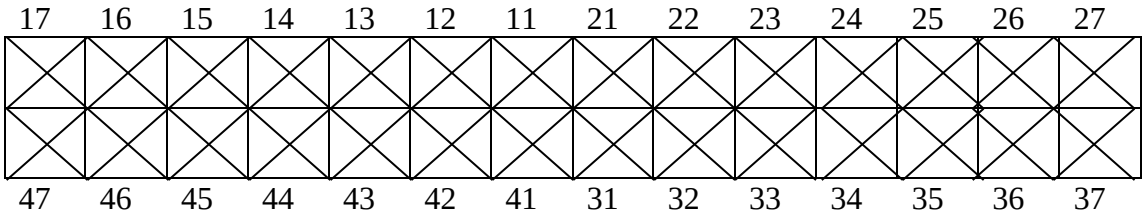
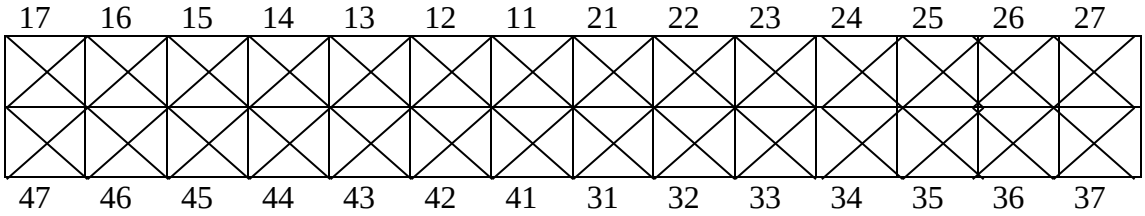


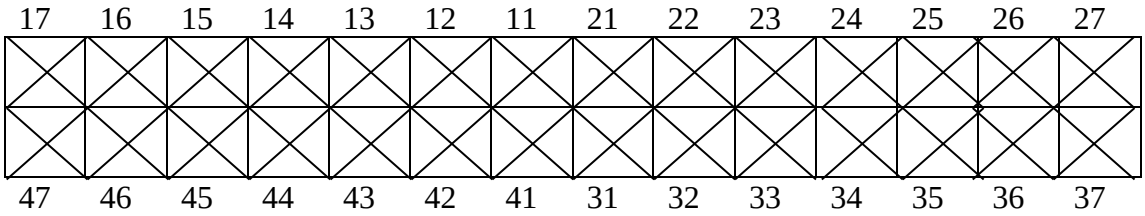
CLINICAL ATTACHMENT LOSS (mm) (BASELINE)



CLINICAL ATTACHMENT LOSS (mm) (3 MONTHS)



CLINICAL ATTACHMENT LOSS (mm) (6 MONTHS)



MOBILITY:

FURCATION INVOLVEMENT CLINICALLY:

Tooth No.	**Baseline horizontal probing depth (with Nabers)**	**3 months horizontal probing depth (with Nabers)**	**6 months horizontal probing depth (with Nabers)**

FURCATION INVOLVEMENT CLINICALLY AFTER FLAP REFLECTION:

Tooth No.	Material used	Clinical Baseline horizontal furcation depth (UNC 15)	Clinical Baseline vertical furcation height (UNC 15)

FURCATION INVOLVEMENT (CBCT):

Tooth No.	Material Used	Baseline Bone Defect Height	Bone Defect Height (6 Months)	Baseline Bone Defect Width	Bone Defect Width (6 Months)	Baseline Bone Defect Depth	Bone Defect Depth (6 Months)

Tooth No.	Baseline Bone Defect Volume	Bone Defect Volume (6 Months)

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(Confidential)
Informed Consent Form
"**Comparative Evaluation of 1% Metformin gel plus Platelet Rich Fibrin and Platelet Rich Fibrin alone in Treatment of Class II Furcation Defects: A Clinico-radiographic Study**"

NAME:
Mr./Master/Mrs./Miss._____
Resident of:
_____
_____aged _____ years, exercising my free will/choice, without any pressure/lure of incentive in any form, hereby give my consent for the project to be conducted by the doctor.

I acknowledge the receipt of "patient's information sheet", and also the doctor has informed me about this research project suitably and sufficiently to my satisfaction.
I agree to undergo this surgical periodontal treatment upon me which includes placing 1% Metformin gel and Platelet Rich Fibrin at one Class II furcation defect and only Platelet Rich Fibrin at another Class II furcation defect in my oral cavity. I authorize placement of gel and PRF in the defects. Potential benefits, risks and complications related to the materials used in surgery have been explained to me.

I agree to let my X-rays, photographs, blood investigations, other investigations to be taken as required.

I consent to the administration of anesthesia or other medications before, during or after the procedure by qualified personnel. I understand that all anesthetics or sedation medications include the very rare potential of risks or complications, such as damage to vital organs including the brain, heart, lungs, liver and kidneys; paralysis; cardiac arrest; and/or death from both known and unknown causes.

I understand that there are potential risks, complications and side effects associated with any dental procedure. Although it is impossible to list every potential risk, complication and side effect, I have been informed of some of the possible risks, complications and side effects of periodontal surgery.

I agree to take part in this project and will not mix any other projects during the period of this trial. I shall report to the dental hospital or other place where called on given appointment dates and time.

I certify that I have read or had read to me the contents of this form.

_____Date_____
Patient /legally authorized representative signature