Original Article

Effect of in-situ application of simvastatin gel in surgical management of osseous defects in chronic periodontitis—A randomized clinical trial

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ABSTRACT

Background: The present randomized controlled clinical study was designed to investigate the effect of in situ application of 1.2 mg Simvastatin (SV) gel in the surgical management of Intrabony defects in chronic periodontitis patients.

Methodology: 20 patients contributing 40 sites were categorized into two treatment groups: Open flap debridement plus 1.2 mg SV gel (Group 1) and Open flap debridement plus Placebo gel (Group 2). Gingival index (GI), Plaque index (PI), Pocket depth (PD) and clinical attachment level (CAL) were recorded at baseline, 3 months, 6 months and 9 months. At baseline and at the end of 6 and 9 months Radiographic evaluation of Intrabony defect fill was done using Image j software.

Results: Significant reduction of GI, PD and gain in CAL was observed at the end of 9 months in both groups. Amount of bone fill and percentage of original defect fill in Group 1 was statistically highly significant than Group 2 at the end of 6 and 9 months.

Conclusion: Higher amount of decrease in GI and PD along with more amount of CAL gain was observed in treatment group than control group. Radiological assessment confirmed that significant intrabony defect fill and percentage fill of original defect in treatment group than controlled group.

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1. Introduction

Periodontitis, an inflammatory disease of periodontium is considered to be caused by bacterial biofilm (Dental plaque), and its onset and progression are modulated by a variety of risk factors, such as systemic conditions and smoking. If Periodontitis left untreated, results into periodontal attachment loss and subsequently tooth loss. Various methods are effective in repairing these periodontal defects by creating space and host bone formation through the use of various periodontal regenerative materials.¹ Autologous bone graft is ideal grafting material for the treatment of periodontal defects and fracture repair.² However, need for a second surgical site is the most important drawback which has led to the development of alternative materials. Growth factors (e.g.: bone morphogenetic proteins) are expensive, may degrade with rapid rate at the treated site and sometimes may elicit immune responses, thus limiting their use.³

Not satisfied with traditional weapons to restore lost alveolar bone support, researchers have turned towards new contemporary methods for periodontal regeneration.⁴ The newer approaches e.g. Extracorporeal shock wave therapy, Photodynamic therapy, polishing with glycine powder to prevent subgingival plaque formation, topical and systemic administration of Simvastatin etc are still in infancy but show promise nonetheless. Pharmacologic compounds e.g. Simvastatin stimulate bone growth by inducing growth factors. This newer contemporary approach may prove to be a cost-effective, non-space occupying way to correct bone defects.⁵

Statins block conversion of hydroxy-3-methyl-glutaryl coenzyme A (HMG-CoA) to Mevalonic acid, required for cholesterol biosynthesis. By this, it reduces cardiovascular mortality and morbidity and helpful in prevention of primary and secondary coronary artery disease.⁶ Other than this primary effect, few cholesterol independent effects have also been shown by statins, called as pleiotropic effect. It consists of effects on vascular tissue, heart, kidney, glucose and bone metabolism.⁷

Recent literatures have shown that SV assists in bone regeneration as well as has the anti-inflammatory effect when delivered systemically or applied locally.⁸ SV also increases the expression of osteocalcin, Type I collagen and bone sialoprotein

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with significant amount of anti-inflammatory effect by reducing the production of interleukin-6 and interleukin-8.⁹ SV is reported to increase vascular endothelial growth factor (VEGF) in dose dependent manner and the authors have suggested that statins may promote osteoblast differentiation by stimulating VEGF expression in bone tissue.¹⁰

Various controlled drug releasing vehicles have been mentioned in literature such as Carbopol, hydroxylpropyl methylcellulose etc.^{11,12} Traditionally in the management of mild to moderate pockets tetracycline fiber, metronidazole gel, chlorhexidine chip etc have been used as local drug delivery agents. Vehicles used for this type of drug delivery should be bio compatible with easy fabrication and predictable biodegradable kinetics. Carbopol has been used extensively in pharma industry, such as the solution of various ophthalmic drug deliveries.¹³

SV is a new pharmacological agent introduced as a host modulatory agent in periodontal therapy. Very few clinical trials have been reported using SV as a local host modulatory agent in the management of periodontal diseases. Keeping the above facts in mind, the present study was carried out as randomized controlled split mouth placebo control single centered clinical trial to investigate clinically and radiologically (bone fill) the effect of in-situ gel application of 1.2 mg SV in the surgical management of osseous defects in chronic periodontitis. Aim of the present study was to evaluate whether SV used in-situ gel after surgical debridement will have any added value in Clinical (Gingival index, Plaque index, Pocket Probing depth, Clinical attachment level) and Radiographic parameter (Bone fill) over the period of 9 months.

2. Materials and methods

2.1. Source of data

Patients for this study were recruited from the outpatient section of the Department of Periodontics, KLE Society's institute of Dental Sciences, Bangalore, Karnataka. Twenty patients, aged between 25–55 years (10 males and 10 females) diagnosed with generalized chronic periodontitis, contributing 40 sites were enrolled for the study by convenience sampling method. It was made clear to the all subjects that participation was voluntary. Written informed consent was obtained from each and every patient, and ethical clearance for the study was received from the university ethical Committee and registered with clinical trial registry of India. (CTRI/2016/11/007428).

2.2. Selection criteria

Patients without any systemic illness having periodontal probing depth of ≥ 5 mm clinically and radiographic evidence of vertical bone loss in the mandibular molar region bilaterally without any history of periodontal therapy and antibiotic administration in last 6 months were included for this study. Patients with history of administration of NSAID drugs, patients with aggressive periodontitis, current smokers patients, patient on systemic statin therapy, patients with immunocompromised medical status, patients with bleeding disorders, pregnant and lactating female patients and patients indicated for multiple extractions or undergone multiple extractions were excluded from the study.

All selected patients, following an initial examination and treatment planning discussion, were given detailed instructions regarding plaque control measures and received preliminary periodontal therapy consisting of scaling and root planing. Clinical parameters, including Gingival Index (GI),¹⁴ Plaque index,¹⁴ Probing pocket depth (PPD) and Clinical Attachment Level (CAL)

were recorded full mouth as well as site specific at baseline and at the end of 3, 6 and 9 months post operatively.

For Probing Pocket Depth (PPD) measurements, a customized acrylic stent was fabricated to reduce the measurement error using cold cure acrylic resin on each patient's study cast to fit over the teeth selected for the study. A metal wire was adapted 1 mm above the gingival margin on the buccal surface of the selected teeth and fused to the stent. The pocket depth was measured using a University of North Carolina No 15 (UNC-15) probe.¹⁵ The customized acrylic stents on the prepared study casts were stored in water to minimize distortion and to record the measurement at 3 months, 6 months and 9 months post –operatively. Clinical Attachment Level (CAL) was measured by considering fused metal wire of customized acrylic stent as the fixed reference point. The distance from the metal wire [Fixed reference point (FRP)] to the free gingival margin (FGM) was recorded. The level of attachment was determined by adding this distance to the probing pocket depth (PPD).¹⁶

2.3. Radiographic assessment of intrabony defects: (Fig. 1)

Intraoral direct digital periapical Radiovisiograph (RVG-Suni Medical Imaging, Inc.) of each defect site was exposed using long cone paralleling technique. The mandibular molar regions with bilateral intrabony defects were the selected site for the study. Exposures were made at 70KVp, 7 ma for 0.2 s. The focus to film distance was 20 cm and film holder (Troll byte plus) was used. The radiographs were taken by the same clinician throughout the study to minimize errors. To estimate magnification, an orthodontic wire with known diameter (0.8 mm) and length (10 mm) was incorporated in a customized occlusal splint.¹⁷ On the digitized images Linear measurements were made using Image J software (Wayne Rasband, National institute of health-USA). Intrabony defect depths were determined by linear measurement analysis of RVG. The following two distances were assessed by RVG for each defect :

- Cementoenamel junction (CEJ) to Alveolar crest (AC)
- Cementoenamel junction (CEJ) to Base of defect (BD)

Base of the defect was defined as the most coronal point where the periodontal ligament showed a continuous width. If identification of periodontal ligament is difficult then the point where the projection of the alveolar crest crossed the root surface was used. If both the structures could be identified at one defect, the landmark defined by the periodontal ligament was taken as the base of the

Fig. 1. Amount of bone fill was measured by Linear measurement analysis of RVG, considering three landmarks: Cementoenamel junction (CEJ), Alveolar crest (AC) and Base of the defect (BD).

defect and the crossing of the silhouette of the alveolar crest with the root surface was defined as the alveolar crest. If several bony contours could be identified, the most apical crossing of the root was defined as the base of the defect and the most coronal as the alveolar crest.¹⁷ Amount of bone fill was estimated by the following formula-

Amount of bone fill = (Distance from CEJ to BD) – (Distance from CEJ to AC)

2.4. Formulation of 1.2% SV in-situ gel

The gel containing simvastatin for local delivery was prepared in the Department of Pharmaceutics, KLE Pharmacy College, Bangalore using Pharma grade materials. Simvastatin was obtained in the purest form from Acme pharmaceuticals, Ahmedabad, India. For the preparation of 1.2% SV gel, 100 ml water was taken in a beaker. 3% w/v carbopol 934 p was added to it. The mixture was then heated to 50°C–60°C and agitated using a mechanical shaker to obtain a homogeneous gel. A pre weighed amount of SV (1.2%) was added to the above mentioned homogeneous solution and dissolved completely to get a homogeneous phase of polymer, solvent, and drug. This homogenization was performed using a lab stirrer at 1300 rpm. 0.1% Methyl paraben (as preservative) was added to the preparation. Prepared gel formulation was sterilized by autoclaving for 30 min at 121°C. The prepared formulations were transferred to 2 ml syringes under strict sterile conditions and dispensed for clinical study.

2.5. Surgical procedure

The selected sites were randomly assigned as either experimental (Group 1) or control site (Group 2) by coin toss method. A Standard open flap surgical procedure was performed and 0.1 ml of placebo gel (Carbopol 934 P without SV) and 0.1 ml (1.2 mg/0.1 ml) 4 of SV gel was placed at exposed defect site at control and experimental site respectively. The surgical site was protected with a non-eugenol periodontal dressing after suturing. The patients were prescribed Paracetamol 500 mg orally every 6th hourly for 3 days. Patients were instructed to rinse the mouth with 0.2% chlorhexidine digluconate mouth rinse twice daily for 2 weeks. The clinical parameters and Radiographic measurements were recorded in the follow up examinations at the end of 3 months, 6 months and 9 months.

2.6. Statistical analysis

All clinical characteristics (GI, PI, PPD and CAL) and Radiographic parameters (Amount of defect fill and percentage of original defect fill) were recorded, tabulated and analyzed at baseline, 3 months, 6 months and 9 months. Student “t” test and Chi-Square (χ^2) tests were employed to evaluate the data. The chi-square was used to determine proportions percentages by independent characteristics while student ‘t’ test was used to determine whether there was a statistical difference between two groups in the parameters measured. In both the test, P values < 0.05 were considered significant and all the statistical calculations were performed through SPSS for Windows (Statistical Presentation System Software, 1999, SPSS Inc, New York, version 10.0).

3. Results

Overall the pharmacological agent SV, in-situ gel application was well tolerated by all the subjects with no adverse reactions

observed at any stage of the study. Randomly assigned all controlled and experimental 40 sites (2 sites per patient) from twenty patients were assessed for all clinical and radiographic parameters.

Evaluation of Gingival and Plaque index:(Table 1)- Comparison of mean gingival index score at baseline, 3 months, 6 months and 9 months between study groups was statistically highly significant at 6 months and 9 months interval but at baseline and 3 months, it was statistically non-significant. While no statistically significant difference was found between two groups for plaque index.

Evaluation of Probing pocket depth: (Table 2)- Comparison of mean probing pocket depth at baseline, 3 months, 6 months and 9 months between study groups was statistically significant only at 9 months interval. When the both study groups were compared to each other from baseline, the reduction in PD was statistically highly significant ($p < 0.001$) with the ‘t’ value 55.183, 70.354 and 34.696 at 3 months, 6 months and 9 months respectively. The observed reduction in PPD from baseline at the end of 9 months in group 1 was 4.85 ± 1.309 mm which is approximately 1.75 times more than group 2 (2.75 ± 0.910 mm).

Evaluation of Clinical attachment level: (Table 3)- Comparison of mean CAL at baseline, 3 months, 6 months and 9 months between study groups was statistically not significant at all-time intervals. When the both groups were compared with each other from baseline, the gain in CAL was statistically highly significant ($p < 0.001$) with the ‘t’ value 25.599, 44.333 and 41.341 at 3 months, 6 months and 9 months respectively.

3.1. Analysis of radiographic parameters

3.1.1. Amount of defect fill: (Fig. 2)

The mean amount of defect fill in group 1 was 0.071 ± 0.221 mm at 3 months, 0.926 ± 0.713 mm at 6 months and 1.67 ± 0.889 mm at 9 months post treatment. In case of group 2, the amount of defect fill at 3 months was -0.114 ± 0.263 mm, 0.193 ± 0.134 mm at the end of 6 months and 0.334 ± 0.175 mm at the end of 9 months post treatment.

The ‘t’ value was 5.790, 20.419 and 43.438 at the end of 3 months, 6 months and 9 months respectively suggesting that there was a statistically highly significant difference in the amount of defect fill at the end of 6 months ($p < 0.001$) and 9 months ($p < 0.001$) with the experimental site showing 5 times better result than control at the end of 9 months.

3.1.2. Percentage of original defect fill: (Fig. 3)

The ‘t’ value was 4.348, 21.503 and 73.310 at the end of 3 months, 6 months and 9 months respectively, suggesting that there was a statistically highly significant difference in the percentage of original defect resolved at the end of 6 months ($p < 0.001$) and 9

Table 1

Comparison of mean gingival index scores at baseline, 3 months, 6 months and 9 months interval.

Visit		N	Mean	SD	Min.	Max.	‘t’ value	‘p’ value
Baseline	Group 1	20	1.325	0.231	1.00	1.75	0.025	0.876
	Group 2	20	1.338	0.272	1.00	2.00		
3 Months	Group 1	20	1.035	0.207	0.50	1.25	1.537	0.223
	Group 2	20	1.125	0.250	0.75	1.75		
6 Months	Group 1	20	0.685	0.212	0.50	1.00	13.675	s0.001*
	Group 2	20	0.963	0.260	0.50	1.50		
9 Months	Group 1	20	0.438	0.197	0.25	1.00	29.646	<0.001*
	Group 2	20	0.800	0.224	0.50	1.25		

* Statistically significant at 5% level of significance ($P < 0.05$).

Table 2

Change from baseline (reduction) in probing pocket depth.

Visit		N	Mean	SD	Min	Max	't' value	'p' value
3 Months	Group 1	20	2.60	.681	2	4	55.183	<0.001*
	Group 2	20	1.25	.444	1	2		
6 Months	Group 1	20	3.95	.826	2	5	70.354	<0.001*
	Group 2	20	2.15	.489	1	3		
9 Months	Group 1	20	4.85	1.309	3	7	34.696	<0.001*
	Group 2	20	2.75	.910	1	4		

*Statistically significant at 5% level of significance ($P < 0.05$).**Table 3**

Comparison of baseline and post-operative changes (gain) in clinical attachment level.

Visit		N	Mean	SD	Min.	Max.	't' value	'p' value
3 Months	Group 1	20	1.85	0.489	1	3	25.599	<0.001*
	Group 2	20	1.10	0.447	0	2		
6 Months	Group 1	20	2.90	0.788	2	4	44.333	<0.001*
	Group 2	20	1.50	0.513	1	2		
9 Months	Group 1	20	3.55	0.999	2	5	41.341	<0.001*
	Group 2	20	1.80	0.696	1	3		

* Statistically significant at 5% level of significance ($P < 0.05$). **Fig. 2.** Comparison of post-operative change in defect fill at 3 months, 6 months and 9 months between Group 1 (Experimental group) and Group 2 (Control group).

months ($p < 0.001$) with the experimental site showing approximately 3 times better result than control at the end of 9 months.

4. Discussion

Anti-inflammatory effect of SV suggests a possible role in host modulation and it might be a newer agent as a host modulator in the management of periodontal disease.⁸ Bradley AD et al.¹⁸ found decrease amount of bone loss and inflammation in experimental periodontitis after local application of SV in rats. Host modulation property might be associated with decrease in pro inflammatory cytokines level in experimental group. Bone anabolic, anti-resorptive and possible host modulation property of SV have a significant potential for improving periodontal treatment outcomes.

Fig. 3. Comparison of percentage fill of original defect from baseline at 3 months, 6 months and 9 months between Group 1 (Experimental group) and Group 2 (Control group).

In the present study, the problems of clinical periodontal probing were minimized by using customized acrylic stents with guiding grooves for reproducible probing sites and directions as given by Rams and Slots.¹⁹ CAL was measured using occlusal acrylic stent with a metal wire incorporated in it as a fixed reference point and UNC 15 probe. To ensure reliability and reproducibility of data collected similar methodology was used by Quintero et al.²⁰ who found an added advantage of using a stent over cemento-enamel-junction as a fixed reference point and probes over silver point.

For Radiographic evaluation, mandibular bilateral molar regions were selected sites for the study as the film could be placed parallel and closer to the tooth and the thick bony trabeculae are aligned horizontally and closer to each other. In maxillary molar region chances of operator errors are more due to the overlapping of the palatal root over the osseous defect and variations in the anatomy of the palatal vault.²¹

In the present study, a significant decrease in gingival bleeding index (GI) has been found from baseline to 6 and 9 months in both the control and experimental site. The mean change in GI from baseline to at the end of 6 months at the experimental site was 0.640 ± 0.238 which was greater than that observed at the control site 0.375 ± 0.152 and statistically highly significant. This was in accordance with the findings of Pradeep AR and Thorat MS⁵ who also reported significant reduction in GI from baseline to at the end of 6 months between study groups. This reduction in experiment group was 2.32 ± 0.80 while in control group it was 0.50 ± 0.68 . A statistically highly significant reduction ($p < 0.001$) in mean GI has been reported over 9 months from baseline between study groups. This reduction in experiment group was 0.888 ± 0.275 while in control group 0.538 ± 0.147 . Better results by SV, suggests the additional anti-inflammatory effect of SV when applied in situ in conjunction with conventional periodontal therapy. A similar anti-inflammatory effect of SV was observed by Lindy et al.²² in patients with chronic periodontitis who were on systemic statin therapy.

A statistically highly significant ($p < 0.001$) reduction in Probing pocket depth were found within both groups compared to baseline at all-time intervals. A mean change of 3.95 ± 0.826 mm at the experimental site indicated a higher reduction in probing pocket depth compared to the control site which showed a mean change of 2.15 ± 0.489 mm over 6 months. This finding was in accordance with the findings of Pradeep AR and Thorat MS⁵ who reported a

higher reduction in probing pocket in experiment group (4.26 ± 1.59 mm) than control (1.20 ± 1.24 mm) over the period of 6 months from baseline. In this present study, a significant reduction in PPD has been reported from baseline over the period of 9 months with the mean change of 4.85 ± 1.309 mm in experiment group and 2.75 ± 0.910 mm in control group.

In context of Clinical attachment level in the present study, both the experimental and control sites showed highly significant ($p < 0.001$) improvement over 6 months with a mean gain of 2.90 ± 0.788 mm at the experimental site and 1.50 ± 0.696 mm at the control site. Again these findings were concurrent with that of Pradeep AR and Thorat MS⁵ who found a trend towards more gain in attachment level in experiment group (4.36 ± 1.92 mm) than control group (1.63 ± 1.99 mm) from baseline over the period of 6 months. A statistically highly significant ($p < 0.001$) gain in CAL from baseline has been found in this study with the mean change of 3.55 ± 0.999 mm at the experiment site and 1.80 ± 0.696 mm over the period of 9 months. These results demonstrated that the experimental site showed a higher clinical attachment gain (approximately 2 times) compared to control at the end of 9 months. These findings were in accordance with Rath A et al.²³ who found a trend towards gain in attachment level in both group with the better result in SV group.

A highly significant ($p < 0.001$) change in amount of defect fill was observed at the control and experimental sites over 6 and 9 months duration. A mean defect fill of 0.926 ± 0.713 mm and 1.67 ± 0.889 mm were observed at the experimental site over the period of 6 and 9 months respectively while, at the control site this value was 0.193 ± 0.134 mm and 0.334 ± 0.175 mm over the same period. The post-operative percentage of defect fill was greater in the experimental site (32.5%) compared to the control site (11.8%) at the end of 6 months, suggesting a possible role of SV in bone formation. These findings were in accordance with the finding of Pradeep AR and Thorat MS⁵ who demonstrated a significant mean percentage fill of original defect in experiment group (32.54%) compared to control (2.16%) from baseline over the period of 6 months. In this study, mean percentage of bone fill in experiment group was 60.7% in comparison to control group which was 20.8% over the period of 9 months. Nassar PO et al.,²⁴ Nyan M et al.,²⁵ Pradeep AR et al.,²⁶ Seto H et al.²⁷ also demonstrated that SV has bone anabolic property and shows osseous regeneration at defect site. This bone anabolic property may be because of increased bone morphogenetic protein-2 expression during bone regeneration,² anti-inflammatory effects⁸ and angiogenesis during wound healing.

Within the scope and limitations of this study, improvements in clinical and radiographic parameters both at experimental (Group 1) and the control (Group 2) sites were observed. However, the experimental site (Group 1) showed better results compared to control (Group 2) which was statistically significant.

5. Conclusion

It was observed that, reduction in gingival index (GI), Pocket depth (PPD) and gain in Clinical attachment level (CAL) were greater in the experimental site compared to control site. Amount of bone fill was also observed in greater amount than control group. These observations have suggested the use of non-space occupying host modulatory pharmacologic agent namely Simvastatin, in the form of in-situ gel as an adjunct to mechanical and surgical treatment approaches may be the future potential application in regenerative periodontal therapy. However, more randomized controlled trial to be conducted with long term follow up and with different vehicles using different concentrations of SV to proclaim the observations of this study.

Conflict of interest

The authors have none to declare.

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