EVALUATION OF GINGIVAL CREVICULAR FLUID ALKALINE PHOSPHATASE IN POSTMENOPAUSAL WOMEN TREATED WITH SUBGINGIVAL SIMVASTATIN

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ABSTRACT

BACKGROUND

Women at menopause are found to be at risk for decreased bone density and chronic periodontitis due to oestrogen deficiency and common risk factors. Statins have anti-inflammatory and anti-resorptive properties and are successfully used in postmenopausal osteopenia. Increased alkaline phosphatase activity is observed in periodontitis. Thus, 1.2 mg Simvastatin gel was used subgingivally in this study. The level of alkaline phosphatase was evaluated in the sites treated as a biomarker.

MATERIALS AND METHODS

20 systemically healthy postmenopausal subjects were sampled. Two bilateral pockets (≥ 4 mm) in each subject were randomised. After phase I therapy, the selected sites were randomised as either site A or site B. The sites received either 1.2 mg Simvastatin in-situ gel or a placebo gel. An assessment of the probing depth and clinical attachment level was made on the baseline and at 3 months interval. Gingival crevicular fluid was tested for alkaline phosphatase levels on baseline, 14th day and 90th day post-operatively. Statistical analysis included descriptives, repeated measure ANOVA, Wilks' Lambda, paired and independent sample t-test.

RESULTS

The test and control sites demonstrated improvements in clinical parameters, which was statistically significant ($p < 0.05$). Further, the test sites showed statistically significant improvement over the control sites. A significant decrease in GCF alkaline phosphatase levels was observed in both test and control sites after 14 days. However, the test sites showed significantly higher values than control sites. Although not significant, the alkaline phosphatase levels further decreased in test sites when evaluated after 90 days.

CONCLUSION

The use of simvastatin in-situ gel in periodontal pockets showed satisfactory clinical improvement owing to its prolonged anti-inflammatory property. It can be a promising adjunct to conventional protocols employed in management of periodontal disease in postmenopausal subjects.

KEY WORDS

Alkaline Phosphatase, Chronic Periodontitis, Gingival Crevicular Fluid, Post Menopause, Simvastatin.

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BACKGROUND

Periodontal disease is a major public health concern with multicentric prevalence studies having reported a prevalence rate of more than 85% in Indian population.¹ Periodontitis is a chronic inflammatory disease, which eventually causes attachment loss and tooth loss. Although, periodontal disease is attributed to microbial infection, the host response and hormonal factors affect its incidence and progression.²

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Oestrogen deficiency in postmenopausal women is a contributing factor to osteopenia and a potential risk factor for periodontal disease. Dental plaque instigates an excessive response, which is elicited clinically as increased bleeding on probing and bone loss.³

Simvastatin is an inhibitor of hydroxymethyl glutaryl coenzyme A reductase primarily developed for treatment of coronary heart disease by serum cholesterol reduction. Statins have other non-cholesterol pleiotropic effects. Due to their anti-resorptive and bone anabolic effects, they constitute a major breakthrough in osteoporosis treatment along with bisphosphonates and oestrogen.⁴ Its anti-inflammatory and anti-oxidant properties are found beneficial in periodontal therapy with studies showing 37% reduction in periodontal pockets in patients on systemic statin therapy.⁵

Alkaline phosphatase is one of the first enzymes isolated from the GCF and recognised as a biomarker for periodontal status. During inflammatory states, it is secreted by the granular leukocytes.⁶ Increased alkaline phosphatase activity in relation to inflammation is observed in gingivitis and

periodontitis.⁷ Postmenopausal women with Chronic periodontitis were shown to have increased levels of ALP in GCF and the levels were found to decrease after periodontal therapy.⁸ A significant decrease in probing depth was documented in chronic periodontitis patients treated with local delivery of 1.2 mg Simvastatin.⁹

Menopause predisposes women to decreased bone density and increases their susceptibility to periodontitis. Simvastatin not only exerts bone anabolic anti-inflammatory effects, but also prevents progressive bone resorption. This research was formulated on these lines and it incorporated an assessment of the local alkaline phosphatase levels as a biomarker.

MATERIALS AND METHODS/ Objective

The evaluation of simvastatin delivered subgingivally as an in situ gel in postmenopausal subjects having chronic periodontitis. The site-specific level of alkaline phosphatase was assessed for its use as a biomarker.

Trial Design

This was a randomised clinical trial. The subjects were evaluated prospectively for 3 months after intervention on two contralateral sites.

Sampling and Randomisation

A study examiner enrolled the subjects selectively. This was done in the Periodontology Department of JSS Dental College. The ethical clearance was obtained from the Institutional Review Board and an informed written consent was taken from the subjects.

Twenty postmenopausal subjects between 45 and 55 years of age with no significant medical history, non-smokers and untreated for periodontitis were selected. In each patient, two contralateral pockets measuring ≥ 4 mm were selected and randomised as site A and site B by toss of a coin.

Clinical Examination

Florida probe was used to record the probing depth and attachment level. This was performed on the baseline and day 90. The gingival crevicular fluid was sampled from the test and control sites at baseline, 14 days and 90 days. This was done by the study examiner.

Intervention

The simvastatin gel and a placebo gel were formulated and sent in matching sealed bottles (A and B) by the pharmaceutical personnel. The study therapist thoroughly performed scaling, root planing and subgingivally delivered the gel A in site A and gel B in site B (Figure 1).

Preparation of 1.2 mg Simvastatin in situ Gel and Delivery

1.2 milligrams of simvastatin powder was dissolved in methanol solvent, to which 8 mL of distilled water and 2.4 grams of polymer were added to prepare the gel. The selected pockets were isolated and 0.1 mL of the Simvastatin in situ gel or the placebo gel was deposited a syringe. The area was covered by a periodontal dressing. Patients received post-operative instructions.

Biochemical Analysis

After isolation, 2 microns of GCF was collected using a 5-micron micropipette from the gingival sulcus (Figure 2) and transferred to sterile vials having 2 mL Tris carbonate buffer.

The vials were sent for estimation of alkaline phosphatase level using an autoanalyzer (CHEM-5 PLUS®). The hydrolysis of p-Nitrophenyl phosphate to yellow coloured p-Nitrophenol and phosphate is catalysed by alkaline phosphatase enzyme at a pH of 10.3. The change in absorbance measured by autoanalyser at 405 nm wavelength is proportional to ALP activity in the sample. This is the Wilkinson's adaptation of the Bessey and Lowry method employed in the estimation of the level of alkaline phosphatase.

Statistical Analysis

The SPSS for Windows version 16.0 was used. The change in the parameters was assessed with descriptive statistics and repeated measure ANOVA test. The change, the multivariate tests, paired "t", Wilks' Lambda tests were used for comparisons over the durations in both the test and the control. The independent samples "t" test was used to compare values between the two groups over the durations. The correlation of alkaline phosphatase levels to probing depth and clinical attachment levels was done using Pearson's correlation test.

RESULTS

Initially, 55 postmenopausal subjects were considered. Only 20 subjects satisfied all the criteria. They underwent the proposed treatment and evaluation [Figure 3]. No patient reported with discomfort. The change in GCF Alkaline Phosphate level from baseline to 3 months was considered in calculation of the power of the study. The group sample sizes of 20 achieved 94% power. The mean change in control group was 8.0 $\pm$ 17.0 and 24.0 $\pm$ 17.0 in test group. Both the groups demonstrated a statistically significant ($P < 0.05$) probing depth decrease and clinical attachment gain from baseline to 90 days [Table 1]. When the test and control groups were comparatively checked for decreased probing depth and clinical attachment level gain from baseline to 90 days, the results were statistically significant in the test (Simvastatin) group [Table 2]. Both test and control groups showed significant decrease in GCF alkaline phosphatase levels after 14 days with the control sites showing significantly higher values than control site. After 90 days, although not significant the alkaline phosphatase levels further decreased in test sites and increased in control sites (Table 3 and 4). The correlation of GCF alkaline phosphatase levels to the clinical parameters namely probing depth and clinical attachment level was found to be statistically insignificant (Table 5).

Parameter	Time Intervals	Simvastatin Sites	Placebo Sites
PPD	Baseline	6.200 $\pm$ 0.52	6.150 $\pm$ 0.59
	3 months	4.450 $\pm$ 0.72	4.900 $\pm$ 0.788
	P-value	<0.001	<0.001
CAL	Baseline	6.250 $\pm$ 0.71	6.200 $\pm$ 0.76
	3 months	4.400 $\pm$ 0.68	4.900 $\pm$ 0.78
	P-value	<0.001	<0.001

Table 1. The PD, CAL and GCF ALP within the groups at Different Time Intervals

Statistically significant at $P < 0.05$. PPD: Probing Pocket Depth, CAL: Clinical Attachment Level.

Parameter	Simvastatin Sites	Placebo Sites	P-value
PPD			
Baseline	6.200 ± 0.52	6.150 ± 0.59	0.778
3 months	4.450 ± 0.72	4.900 ± 0.788	0.062
CAL			
Baseline	6.250 ± 0.71	6.200 ± 0.76	0.833
3 months	4.400 ± 0.68	4.900 ± 0.78	0.038

Table 2. The PPD and CAL between the groups over Different Time Intervals

Statistically significant at $P < 0.05$. PPD: Probing Pocket Depth, CAL: Clinical Attachment Level

Parameter	Simvastatin Sites	Placebo Sites	P value
GCF ALP			
Baseline	32.634 ± 12.87	30.992 ± 11.97	0.678
14 days	10.310 ± 4.33	16.970 ± 8.16	0.003
3 months	8.597 ± 3.18	22.95 ± 14.96	<0.001

Table 3. The GCF ALP between the groups over Different Time Intervals

Statistically significant at $P < 0.05$. GCF ALP: Gingival Crevicular Fluid Alkaline Phosphatase.

Parameter	Time Intervals	Simvastatin Sites	Placebo Sites
GCFALP	Baseline	32.634 ± 12.87	30.992 ± 11.97
	14 days	10.310 ± 4.33	16.970 ± 8.16
	90 days	8.597 ± 3.18	22.95 ± 14.96
	P-value	<0.001	<0.001

Table 4. The GCF ALP within groups at Different Time Intervals

Statistically significant at $P < 0.05$. GCF ALP: Gingival Crevicular Fluid Alkaline phosphatase.

Group	GCF Alkaline Phosphate Versus	PPD	CAL
Control	Pearson Correlation	0.052	-0.471
	'p' value	0.826	0.036
Test	Pearson Correlation	0.042	0.184
	'p' value	0.860	0.437

Statistically significant at $P < 0.05$. PPD: Probing Pocket Depth, CAL: Clinical Attachment Level. GCF ALP: Gingival Crevicular Fluid Alkaline phosphatase.

Figure 1. Subgingival Delivery of In Situ Gels

Figure 2. Collection of GCF from Periodontal Pocket Site for ALP Estimation

Figure 3. Consort Flow Chart for Patient Enrolment, Allocation, Follow-Up and Analysis

DISCUSSION

The present randomised controlled trial was conducted in a group of postmenopausal women to assess the benefits of simvastatin in situ gel delivered locally into periodontal pockets.

The pathogenesis of periodontitis is explained by the interaction of microbial dental plaque and host defence mechanisms. Other host associated mechanisms and hormonal status can affect the course of periodontal disease by inflammatory response to infection causing bone loss.² The variations in secretion of sex hormones during puberty, menstruation, pregnancy and menopause are found to be associated with increased gingival inflammation.¹⁰ Oestrogen deficiency after menopause is found to be a dominant pathologic factor for osteopenia or decreased bone mineral density. This may cause decreased alveolar density and increased susceptibility to bone resorption due to periodontal inflammation.¹¹ Studies in oestrogen deficient postmenopausal women reported a 3-fold increase in alveolar resorption and systemic oestrogen therapy resulted in decreased gingivitis.¹² Since prevalence of osteopenia and tooth loss increase with advancing age and they share risk factors the postmenopausal period remains vulnerable to periodontal disease progression. Thus, the study was designed to benefit the postmenopausal women.

A split mouth design was employed to randomise the two selected pocket sites in each of the 20 patients. This protocol prevents intra-patient variations, as the sites are treated by random selection in the same session.¹³

The goal of periodontal therapy is to eliminate inflammation, arrest worsening of the disease process and create an environment conducive to maintenance of health. Scaling and root planing constitute the corner stone of periodontal therapy, which reduces inflammation pocket depth and increases CAL. Adjunctive use of antibiotics suppress the remaining pathogens.¹⁴ Local drug delivery in the subgingival target area is a reservoir that can provide higher therapeutic doses of the agent with fewer applications over longer periods with better patient acceptance when compared to systemic therapy.^{15,16}

Simvastatin is a synthetic statin widely used for hypercholesterolemia and osteopenia. It is found to inhibit bone resorption and increase osteoblast activity by increasing the expression of bone morphogenetic protein 2.¹⁷ Simvastatin inhibits isoprene modification of signal transducers of inflammation, thus expressing its anti-inflammatory effect. It decreases production of many inflammatory cytokines and matrix metalloproteinases.¹⁸ Local application of Simvastatin is known to upregulate periodontal regeneration through the osteoblastic differentiation of human periodontal ligament cells.¹⁹ Statin medication in osteopenic patients reported fewer periodontal pockets.⁵

In an attempt to harness the local effects of simvastatin, it was used as a subgingivally delivered in situ gel here. Another research indicated that 1.2 mg% could be optimal through clinical and laboratory assessment.⁹ Similarly in this study, the statin sites showed statistically significant improvements when compared to placebo sites.

Simvastatin gel has been used as a local delivery agent in Type II diabetic patients with chronic periodontitis with beneficial results.¹⁹ The local delivery of simvastatin in grade II furcation defects and in periodontal pockets enhances the

outcome of mechanical therapy resulting in reduced pocket depths, improved attached gain and bone fill.^{20,21}

Alkaline phosphatase has been measured in GCF to examine the relationship between periodontal conditions and disease activity. Increased alkaline phosphatase activity in relation to inflammation is observed in gingivitis and periodontitis.²² Since alkaline phosphatase alterations in its GCF levels possibly provides a site specific indication of periodontal disease activity, in this study we have used GCF ALP as a biomarker to assess periodontal condition. The test and control groups showed significant decrease in GCF alkaline phosphatase levels 14 days with the test site showing significantly higher values than control site. After 90 days, although not significant the alkaline phosphatase levels further decreased in test sites and increased in control sites. A previous study on GCF alkaline phosphatase levels in postmenopausal women demonstrated significantly higher GCF alkaline phosphatase levels in periodontitis group. The levels markedly reduced after phase I therapy with a positive correlation between GCF alkaline phosphatase and probing depth.⁸

CONCLUSION

The results prompt that the subgingival delivery of 1.2 mg simvastatin in-situ gel is a promising and cost effective adjunctive to scaling and root planing, as it showed significant improvements in periodontal health. The results also support the assessment of GCF ALP as a diagnostic marker of periodontal disease activity. The study is limited by the relatively small sample size and shorter duration of assessment. This encourages the prospects of a bigger research for more conclusive outcomes.

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Abbreviations

ALP: Alkaline Phosphatase.

CAL: Clinical Attachment Level.

GCF: Gingival Crevicular Fluid.

PD: Probing Depth.

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