

A RADIOLOGICAL, MICROBIOLOGICAL AND CLINICAL EVALUATION OF THE REGENERATIVE POTENTIAL OF XENOGRAFT ALONE AND IN COMBINATION WITH 1% ALENDRONATE AND 1% METFORMIN GELS FOR INFRABONY PERIODONTAL DEFECTS- A RANDOMIZED CONTROL TRIAL**¹Mannepalli Madhusudhan MDS and ^{2*}Jammula Surya Prasanna MDS**¹Sr. Lecturer, Dept of Periodontics, Panineeya Institute of Dental Sciences and Research Centre. Dilsuck Nagar, Hyderabad.²Professor, Dept of Periodontics, Panineeya Institute of Dental Sciences and Research Centre. Dilsuck Nagar, Hyderabad.***Corresponding Author: Dr. Jammula Surya Prasanna MDS**

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ABSTRACT

Objectives: The main aim of this study was to assess the competency of Xenograft (XG) alone and in combination with 1% ALN and 1% MF in the regeneration of periodontal bone defects. **Materials and Methods:** In this randomized interventional study, a total of 36 infra bony defects were randomly assigned to three groups, each group comprised of 12 defects: Group I with 1% ALN gel + XG (test), Group II with 1% MF gel + XG (test) and Group III with XG alone (active control). This was a split-mouth study. Clinical criteria like pocket depth (PD), relative vertical clinical attachment level (RVCAL) were assessed. Radiographic parameter like linear bone growth (LBG) was measured with the help of a grid. The microbial count (MC) was estimated by plaque sample. All the parameters were calculated prior to the beginning of the study and at six months. Statistical analysis was performed using the Chi-square and paired t-test. **Results:** The mean reduction of PD, gain in RVCAL, LBG and MC were statistically significant within the groups ($p < 0.05$) from baseline to six months. However, MC was insignificant in group III ($p = 0.294$) within the group, but was significant on the intergroup comparison. **Conclusions:** Using XG alone as well as in combination with 1% ALN and 1% MF has given appreciable results.

KEYWORDS: Alendronate, Grid, Metformin, Regeneration, Vertical bone defects, Xenograft,**INTRODUCTION**

Alveolar bone formation and resorption is a methodical process. Any disruption to this sequence could result in excess bone formation or bone loss. Periodontitis is one such destructive process leading to alveolar bone loss. The logical hypothesis is that therapeutic agents which restrain the bone resorption or hasten bone formation could protect against alveolar bone loss.

Mechanical therapy is the gold standard to eradicate oral bacteria from periodontal pockets, but complete eradication is not possible because of the sustenance of some exceptional morphological variations and bacterial strands encircling the tooth structure.^[1] As an adjunctive therapy, systemic antimicrobials are sufficiently effective to some extent, but they have their deleterious effects like allergy, systemic toxicity, development of bacterial resistance etc. To overcome these adverse effects extensive research was accomplished and many systematic reviews were thoroughly scrutinized about the efficacy of antimicrobial drug delivery.^[2-5] The local drug delivery systems are site-specific, with excellent

antimicrobial activity and amplified intra sulcular drug absorption.^[6] without the detrimental effects seen with the systemic delivery,

Among the available drugs, Bisphosphonates (BP) are one of the most important and effective group of anti-resorptive drugs. Alendronate (ALN) is the classic drug among the BP group. ALN has osteo-stimulative properties and hence acts as a dynamic inhibitor of bone resorption.^[7] ALN has been postulated to help as a supportive treatment of chronic periodontitis (CP).^[8] Metformin (MF) is a second-generation biguanide used to regulate type 2 diabetes mellitus. Studies have also proven the positive effect of MF on bone formation.^[9] Marked up regulation of osteoblastic activity and down regulation of osteoclastic activity is the important function of MF thus accelerating the bone formation. Considering this beneficial property, MF is also being considered in the periodontitis treatment.^[9-11] Bone grafts are bioresorbable agents which facilitate the bone genesis as well as wound healing by its space maintenance and scaffolding action. New bone formation is initiated by the mineral reservoir

present in grafts. Grafts that are obtained from non-human species i.e. animals are Xenografts which are osteoconductive in nature with limited resorptive potential. Generally used Xenograft in periodontal regeneration is deproteinized bovine bone mineral.^[12-13]

It is a proven fact that ALN and MF are effective bone-forming agents when administered systemically. However their effect when delivered locally in combination with a xenograft is not yet proven. Thus, in this study, based on null hypothesis we aimed to assess which of the two agents when used with xenograft were more effective in bone formation.

MATERIALS AND METHODS

This randomized, triple masked interventional analysis was conducted in the outpatient Department of Periodontology, Hyderabad. The study was done from January 2017 to July 2018. Patients of both sexes, having chronic periodontitis, aged between 35-55 years participated. After an extensive explanation of the procedure, a written approval was acquired from all the patients. The institutional Ethical Committee and Review Board, assessed this study, and also this study was registered in <http://ctri.nic.in> as CTRI/2018/02/011881. Consolidated Standards of Reporting Trials (CONSORT) standard procedure as well as the Helsinki Declaration for human research^[14], was followed for conducting this study. A complete intraoral soft-tissue examination was performed to register the oral mucosal condition at the inception of the study so that any changes in the course of the study could be identified and assessed. Healing was adequate without any significant visual inflammatory signs, and no detrimental or untoward effects were seen throughout the study.

The inclusion criteria for this study were as follows: CP patients with angular intrabony defects with pocket depth (PD) \geq 5mm or Clinical Attachment Loss (CAL) \geq 2 mm subsequent to scaling and root planing (SRP). At least three defects had to be present in each patient. The exclusion criteria for this study was: subjects with established systemic diseases (even suffering from a common cold), with obvious or established sensitivity to ALN and MF, systemic or local administration of ALN or any of the BP family drugs for any other reason, using any form of tobacco products and alcohol, previous six months of periodontal treatment history and antibiotic therapy, pregnant and nursing mothers, immunocompromised and mentally challenged patients, aggressive forms of periodontitis and patients taking any other medications even for any simple reason (e.g.: paracetamol).

Calculations of radiographic bone height with the grid: a radio-opaque mesh or a grid with calibrations of 1x1 mm² was positioned in between the exposed object and the radiographic film during the time of exposure. The adjoining two parallel grid lines should be at the equidistant of the film. Following formula was used for measurements:

$$\frac{\text{The actual span between two points (grid)}}{\text{The measured span between two points (grid)}} = \frac{\text{Actual span between two points (anatomic)}}{\text{Measured span between two points (anatomic)}}$$

According to the selection criteria, initially a total of 60 patients with 120 periodontal defects were selected, a few dropped out due to personal reasons and some could not fulfil the criteria. Among 120 defects only 90 defects were finalized and included in the study. Among these 36 defects from 24 CP patients was selected, and divided equally into three groups, each group comprised of 12 defects (Figure 1).



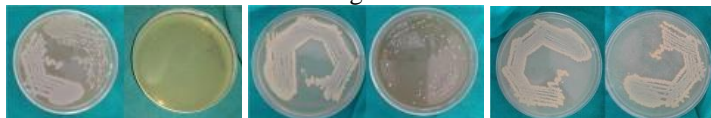
All the subjects underwent thorough SRP at baseline. **Group I-** Alendronate group (ALN) - treated with 1% alendronate gel along with Xenograft. **Group II-** Metformin group (MF) - treated with 1% metformin gel along with Xenograft. **Group III-** Xenograft group (XG) - treated with Xenograft alone. Grouping was done by computer randomized allocation. All three groups were assessed in the same patient and any dissimilarity was evaluated.

Clinical measurements like pocket probing depth (PD) gain and relative vertical clinical attachment level (RVCAL) gain were assessed. A radiographic parameter like linear bone growth (LBG) was measured with the help of a grid. Microbial count approximated with the collected subgingival plaque specimens. All the criteria were assessed at the initial stage of the study and again after six month intervals. Williams graduated probe (Hu-Friedy, Chicago, IL.) was used to measure all the parameters. Radiographs were taken along with GRID (Blue dent India, Ullagaram, Nanganallur, Chennai, India). All surgical procedures were done by a single operator, without any knowledge of the group allocation and the type of medication being used in the periodontal pocket, thus making the study completely randomized.

Measurements of the vertical distance (VD) was done by counting the squares of grid from the Cemento enamel junction (CEJ) to the Level of alveolar crest (AC) and the defect base (DB). Defect fill was determined by the discrepancy in the difference from the CEJ to AC and to BD. The level of bone gain was calculated from baseline to six months (Figure 2, 3, 4)



Microbial Count was carried out with the help of sterile paper points (pink blue, Bangalore, India), subgingival plaque was collected and transferred to preheated cooked meat medium 2 ml (Robertson's) for culture. Before the sample collection supra gingival plaque was removed and the site was isolated with cotton roles, to prevent contamination. An anaerobic culture was performed to evaluate the microbial growth at baseline and six months (Figure 5, 6, 7).



Formulation of Gel: Alendronate

A weighed quantity of corbopol with 1.5% concentration was dispersed in 25 ml of distilled water, temperature static at 95°C. At this temperature the diffusion was agitated/ stirred with a magnetic stirrer for 20 min to accelerate hydration of corbopol gel. To the above formulation, a calculated volume of Alendronate (1%) and preservative was added slowly with constant stirring. The dispersion was diluted with tri-ethanolamine to adjust the pH and kept at 4°C overnight to form a clear gel.

Analysis Formula

Absorbance=0.273; Wave length=264nm

$$\text{Drug content} = \frac{\text{Absorbance}}{\text{Slope}} \times \frac{1}{1000} \times \frac{100}{\text{Dose}} \times 100$$

$$= \frac{0.273}{0.003} \times \frac{1}{1000} \times \frac{100}{9.4} \times 100 = 96.46\%$$

Metformin gel

A measured volume of Gellan gum with 1% concentration was disseminated in 25 ml of clarified water at 95°C. At this temperature the diffusion was agitated/ stirred with a magnetic stirrer for 20 min to promote dampness of Gellan gum. To the above formulation a measured volume of metformin (1%) and preservative was added slowly with constant stirring. The dispersion was neutralized with triethanolamine to adjust the pH and kept at 4°C overnight until a clear gel formed.

Analysis Formula

Absorbance=0.111; Wave length=233nm

$$\text{Drug content} = \frac{\text{Absorbance}}{\text{Slope}} \times \frac{1}{1000} \times \frac{100}{\text{Dose}} \times 100$$

$$= \frac{0.111}{0.071} \times \frac{1}{1000} \times \frac{100}{15.5} \times 100 = 100.64\%$$

Standardization of the drug delivery: An insulin syringe of 1 ml with 40 unit gradations (U-40 INSULIN) was used to standardize the quantity of the drug. 25 µl was delivered along with Xenograft. 40 units = 1 ml. Each unit = 1/40 ml i.e. 25 µl 0.025 ml). 1% = 1gm in 100mL =>10mg in 1 ml .So 1ml contains 10mg of drug => 1µl contain 10 µg. 10µl contain 100 µg i.e., 0.1mg of drug. 1µl contains 10 µg of drug, hence 25µl contains 250 µg i.e., 0.25mg of drug.

In this analysis, 1% of Alendronate gel was prepared, but a standardized 25µl quantity of the drug was delivered into the defect along with a Xenograft.

All the patients, underwent an initial examination and were thoroughly briefed about the treatment protocol (phase I periodontal therapy i.e. SRP and plaque control measures) during the study period.

The surgical procedure was performed under local anesthesia. A full thickness flap was raised and after thorough debridement, sites which fulfilled the inclusion criteria received the drug. The standardized injecting method was followed with the same quantity of drug delivered in all the defects. After approximation of the flaps with suture, a proper pocket seal was given with periodontal dressing. All the parameters were compared at baseline as well as at six months. The entire procedure was masked.

Primary and secondary aftermaths were measured, the primary aftermath of the study was to measure the change in radiographic LBG and microbiological count from baseline to 6 months. Secondary aftermaths measured were PD and RVCAL.

A power analysis was performed to estimate the sample size, based on a significance level of 0.05 and power of 80 %. Design of the study was a randomized, prospective, parallel-arm, interventional clinical trial. One-way analysis of variance (ANOVA) was used; the repeated measure ANOVA test was used to calculate the statistical significance of the mean change from baseline

to follow-up interval within the groups as well as to determine the significance of differences among the groups. Intragroup comparison was done by using a paired t-test. Categorical variables were compared using the Chi-square test.

RESULTS

When PD was compared in all the groups, PD reduction was statistically significant from baseline to six months, (6.50 ± 1.09 & 3.42 ± 1.08 ; 6.50 ± 0.90 & 3.42 ± 0.90 ; 7.58 ± 1.78 & 3.67 ± 0.49) in all the groups, p-value was (<0.001). RVCAL also reduced from baseline to six months, (12.17 ± 2.04 & 9.08 ± 1.44 ; 11.92 ± 1.38 & 9.75 ± 3.05 ; 12.42 ± 1.51 & 8.42 ± 0.90) p-value was <0.001 in group I&III, 0.044 in group II. Bone level (LBG), alveolar bone gain was also statistically significant. (13.33 ± 2.67 & 11.25 ± 2.49 ; 12.75 ± 1.22 & 10.83 ± 0.72 ; 12.83 ± 1.53 & 10.67 ± 1.15) p-value was (<0.001 in all the groups). Reduction was significant in

MC (227.4 ± 22.3 & 95.8 ± 18.1 ; 227.1 ± 16.2 & 183.9 ± 11.1 ; 218.1 ± 13.1 & 207.5 ± 37.4) in the group I&II which was observed from baseline to six months, but there was no significance in group III (p-0.294) (Table 1).

When the comparison was done between groups, there was no significant difference of PD, RVCAL and LBG at baseline as well as at six months. But when MC was compared in between groups reduction was significant at baseline (p-0.04) as well as at six months (0.000) (Table 1).

The results of this study have shown that the action of 1% ALN gel + XG, 1% MF gel + XG, and XG alone, were similar on all parameters from baseline to six months. But more precise action was seen in relation to MC. Significant reduction of MC was seen in both inter and intragroup comparisons (Table 1).

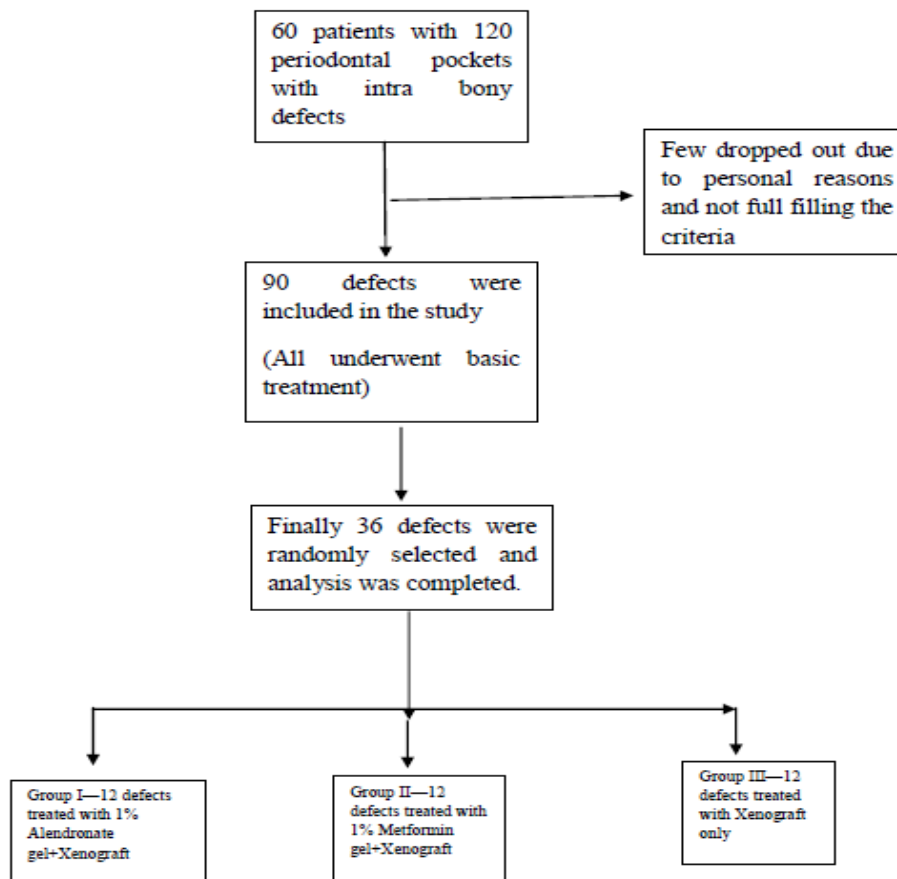


Figure 1: Study selection criteria.

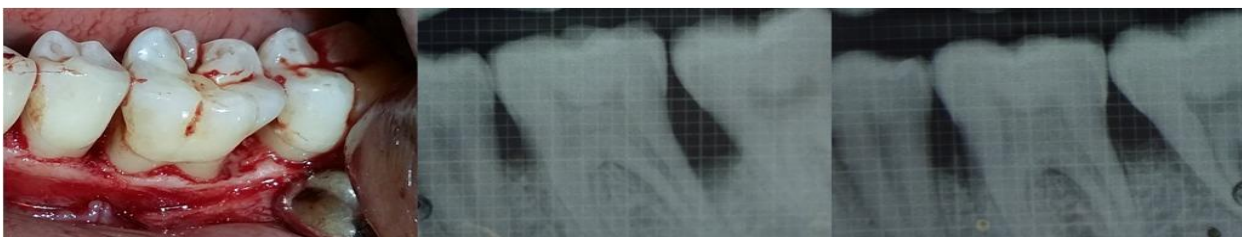


Figure 2: Linear bone growth (LBG) in group I (ALN GROUP) from base line to six months.



Figure 3: Linear bone growth (LBG) in group II (MF GROUP) from base line to six months.

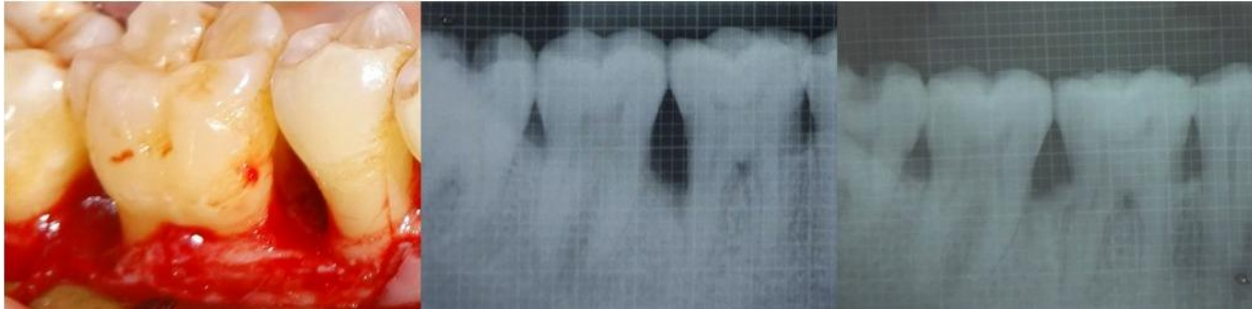


Figure 4: linear bone growth (LBG) in group III (XG GROUP) from base line to six months.

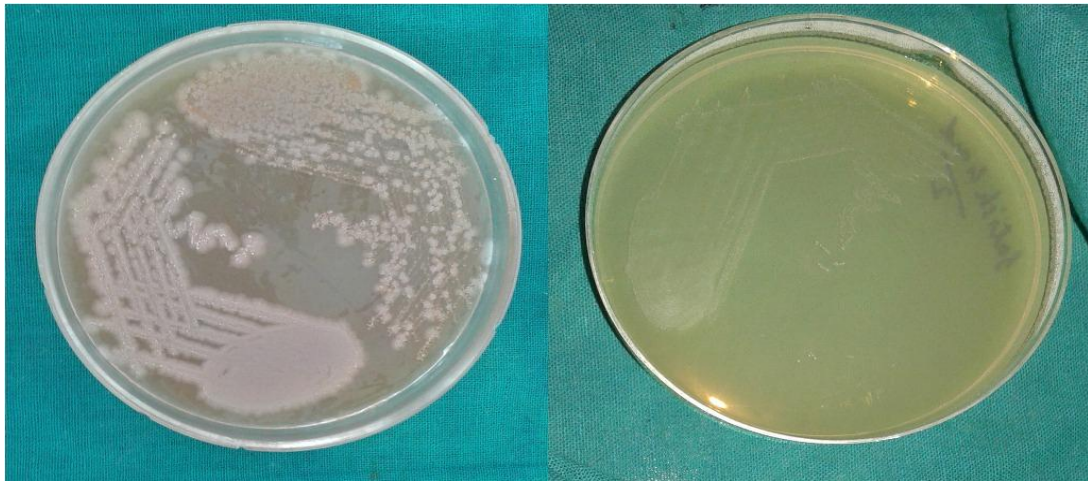


Figure 5: Microbial count (MC) in group I (ALN GROUP) from base line to six months.

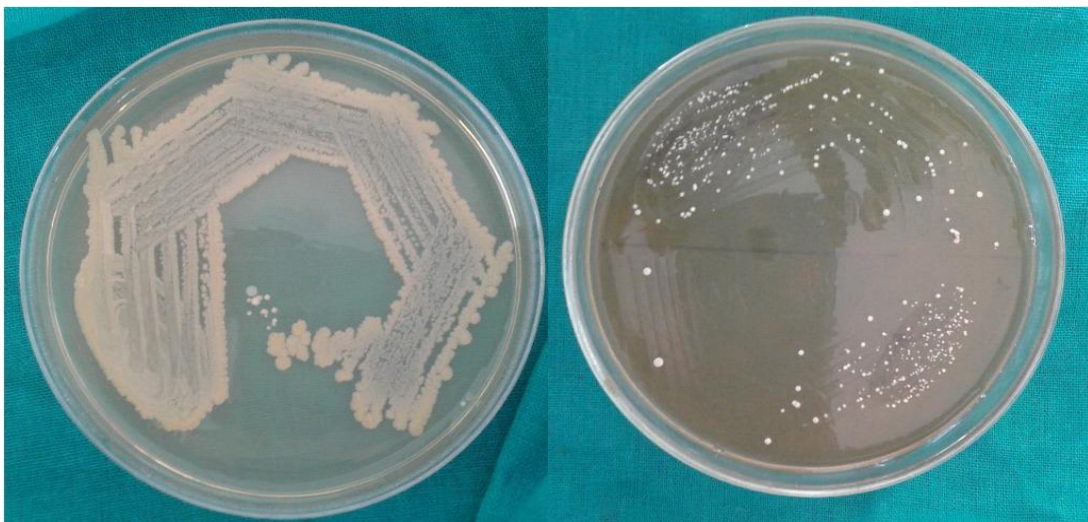


Figure 6: Microbial count (MC) in group II (MF GROUP) from base line to six months.

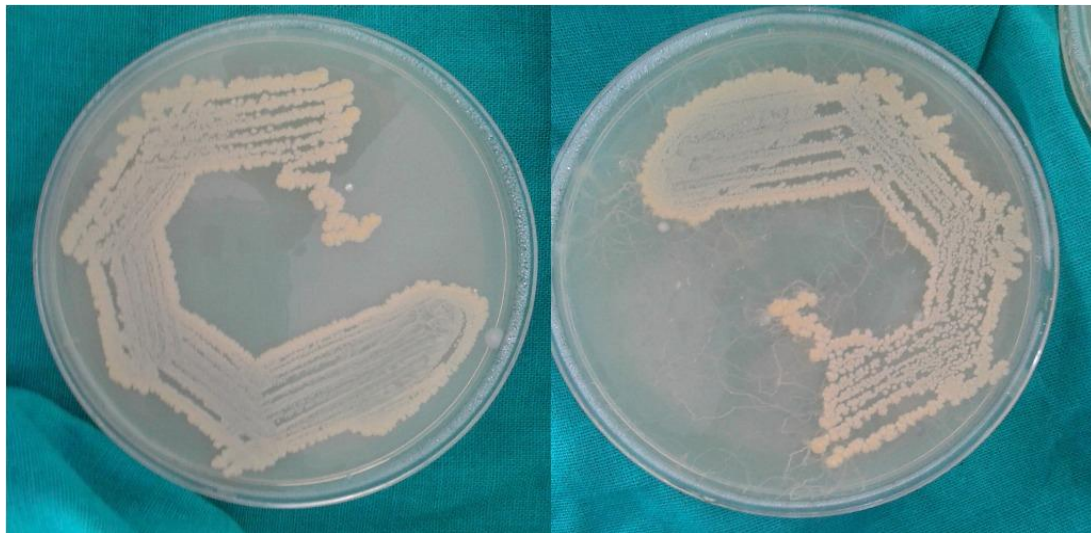


Figure 7: Microbial count (MC) in group III (XG GROUP) from base line to six months.

Table 1; Intra and Inter group comparison of all the parameters at baseline to six months.

Variables		Group						p-value
		Group 1 1% ALN gel + XG		Group 2 1% MF gel + XG		Group 3 XG		
		Mean	SD	Mean	SD	Mean	SD	
PD (n=12)	Baseline	6.50	1.09	6.50	0.90	7.58	1.78	0.18
	6 months	3.42	1.08	3.42	0.90	3.67	0.49	
p-value		<0.001*		<0.001*		<0.001*		
RVCAL (n=12)	Baseline	12.17	2.04	11.92	1.38	12.42	1.51	0.765
	6 months	9.08	1.44	9.75	3.05	8.42	0.90	
p-value		<0.001*		0.044*		<0.001*		
Bone level(LBG) (n=12)	Baseline	13.33	2.67	12.75	1.22	12.83	1.53	0.723
	6 months	11.25	2.49	10.83	0.72	10.67	1.15	
p-value		<0.001*		<0.001*		<0.001*		
Microbial count (MC) (n=36)	Baseline	227.4	22.3	227.1	16.2	218.1	13.1	0.04*
	6 months	95.8	18.1	183.9	11.1	207.5	37.4	
p-value		0.000*		0.000*		0.294		

SD –Standard Deviation, PD-Pocket Depth, RVCAL-Relative Vertical Clinical Attachment Level, LBG-Leaner Bone Growth, ALN-Alendronate, MF-Metformin, XG-Xenograft, *Significant, n-Number, p-value-Calculated Probability(p<0.05 significance)

DISCUSSION

Progression of periodontal disease causes increased pocket depths as well as alveolar bone destruction. To arrest the disease process, treatment is imperative. The eventual objective of therapy is to gain new attachment by reducing the PD and augmenting the bone height.^[15]

Various treatment approaches exist to treat periodontitis such as non -surgical therapy (NST), surgical therapy (ST) and an ancillary application of pharmacological agents.^[16]

Although mechanical NST and ST have shown benefits as alternative remedies in the periodontal disease treatment approaches, their failure to completely annihilate the reestablished pathogens from externalities of other niches of the oral cavity may cause reinfection. Concomitant application of chemotherapeutic agents in different modalities may overcome these impediments.^[17-18] Usage of specific medications like

antimicrobials and host modulating agents help in the control of microbial flora of periodontitis. Additional benefits of these agents might be diminution of excessive pro inflammatory enzyme levels by curtailing the activity of cytokines, prostaglandins, and osteoclasts.^[19]

The advantages of local drug delivery systems over the systemic therapy are attaining high intra sulcular drug concentrations at smaller doses, and a sustained drug action to the targeted area. Moreover, dose reduction leads to increased patient compliance.^[20] Further, the need for periodontal surgery might be prevented if intra -pocket medicaments are used along with SRP.^[21]

BP's are the hailed medicaments in the present pharmacological weaponry against osteoclast-mediated bone loss. BP can offer consequential clinical amelioration where disproportionate bone formation and resorption takes place due to osteoblast and osteoclast

hostility during predisposed disease pathology.^[22-23] ALN is a second generation nitrogen-containing BP, which promotes osteoclast apoptosis and is perspicuous from that of the non-nitrogen-containing BP.^[24] Systematization of osteoclast activities inclusive of stress fiber assembly, membrane ruffling, and survival are inhibited by BP's, ultimately leading to osteoclast apoptosis.^[24] The main advantage of BP is binding capacity with hydroxyapatite, the important inorganic material found in the bone.^[25] Research suggests that BP's reciprocate the actions synchronously with osteoclasts and osteoblasts so that not only resorption is minimized but also early bone formation is observed.^[26] Additionally, BP counteracts the functions of various matrix metalloproteinases (MMPs) involved in the periodontal connective tissue breakdown.^[27-28] Ko FC et al, found BP withdrawal affected bone formation.^[29]

MF is a first-line therapy for type-2diabetes mellitus. In vitro studies delineated that MF is osteogenic, can also induce osteoblastic cell differentiation and bone matrix synthesis. Apart from this Bone Morphogenic Protein-2 (BMP-2) was expressed by MF, which helps in the regeneration of bone in irradiated tissues. Further cartilage formation and bone induction are carried out.^[30-31] MF stimulates osteoblastic bone formation by interacting with the transcription factor Runt-related transcription factor 2 (Runx2).^[32] Possibly via stimulation of Runx2. MF increases osteoblastic proliferation, alkaline phosphatase activity leads to form mineralized nodules in osteoblasts, further the action of MF on bone marrow mesenchymal cell progenitors (BMPCs) will have an osteogenic effect.^[33-35]

XG is a graft taken from a non human species. Currently organic, bovine, equine-derived and coral skeleton bones are being extensively used for periodontal regeneration. After different purification procedures, all organic components were removed and an uninterrupted inorganic form of the graft is made in different particle sizes, both cancellous and cortical bone forms. The only constraint of XG is that it is only osteoconductive in nature and does not have osteogenic and osteoinductive properties.^[36]

On intragroup comparison, MC in group 3 was not significant, but when we compared inter- group MC was significant and remaining parameters were not significant.

These study results are in accordance with the study done by Ipshita et al,³⁷ when they had tried 1% ALN alone in furcation defects significant results in a reduction of PD, gain in RVCAL were seen. Meta-analysis and a systematic review, done by Chen et al³⁸ and Akram et al³⁹ about alendronate show their conclusions were also in accordance to this study results. In another systematic review by Akram et al⁴⁰ and Najeeb et al⁴¹ metformin has given significant results after scaling which is in accordance to this study results except for RVCAL. This

study results were also partially in accordance with two studies done by Pradeep et al.^[10-11]

Above named materials, when compared individually show conflicting results. As very minimal research was done in combination therapy, drawing results are inconclusive. The said and done studies compared only clinical parameters but are not relevant to microbes. The fascinating feature in this article is the combination therapy used and evaluating its effect on the microbial count. The distinguished impediments found were a smaller extent of the study samples and the drug release period was not calculated. Sustainability of the gel in the pockets was mysterious. Since conventional radiographs were taken manual errors and misinterpretation could be possible.

CONCLUSION

Regeneration of intrabony defects is being carried out with a plethora of agents for many years. MF and ALN were shown to enhance osseous regeneration by their osteoblast-promoting and osteoclast-inhibiting actions. In this frame of reference, MF and ALN were used as additives to the xenograft and delivered at the site of interest as a combination therapy. Delivering the drug at the specific site would minimize the adverse effects seen with the systemic delivery and also has added benefits of controlled drug release, sustained action, and more patient compliance. The results yielded insignificant outcomes on the bone regeneration though there was a significant reduction in the microbial counts. Future research should focus on various other combination therapies for the regeneration of deeper angular defects with more patient compliance.

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