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Comparative Clinical Evaluation of Modified Coronally Advanced Flap with and without Platelet Rich Fibrin in the Treatment of Multiple Adjacent Miller's Class I and Class II Gingival Recession Defects: A Randomized Clinical Trial

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ABSTRACT

Background and aim: Coronally advanced flaps, along with their modifications, are the simplest and most predictable procedures for root coverage. The addition of an inter-positional material is considered to achieve better outcomes. Therefore, this study aimed to assess the potential benefits of adding platelet-rich fibrin (PRF) membrane to the modified coronally advanced flap (MCAF) procedure in terms of change in vertical recession depth (VRD) and mean root coverage percentage (MRC%).

Materials and methods: A total of twelve systemically healthy subjects with thirty-four Miller's class I and II gingival recession defects were randomly assigned to a control (MCAF) or test (MCAF + PRF) group with seventeen defects in each. Vertical recession depth, gingival thickness (GTH), width of keratinized gingiva (WKG), probing depth (PD), and clinical attachment level (CAL) were recorded at baseline, one month and three months, mean root coverage percentage (MRC%) at one month and three months and VAS score for pain at ten days post-operative.

Results: MRC% was 75.96±21.01 and 83.23±18.28 % in the control and test groups at three months with no statistically significant difference ($p>0.05$). However, a statistically significant difference ($p<0.05$) was obtained concerning GTH gain at one and three months and decreased VAS score for pain at ten days in the test group compared to the control group.

Conclusions: Increase in GTH appears to justify using PRF and MCAF for treating multiple adjacent Miller's class I and II gingival recession defects. PRF membrane might be an alternative to different grafting materials in root coverage procedures.

1. Introduction

Gingival recession is the exposure of the root surface by an apical shift in the position of the gingiva.[1] A higher prevalence of gingival recession (60% to 90%) has been reported in the literature, and it has increased with advancing age.[2] Surgical root coverage procedures aim to reduce dentinal hypersensitivity, improve gingival esthetics, prevent root caries and facilitate plaque control efforts. Among several surgical techniques proposed to treat gingival recession, coronally advanced flap (CAF) is one of the most reliable and widely used surgical techniques for treating Miller's class I and II gingival recession defects.[3] Furthermore, Zucchelli and de Sanctis 2000 modified the CAF procedure, avoiding the need for a vertical releasing incision.[4] Coronally advanced flap with its modifications is an effective and predictable technique for treating isolated and multiple gingival recession defects.[5] However, treatment of multiple gingival recession is less predictable and effective than isolated recession defects.[6, 7] Even though the combination of CAF with connective tissue grafts (CTG) has been considered the gold standard in the achievement of predictable root coverage,[6, 7] there are several limitations related to the harvesting of soft tissue autografts.[5] Therefore, the newer approaches that combine CAF with autologous platelet-rich fibrin (PRF) membranes are considered a feasible substitute for the CAF+CTG technique for treating gingival recession defects.[8, 9] PRF, first developed in France by Choukroun, is a second-generation platelet concentrate with

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simplified processing, which requires neither anticoagulant nor bovine thrombin for its preparation.[10] Thus, it is non-toxic, nonimmune-reactive, and is known to accelerate wound healing and hard and soft tissue maturation when used in conjunction with various root coverage procedures.[11] Based on the available literature, there appears to be large variability and conflict in the outcomes of the studies comparing coronally advanced flaps with and without the use of PRF. Therefore, this study aimed to determine whether adding autologous platelet-rich fibrin (PRF) membrane to modified coronally advanced flap (MCAF) would provide additional benefit in root coverage of adjacent Miller's class I and class II gingival recession defects compared to MCAF alone.

2. Material and methods

This randomized, single-blinded, controlled clinical trial was approved by the Institutional Review Committee and registered as a clinical trial by the National Institute of Medical Statistics (India Council of Medical Research); the Clinical Trial identifier no. CTRI/2019/08/020857. This clinical study adheres to the principles of per Helsinki Declaration of 1975, as revised in 2013. A total of 34 gingival recession defects among 12 systemically healthy patients of either gender (males and females) visiting the out-patient section of the Department of Periodontology and Oral Implantology and satisfying the following inclusion criteria were enrolled; multiple adjacent Miller's Class I and class II gingival recession in maxillary anterior and premolars, the width of keratinized gingiva $\geqslant$ 3 mm in patients of age between 20 to 35 years (of either gender) with at least 20 natural teeth within both jaws. Exclusion criteria included; inflammatory periodontal disease, probing depth (PD) >3mm, previous surgical attempt to correct gingival recession, patient with known systemic disease or severe immune deficiency, pregnancy, smokers, patients receiving treatment with any medications known to affect periodontal health and malpositioned tooth. Written informed consent was obtained prior to the study. Sample size calculation was done in the literature by Sofia Aroca et al. in 2009,[12] using the mean ± SD of root coverage percentage as a clinical parameter among test and control groups which was 80.7 ± 14.7 and 91.5 ± 11.4, respectively. Considering the minimum difference of 10.8 in root coverage percentage in between the groups and minimum standard deviation as 11.4, the sample size has been calculated for two means of comparison. If each group is normally distributed, we need to enroll 17 defects in each group to be able to reject the alternate hypothesis that both the means are equal with 80% power and 95% confidence interval (CI). Using the formula:

$$\frac{(Z_{1-}\alpha/_2+Z\beta)^2\, 2\sigma^2}{(\overline{X_1}-\overline{X_2})^2} = \text{(in each group)}$$

Where $Z_{1-}\alpha/_2$ at 95%CI=1.96,
$Z\beta$at 20%=0.84,
σ= 11.4
X_1= 80.7
X_2= 91.5

Substituting the values,

$$\frac{2\,(1.95+0.84\,)^2(11.4)^2}{(91.5-80.7)^2}$$

= 17.47
≈17 gingival recession defects in each group.

Simple randomization for allocating subjects into either the control group (MCAF) or test group (MCAF + PRF membrane) with 17 gingival recession defects in each was done using computer-generated randomization. In all the enrolled patients, routine radiographic and blood investigations were done. The initial therapy consisted of nonsurgical periodontal therapy, as indicated. Periodontal re-evaluation was performed four weeks after phase I therapy. A single examiner (a periodontist other than the principle investigator) recorded all the clinical parameters at different time intervals (baseline, one month, and three months) who was masked about the treatment groups. Our primary objective was to assess and compare the postoperative change in vertical recession depth and mean root coverage percentage. In contrast, our secondary objective was to assess and compare the change in gingival thickness, the width of keratinized gingiva, probing depth, clinical attachment level, and postoperative pain among the test and control groups.

Analyzed variables

The clinical parameters recorded for both groups at baseline, one month, and three months post-operative were; VRD, measured from the cementoenamel junction (CEJ) to gingival margin at mid-facial point, WKG, the distance between the margin of the gingiva and mucogingival junction, PD, measured from the crest of gingival margin to the base of the gingival sulcus, CAL, measured from CEJ to the base of gingival sulcus and GTH, measured at midfacial point, 3 mm below the gingival margin. The mucosal surface was pierced with a no.15 endodontic reamer at a 90^o angle with slight pressure until hard tissue was reached. After sliding the silicone stop on the reamer until it was in close contact with the gingiva, it was removed, and the distance between the reamer's tip and the silicone stop's inner border was measured to the nearest 0.1 mm with vernier calipers. Similarly, the Visual Analog Scale (VAS) was recorded ten days post-operative. The VAS is a straight, 100-mm line (10cm), where the left end of the line (i.e., zero) represents "no pain," while the other end (i.e.10) denotes "worst pain experienced ever." At one month and three months post-operative, MRC% was calculated according to the following formula:

([VRD preoperative - VRD postoperative] / VRD preoperative) × 100%.

Surgical procedure

The principle investigator performed all the surgical procedures in both groups. Following local anesthesia, surgical procedures were performed on both the test and control groups. Gracey curettes were used to scale recession defects thoroughly. Root conditioning was not applied, and used the modified Coronally Advanced Flap technique. In contrast to the conventional technique, this Zucchelli's modification of the CAF procedure avoids the need for a vertical releasing incision. The flap design was as follows: Oblique sub-marginal incisions were made in the interdental areas connecting intra-crevicular incisions at recession defects (Figs. 1b, 2b). The incisions were extended to include one tooth on each side of the teeth that needed treatment to enable coronal repositioning of the flap. Coronal-apical directions were made using the split-full-split flap incisions (Fig. 1c). The most apical portion of the flap was split-thickness, whereas the gingival tissue adjacent to the root defect and the interproximal bone was increased to full thickness to provide maximum soft tissue thickness of the flap to be positioned coronally over the denuded root. These made it possible to adjust the flap coronally without tension. All papillae were de-epithelialized to make a bed of connective tissue. In the test group, a prepared PRF membrane was positioned over the recession defects, just below the CEJ (Fig. 2c). The gingival flap was repositioned and secured with its margin 1-2 mm coronal to CEJ by sling sutures around the

contact points on both the control and test sites using a 4-0 silk suture (Figs. 1d, 2d). Gentle pressure was applied for five minutes to stabilize the blood clot. The periodontal dressing was completed in the end.

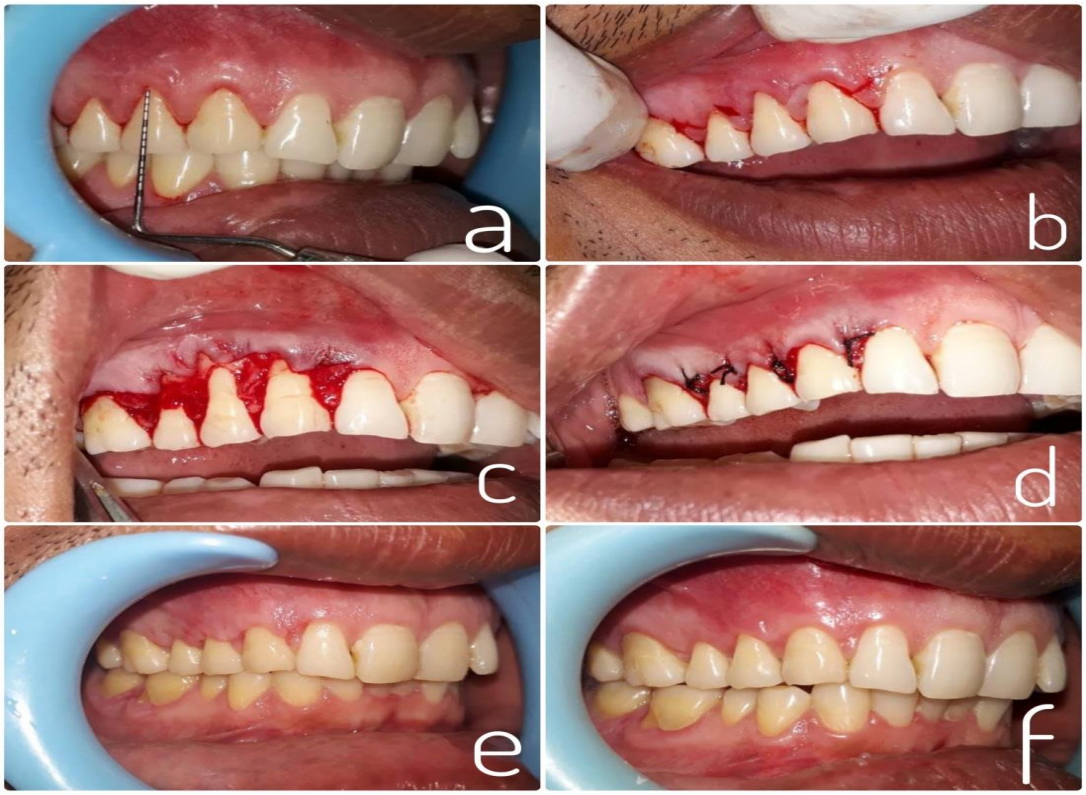

Fig. 1. Preoperative, intraoperative, and postoperative pictures of control (Modified coronally advanced flap) group: (a) Preoperative view, (b) Incision design, (c) Flap elevation, (d) Flap sutured after coronal advancement, (e) One month postoperative, (f) Three months postoperative.

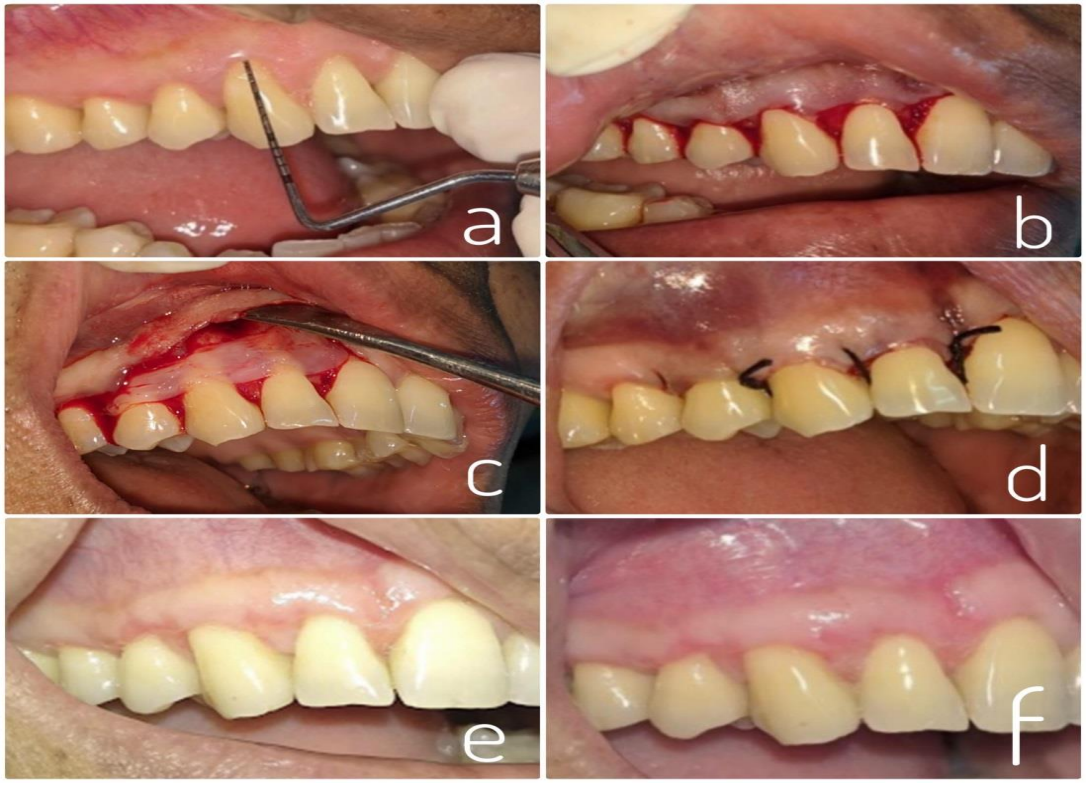

Fig. 2. Preoperative, intraoperative, and postoperative pictures of test (Modified coronally advanced flap with PRF membrane) group: (a) Preoperative view, (b) Incision design, (c) PRF membrane placed over recession defect, (d) Flap sutured after coronal advancement, (e) One month postoperative, (f) Three months postoperative.

Preparation of PRF membrane

A 10 ml syringe was used to collect intravenous blood (by venipuncture of the antecubital vein) from the patient, which was transferred to a test tube and centrifuged immediately without the addition of anticoagulant at 3000 rpm for 10 minutes in a PRF machine[13] (REMI-R-8C laboratory centrifuge). The fibrin clot formed in the middle part of the tube was easily separated from the lower part of the centrifuged blood and kept in the PRF box (GDC). PRF membrane of uniform thickness was then prepared. The patients were advised to take a soft diet, avoid biting and brush the operated site for two weeks. Patients were prescribed analgesics for postoperative pain and discomfort. Tablet Ibuprofen 400mg TID for two days, then SOS, and 0.2% chlorhexidine oral rinse twice daily for two weeks for its antiseptic and plaque control properties. Patients were recalled after ten days for suture removal and recording postoperative pain using a VAS score. All patients were examined and instructed on mechanical tooth cleaning in the operative areas using a soft toothbrush and a roll technique two weeks after surgical treatment. The postsurgical assessment of clinical parameters (VRD, MRC%, GTH, WKG, PD, CAL) at one month and three months was done by the same trained examiner blinded for the two study groups, and change in clinical parameters was determined by comparing the postsurgical measurements at each site with the baseline values.

Statistical analysis

Statistical Package for Social Sciences was used to conduct the statistical analysis (SPSS, version 13). Percentage distribution of subjects with Miller's class I and class II gingival recession was tabulated in each group and with the change in vertical recession depth (VRD) after periodontal surgery. Descriptive statistics were calculated to explore numeric data characteristics and calculate mean, median, SD, rank, and range. The normality of variables was examined. The Mann-Whitney U-test was used to compare the clinical parameters between the two groups. Friedman test was used to compare the data within the group in case of repeated measures. Wilcoxon's signed-rank test was used to compare the variables within the same groups in each follow-up and baseline. Confidence intervals were set at 95%, and the probability of significance was set at a 5% significance level.

3. Results

Total of thirty-four Miller's class I and class II multiple adjacent gingival recession defects present on the maxilla of twelve patients within the age range between 26 and 32 years were included in the study. Patients reported no complications during the healing phase. All patients complied with the three months follow-up visits. Baseline parameters, i.e., VRD, GTH, WKG, PD, and CAL, were similar in both the test and control groups, with non-significant statistical differences (p>0.05) (Table 1).

Table 1. Intergroup comparison of parameters at baseline in control and test group.

Parameters at Baseline	Groups	Mean ±SD	Median	Mean Rank	P-value†
VRD (mm)	Control	2.29 ±0.86	2	18.76	0.44
	Test	2.02 ±0.81	2	16.24	
GTH (mm)	Control	1.11 ±0.28	1	15.62	0.16
	Test	1.23 ±0.31	1	19.38	
WKG(mm)	Control	3.58 ±0.79	3	19.24	0.21
	Test	3.26 ±0.50	3	15.76	
PD (mm)	Control	1.32 ±0.52	1	16.47	0.50
	Test	1.41 ±0.44	1.5	18.53	
CAL (mm)	Control	3.55 ±1.04	3.5	17.74	0.88
	Test	3.44 ±0.84	3.5	17.26	

† ***Mann-Whitney U test, SD-Standard Deviation.***
VRD: vertical recession depth, GTH: gingival thickness, WKG: width of keratinized gingiva, PD: probing depth, CAL: clinical attachment level.

Intergroup comparison showed statistically non-significant differences for VRD reduction, MRC %, WKG increase, PD reduction, and CAL gain (p>0.05), with an exception in GTH, which showed a statistically significant (P<0.001) increase in the test group at both one month and three-month comparison (Table 2). VAS scores for pain experienced by subjects were also found to be significantly less in the test group compared to the control group when recorded at ten days post-operative (Table 2).

Table 2. Intergroup comparison of parameters between the control and test groups at one month and three months.

Clinical Parameters		Control Group			Test Group			P-value†
		Mean± SD	Median	Mean Rank	Mean± SD	Median	Mean Rank	
VRC (mm)	1 month	0.97± 0.64	1.0	18.68	0.82 ± 0.55	1.0	16.32	0.49
	3 months	0.67 ± 0.66	0.5	19.44	0.41 ± 0.53	0.5	15.56	0.25
MRC %	1 month	62.38±19.51	50	16.97	64.76±21.44	50	18.03	0.76
	3 months	75.96±21.01	75	15.74	83.23±18.28	75	19.26	0.30
GTH (mm)	1 month	1.14 ± 0.29	1.0	11.97	1.55±0.30	1.5	23.03	<0.001
	3 months	1.08 ± 0.19	1.0	10.88	1.67±0.39	1.5	24.12	<0.00
WKG (mm)	1 month	3.85 ± 0.76	3.5	19.65	3.52±0.57	3.5	15.35	0.19
	3 months	3.94 ± 0.82	4	18.03	3.79±0.56	3.5	16.97	0.74
PD (mm)	1 month	1.029 ± 1.12	1	18	1.00±0.00	1	17.0	0.31
	3 months	0.88 ± 0.21	1	17.50	0.88±0.21	1	17.50	1.0
CAL (mm)	1 month	2.0 ±0.70	2	18.62	1.82 ± 0.55	2.0	16.38	0.51
	3 months	1.55 ± 0.72	1.5	18.82	1.41 ± 0.50	1.5	16.18	0.45
VAS score	10^{th} day	5.82± 0.72	6	24.59	4.47±0.51	4	4. 10.41	5. <0.001

P-value †Mann-Whitney U test.

VRD: Vertical recession depth, MRC%: Mean root coverage percentage, GTH: Gingival thickness, WKG: Width of keratinized gingiva, PD: Probing depth, CAL: Clinical attachment level, VAS: Visual analog scale.

Intragroup comparison within both the control and test group showed statistically significant differences (P<0.05) at one month and three months when compared to baseline values for all the clinical parameters (VRD, KWG, PD, CAL) except for GTH in the control group (Tables 3, 4). Interestingly, a significant increase in GTH was found at both follow-up visits only in the test group (Table 4). An increment in MRC % from one month to three months was statistically significant in both the control and test group (P-value<0.05) (Table 5).

Table 3. Intragroup comparison of parameters within control group between baseline to 1 month, one month to 3 months, and baseline to 3 months.

Clinical Parameters		Median (Range)	Mean Rank	P-value‡
VRD (mm)	Baseline	2 (1-4)	9	<0.001[a]
	1 month	1 (0-2)	5.5	0.002[b]
	3 months	0.5 (0-2)	9	<0.001[c]
GTH (mm)	Baseline	1 (1-2)	2	0.56[a]
	1 month	1 (1-2)	1.5	0.15[b]
	3 months	1 (1-1.5)	3	0.65[c]
WKG (mm)	Baseline	3 (3-5)	4.5	0.007[a]
	1 month	3.5 (3-5.5)	3	0.18[b]
	3 months	4 (3-5.5)	5	0.003[c]
PD (mm)	Baseline	1 (0.5-2)	4.42	0.028[a]
	1 month	1 (1-1.5)	3	0.025[b]
	3 months	1 (0.5-1)	5	0.006[c]
CAL (mm)	Baseline	3.5 (2-5.5)	9	<0.001[a]
	1 month	2 (0-3.5)	5.5	0.005[b]
	3 months	1.5 (0-3)	9	<0.001[c]

‡ Wilcoxon-signed rank test, [a] baseline Vs. 1 month, [b] 1month Vs. 3 months, [c] baseline Vs. 3months.

VRD: Vertical recession depth, GTH: Gingival thickness, WKG: Width of keratinized gingiva, PD: Probing depth, CAL: Clinical attachment level.

Table 4. Intragroup comparison of parameters within test group between baseline to 1 month, 1 month to 3 months, and baseline to 3 months.

Clinical Parameters		Median (Range)	Mean Rank	P-value‡
VRD (mm)	Baseline	2(1-4)	9	<0.001[a]
	1 month	1(0-2)	6.5	0.002[b]
	3 months	0.5(0-2)	9	<0.001[c]
GTH (mm)	Baseline	1(1-2)	5.5	<0.001[a]
	1 month	1.5(1-2)	2.5	0.002[b]
	3 months	1.5(1-2.5)	7.5	<0.001[c]

KG (mm)	Baseline	3(3-4.5)	6	0.001[a]
	1 month	3.5(3-5)	6	0.001[b]
	3 months	4(3.5-5)	9	<0.001[c]
PD (mm)	Baseline	1.5(1-2)	5	0.028[a]
	1 month	1(1-1.5)	2.5	0.025[b]
	3 months	1(0.5-1)	7	0.006[c]
CAL (mm)	Baseline	3.5 (2.5-6)	9	<0.001[a]
	1 month	2 (0-3)	6	0.003[b]
	3 months	1(0-3)	9	<0.001[c]

‡Wilcoxon-signed rank test, [a] baseline Vs. 1 month, [b] 1month Vs. 3 months, [c] baseline Vs. 3months.
VRD: Vertical recession depth, GTH: Gingival thickness, WKG: Width of keratinized gingiva, PD: Probing depth, CAL: Clinical attachment level.

Table 5. In the control and test group, there was a comparison of MRC % between 1 and 3 months.

Group		Mean ±SD	Median (Range)	Mean Rank	P-value‡
Control group (n=17)	1 month	62.38± 19.51	50 (42.8-100%)	5.5	0.005[a]
	3 months	75.96± 21.01	75 (50-100%)		
Test group (n=17)	1 month	64.76 ±21.44	50 (40-100%)	6	0.003[a]
	3 months	83.23 ± 18.28	80 (50-100%)		

‡Wilcoxon-signed rank test, [a] 1month Vs. 3 months.
MRC%: Mean root coverage percentage.

4. Discussion

Treatment of multiple adjacent gingival recession defects presents more challenges due to factors like a need for larger avascular root surfaces to be treated during single sessions, more complicated hard and soft tissue management, and other restricting anatomical variations. CAF, MCAF, and modified coronally advanced tunnel techniques are the techniques preferred for treating multiple adjacent gingival recession defects. In this study, the MCAF technique was used as it avoids the vertical releasing incisions and ensures adequate lateral blood supply to the repositioned flap, provides stability to the flap during the healing phase, and avoids unaesthetic scars. Similarly, a split-full-split flap allows for adequate coronal advancement and ample blood supply to the surgical interdental papillae.[4] Platelet Rich Fibrin membrane was used in combination with MCAF in the test group as it has been claimed that it aids in the promotion of initial stabilization and revascularization of flaps and grafts in root coverage procedures.[13-16] This membrane consists of a fibrin-based, three-dimensional polymerized matrix in a specific structure incorporating platelets, leukocytes, growth factors, and the presence of circulating stem cells that are released relatively at a constant concentration over seven days.[16] In both groups, statistically significant improvement was found in all clinical parameters from baseline to three months, except for GTH in the control group. These could be attributed to stringent inclusion and exclusion criteria, adequate baseline width of keratinized gingiva, split full split design of flap, passive tension-free placement of flap at least one millimeter coronal to the CEJ, increased stability due to the application of both sling sutures and rigorous maintenance regimen. In the test group, the GTH increase was statistically significant in the control group. These results are in agreement with the study by Aroca et al.[12] In our study, there was an increase in gingival thickness from 1.23 ± 0.31 at baseline to 1.67 ± 0.39 at three months, with an increment of 0.44mm in the test group. This result is comparable to the findings by Thamaraiselvan

M et al.[15] who reported the mean increment of 0.30 mm gingival thickness from baseline (0.95±0.14) to sixth month (1.25±0.23) with the addition of PRF to CAF. This increase in gingival thickness in the test group may be caused by the spacing effect of the PRF membrane or by the proliferative influence of growth factors released from the PRF membrane on gingival and PDL fibroblasts.[12] Since thick tissue resists trauma and subsequent recession, enables tissue manipulation, promotes creeping attachment, and exhibits less clinical inflammation, this increase in GTH should be considered clinically significant.[19]

In our study, there was no statistically significant increment ($p>0.05$) in MRC% in the test group compared to the control group at one and three months. Similar findings were also reported by Thamaraiselvan M et al.,[15] Gupta S et al.,[16] and Kuka S et al.[19] in their studies. The inferior root coverage obtained in a study by Aroca et al.[12] was attributed to using a single-layer PRF membrane for multiple recession defects. The same reason could be attributed to the non-significant increment in MRC% with the addition of PRF membrane in our study. Also, It would have been appropriate to use more than one membrane placed in the opposite direction to have a uniform effect throughout the full recession faults since the PRF membrane is an inhomogeneous matrix with leukocytes and platelet aggregates concentrated within different ends. However, the MRC% obtained in the test group at three months (83.23±18.24%) was higher than the control group (64.76±21.44%). These may be attributed to creeping attachment and the healing potential provided by PRF.[20] Both the treatment groups showed a statistically significant reduction in PD and gained in CAL at one month and three months ($p<0.05$); however, it was not significant between the groups ($p>0.05$). The decrease in PD and increased recession coverage is responsible for the increase in clinical attachment. It is also possible that this resulted in forming new connective tissue attachments. However, the type of attachment cannot be defined without histological evidence. The growth factors secreted by the PRF membrane in the test group may have improved the attachment of cells between the overlaying flap and the underlying root surface, preventing the flap from shrinking.[16-18] There was a statistically significant increase in WKG at one month and three months in the test group but only at one month in the control group compared to the baseline. The mechanism behind this increase in both groups could be the reversion of mucogingival junction towards its original level as it is genetically determined and has been shown to re-establish itself to the original position.[21-22] In our study, the result in the control group agrees with the study by Gürgan et al.[23] who showed the greatest amount of reversion of mucogingival line occurring between baseline and one month after the coronally advanced flap technique. In the patient-centered outcome, postoperative pain experienced by patients in the test group scored significantly less compared to the control group ($p<0.05$). The result of our study is in accordance with the studies by cheng al.[24] The beneficial effect of PRF in diminishing postoperative pain and discomfort might be attributed to the release of growth factors and cytokines immersed in platelets, leukocytes, and the fibrin mesh. Clinically, a reduction in postoperative pain results in decreased postoperative discomfort, lower demand for postoperative emergency appointments, and increased patient satisfaction during the healing phase.

5. Conclusion

The MCAF and MCAF+PRF approaches can be successful in root coverage of multiple adjacent Miller's class I and II gingival recessions. Although the PRF membrane as an inter-positional material in MCAF did not provide additional benefit in terms of root coverage when compared with MCAF alone, it significantly increased gingival thickness. Also, the addition of PRF membrane showed significantly reduced postoperative pain experienced by the patient. For the treatment of multiple gingival recessions, PRF might be an alternative to various grafting materials; however, more randomized and controlled clinical studies with long-term follow-ups are required to shed more insight on the insight.

Conflict of Interest

The authors declared that there is no conflict of interest.

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