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Efficacy and Release Profile of Subgingivally Delivered Simvastatin Utilizing In Situ forming Implant as an Adjunctive in Treatment of Severe Chronic Periodontitis (A Randomized Controlled Clinical Trial)

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ABSTRACT

Introduction: This study is to compare the clinical efficacy; the release profile in gingival crevicular fluid and the bone fill following subgingival delivery of simvastatin using methyl cellulose gel or in situ forming implant as an adjunctive to periodontal debridement in the treatment of severe chronic periodontitis patients.

Subjects and methods: Twelve patients diagnosed with localized chronic severe periodontitis where two deepest pockets where selected at contralateral sides in a split mouth study. The patients were divided into two groups were Group I include 12 sites that will received 1.2mg simvastatin loaded in methyl cellulose gel as a local delivery system and Group II include 12 sites that will received 1.2mg simvastatin loaded in in situ forming implant local delivery system**.** Clinical and radiographic effectiveness the drug release profile into the GCF were observed.

Results: The obtained data revealed that Group II showed higher clinical improvement and more sustained release of the drug while both groups showed no significant difference in bone fill radiographically. Conclusion: It was concluded that in situ implant showed more sustained release, improved clinical with the same percentage of bone fill as methyl cellulose gel.

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Introduction:

Periodontal diseases occur as a result of the host immune inflammatory response to oral pathogens, that produces harmful byproducts and enzymes that break down extracellular matrices, as well as host cell membranes and lead to bone resorption, creating bony defects that may cause tooth loss [1()].

Non-surgical treatment; periodontal pocket debridement combined with personal plaque control is the treatment of periodontitis that have been validated to help in reduction of clinical parameters in 3 months. Pharmacotherapeutics may have an adjunctive role in the management of periodontitis either to slow the progression of the disease or to improve periodontal status in certain patients where it could be delivered systemically or locally [(2)]. Local application of chemotherapeutic agents into periodontal pocket suggested having the following advantages; in terms of rising drug concentration directly in the action site, preventing systemic side effects, and facilitating prolonged and controlled drug delivery to improve clinical signs of periodontitis [(3)].

Statins are specific inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A reductase and widely used to lower blood cholesterol levels**.** Recent studies showed that locally delivered statin pleiotropic properties including bone regeneration capacity as well as their anti-inflammatory effect when delivered or applied locally [(4)]. Various local drug delivery system for treating periodontitis have been introduced including; fibers, films, injectable systems, gels, strips, compacts and vesicular systems. The development of new injectable drug delivery systems has received considerable attention over the past few years [(5)].

This study was conducted to investigate the effectiveness of local application of 1.2mg simvastatin delivered in in situ forming implant system in terms of periodontal clinical parameters and radiographic bone fill and to monitor the drug release profile into the gingival crevicular fluid.

Subjects and methods:

Subjects selection:

Twenty four patients were recruited from the outpatient clinic of Oral Medicine, Periodontology and Oral Diagnosis department, Faculty of Dentistry, Ain Shams University and diagnosed with localized chronic severe periodontitis having stage III periodontitis, grades A with at least two teeth exhibiting probing depth (PD) ≥6 mm and clinical attachment loss (CAL) ≥4[(6)] where two deepest pockets where selected.

Simvastatin formulas preparation:

1) Preparation of simvastatin in methylcellulose gel (Sim/gel):

A 4% methyl cellulose 4000 C***ps*** gel was prepared by dispersing 2 gm of methyl cellulose powder in 50 ml of hot distilled water 50 - 60 ^{o}C. Then SMV was added to distilled water to produce 1.2% concentration of the drug in the gel. Continuous stirring is performed until cooling to obtain the gel form of homogeneous mixture of polymer [(7)]**.**

2) Preparation of simvastatin In-situ implant (Sim/insitu):

PLGA (15 % w/w) was dissolved in PEG 400 at 25 ^{o}C for 30 minutes under stirring in a glass vial and then simvastatin was added. The mixture was vortexed for 3 minutes, followed by 3 hours standing at 25 ^{o}C. The formulation was then sonicated for 10 min to remove air bubbles. The formulation was then stored at – 20^{o}C [(8)].

Patients grouping:

In a **randomized, comparative, contra lateral split mouth clinical trial**; two sites were selected in each enrolled patient representing the deepest pocket using randomized computer generated list:

Group I (sim/gel): Included 12 sites that received closed periodontal pocket debridement and application of 1.2 mg simvastatin loaded in methyl cellulose gel local delivery system.

Group II (sim/insitu): Included 12 sites that received closed periodontal pocket

debridement and application of 1.2mg simvastatin loaded in in situ forming implant as a local delivery system. (Fig.1)

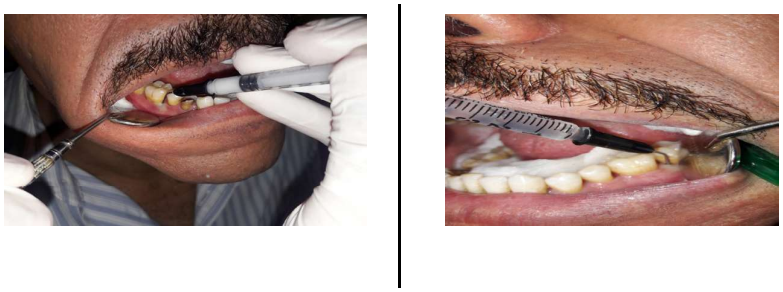

Fig (1). Injection of interventions in study sites

Radiographic evaluation:

Bone fill was measured on the radiograph by measuring the difference in vertical distance from the CEJ to the base of the defect by Digora software. Radiographic evaluation was carried **at baseline** and **after 6 months** from treatment. (Fig.2)

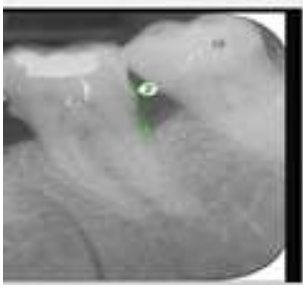

Fig. 2 picture showing radiographic evaluation of bone height

Release Profile Assessment:

Samples from the gingival crevicular fluid was collected from all selected sites at 1 day, 3, 7, 12 and 18 days after local application of the drug using standardized sterile periopaper ***(PerioPaper Strips, Oraflow, Plainview, NY).*** The sterile periopaper strips were inserted into the deepest part of each periodontal pocket for 15 seconds; then placed in Eppendorf tubes and stored in -80° C to be analyzed. [9] (Fig.3)

Fig. 3 Sample taking by periopaper

Simvastatin concentration was measured using High performance liquid chromatography:

HPLC device (Waters 2695 LC) separation module that includes: Pump with low pressure mixing system, vaccum degasser, water 996 PDA detector (200-600nm), auto sampler with a sample loop of 100 micro liter and a capacity of 120 vials and empower software.

Results:

Clinical assessment:

Plaque index (PI) & Gingival index (GI):

In both groups mean PI increased from baseline to 3 months. Reduction of plaque index occurred from 3 to 6 months the same value at 6 month interval in both groups. In both groups there was an increase in gingival index from baseline to 3 months however, in Group I the difference was significant. Reduction of gingival index occurred from 3 to 6 months with Group II showing lower value.

Table (1): ***Comparison between mean plaque index and gingival index in two groups and the changes by time within each group***

	Visit	Group I	Group II	P-value
PI	Baseline	0.42 (0.51)	0.58 (0.51)	0.218
	3 months	1.17 (0.58)	0.92 (0.51)	0.138
	6 months	0.75 (0.45)	0.75 (0.62)	0.500
GI	Baseline	0.08 (0.29)	0.08 (0.29)	0.500
	3 months	1.00 (0.60)	0.58 (0.51)	0.041
	6 months	0.75 (0.45)	0.67 (0.65)	0.360

Probing depth& Clinical attachment level (CAL):

In both groups after 3 months there was a decrease in mean PD with no statistically significant however, after 6 months Group II showed significant lower mean value than Group I. In both groups after 6 months there was a statistically significant reduction from baseline values. On comparing baseline values in CAL they decreased in mean values from baseline to 6 months.

Table (2): Comparison between probing depth and Clinical Attachment Level Gain in two groups and the changes by time within each group:

	Visit	Group I	Group II	P-value
PD	Baseline	7.50 (0.90)	7.08 (0.51)	0.090
	3 months	5.83 (0.94)	5.67 (1.15)	0.351
	6 months	5.25 (0.62)	4.75 (0.45)	0.017
CAL	Baseline	7.25 (1.60)	7.42(1.38)	0.394
	3 months	5.58(1.83)	6.00(2.04)	0.302
	6 months	5.00 (1.28)	5.08(1.16)	0.434

II)Evaluation of bone fill (marginal bone height):

By comparing the percentage of change in marginal bone height in both groups it was found that the percentage was slightly higher in Group I than Group II, yet statistically there was no significant difference.

Table (3): *Comparison of radiographic bone fill percentage between two groups:*

Radiographic results	%change	P-value
Group I (sim/gel)	20.55(6.52)	0.0000*
Group II(sim/insitu)	19.82(4.08)	0.0000*

IV) Simvastatin release profile:

Simvastatin release decreased from day 1 to day 18 in Group I and increased significantly in Group II but the difference in concentration was significant only at day 1. After 3 days the simvastatin release in Group I was significantly **lower** than in Group II. There was no statistically significant difference between both groups after 7, 12 and 18 days.

Table (4): *Simvastatin release profile the changes by time within each group*

	Group I (sim/gel)		Group II (sim/ insitu)		*P- value*
	Mean(µm)	*St Dev*	*Mean(µm)*	*St Dev*	
day 1	46.14[a] (8.10)		30.83[a] (6.01)		0.0000
day 3	33.26[a] (8.09)		38.90[a] (4.54)		0.024
day 7	34.68[b] (8.47)		34.16[b] (7.97)		0.439
day 12	35.08[b] (8.02)		41.81[a] (15.59)		0.098
day 18	36.60[a] (5.18)		38.16[a] (4.10)		0.210

Discussion:

Simvastatin drug was proved to have an anti-inflammatory effect by inhibiting expression of IL-6, IL-8, IL-1 ß and TNF-∂ level and an osteopromotive effect by inhibiting receptor activator of nuclear factor kB (RANK), RANK ligand (RANKL), promote the level of osteoprotegerin (OPG) and encouraging differentiation of osteoblasts and promoting neovascularization through its effect on BMPs and endothelial growth factor so it could promote regeneration of bone and healing and soft tissues [(10)].

Therefore, the present study was designed to evaluate the effect of injecting simvastatin locally in the periodontal pocket using two different local delivery systems as an adjunct for non-surgical periodontal treatment. Both groups showed significant improvement in clinical parameters, radiographic bone fill after 6 months of follow up. On comparing the mean concentration of the drug in GCF in **Group II (sim/insitu)** it showed more sustained release than in **Group I (sim/gel)**

Conclusion:

Both simvastatin in situ implant and gel delivery systems showed significant reduction in all measured clinical and radiographic parameters through the 6 months of follow up.

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