Research Article

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Hyaluronic Acid: A Promising Mediator for Periodontal Regeneration

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ABSTRACT

Background: Periodontitis or Periodontal disease is an inflammatory condition of the periodontium, which evokes an immune response thus resulting in loss of supporting tissues of the teeth. The periodontium represents a system, where epithelial, mineralized and non mineralized tissues exist in harmony with each other which provides an effective barrier against microbial invasion and prevents the destruction of the underlying periodontal tissues by bacterial toxins and enzymes. However, an imbalance can occur within the periodontium during chronic inflammation stage thereby leading to detrimental effects on collagen, proteoglycans and glycosaminoglycans.

Keywords: hyaluronic acid, periodontology, periodontal regeneration.

INTRODUCTION

Periodontitis or Periodontal disease is an inflammatory condition of the periodontium, which evokes an immune response thus resulting in loss of supporting tissues of the teeth.[1] The periodontium represents a system, where epithelial, mineralized and non mineralized tissues exist in harmony with each other which provides an effective barrier against microbial invasion and prevents the destruction of the underlying periodontal tissues by bacterial toxins and enzymes. However, an imbalance can occur within the periodontium during chronic inflammation stage thererby leading to detrimental effects on collagen, proteoglycans and glycosaminoglycans. [2]

Gingival Recession is a common condition and its prevalence increases with age. Patients often have esthetic concerns because of it and face a problem of dentinal hypersensitivity. Thus, various treatment modalities have been used for the correction of gingival recession defects such as free gingival graft (FGG), sub epithelial connective tissue graft (SCTG), coronally advanced flap (CAF). SCTG being the gold standard has been used quite frequently in gingival recession.

The primary etiology of periodontal disease is dental plaque. Root surfaces which are affected by periodontitis are hypermineralized and contaminated by bacteria and their products such as endotoxins. [3] As a result of this, they are not compatible with the adjacent healthy periodontal tissues, the proliferation of which is necessary for regeneration of periodontium. [3] Therefore, modifying the contaminated root surfaces by disinfecting them, allows for healthy attachment of periodontal tissues and thus aiding in regeneration. [4]

Scaling and root planing are quite effective in removal of bacterial deposits and endotoxins from the exposed root surface. However, by mechanical means alone, it is not possible to completely decontaminate a periodontitis-affected root surface. [5] A smear layer is then formed on to the root surfaces which later affects fibroblastic adaptation in healing of periodontal tissues. [6] Smear layer is composed of remnants of dental calculus, cementum

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Fig 1: Gengigel 0.2%

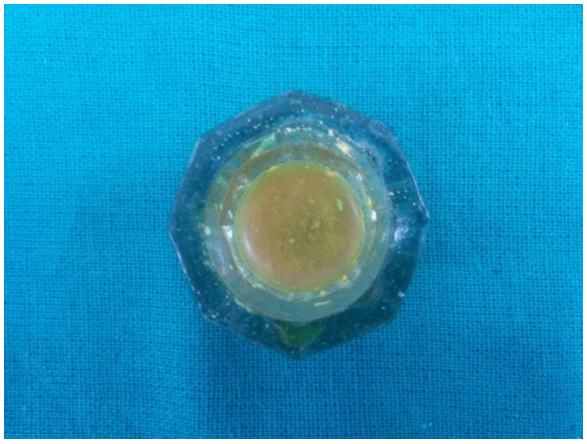


Fig 2: A slurry of tetracycline with normal saline.

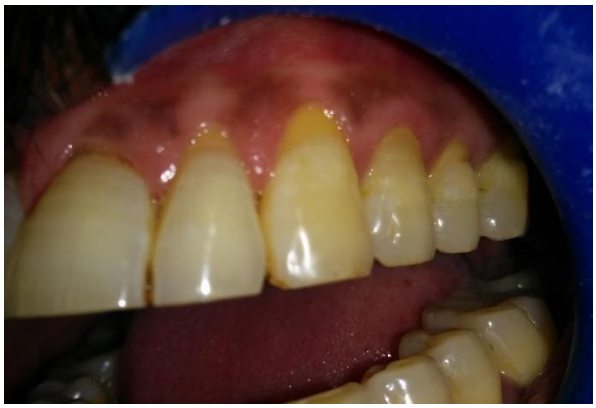


Fig 3: Group 1 pre operative.

fragments, subgingival plaque etc which acts as a barrier between periodontal tissues and root surface, thus inhibiting new attachment. [7] Furthermore, the smear layer is resistant to saline rinsing. [7,8]

Thus, root biomodification can be used as an adjunct to mechanical root surface debridement so as to

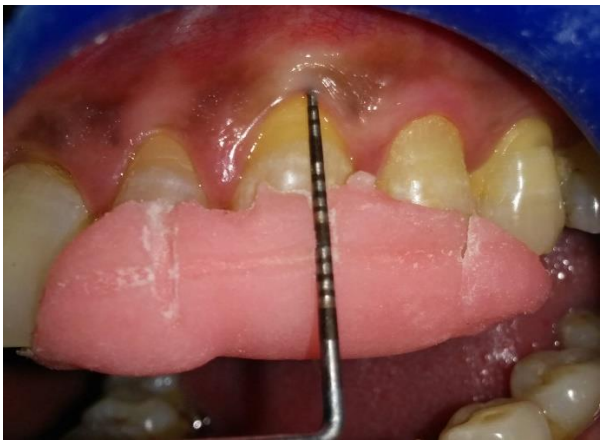


Fig 4: Group 1 probing depth.

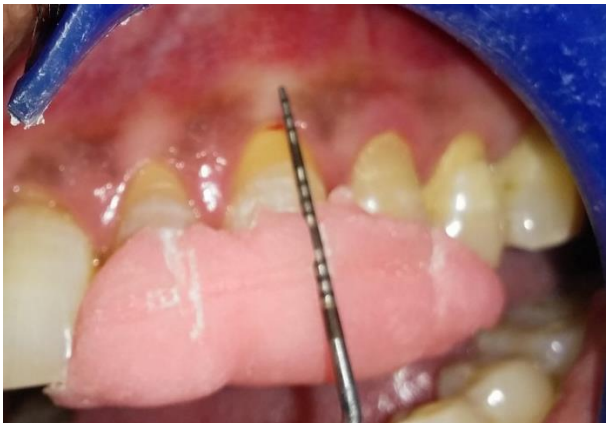


Fig 5: Group 1 Width of keratinized gingiva.

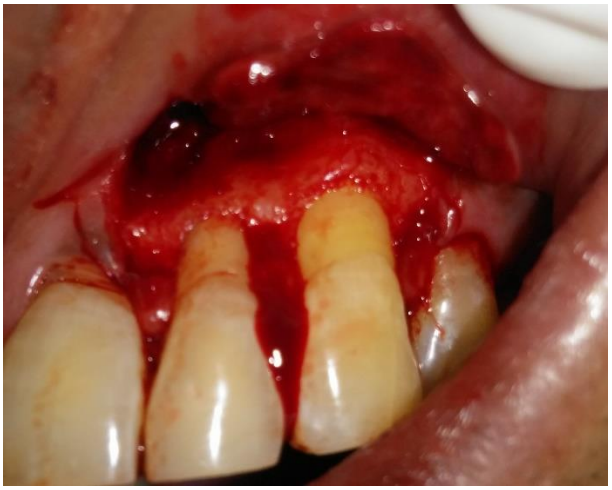


Fig 6: Group 1 Raising of the flap.

facilitate new attachment on the dentin surface. [9,10] Various methods have been attempted for root conditioning prior to root coverage procedures such as citric acid, tetracycline hydrochloride, EDTA, fibronectin, laminin, doxycycline, emdogain, growth factors, lasers and so on.

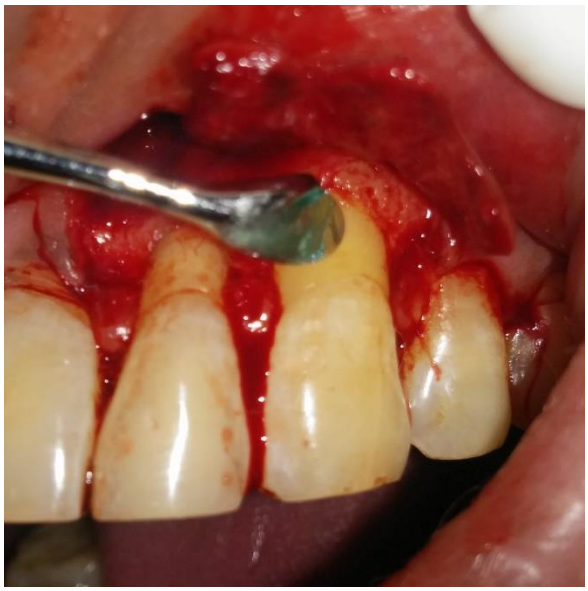


Fig 7: Group 1 Application of Gengigel.

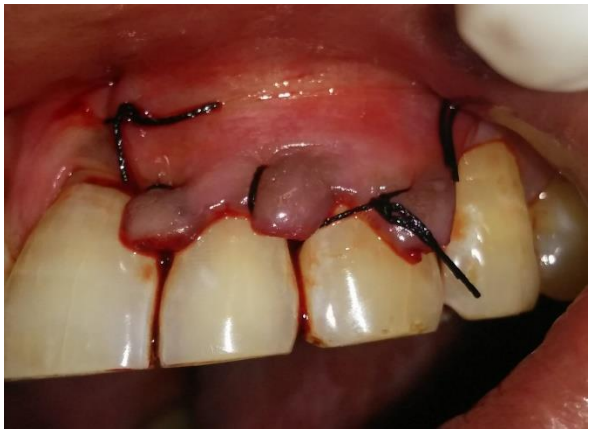


Fig 8: Group 1 Sutures placed.

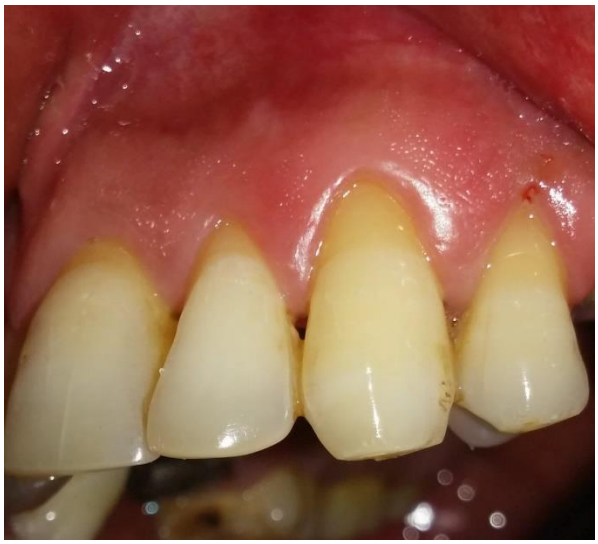


Fig 9: Group 2 Pre operative.

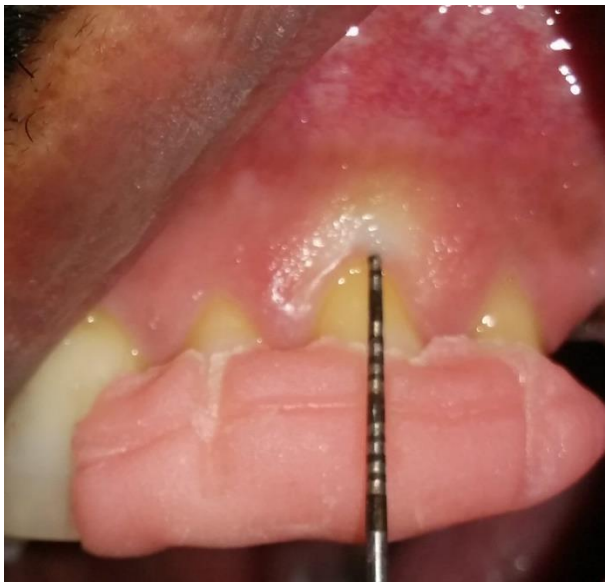


Fig 10: Group 2 Recession height.

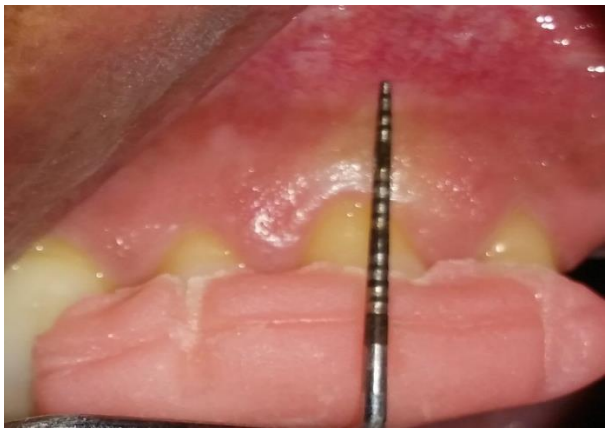


Fig 11: Figure 11 - Group 2 Width of keratinized gingiva.

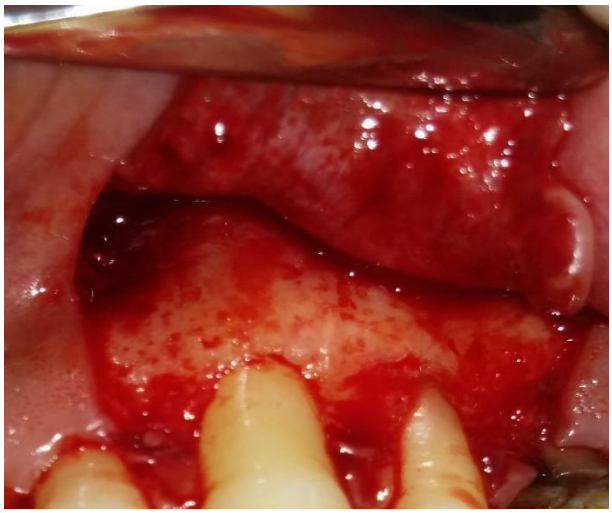


Fig 12: Group 2 Raising of the flap.

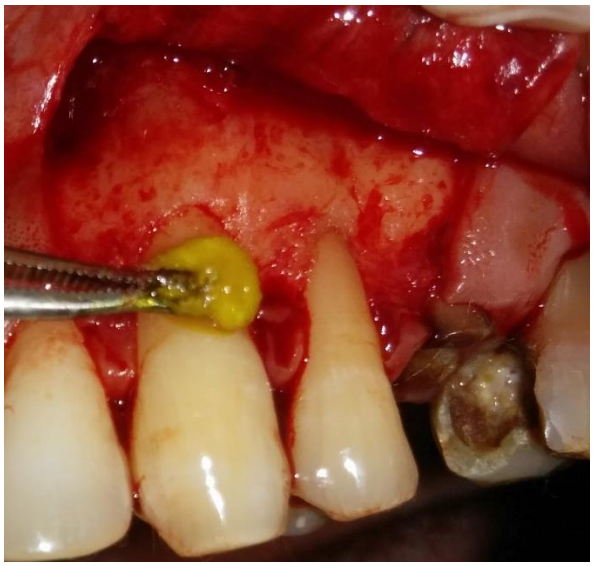


Fig 13: Group 2 Application of Tetracycline.

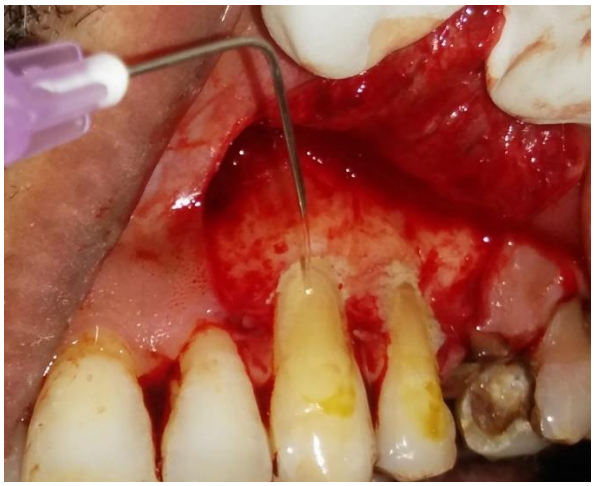


Fig 14: Group 2 Rinsing off Tetracycline.

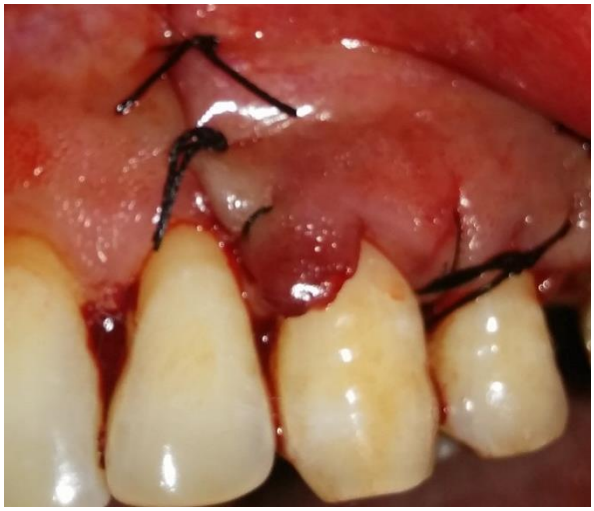


Fig 15: Group 2 Sutures placed.

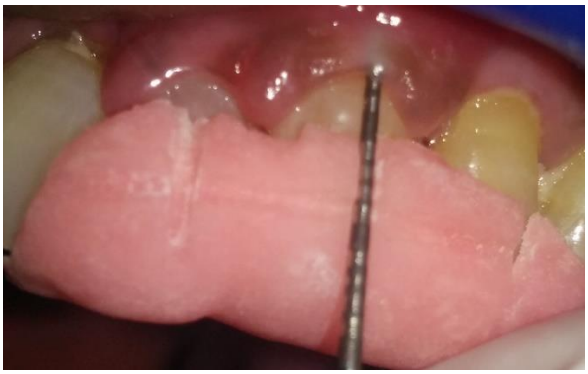


Fig 16: Group 1 Post operative Recession height at 3 months.

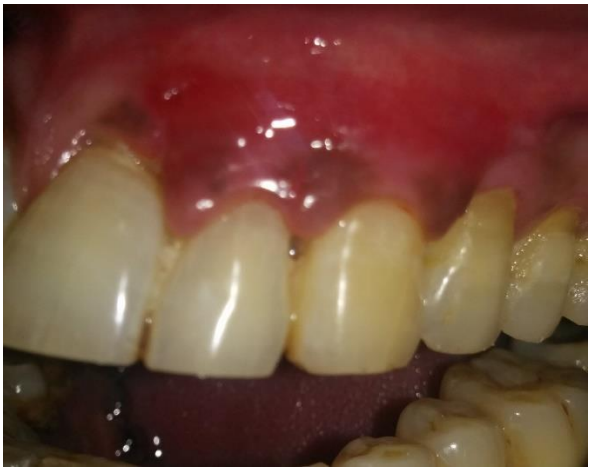


Fig 17: Group 1 Post operative at 3 months.

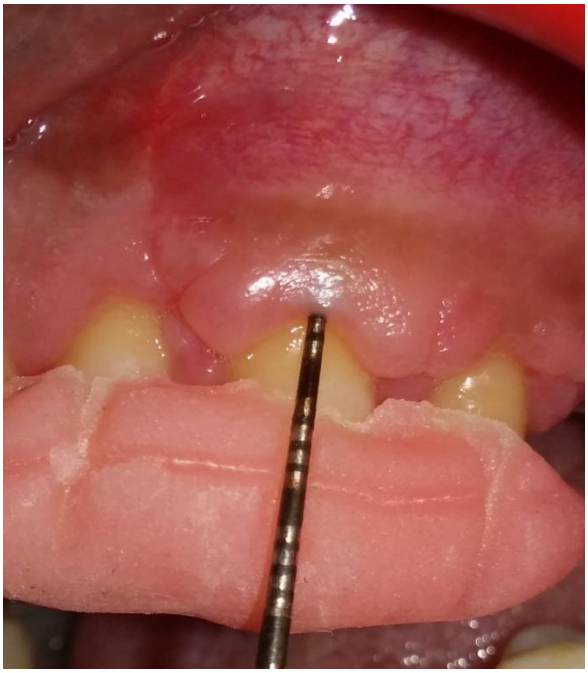


Fig 18: Group 2 Post operatve Recession height at 3 months.

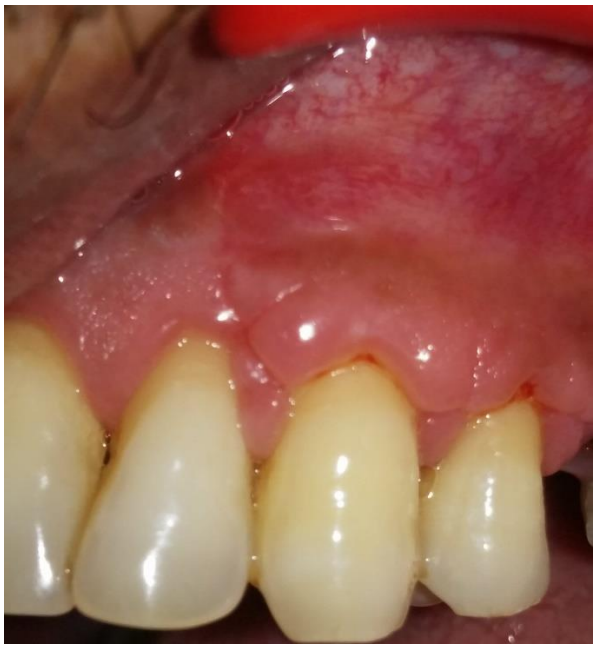


Fig 19: Group 2 Post operatve at 3 months.

Hyaluronan has been identified in all periodontal tissues, predominantly in nonmineralized tissues, such as gingiva and periodontal ligament, as compared to mineralized tissues, such as cementum and alveolar bone. [10] Though HA is mostly advocated in dentistry for topical application, some of the recent studies were done using hyaluronan as a regenerating material.

Gengigel (Ricerfarma, Milano, Italy), a commercially available form of hyaluronic acid has been used as an adjunct to scaling and root planing which is a topically applied anti inflammatory agent. [11] Also it has been used in root coverage procedures.

Hyaluronic acid is a biologically acceptable product therefore it can be used safely, without any evidence of cytotoxicity. [12] Gengigel as a product for oral use has been evaluated by various tests such as skin irritation test and percutaneous absorption test and has been proved to be a safe non irritant product. [12] It can be also be used by children and pregnant women without any restriction. Hyaluronic acid injections, gel or oral (by mouth) is contraindicated in allergic patients.

Tetracycline possesses several unique antibacterial properties that may contribute to their effectiveness in periodontal therapy such as anti- inflammatory effects, collagenase inhibition, inhibition of microbial attachment and root surface conditioning. [13,14] The tetracycline used was most often applied in concentration of 100mg/ml to root surfaces for 3-5 minutes.

It helps in the demineralization of root surfaces, eliminates the smear layer, aids in opening of the dentinal tubules, and exposes some components of the matrix like type I collagen. [15] Also it helps in fibrin clot stabilization, increased chemotaxis, adhesion and growth and proliferation of fibroblasts on the root surface and inhibition of tissue degrading enzymes such as matrix metalloproteinases. [16,17]

Thus the aim of the present study was to compare and evaluate the clinical efficacy of Gengigel versus Tetracycline in conjunction with Coronally Advanced Flap in management of Miller's class I and class II recession.

MATERIALS AND METHOD

A study was conducted in the Outpatient Department of Periodontology and Oral Implantology at I.T.S Dental College, Muradnagar, Ghaziabad. It was a randomized controlled clinical trial. A total number of 20 sites of Miller's class I and class II gingival recession were included in the study. All the patients were randomly divided into two groups i.e Group 1 (CAF + Gengigel) and Group 2 (CAF+Tetracycline hydrochloride) with the toss of a coin.

Group 1 included hyaluronan gel (Gengigel 0.2% gel - 0.2% Hyaluronan gel marketed by Ricerfarma Pharmaceuticals, Milan, Italy) with CAF (Figure A.1) and Group 2 included CAF with Tetracycline hydrochloride (Figure A.2).

All subjects were systemically healthy, non-smokers between 18-50 years, who had not received any kind of periodontal therapy for last two years and who had Miller's class I and class II gingival recession and those who can understand and follow oral hygiene instructions properly were included in the study.

Medically compromised patients, with any history of allergy to the material used in this study, who have undergone antimicrobial therapy in previous 6 months or are on any drug therapy which is known to influence the periodontium, pregnant or lactating females and patients who had undergone any type of periodontal flap surgical procedure or regenerative therapy 6 months prior to the initial examination were excluded from the study.

A detailed dental and medical history was recorded for every patient. The study was thoroughly explained to all the patients and an informed consent was obtained.

Occlusal stents were made of cold cure acrylic for positioning the probe and measuring the defects from a fixed reference point for the standardization of clinical measurements. UNC-15 probe was used to measure the clinical parameters.

The following clinical parameters were measured using UNC 15 probe at baseline and then at 3 months :

- Gingival index (GI) (Loe and Silness in 1963) [18]
- Plaque index (PI) (Turskey- Gilmore- Glickman modification of Queigly- Hein Plaque index in 1970) [19]
- Probing depth (PD in mm)
- Relative attachment level (RAL in mm) (from the fixed reference point to the base of the sulcus)
- Recession height (RH) (distance between CEJ and the most apical point of the gingival margin)
- Width of attached gingiva (WAG) (distance between the most apical point on the gingival margin and mucogingival junction at the same point as PD, CAL and RH)

Clinical examination was performed by a single examiner with a UNC 15 probe. All the parameters were recorded at baseline and at 3 months after obtaining the patients consent form.

SURGICAL TECHNIQUE

Two weeks prior to the surgery, all the patients received thorough supra and subgingival scaling and root planing and detailed oral hygiene instructions were given.

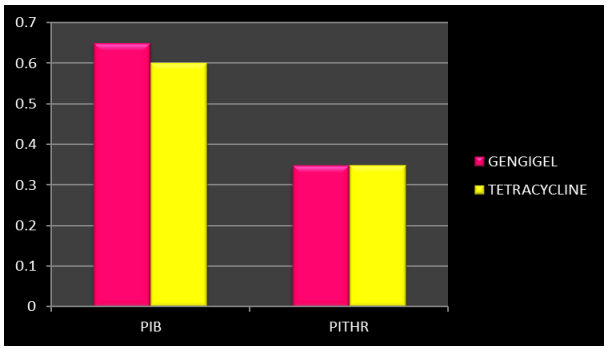
All periodontal surgical procedures were performed under aseptic conditions. Standardized surgical procedure for both the groups were performed as follows : Local anesthetic was given in the surgical area (2% lignocaine with adrenaline 1:80,000). After local anesthesia and before the elevation of the flap, root planing was done with help of Gracey curettes to reduce root convexity. After that the root surface was washed with normal saline. Intrasulcular incisions were then made with 15 no. blade on the buccal aspect of the involved tooth. This incision was then horizontally extended to the adjacent papillae without involving the gingival margin of adjacent teeth. Two oblique vertical releasing incisions were then given from the mesial and distal extremities of the horizontal incision which was extended beyond the mucogingival junction. A trapezoidal full-thickness flap was raised with a periosteal elevator. Then a partial-thickness dissection was carried out apically leaving the underlying periosteum. In Additionally, a dissection parallel to the vestibular lining mucosa was performed with a blade so as to release the muscle tension and to facilitate the passive coronal displacement of the flap. The papillae adjacent to the involved tooth were de-epithelized so as to create the connective tissue beds. The flap was then coronally advanced and adapted properly to cover the cementoenamel junction. [20] (Figure A.3- A.6 and A.9- A.12)

In the Group 1, hyaluronan gel (gengigel 0.2%) was applied on the root surface using a sterile instrument (Figure A.7). The flap was then passively advanced coronally and sutured (Figure A.8) whereas in Group 2, a cotton pellet dipped in a slurry of tetracycline hydrochloride was burnished onto the root surface for 1 minute (Figure A.13). After that the root surface was thoroughly rinsed with help of normal saline (Figure A.14). The flap was advanced coronally and sutured (Figure A.15). Oblique releasing incisions were also sutured using interrupted sutures, with 5-0 ethicon nonresorbable sutures, while the coronal mesial and distal extremities of the flap were adapted in place using sling sutures.

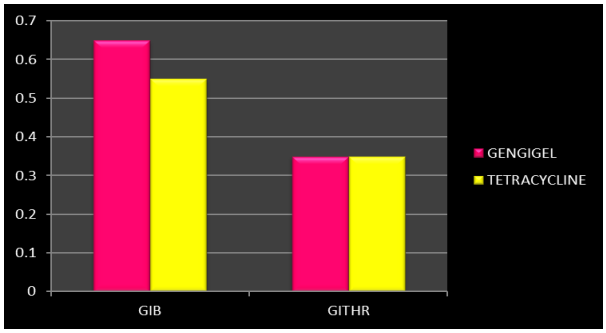
All the clinical parameters were recorded again after 3 months.

POSTSURGICAL CARE

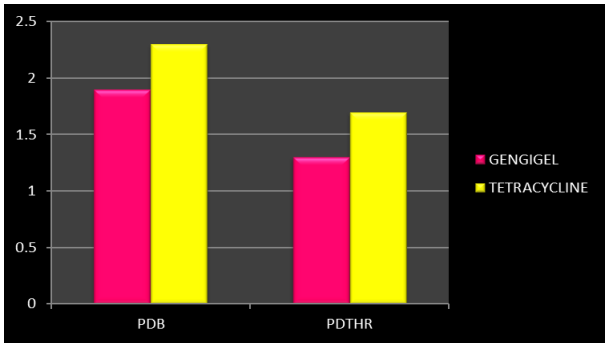
All the patients were given instructions to discontinue tooth brushing, avoid trauma at the surgical site. Postoperative instructions were given. Amoxicillin, 500 mg, three times a day (t.i.d), for 5 days, and analgesics Ibuprofen, 400 mg, t.i.d for 3 days were given. Patients were advised to avoid hard chewing in the surgical areas and use 0.12% solution of chlorhexidine digluconate to rinse twice daily for 4 weeks. Sutures were removed after 2 weeks.



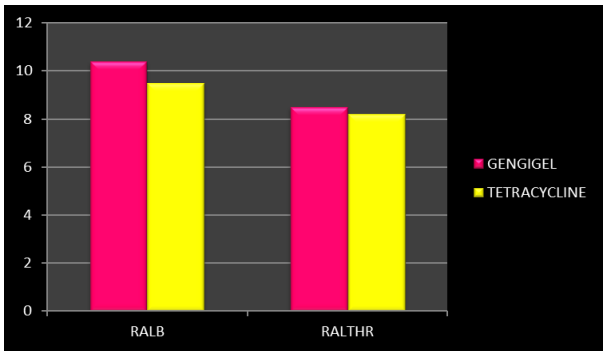
Graph a.1: intergroup comparison of plaque index at baseline and at 3 months.



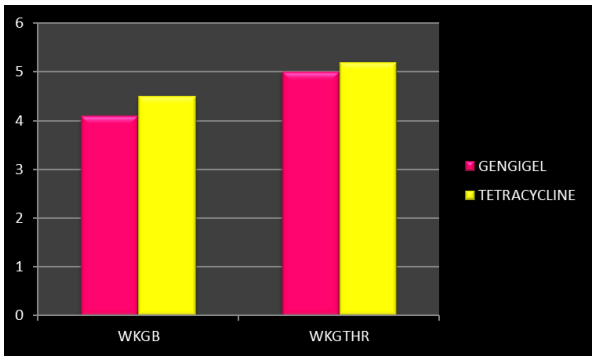
Graph a.2: Intergroup comparison of gingival index at baseline and at 3 months.



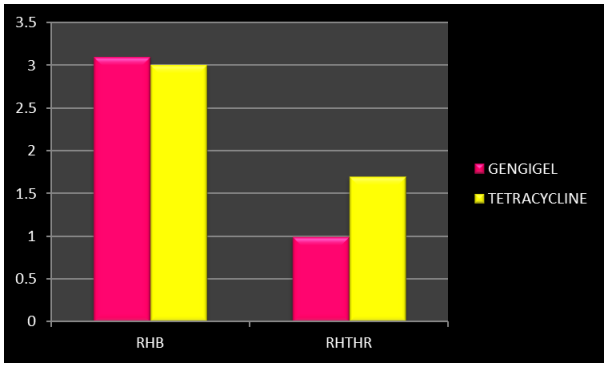
Graph a.3: Intergroup comparison of probing depth at baseline and at 3 months.



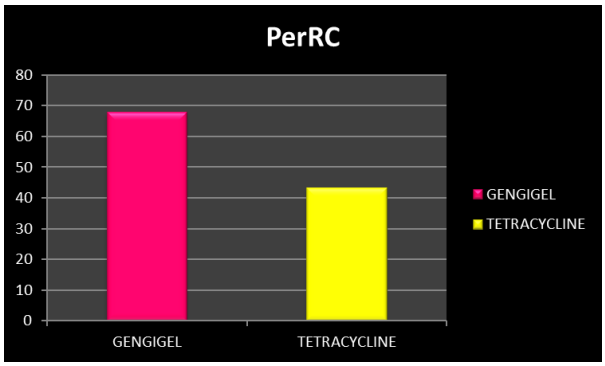
Graph a.4: Intergroup comparison of relative attachment level at baseline and at 3 months.



Graph a.5: Intergroup comparison of width of keratinized gingiva at baseline and at 3 months



Graph a.6: Intergroup comparison of recession height at baseline and at 3 months.



Graph a.7: Intergroup comparison of percentage of root coverage at baseline and at 3 months.

STATISTICAL ANALYSIS

A total of 20 gingival recession defects were considered for the study. The values obtained were analyzed statistically using the software Statistical Package for Social Sciences (IBM, SPSS, Statistics 20). Paired t-test was used for the statistical evaluation of changes in all the clinical parameters from baseline to 3 months. For comparisons between the groups, the independent t-test was used. This was followed for both the treatment groups.

Table a.1: Inter group comparison of all the clinical parameters at baseline and at 3 months.

	GROUPS	Mean	Std. Deviation	P value
PIB	1	.6500	.13693	.580
	2	.6000	.13693	
PITHR	1	.3500	.13693	1.000
	2	.3500	.13693	
GIB	1	.6500	.13693	.397
	2	.5500	.20917	
GITHR	1	.3500	.13693	1.000
	2	.3500	.13693	
PDB	1	1.900	1.2450	.518
	2	2.300	.4472	
PDTHR	1	1.30	1.037	.471
	2	1.70	.570	
RALB	1	10.400	1.5166	.232
	2	9.500	.3536	
RALTHR	1	8.500	1.0000	.536
	2	8.200	.2739	
WKGB	1	4.100	1.1402	.572
	2	4.500	1.0000	
WKGTHR	1	5.000	.7906	.856
	2	5.100	.8944	
RHB	1	3.100	.2236	.608
	2	3.000	.3536	
RHTHR	1	1.000	.0000	.000
	2	1.700	.2739	
PerRC	1	67.64	2.109	.000
	2	43.22	7.087	

Table a2: Intra group comparison of all the clinical parameters of group 1 (coronally advanced flap and gengigel) at baseline and at 3 months.

		Mean	Std. Deviation	P value
1	PIB	.6500	.13693	.004
	PITHR	.3500	.13693	
2	GIB	.6500	.13693	.004
	GITHR	.3500	.13693	
3	PDB	1.900	1.2450	.145
	PDTHR	1.30	1.037	
4	RALB	10.400	1.5166	.005
	RALTHR	8.500	1.0000	
5	WKGTHR	5.000	.7906	.009
	WKGB	4.100	1.1402	
6	RHB	3.100	.2236	.000
	RHTHR	1.000	.0000	
7	PerRC	67.64	2.109	.000
	VAR00001	.0000	.00000	

Fig a3: Intra group comparison of all the clinical parameters of group 2 (coronally advanced flap and tetracycline) at baseline and at 3 months.

		Mean	Std. Deviation	P value
1	PIB	.6000	.13693	.016
	PITHR	.3500	.13693	
2	GIB	.5500	.20917	.004
	GITHR	.3500	.13693	
3	PDB	2.300	.4472	.004
	PDTHR	1.70	.570	
4	RALB	9.500	.3536	.000
	RALTHR	8.200	.2739	
5	WKGTHR	5.100	.8944	.033
	WKGB	4.500	1.0000	
6	RHB	3.000	.3536	.000
	RHTHR	1.700	.2739	
7	PerRC	43.22	7.087	.000
	VAR00001	.0000	.00000	

RESULTS

In this randomized controlled clinical trial, all the clinical parameters were recorded at baseline and then at 3 months postoperatively.

1. Inter group comparisons were done for the changes in mean plaque and gingival scores, clinical attachment levels, probing depth, recession height, width of keratinized tissue and percentage of root coverage values by applying independent student t test. Comparisons were drawn for baseline and 3 month values between group 1 and group 2. (Table A.1)

2. Intra group comparisons were drawn for the changes in mean plaque and gingival scores, relative clinical attachment levels, probing depth, recession height, width of keratinized tissue and percentage of root coverage. Pairwise comparisons within the groups were done by applying paired sample t test. Comparisons were drawn between baseline and 3 month values. (Table A.2 and A.3)

PLAQUE INDEX

Group 1 - For intragroup comparison, the mean±SD at baseline was 0.65±0.136 which was reduced to 0.35±0.136 at 3 months. These changes were found to be statistically significant (P≤0.05) (Table A.2). Group 2 – For intragroup comparison, the mean±SD at baseline was 0.60±0.209 which was reduced to 0.35±0.136 at 3 months. These changes were found to be statistically non significant (P≥0.05) (Table A.3). For intergroup comparison, non significant results were seen (P≥0.05) (Table A.1) (Graph A.1).

GINGIVAL INDEX

Group 1 - For intragroup comparison, the mean±SD at baseline was 0.65±0.136 which was reduced to 0.35±0.136 at 3 months. These changes were found to be statistically significant (P≤0.05) (Table A.2). Group 2 – For intragroup comparison, the mean±SD at baseline was 0.55±0.209 which was reduced to 0.35±0.136 at 3 months. These changes were found to be statistically non significant (P≥0.05) (Table A.3) For intergroup comparison, non significant results were seen (P≥0.05) (Table A.1) (Graph A.2).

PROBING DEPTH

Group 1 - For intragroup comparison, the mean±SD at baseline was 1.90±1.24 which was reduced to 1.30±1.03 at 3 months showing statistically significant results (P≤0.05) (Table A.2). Group 2 – For intragroup comparison, the mean±SD at baseline was 2.30±0.44 which was reduced to 1.70±0.57 at 3 months again showing a statistically significant difference (P≤0.05) (Table A.3). For intergroup comparison, non significant results were seen (P≥0.05) (Table A.1) (Graph A.3).

RELATIVE ATTACHMENT LEVEL

Group 1 - For intragroup comparison, the mean±SD at baseline was 10.4±1.51 which was reduced to 8.50±1.00 at 3 months. These changes were found to be statistically significant (P≤0.05) (Table A.2). Group 2 – For intragroup comparison, the mean±SD at baseline was 9.50±0.35 which was reduced to 8.20±0.27 at 3 months again showing a statistically significant difference (P≤0.05) (Table A.3). For intergroup comparison, non significant results were seen (P≥0.05) (Table A.1) (Graph A.4).

WIDTH OF KERATINIZED TISSUE

Group 1 - For intragroup comparison, the mean±SD at baseline was 5.00±0.79 which was reduced to 4.10±1.14 at 3 months. These changes were found to be statistically non significant (P≥0.05) (Table A.2). Group 2 – For intragroup comparison, the mean±SD at baseline was 5.10±0.89 which was reduced to 4.50±1.00 at 3 months. These changes were found to be statistically non significant (P≥0.05) (Table A.3). For intergroup comparison, non significant results were seen (P≥0.05) (Table A.1) (Graph A.5).

RECESSION HEIGHT

Group 1 - For intragroup comparison, the mean±SD at baseline was 3.10±0.22 which was reduced to 1.00±1.14 at 3 months. These changes were found to be statistically significant (P≤0.05) (Table A.2) (Figure A.16). Group 2 – For intragroup comparison, the mean±SD at baseline was 3.00±0.35 which was reduced to 1.70±0.27 at 3 months. These changes were found to be statistically significant (P≤0.05) (Table A.3). For intergroup comparison, significant results were seen for Group 1 (P≤0.05) (Table A.1) (Graph A.6) (Figure A.18).

PERCENTAGE OF ROOT COVERAGE

Group 1 – For intragroup comparison, the percentage of root coverage was 67.64% which was found to be statistically significant (P≤0.05) (Table A.2) (Figure A.17) and for Group 2, it was 43.22% which was again statistically significant (P≤0.05) (Table A.3) (Figure A.19). For intergroup comparison, significant results were seen for Group 1 (P≤0.05) (Table A.1) (Graph A.7).

DISCUSSION

HA is an essential component of extracellular matrix. It has a major role in tissue morphogenesis, cell migration, differentiation, and adhesion. [21,22,23] Hyaluronan facilitates extracellular matrix cell infiltration into the inflamed area with the help of fibrin clot, thereby providing structural framework to the tissues. Pro-inflammatory cytokines are produced by various cells such as fibroblasts, keratinocytes, cementoblasts and osteoblasts which promotes the inflammatory response. Also it leads to the activation of inflammatory cells, such as neutrophils and macrophages. During granulation phase, organization occurs by cellular proliferation and migration of matrix cells into granulation tissue matrix. In the granulation phase, hyaluronan synthesis stops and existing hyaluronan is degraded by hyaluronidases resulting in the formation of lower molecular weight hyaluronan molecules, which promote angiogenesis in the wound area and accelerates bone regeneration. [24]

The results of the present study showed that the mean % of Root coverage in Group 1 (Gengigel + CAF) was more i.e 67.64% than Group 2 (Tetracycline + CAF) which was 43.22%. The results of which were in accordance with a study done by Kumar et al, 2014 where the mean % of Root coverage with Gengigel + CAF was 68.33%. However contrasting results were shown by Bouchard et al, 1997 when Tetracycline was used along with CAF where the mean % of Root coverage came out to be 79.3 %.

In the present study, the Recession height was reduced from 3.1 mm to 1.00 mm in Group 1 which showed statistically significant results. The results of our study were similar to a study done by Kumar et al, 2014 where the Recession height was reduced from 3.2 mm to 1.10 mm with Gengigel. However with the use of Tetracycline, the Recession height reduced from 3.00 to 1.7 mm. In contrast Bouchard et al, 1997 showed the mean surface area of root exposure was reduced from 11.53 mm^2 to 0.34 mm^2 with the use of Tetracycline.

Effect of hyaluronic acid in the treatment of periodontitis is well studied by Xu Y et al. [25] Ballini et al examined Esterified Hyaluronic Acid and Autologous Bone in the Surgical Correction of the Infra Bony Defects where he had demonstrated regeneration. [26] De Araujo Nobre studied effect of

hyaluronic acid in the treatment of peri implant pockets. [27] Nobre et al compared the effect of chlorhexidine and hyaluronan on immediate functional implants inside maintenance protocol. [28] Klinger and Ratevatalla et al studied the effect of hyaluronan at bone implant interface. [29,30] Prato used hyaluronic acid as an autologous graft material for gingival augmentation procedures. [31]

Hyaluronan has been studied and used widely in the field of dentistry, but to the best of our knowledge, there is no published data on the comparison of HA and Tetracycline in the treatment of gingival recession. Hence, it is premature at this point to make a comparison of the results of the present study with previous studies.

However, this study has a certain drawbacks. The cases were followed for a period of 3 months. A longer follow-up is required to know the long-term stability of the treatment and the added advantage of using Gengigel as an adjunct to root coverage. It is entirely based on clinical parameters and the exact nature of periodontal regeneration can be made only by histological evaluation. Studies with a larger sample size and higher statistical power are required in the future to further explore the use of hyaluronan as an adjunct to periodontal surgeries.

CONCLUSION

The adjunctive use of 0.2% Hyaluronic Acid gel (Gengigel) was evaluated in this study and it was proved to be safe and provided statistically significant results. It shows that hyaluronan affects the treatment outcome when used as an adjunct to CAF procedure in root coverage procedures. Thus it can be concluded that Hyaluronan can be easily combined with CAF procedures in compromised cases and in situations where more stable results are desired.

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CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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