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Amniotic Allograft with Coronally Advanced Flap for Treatment of Gingival Recession: A Randomized, Controlled Clinical Trial

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A B S T R A C T

Objectives: Amniotic membrane (AM) is an allograft obtained from humans that contains a variety of growth factors, and has outstanding healing qualities. This study aimed to assess the efficacy of amniotic allograft with coronally advanced flap (CAF) for root coverage.

Materials and Methods: This randomized controlled clinical trial comprised 24 individuals with Miller's Class I and II root resorption defects. Each patient received treatment using the CAF approach, with the test group receiving a combined therapy using an AM. Measurements were made at baseline and 6 months after surgery for gingival biotype (GB), dentin hypersensitivity (DH), recession depth (RD), recession width (RW), gingival recession total surface area (GRTSA), keratinized tissue width (KTW) and probing depth (PD).

Results: There was a statistically significant reduction in RD (from 2.83 mm to 0.92 mm), a significant increase in KTW (from 3.17 mm to 4.25 mm), improvement in GB, and a reduction in DH in the test group compared to the control group ($P<0.05$).

Conclusion: Miller's Class I and II root recession defects can be effectively treated with a CAF. Application of AM under the CAF improved root coverage and GB, and further increased the KTW after 6 months, compared to CAF alone.

Keywords: Amnion; Surgical Flaps; Gingival Recession; Tooth Root; Surgery

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INTRODUCTION

Gingival recession is a common esthetic and functional concern associated with the periodontal tissues. Gingival recession by at least 1 mm is often present in one or more sites in over 50% of the population [1]. Also, it has been reported that 88% of the elderly over the age of 65 years and 50% of individuals between 18-65 years have gingival recession at one or more sites [1].

Several surgical approaches may be adopted for treatment of gingival recession such as free soft tissue grafts like free gingival graft, connective tissue graft, and combination procedures, and pedicle flaps such as laterally relocated flap, coronally advanced flap (CAF), and double papilla flap [2]. Some novel approaches such as the guided tissue regeneration, enamel matrix proteins, and vestibular incision have also been used in recent years [2]. Gingival recession may be treated by the subperiosteal tunnel access technique, pinhole technique, pouch technique, and tunnel procedures [2]. Of the abovementioned approaches, the most reliable method is thought to be the subepithelial connective tissue graft in conjunction with a CAF [3]. Autografts have

benefits, but they have certain disadvantages as well. For example, there is a limited supply, and the process of their procurement prolongs the surgical time and considerably increases the patient morbidity [4]. It has been proposed that soft tissue allografts may be used instead of autogenous tissue grafts.

The amniotic membrane (AM) allograft was recently introduced [5]. It is an allograft of alternative origin that has been recommended for treatment of numerous medical and dental conditions. It is derived from the human amnion tissue, which is the innermost lining component of the placental membrane [5]. It is an immunotolerant composite membrane made of pluripotent cellular components in a semipermeable membrane [6-8]. Furthermore, it is a good candidate for guided tissue regeneration since it contains pluripotent stem cells capable of differentiating into other periodontal cellular components. Optimal revascularization capacity of the AM is another outstanding feature of this biological scaffold [9,10]. It also contains numerous growth factors that may promote fibroblast development and neovascularization, potentially leading to granulation tissue formation [5,10,11]. However, the number of studies on the effectiveness of AM for treatment of gingival recessions is limited. Therefore, this study aimed to assess whether the addition of an AM allograft to CAF would improve root coverage of Miller's Class I or II gingival recession defects compared to CAF alone.

MATERIALS AND METHODS

Study design and participants:

This prospective, randomized, single-blind, controlled clinical trial with a parallel design was carried out at a single center between March 2016 and May 2017, and was registered at ClinicalTrials.gov under the registration number CTRI/2017/07/009126. The study protocol was also approved by the institutional review board (IEC/VDC/MDS15 PERIO 1). The CONSORT guidelines were followed in the conduct and reporting of this trial.

Sample size:

The sample size was calculated using the Openepi software, considering α=0.05, a power (1-β) of 80%, and effect size of 0.5. A total of 46 patients were assessed for eligibility, and 16 patients were excluded for not meeting the inclusion criteria and 2 patients were not willing to participate in the study. Twenty-four patients with Miller's Class I and II recession defects with random allocation ratio 1:1 (12 in each group) were included in the study.

Eligibility criteria:

The inclusion criteria were minimum age of 18 years, presence of Miller's Class I or II gingival recession defects, and presence of at least 3 mm of keratinized tissue width (KTW) apical to the recession. Patients with recessions associated with caries, severe abrasions, or restorations, patients with active periodontal or gingival disease, patients with systemic disorders that could impede healing, patients with teeth that had a prominent root, smokers, and those with poor oral hygiene were excluded. Each patient gave written informed consent after receiving comprehensive information about the surgical procedure. Any additional dental care required was provided in addition to the trial procedure. Two weeks before the surgery, a full-mouth scaling and dental prophylaxis was performed. In the test group, a CAF was performed along with using an AM allograft while the control group only received the CAF.

Interventions and outcomes:

Surgical procedure:

The surgery was carried out under local infiltration anesthesia with 2% lidocaine mixed with 1:80,000 adrenaline (Indoco remedies Ltd., India) administered at the surgical site. To prevent operator bias, the same surgeon carried out the test and control surgeries. A CAF was performed, along with AM at the test site (Fig. 1). The surgical site was outlined by sulcular incisions surrounding the affected teeth and two oblique releasing incisions at the distal and mesial sides. A full-thickness flap was elevated to expose a minimum of 3mm of the marginal bone apical to the dehiscence site. A horizontal releasing incision was made in the periosteum at the flap's base to enable tension-free coronal repositioning. De-epithelialization of each papilla was performed to create a

connective tissue bed. The exposed root surfaces were meticulously planed and scaled using Gracey curettes (Hu-Friedy, USA), without root conditioning. To determine the required AM width for the surgical procedure, a template of the recession defect was fabricated using sterilized tin foil. After trimming of the AM (obtained from the TATA Memorial Hospital Tissue Bank, India) to fill the recession defect area, the flap was coronally positioned over the membrane and sutured with 4-0 bioabsorbable sutures (VICRYLTM, Johnson & Johnson Meditech, USA) utilizing sling sutures. Next, two direct interrupted sutures were placed on either side of the vertical incisions. For optimal wound stabilization and patient comfort, a non-eugenol periodontal dressing was applied after placing a tin foil of the appropriate size on the buccal side. All patients in the control group underwent a similar surgical procedure without the use of an AM.

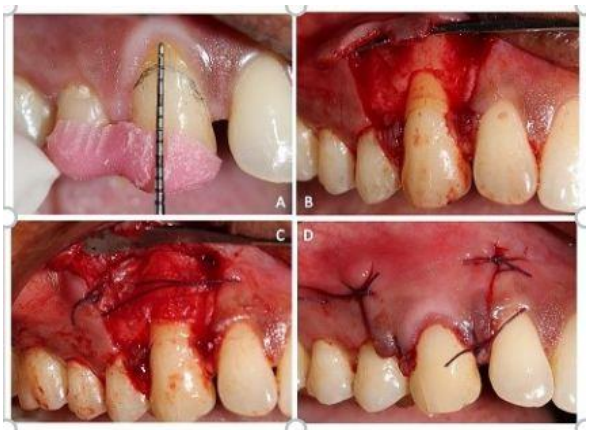

Fig 1. Test procedure: (A) preoperative view of the maxillary right anterior teeth (test side); (B) elevated full-thickness mucoperiosteal flap; (C) application of AM; (D) flap sutured coronally

Postoperative care:

Postoperative analgesics (400mg ibuprofen and 325mg paracetamol three times a day for three days) were given to each patient. Postoperative instructions were also provided. The patients were instructed to rinse their mouth with 0.12% chlorhexidine gluconate mouthwash two times a day for 4 weeks and not to brush their teeth at the surgical site for 2 weeks. After surgery, all patients received instructions on how to clean their teeth mechanically at the operated areas using the roll technique with a soft toothbrush 15 days later (Fig. 2).

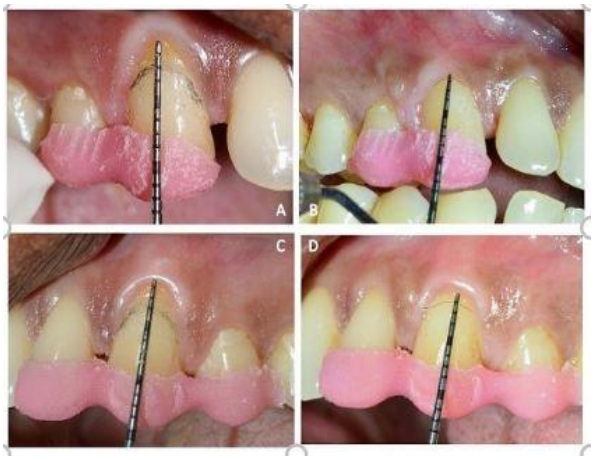

Fig 2. (A) Test site at baseline, (B) Test site at 6 months postoperatively, (C) Control site at baseline, (D) Control site at 6 months postoperatively.

Measurement of clinical parameters:

The following clinical assessments were performed immediately after surgery and also 6 months later: plaque index (PI), gingival index (GI) [12], KTW measured from the mucogingival junction to the gingival margin, probing depth (PD), dentin hypersensitivity (DH), and gingival biotype (GB). The recession depth (RD) was measured from the cementoenamel junction to the gingival margin at the mid-buccal point of the involved teeth. The recession width (RW) was measured at the cementoenamel junction. Gingival recession total surface area (GRTSA) was also calculated. GB was evaluated using the probe transparency approach. A customized acrylic stent was fabricated to ensure reproducibility of the clinical data, according to the guidelines by Lekovic et al [13]. The custom-made acrylic stent featured a groove designed to ensure reproducible probe positioning, to ensure that the preoperative and postoperative measurements are made at the same location and angle. This groove's apical end was used as a fixed reference point. One single examiner used a UNC-15 periodontal probe (Hu-Friedy, USA) to perform all the clinical measurements during the trial,

minimizing individual variability. Six months after surgery, patient-centered outcomes were assessed for the potential adverse effects on comfort, tooth sensitivity, and esthetics.

Statistical analysis:

SPSS version 20 (SPSS Inc., Chicago, IL, USA) was used to analyze the recorded data. For each parameter, a subject-level analysis was carried out. Descriptive statistics were presented as mean ± standard deviation. The data distribution normality was tested with the Shapiro-Wilk test. Accordingly, non-parametric comparative statistical tests were applied. Intragroup differences were assessed using paired t-test, while intergroup differences were analyzed with the unpaired t-test. Statistical significance was established at $P<0.05$.

RESULTS

There were 7 female patients and 17 male patients between 20 to 50 years, with a mean age of 35±8.79 years. There was no drop outs (Fig. 3).

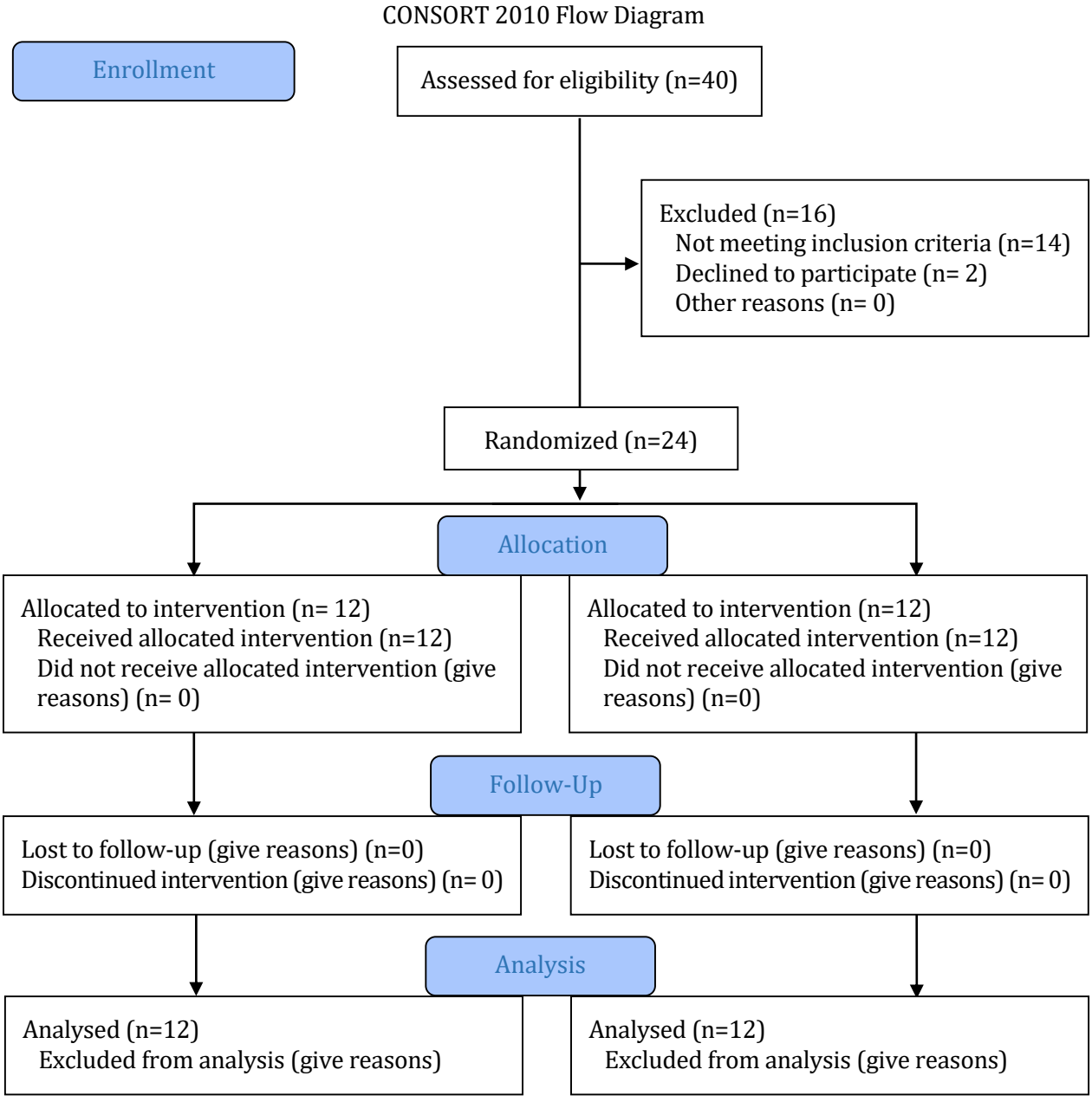

Fig 3. CONSORT flow-diagram of the study

Harms:

None of the patients experienced any postoperative complication, such as tissue necrosis, severe discomfort, or bleeding from the surgical site.

Measurements were made both before the surgical procedure and 6 months later. Before the surgical procedure, there was no statistically significant difference in the type of recession defects between the two groups (P>0.05). Table 1 provides information on age, sex, recession site, and recession type distribution. Both groups exhibited a time-dependent reduction in GI and PI. However, no significant differences were observed between baseline and 6 months in any of the study groups (P>0.05).

After 6 months, complete root coverage was attained at 25% of the test sites, compared to only 16.6% of the control sites. In both the test and control groups, one patient (8.3%) did not exhibit any root coverage. Eight cases (66.6%) out of 12 in the test group, and 9 cases (75%) out of 12 in the control group had partial root coverage.

After 6 months, the mean RD in the test group decreased from 2.83mm at baseline to 0.92mm, with a mean difference of 1.5mm (P=0.0003, Table 2). Both groups showed a significant reduction in RD compared to baseline. In the control group, the mean RD dropped from 2.58mm at baseline to 1.33mm after 6 months, resulting in a mean difference of 1.25mm (P=0.000). Additionally, RW significantly decreased in both groups, with mean differences of 1.58mm in the test group and 1.09mm in the control group (P<0.05). The intergroup comparison revealed a significant difference as well (P=0.000). The GRTSA values significantly dropped at 6 months in both the test and control groups, with mean differences of 7.5 and 5.67mm, respectively (P<0.05). However, a comparison of the two groups revealed no statistically significant difference (P=0.22, Table 3).

Table 1. Demographic characteristics of the participants and recession sites

Variable		CAF+AM	CAF	Total
Sex	Male	9	8	17
	Female	3	4	7
Age (yrs.)	Mean	35.42	34.58	35
	Standard deviation	8.35	9.56	8.79
Recession site	Maxillary lateral incisors	1	0	1
	Maxillary canines	2	2	4
	Maxillary premolars	5	6	11
	Maxillary molars	3	3	6
	Mandibular central incisors	0	1	1
	Mandibular premolars	1	0	1
Recession type	Miller's Class I	9	10	19
	Miller's Class II	3	2	5
Total		12	12	24

Table 2. Intragroup comparison of parameters between baseline and 6 months in the test (CAF+AM) group

Parameter	Time Point	Mean	SD	Mean Diff.	SD Diff.	% of Change	Paired t	P-value
RD	Baseline	2.83	0.58	1.5	0.31	112.5	5.20	0.0003*
	6 months	0.92	0.79					
RW	Baseline	3.5	0.67	1.58	0.44	82.29	5.0616	0.0004*
	6 months	1.92	1.38					
GRTSA	Baseline	9.92	2.71	7.5	1.06	310.34	9.5743	0.0001*
	6 months	2.42	2.47					
KTW	Baseline	3.17	0.39	-1.08	0.21	-25.41	-7.2879	0.0001*
	6 months	4.25	0.62					

RD: recession depth, RW: recession width, GRTSA: gingival recession total surface area, KTW: keratinized tissue width
*P<0.05 was statistically significant, paired t-test

Table 3. Intergroup comparison of parameters between the test and control groups at 6 months

Parameter	Groups	Mean	SD	Mean Diff.	SD Diff.	% of Change	Paired t	P-value
RD	Test	0.92	0.79	0.42	0.34	45.45	1.1007	0.2945
	Control	1.33	0.89					
RW	Test	1.92	1.38	1.58	0.44	82.29	5.0616	0.0004*
	Control	3.5	0.67					
GRTSA	Test	2.42	2.47	1.42	1.10	58.62	1.2993	0.2204
	Control	3.83	2.87					
KTW	Test	4.25	0.62	-0.92	0.26	-21.57	-3.5269	0.0047*
	Control	3.33	0.65					

*P<0.05 was statistically significant, unpaired t-test
RD: recession depth, RW: recession width, GRTSA: gingival recession total surface area, KTW: keratinized tissue width, SD: Standard deviation

With a mean gain of 1.08mm, KGW in the test group increased significantly after 6 months (P<0.05), but there was no statistically significant change in the control group (P>0.05). The intergroup comparison was statistically significant (P=0.004). Patient-centered outcomes were extremely satisfactory in 9 patients, and satisfactory in 12 patients (Table 4).

Table 4. Patient-centered outcomes

Category	Test group	Control group	Total no. of patients
Extremely satisfactory	6(50%)	3(25%)	9(37.5%)
Satisfactory	5(41.6%)	7(58.3%)	12(50%)
No difference	1(8.3%)	2(16.6%)	3(8%)
Condition worsened	0	0	0

There was a significant decrease in DH in both the test and control groups at 6 months compared with baseline; however, the test group had a higher number of patients with reduced DH. In terms of GB, all sites in the test group with a thin biotype transformed to a thick biotype by the end of 6 months; among which, 7 of the test group's 12 sites and 3 of the control group's 12 sites had been initially classified as thin.

DISCUSSION

This study evaluated the additional therapeutic advantage of AM to CAF in treatment of Miller's Class I and II single recession defects after 6 months. The results showed that the CAF + AM and CAF alone were equally successful in treating the gingival recession defects. This surgical approach does not require a second surgical site and satisfies patient expectations by not causing any discomfort in the palate as do the free gingival grafts or connective tissue grafts. It has been demonstrated that the CAF approach is a reliable technique to cover gingival recession defects with clinically acceptable outcomes [14,15].

The efficacy of root coverage procedures is assessed by improvements in RW, RD, GB, and esthetics. The present study assessed these parameters, and found that patient-centered outcomes were satisfactory after surgery. Additionally, the soft tissue recovery in both groups proceeded with no complication. Nevertheless, the CAF+AM group's results at the 6-month follow-up were superior to those of the CAF-alone group. According to these findings, the AM is a reasonably safe material for therapeutic settings. In patients with refractory non-healing wounds, dehydrated human AM allografts have been utilized to promote healing with excellent results and no recurrence of wounds in the long-term [16]. Thus, it appears that AM has exceptional therapeutic properties.

Throughout the trial period, there was no significant change in PD of patients in either group, and the GI and PI scores remained acceptable in both groups. This can be the result of reinforcement of oral hygiene recommendations. From baseline to 6 months, the test group's mean difference in PI and GI was comparable to that in the control group. These changes were in line with the findings of a previous study that used AM [17].

Significant reductions in RD were observed in both groups; however, the difference between the two groups was not statistically significant. In the test group, the mean RD reduction from baseline to 6 months was 1.92 mm. These findings were consistent with the results of another study that compared subepithelial connective tissue graft and AM and found that the mean RD after 6 months was 2.3mm [18]. From baseline to 6 months, there was a significant reduction in both groups' RW: 1.58mm for the test group and 1.09mm for the control group. RW in the test group exhibited a larger drop, much like RD. These findings imply that application of an AM allograft in conjunction with CAF resulted in greater root coverage. These outcomes matched those of a study that compared AM to platelet-rich fibrin, and the AM group showed a significant decrease in RD from 2.8mm to 1.0 mm [19]. A case series used AM and showed a mean reduction in RD by 2.81mm and in RW by 3.65mm at 6 months after surgery [20].

Complete root coverage was achieved in 25% of the test sites and 16.6% of the control sites. During the trial, despite the sites having initial coverage with CAF, there was an increased tendency for recurrence of defects. The same results were reported in an earlier study, which used a dehydrated AM allograft and obtained good results in terms of root coverage, increased tissue thickness, and increased attached gingival tissue. They also found that 4 out of 5 patients had complete root coverage, with the outcome favoring CAF+AM with new gingival tissue gain of 3.2mm (±1.71) showing 97% (±0.5) recession defect coverage [21]. To the best of the authors' knowledge, only a small number of randomized clinical trials have assessed the effectiveness of AM in management of gingival recessions. In AM-treated areas, there was 97% (3.2±1.73mm) root coverage, which is similar to a study by Gurinsky [22].

While the CAF-treated sites confirmed a trend for recession recurrence, the AM-grafted sites over time demonstrated a tendency for coronal displacement of the gingival margin. This could be because AM contains vascular growth factors, and induces fibroblast proliferation, which could hasten angiogenesis and tissue maturation, which could be the reason while the coronal section of the flap was not necrotized, and can promote enhanced healing and more creeping attachment [18]. Consequently, it stands to reason that placement of an AM below the CAF could enhance the result of full root coverage by stabilizing the gingival edge and lowering the likelihood of flap dehiscence throughout the healing process [23]. Another case report described a patient with bilateral gingival recessions; full root coverage (100%) was observed 7 months after surgery in gingival recession defects treated with AM and platelet-rich fibrin [24].

Thin and sensitive marginal tissue may be one of the most significant causes contributing to gingival recession [25]. As a result, increased GT and KTW should be the outcomes of root coverage surgery. In the present study, the keratinized tissue width was significantly greater in the CAF+AM group than in the CAF group. The mean KTW in the CAF+AM group increased from 3.17mm at baseline to 4.25mm after 6 months. The greater usage of AM beneath the flap, which can produce keratinocyte growth factor, encourages the keratinization of epithelial cells and aids the mucogingival junction to maintain its location, and explains this difference [25]. The increase in KTW may be partially responsible for the gingival margins' long-term stability at CAF+AM treated sites compared with CAF alone [23,26].

In the CAF+AM group, there was a significant shift in the GB from thin to thick. In the CAF and CAF+AM groups, there were 3 and 7 cases with thin GB at baseline, respectively. In the CAF group, the number of cases that changed to thick biotype after 6 months remained unchanged, but in the CAF+AM group, all 12 cases had a thick biotype, yielding a 100% positive result. Utilization of the AM allograft is responsible for this clinically and statistically significant alteration. Collagen types I, III, IV, V, and VII, as well as laminins and fibronectins are found in AM and contribute to improved connective tissue proliferation and repair [27]. Since thick gingival tissue is resilient to injury and recession, it encourages creeping attachment

and produces more stable, predictable results over long periods of time. In a study that compared AM with chorion membrane, 9 out of 12 treated recession defects exhibited 100% root coverage at the 6-month follow-up, and 10 out of 12 recession sites that had a thin biotype at baseline developed a thick biotype [28].

Additionally, from baseline to 6 months, there was a significant decrease in DH in both groups in the current study; however, the CAF+AM group had a higher number of cases with DH reduction. This can be explained by the CAF group's gingival margin shifting apically and the test group's greater root coverage.

To assess the perspective of patients, the treatment's effectiveness was evaluated based on the patients' level of happiness and comprehension of the root coverage's ultimate goal.

Both the CAF+AM and CAF-alone treatments produced varying degrees of root coverage within the parameters of this investigation. On every measure, nevertheless, the CAF+AM group significantly outperformed the CAF alone. Small sample size and inclusion of teeth with variable morphological characteristics (molars and single-rooted teeth) may have contributed to significant differences in the findings, which were the main limitations of this study.

Additional research is required to determine the impact of AM through long-term clinical trials with large sample size and patient-based outcome assessments.

CONCLUSION

The results showed that coverage of denuded roots with CAF alone or in conjunction with AM was successful. Our 6-month data comparing the combined CAF+AM approach to CAF alone showed extra benefits in the CAF+AM group in terms of GB change, greater KTW, and mean root coverage. This data, however, is still insufficient to support AM's actual clinical benefit when treating recession defects with CAF.

CONFLICT OF INTEREST STATEMENT

None declared.

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