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Comparative evaluation of efficacy of subgingivally delivered simvastatin and placebo gel in the treatment of stage ii periodontitis as an adjunct to scaling and root planing and its effect on porphyromonas gingivalis: A clinico-microbiological study

Vedika Halan
M.D.S. Periodontics, Bharati Vidyapeeth (Deemed to be University) Dental College and Hospital, C.B.D. Belpada, Sector 7, Navi Mumbai, 400614, Maharashtra, India.
*Corresponding author email: drvedikahalan22@gmail.com

Sneh Agrawal
Associate Professor, Dept. of Periodontology, Bharati Vidyapeeth (Deemed to be University) Dental College and Hospital, C.B.D. Belpada, Sector 7, Navi Mumbai, 400614, Maharashtra, India
Email: snehmds@gmail.com

Varsha Rathod
Professor & H.O.D, Dept. of Periodontology, Bharati Vidyapeeth (Deemed to be University) Dental College and Hospital, C.B.D. Belpada, Sector 7, Navi Mumbai, 400614, Maharashtra, India
Email: drvarshavora92@gmail.com

Palak Dwivedi
M.D.S Periodontics, Dept. of Periodontology, Bharati Vidyapeeth (Deemed to be University) Dental College and Hospital, C.B.D. Belpada, Sector 7, Navi Mumbai, 400614, Maharashtra, India
Email: palak.incantation@gmail.com

Aishwarya Kubal
M.D.S Periodontics, Dept. of Periodontology, Bharati Vidyapeeth (Deemed to be University) Dental College and Hospital, C.B.D. Belpada, Sector 7, Navi Mumbai, 400614, Maharashtra, India
Email: kubalaishwarya@gmail.com

Anirudh Rao
PG student, Dept. of Periodontology, Bharati Vidyapeeth (Deemed to be University) Dental College and Hospital, C.B.D. Belpada, Sector 7, Navi Mumbai, 400614, Maharashtra, India
Email: anirudhr6@gmail.com

Abstract---Aim: To evaluate the clinical efficacy of subgingivally delivered SMV and Placebo gel and compare in the treatment of stage II periodontitis when used as an adjunct to SRP and their antimicrobial effect on the Porphyromonas gingivalis bacteria. Materials & Methods: In a split mouth study design, 10 systemically healthy patients with stage II periodontitis were included in this study based on the inclusion and exclusion criteria. Two sites with deepest pockets were selected. Plaque samples were collected from these pockets at baseline followed by full mouth scaling and root planning, In Group I 1,2% SMV gel and in Group II Placebo gel was placed in the subgingival pockets. Microbiological analysis for Colony Forming Units of P.g was done at baseline after 3 months, Clinical parameters (GI, PI, PD, CAL, mSBI) were evaluated at baseline (before SRP), 1 month and 3 months. Result: Both the groups showed significant reduction in GI, PI, PD, CAL, mSBI and CFU of Pg but Group I showed more significant reduction than Group II. Conclusion: Subgingivally delivered 1.2% SMV gel when used as anadjunct to scaling and root planing had more encouraging results in clinical as well as microbial parameter in patients with stage II periodontitis.

Keywords---simvastatin, placebo gel, porphyromonas gingivalis.

Introduction

Periodontitis is an inflammatory disease of infectious origin characterized by progressive destruction of periodontal soft and hard tissues resulting in tooth loss.[1] The pathogenesis of periodontal disease involves a complex interaction of immune and inflammatory cascades initiated by bacteria of the oral biofilm.[2] Scaling and root planing (SRP) alone may fail to eliminate the putative pathogens from the pockets completely due to the invasion of these organisms within the gingival tissue or in deeper areas inaccessible to periodontal instrumentations. Thus, it may result in recurrence of periodontal disease. Therefore, the selective removal or inhibition of pathogenic microbes with systemic or locally delivered (LDD) antimicrobial and host modulating agents, in combination with SRP, is often considered as an effective approach at specific disease active sites.[3-5] Statins are therapeutic drugs used for locking the synthesis of cholesterol as it inhibits 3-hydroxy-3-methylglutaryl coenzyme A reductase. Statins also possess multiple pleiotropic actions such as anti-inflammatory, antioxidant, antithrombotic, angiogenesis promotion, immunomodulatory and increase in bone formation.[6]

Systemic administration of statin is observed to be associated with fewer signs of periodontal inflammation, beneficial effects on alveolar bone, decreased tooth

mobility and reduced risk of tooth loss in patients with periodontal disease when compared with SRP alone.[7-9] Simvastatin (SMV) is a semi synthetic statin which is produced by direct alkylation of lovastatin that is obtained by natural fermentation of fungus, Aspergillus terrus.[10] SMV is the optimal statin for controlling periodontal pathogens, such as Porphyromonas gingivalis (Pg) and Aggregatibacter actinomycetemcomitans (Aa) and can be used as a local delivery to acheive the periodontal health by periodontal regeneration.[11] However, clinical trials investigating the effects of different statins on adjuvant treatment of chronic periodontitis (CP) are limited. Pg is recognized as the keystone pathogen for initiation and progression of destruction of tooth supporting structures. Studies have demonstrated an increased risk of periodontal breakdown in Pg positive sites and better post treatment results in their absence.[12] Promoting periodontal regeneration by non-surgical therapy using SMV as a local drug delivery is always better than surgical intervention to restore periodontal health. The aim of the present study was to compare and evaluate the clinical efficacy of subgingivally delivered SMV and Placebo gel and compare in the treatment of stage II periodontitis when used as an adjunct to SRP and their antimicrobial effect on the Pg bacteria.

Materials and Methods

This is a randomized, single blind, comparative, split mouth clinical and microbiological study. The Institutional Ethics Committee approval was obtained from Bharati Vidyapeeth Dental College and Hospital, Navi Mumbai and the study was conducted. Patients, who reported to the Department of Periodontology of the institution, were screened, and enrolled in the study based on the inclusion and exclusion criteria. A total of 10 patients were selected and evaluated clinically and microbiologically at specific time intervals as described. The inclusion criteria included, (1) Subjects aged 18-60 years of either sex, (2) Subjects with stage II periodontitis (probing depth [PD] greater than or equal to 5mm but not more than 6mm and clinical attachment loss [CAL] of 3 to 4 mm) in at least 2 sites in different quadrants of the mouth. (3) Subjects who had not received periodontal therapy within preceding 6 months. The exclusion criteria included (1) Subjects with known history of any systemic disease. (2) with known or suspected allergy to the SMV group. (3) Subjects on systemic statin therapy. After enrollment of 10 patients with Stage II periodontitis, case history was recorded. As per the inclusion criteria, two sites with the deepest pocket were selected. They were randomly divided into two groups using a coin toss method.

- Group I: 1.2% SMV gel
- Group II: Placebo gel

Acrylic stents for the selected sites were prepared. Clinical parameters were checked, and plaque samples were collected at baseline before SRP.

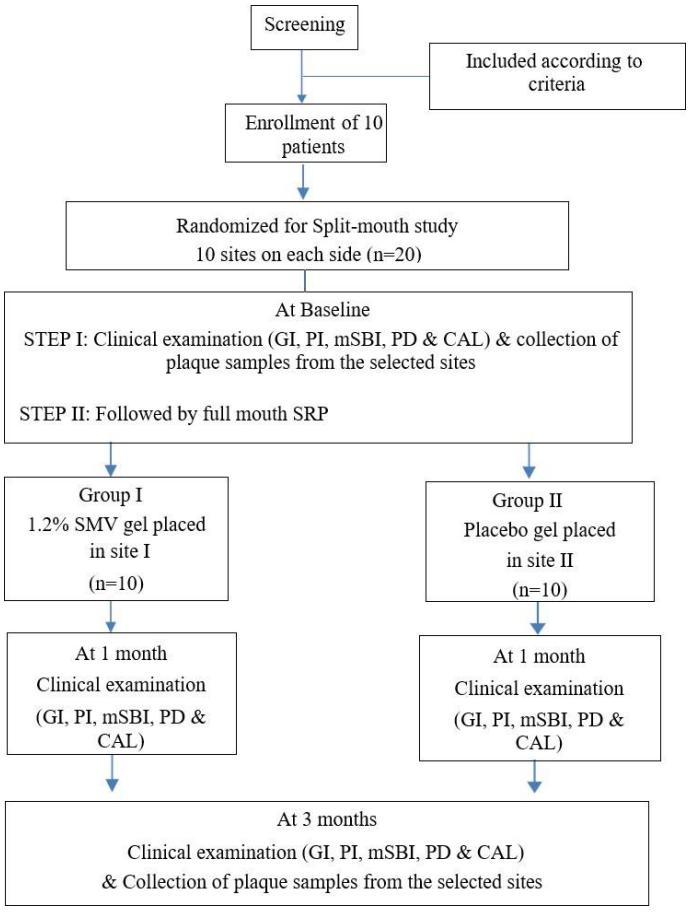
Clinical Parameters

- Full mouth Plaque Index (Silness & Loe - 1964)
- Full mouth Gingival Index (Loe & Silness - 1963)
- Site specific modified Sulcus Bleeding Index (Mombelli et al - 1987)

- Site specific Periodontal Status – Pocket Depth & Clinical Attachment Level

Microbiological Analysis

Site specific microbiological analysis will be done by taking plaque samples from both study group at the baseline visit and after 3 months. Analysis will be done to determine the total number of Colony Forming Units (CFU) of Porphyromonas gingivalis present inthe plaque samples.



Sample collection

- Excess saliva was removed using a sterile gauze pad to minimize the collection of transient contaminating bacteria from an exogenous source. Any apparent supragingival plaque was removed from the surface of the tooth to be sampled using a Gracey curette.
- A sterile, fine endodontic paper point was placed, using cotton pliers, in the sulcus of each site for each tooth to be tested until resistance was felt and then left in place for 20s. (Fig.1)
- Paper points were then placed in a plastic tube containing 1.0 mL of pre-reduced, anaerobically sterilized reduced transport media and then

transported to the laboratory at Maratha Mandal's Nathajirao Halgekar Institute of Dental Sciences & Research Centre for processing.

- Sample received in the Transport medium (RTF) were first vortexed, then it was diluted in RTF 1:10 proportion and inoculated in the culture medium according tothe requirement in enriched and selective medium.
- After a brief vortex for 30secs, a series of 10-fold serial dilutions of each sample was prepared. The suspended bacteria were streaked onto Blood agar fortified with Hemin and Vitamin K media.
- The plates were then be placed at 37°C in an anaerobic chamber with an atmosphere of 5% CO_2, 10% H_2 and 85% N_2.

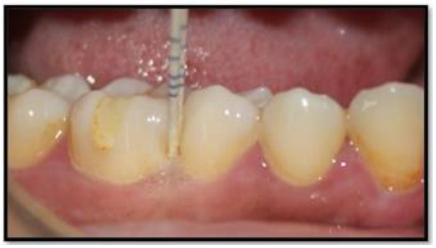


Fig 1. Collection of plaque samples

Identification of P. gingivalis isolates

Colonies that were black pigmented after 5–7 days of incubation were identified as P. gingivalis (Fig 2) and the colony count was done for quantification on colony counter.

Fig 2. Black pigmented colonies of P.gingivalis

Phase I therapy

SRP was performed at baseline. No antibiotics or antiplaque and anti-inflammatory agents were prescribed after treatment locally or systemically.

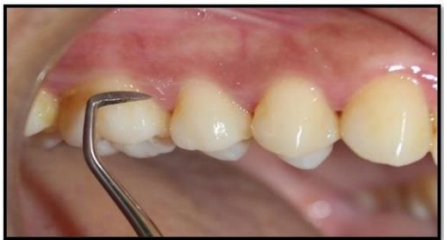


Fig 3. Phase I therapy

Formulation of 1.2% SMV in Situ Gel

After intensive in vitro investigations for optimization and stability, the SMV gel was developed at Dr. D. Y. Patil School of pharmacy, Navi Mumbai as described by A.R. Pradeep et al.[13] Methylcellulose in situ gel was prepared by adding the required amount of biocompatible solvent to an accurately weighed amount of methylcellulose. The vial was heated to 50-60 degree C and agitated using a mechanical shaker to obtain a clear solution. A weighed amount of SMV was added to the above solution and dissolved completely to obtain a homogeneous phase of polymer, solvent, and drug. The final product of SMV in situ gel was prepared with a concentration ;1.2%. The placebo gel was also prepared by the same technique except that SMV was not added.

Local Drug Delivery

For standardization, 0.1 ml of prepared SMV gel (1.2 mg/0.1 ml) was placed in test group (Fig 4) and 0.1 ml of Placebo gel was placed in control group using a sterile disposal syringe with a blunt cannula. No periodontal dressing was applied after delivery of the drug because the prepared formulation decreases in viscosity, which causes swelling and occlusion of the periodontal pocket. After placement of the in-situ gel, patients were instructed to refrain from chewing hard or sticky foods, brushing near the treated areas, or using any interdental aids for 1 week. Any discomfort or adverse effects were checked at recall visits.

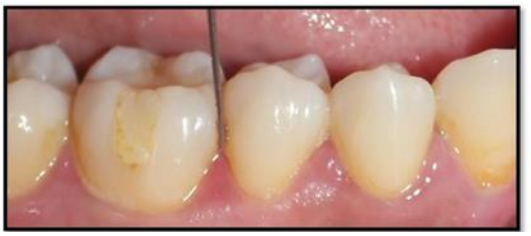


Fig 4. LDD using 1.2% SMV gel

Clinical parameters were repeated after 1 month and 3 months from the baseline after full mouth SRP. Microbiological parameters were repeated after 3 months from the baseline after a full mouth SRP.

Fig 5. PD at site I (baseline-5mm)

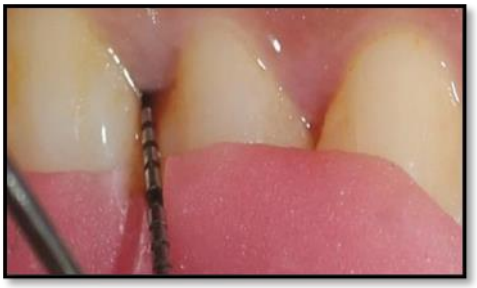


Fig 6. PD at site II (baseline-4mm)

Fig 7. PD at site I (3months-2mm)

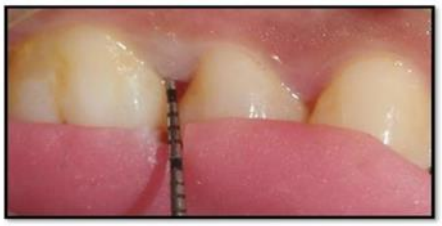


Fig 8. PD at site II (3months-3mm)

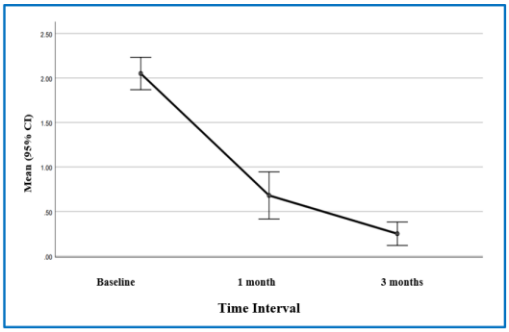
Results

Data of PD, GI, PI, SBI and CAL and CFU/ml were tested for normality using the Kolmogorov-Smirnov test for normal distribution. If the data was normal, differences between the two groups (at each time point) were analysed for differences using a paired-sample t-test. If data was non-normal, it was analyzed for differences using the Wilcoxon signed rank test (non- parametric). Within group analyses was done using the repeat measures analysis of variance (ANOVA) for each group followed by individual pairwise comparisons using the Bonferroni method. If data was non-normal, within group analyses was done using the Friedman test followed by individual pairwise comparisons using the Conover method. Data analysis was done using IBM SPSS software (Version 20.0 Chicago IL, USA)

All testing were done using two-sided tests at alpha 0.05 (95% confidencelevel). Thus, the criteria for rejecting the null hypothesis were a 'p' value of <0.05. Significant reduction in Mean PI score and Mean GI score from baseline to 1 month and from baseline to 3 months. ($p \leq 0.001$) The decrease in mean difference in the PI and GI scores from 1 month to 3 months was also statistically significant. ($p \leq 0.001$).

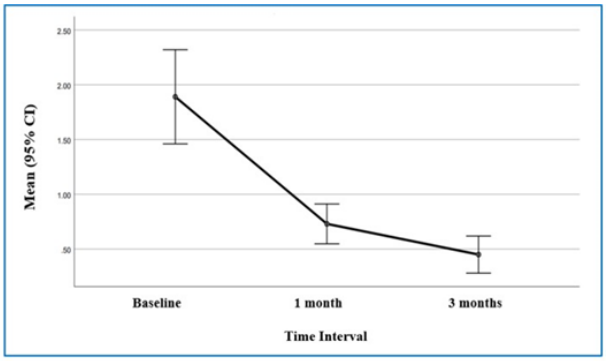
Table-4A: Mean PI Score at baseline, 1 month and 3 months	
PI	Mean ± SD
Baseline	2.05 ± 0.08
1 month	0.68 ± 0.11
3 months	0.25 ± 0.05

Table-4B: Comparison of PI at baseline, 1 month and 3 months				
PI		Mean Difference	Std. Error	P value
Baseline	1 month	1.37	0.06	0.001*
	3 months	1.80	0.07	0.001*
1 month	3 months	0.43	0.10	0.001*



Graph 1: Plot diagram showing decrease in PI from baseline (pre-treatment), to 1 month and 3 months (post-treatment)

Table 5A: Mean GI Score at baseline, 1 month and 3 months	
GI	Mean ± SD
Baseline	1.89 ± 0.60
1 month	0.73 ± 0.25
3 months	0.45 ± 0.23



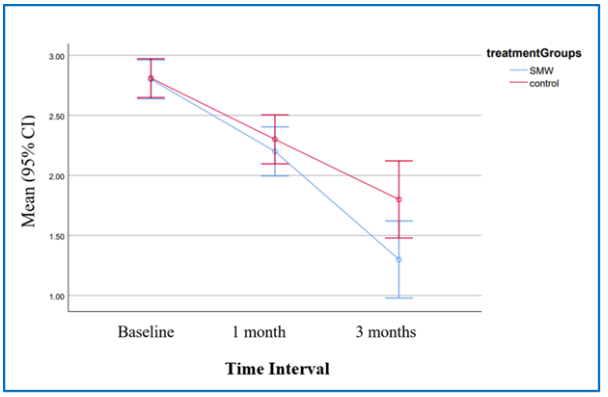
Graph 2: Plot diagram showing decrease in gingival index from baseline (pre-treatment), to 1 month and 3 months (post-treatment)

Table 5B: Comparison of GI at baseline, 1 month and 3 months				
GI		Mean Difference	Std. Error Mean	P value
Baseline	1 month	1.16	0.14	0.001*
	3 months	1.44	0.13	0.001*
1 month	3 months	0.28	0.08	0.02*

A significant reduction in mean modified sulcus bleeding index scores from baseline to 1 month and from baseline to 3 months for Group 1 and Group 2 was seen. (p≤0.001) The mean difference in modified sulcus bleeding index from 1 month to 3 months for Group 1 and Group 2 was statistically significant. (p≤0.001) Group 1 showed greater reduction in bleeding on probing than Group 2.

Table 6A: Mean mSBI Score at baseline, 1 month and 3 months		
mSBI	Mean ± SD	
	Group 1 (SMV)	Group 2 (Placebo)
Baseline	2.8 ± 0.25	2.8 ± 0.22
1 month	2.2 ± 0.34	2.3 ± 0.25
3 months	1.3 ± 0.48	1.8 ± 0.48

Table 6B: Comparison of mSBI in Group 1 and Group 2 at baseline, 1 month and 3 months							
mSBI		Group 1 (SMV)		Group 2 (Placebo)			
		Mean	Std. Error Mean	Mean	Std. Error Mean	P value	
						Group 1	Group 2
Baseline	1 month	0.6	0.10	0.5	0.08	0.001*	0.001*
	3 months	1.5	0.12	1.0	0.14	0.001*	0.001*
1 month	3 months	0.9	0.12	0.5	0.14	0.001*	0.02*



Graph 3: Plot diagram showing decrease in mSBI from baseline (pre-treatment), to 1 month and 3 months (post-treatment)

A significant decrease in mean probing depth from baseline to 1 month and from baseline to 3 months in for Group 1 and Group 2 was observed. (p ≤ 0.001) The

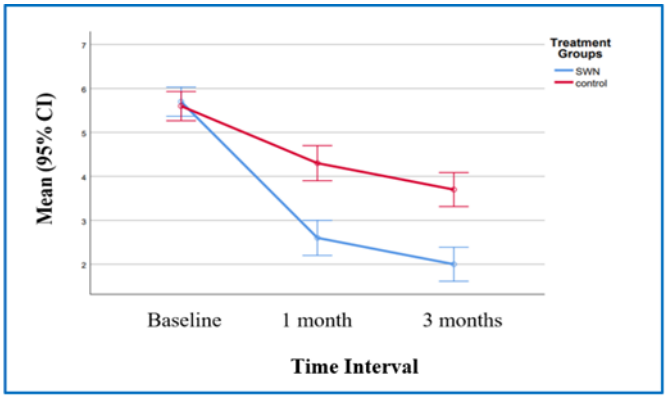
difference in mean probing depth from 1 month to 3 months Group 1 and Group 2 was statistically significant. ($p \leq 0.001$) However, Group 1 showed greater reduction in PD than Group 2.

Table 7A: Mean PD Score at baseline, 1 month and 3 months

PD	Mean ± SD	
	Group 1 (SMV)	Group 2 (Placebo)
Baseline	5.7 ± 0.48	5.6 ± 0.51
1 month	2.6 ± 0.51	4.3 ± 0.67
3 months	2.0 ± 0.47	3.7 ± 0.67

Table 7B: Comparison of PD in Group 1 and Group 2 at baseline, 1 month and 3 months

PD		Group 1 (SMV)		Group 2 (Placebo)			
		Mean	Std Error Mean	Mean	Std Error Mean	P value	
						Group 1	Group 2
Baseline	1 month	3.10	0.10	1.3	0.15	0.001*	0.001*
	3 months	3.7	0.15	1.9	0.23	0.001*	0.001*
1 month	3 months	0.6	0.16	0.6	0.22	0.01*	0.07



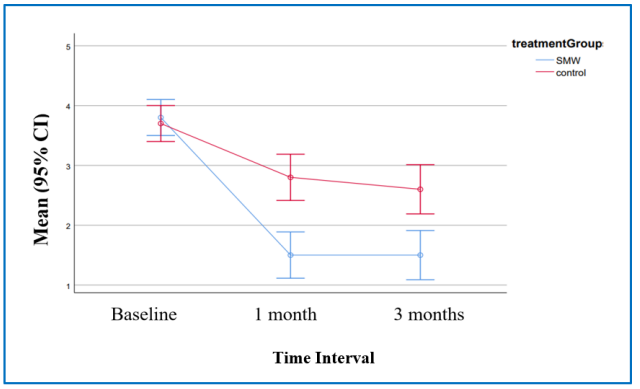
Graph 4: Plot diagram showing decrease in PD from baseline (pre-treatment), to 1 month and 3 months (post-treatment)

Mean clinical attachment level from baseline to 1 month and from baseline to 3 months for Group 1 and Group 2 significantly increased. ($p \leq 0.001$) The mean CAL gain from 1 month to 3 months for Group 1 and Group 2 was statistically non-significant. ($p > 0.05$) There was a significant CAL gain in Group 1 compared to Group 2.

Table 8A: Mean CAL Score at baseline, 1 month and 3 months

CAL	Mean ± SD	
	Group 1 (SMV)	Group 2 (Placebo)
Baseline	3.8 ± 0.42	3.7 ± 0.48
1 month	1.5 ± 0.52	2.8 ± 0.63
3 months	1.5 ± 0.70	2.6 ± 0.51

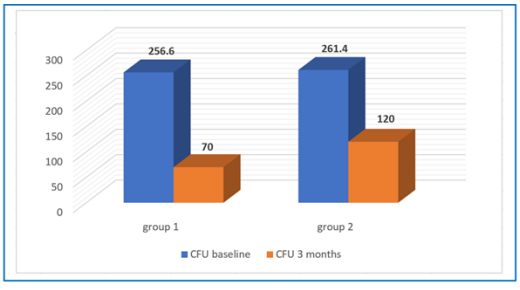
Table 8B: Comparison of CAL in Group 1 and Group 2 at baseline, 1 month and 3 months							
CAL		Group 1 (SRP + SMV)		Group 2 (SRP + Placebo)			
		Mean	Std Error Mean	Mean	Std Error Mean	P value	
						Group 1	Group 2
Baseline	1 month	2.3	0.15	0.9	0.10	0.001*	0.001*
	3 months	2.3	0.30	1.1	0.10	0.001*	0.001*
1 month	3 months	0.0	0.33	0.2	0.13	1.00	0.50



Graph 5: Plot diagram showing increase in CAL from baseline (pre-treatment), to 1 month and 3 months (post-treatment)

The decrease in mean bacterial load of P. gingivalis from baseline to 3 months for Group 1 and Group 2 was statistically significant. (p≤0.001) Group 1 showed greater reduction in bacterial load than Group 2.

Table 9: Comparison of CFU of P. gingivalis in Group 1 and Group 2 at baseline (pre-treatment) and 3 months (post-treatment)						
CFU (10^3CFU/ml)	Group 1 (SMV)		Group 2 (Placebo)			
	Mean ± SD	Std Error Mean	Mean ± SD	Std Error Mean	P value	
					Group 1	Group 2
Baseline	256.6 ± 37.4	11.8	261.4 ± 28.4	8.9	0.72	0.9
3 months	70.0 ± 42.5	13.4	120.0 ± 24.4	7.7	0.72	0.9
Change in CFU	186.6 ± 52.9	16.7	141.4 ± 36.7	11.6	0.001*	0.001*



Graph 6: Colony forming units of P. gingivalis at baseline (pre-treatment) and after 3 months (post-treatment) of Group 1 and Group 2

Both the groups did not show any significant difference in all the clinical and microbial parameters as p>0.05 at baseline (pre-treatment)

Table 10: Comparison of PD, CAL, mSBI and CFU in Group 1 and Group 2 at baseline (pre-treatment)

	Group 1 (SMV)		Group 2 (Placebo)				
	Mean ± SD	Std Error Mean	Mean ± SD	Std Error Mean	Mean Diff	SEM	P value
PD	5.7 ± 0.48	0.15	5.6 ± 0.51	0.16	0.1	0.22	0.66
CAL	3.8 ± 0.42	0.13	3.7 ± 0.48	0.15	0.1	0.20	0.62
mSBI	2.8 ± 0.25	0.81	2.8 ± 0.22	0.72	-0.01	0.10	0.92
CFU	256.6 ± 37.4	11.8	261.4 ± 28.4	8.9	-4.8	14.8	0.75

There was a highly significant difference between Pocket depth reduction and CAL gain in both the groups at 1 month post treatment (p≤0.001).

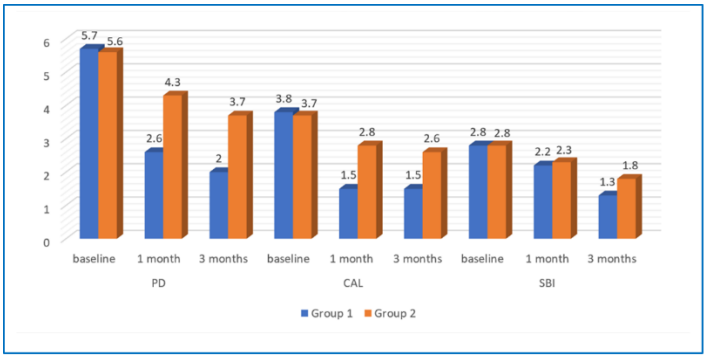
Table 11: Comparison of PD, CAL, mSBI and CFU in Group 1 and Group 2 at 1 month (post-treatment)

	Group 1 (SMV)		Group 2 (Placebo)				
	Mean ± SD	Std Error Mean	Mean ± SD	Std Error Mean	Mean Diff	SE M	P value
PD	2.6 ± 0.51	0.16	4.3 ± 0.67	0.21	-1.7	0.26	0.001*
CAL	1.5 ± 0.52	0.16	2.8 ± 0.63	0.20	-1.3	0.26	0.001*
mSBI	2.2 ± 0.34	0.11	2.3 ± 0.25	0.08	-0.1	0.13	0.47

There was a highly significant difference between PD reduction, CAL gain,Bleeding on probing and colony forming units of p.gingivalis in both the groups post treatment at 3 months (p≤0.001).

Table 17: Comparison of PD, CAL, mSBI and CFU in Group 1 and Group 2 at 3 months (post-treatment)

	Group 1 (SMV)		Group 2 (SRP + Placebo)				
	Mean ± SD	Std Error Mean	Mean ± SD	Std Error Mean	Mean Diff	SE M	P value
PD	2.0±0.47	0.11	3.7±0.67	0.21	-1.7	0.26	0.001*
CAL	1.5±0.70	0.22	2.6±0.51	0.16	-1.1	0.27	0.001*
mSBI	1.3±0.48	0.15	1.8±0.48	0.15	-0.5	0.21	0.03*
CFU	70±42.58	13.46	120±24.49	7.74	-50.0	15.5	0.001*



Graph 7: Comparison of PD, CAL and SBI at baseline (pre-treatment), 1 month and after 3 months (post-treatment) of Group 1 and Group 2

Discussion

Periodontitis is a microbial disease characterized by an inflammatory breakdown of the tooth-supporting structures. Dental plaque illustrates a classic example of both a biofilm and a microbial community.[14] The primary goal of a periodontal therapy is to inhibit the progression of the disease. This is achieved by the alteration of the oral microbiota attached to the root surfaces. Most treatments used for the control of chronic periodontitis are mechanical in nature. It has been shown that with subgingival debridement the total viable bacterial counts and pocket depths can be reduced.[15]

Scaling and root planing (SRP) is the most common form of mechanical therapy which includes removal of supra and subgingival plaque, necrotic cementum, and calculus deposits. The successful clinical effects of SRP are well documented (Morrison et al. 1980, Badersten et al. 1981, Lindhe et al 1983ab, Pihistrom et al.1983, Ramfjord et al. 1987, Kaldahl et al, 1993). These studies showed that SRP decreased clinical probing pocket depth and improved attachment level measurements particularly at sites where surgical access is difficult and areaswith deep pockets. The most desirable outcome of periodontal therapy is regeneration of the periodontal tissues lost during the course of periodontal disease.[16]

Cholesterol is a natural product of the liver; which sometimes can produce excess of cholesterol. Statins block the enzyme linked to the liver's cholesterol production, HMG-CoA reductase, hence, inhibiting the liver's ability to produce LDL. This leads to an increase in the number of LDL receptors on the surface of liver cells, resulting in more cholesterol being removed from the bloodstream and a reduction in risk for high cholesterol-related diseases. Thus, it is responsible for a large proportion of the pleiotropic effects of these drugs, such as improving endothelial function, immunomodulation, antioxidant activity and antimicrobial property.[17] Yazawa et al. studied the effect of SMV on human periodontal (PDL) cells in vitro and showed that, at a low concentration, SMV exhibits a positive effect on the proliferation and osteoblastic differentiation of human PDL cells.[18]

Advantages of using the subgingival drug-delivery system include achieving high intrasulcular drug concentrations, avoiding its systemic side effects, and better patient compliance.[19,20] Therefore, in our in vivo study SMV in situ gel

formulation was used to assess its benefits as a local drug-delivery system which has a controlled drug release. The dose of SMV used was 1.2 mg/0.1 ml injected per site in our study. It is well documented that the topical application of 1.2 mg of simvastatin (0.15% of the maximum recommended daily dose) will not produce an allergic reaction. A single topical dose of 1.2 mg simvastatin (0.15% of the maximum recommended daily dose) was administered to healthy adult subjects, so we did not anticipate any toxicity issues.[21] Stein et al. demonstrated that by reducing the SMV dose from 2.2 to 0.5 mg, there was a decrease in the inflammation to a more clinically acceptable level.[22]

In our present study there was decreased gingival bleeding index from baseline to 3 months in both the groups (test and control) however Group I (test group) showed greater reduction than Group II (control group) in the treatment of CP suggesting an anti-inflammatory effect of SMV. A similar anti-inflammatory effect of SMV was observed by Lindy et al. in patients with chronic periodontitis where patients on systemic statin therapy had 37% fewer pathologic periodontal pockets than those not taking statin medication.[8] The oral hygiene status depicted by plaque scores and gingival scores can influence the treatment outcome. In our study comparison between pre- and post- operative plaque scores was made using Silness and Loe (1964) Plaque Index.[23] The results in PI improved from baseline to 1 month and 3 months post-operative in all the CP patients and was statistically significant. The improvement in plaque index could be because of the SRP performed. The results are in accordance with studies done by Rajeev Ranjan (2017) and Pradeep et al (2010).[24,13]

The comparison between pre- and post-operative gingival scores was made using Loe and Silness (1963) Gingival Index.[32] In our present study, GI improved from baseline to 1 month and 3 months post-operative in all CP patients and was statistically significant. The improvement in gingival index could be because of the SRP performed. The results are in accordance with studies done by Ruoyan Cao et al (2019), N S Rao et al (2013) and Pradeep et al (2013).[11,25,26] Bleeding from the sulcus is the earliest clinical sign of gingivitis. It gives us the indication of the disease activity, and thus can be used to assess gingival health. Therefore, in our present study, site specific assessment of bleeding was done using criteria given by Modified Sulcus Bleeding Index (mSBI) (Muhlemann and Son, 1987).[27] Site specific modified sulcus bleeding assessment improved within the two groups from baseline to 1 month and 3 months post- operatively. The reduction from baseline to 1 month and 3 months within the groups indicate that each treatment was effective. The reduction in SMV group was statistically significant at

1 month however no statistically significant difference was seen at 3 months and in accordance with the studies by Ruoyan Cao et al (2019), Rajeev Ranjan (2017) and Pradeep et al (2010).[11,24,13] Clinical Probing depths in our study reduced in both the groups from baseline to 1 month and 3 months. This was in accordance with the studies conducted by Swati Agarwal et al (2016), Gayathri et al (2017) and Priyanka et al (2017).[5,28,29] In control group, the probing pocket depth reduced because of the beneficial effects of scaling and root planing. However, in SMV group, the probing pocket depths showed more reduction when used as an adjunct to SRP. This indicates that there was an enhanced benefit of SMV when used as an adjunct to scale and root planning in CP patients.

Shabnam Tahamtan (2020), stated that statins can accelerate epithelization and the rate of wound closure by inhibiting adhesion and extravasation of leukocytes into the site ofinflammation, which can result in reduced co-stimulation of T-cells and a reduction in inflammatory cytokines. These processes both facilitate wound healing during the early stages of wound repair and clinically reduce PD and gingival bleeding.[30] Clinical Attachment Level being the "gold standard" for evaluating the success of periodontal therapy. CAL gain was seen in both the groups from baseline to 1 month and 3 months. However, SMV group showed more CAL gain when compared to the control group. This was in accordance with the studies conducted by Ruoyan Cao et al (2019), Rajeev Ranjan (2017) and Pradeep et al (2010).[11,24,13]

C. Bodet (2007) stated that Porphyromonas gingivalis is a gram-negative anaerobic micro- organism involved in the pathogenesis of periodontitis.[31] Porphyromonas gingivalis was selected for micobiological analysis in our study as chronic periodontitis is a polymicrobial disease involving keystone pathogen such as Porphyromonas gingivalis (P.g) that can take over the adaptive immune response.[32] Therefore, elimination of this periodontal pathogen is the cornerstone of periodontal treatment. In our study Colony forming units of P.g was performed to analyze and quantify P.g count in subgingival plaque samples of CP patients as it is one of the most reliable methods for identification and quantification of microorganisms in accordance with Boutaga et al (2003).[33] Greater reduction in Porphyromonas gingivalis count was found in SMV group compared to control group from baseline to 3 months which was statistically significant. Thus, the result was in accordance with the properties of SMV attributing its antibacterial, anti-inflammatory, and inhibitory effects on periodontal pathogens involved in progression of chronic periodontitis.

For maintaining membrane integrity, cholesterol is an integral component needed by bacteria. Statins can counter bacteria by inhibiting the intermediate in the isoprenoid biosynthesis pathway necessary for membrane stability, which is substituted by cholesterol and protects bacteria from the toxic effect of statins. Statins, therefore, kill bacteria directly and by lowering accessible host cholesterol content for bacterial growth and protection. Such effects may be due to the disruption of teichoic acid structures reducing biofilm formation.[54] The hydrophobic nature of simvastatin may explain its antibacterial activity against periodontal pathogens where it disrupts the bacterial membrane in a "soap-like" manner causing its death.This explains the antibacterial property of SMV group.[34]

Hua Xie et al observed a significant correlation between probing depth and *P. gingivalis* degree of invasion. As the pocket depth increases there are ecological changes in the pocket, which then result in changes within the biofilm. The biofilm interactions present at shallow probing depths may have an inhibitory effect on the invasive capabilities of *P. gingivalis*, whereas at deeper probing depths the bacteria present may assist the survival of more invasive *P. gingivalis* strains. In addition, host mechanisms may be present that select for strains which are more invasive at deeper probing depths and that may not be present in shallow pockets. Thus, biofilm interactions may trigger changes that modulate the colonization of a particular P. gingivalis strain at deeper pocket depths, which

may shift the predominant strains to those that are more invasive. This justifies the reduction in pathogenicity of p.gingivalis after 3 months.

Our study showed significant difference in the plaque score, gingival index, sulcus bleeding index and sufficient decrease in the clinical probing depth and gain in clinical attachment level after using SMV gel as a local drug delivery agent when used as an adjunct to SRP. (P <0.001). However, further long-term trials of more than 3 months with larger sample size are needed to prove the efficacy of 1.2% SMV gel when used as an adjunct to non-surgical periodontal therapy. In future, other red complex bacteria involved in CP can also be included for the microbiological analysis.

Conclusion

Thus, it can be concluded that subgingivally delivered 1.2% SMV gel when used as an adjunct to scaling and root planing had more encouraging results in clinical as well as microbial parameter in patients with stage II periodontitis.

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