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Original Article

Effect of atorvastatin gel on clinical parameters and bone histology in alveolar ridge preservation: A randomized clinical trial

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ABSTRACT

Background: The aim of the study was to evaluate the dimensional changes of the alveolar ridge in postextraction sockets filled with 1.2% atorvastatin (ATV) gel covered with a collagen membrane.
Materials and Methods: This study is a split-mouth randomized clinical trial. A total of 30 postextraction sockets of single-root teeth of 15 patients were randomly allocated into two groups: (a) socket filling with 1.2% ATV gel and covered with an absorbable collagen membrane (n = 15) and (b) socket covered with a collagen membrane (n = 15). Four clinical indices – the width and height of sockets and the width and thickness of keratinized gingiva – were measured at baseline and 2 months after the intervention. Two histological parameters, namely the percentage of osteogenesis and the thickness of bone trabeculae, were also measured 2 months after the intervention. Data were analyzed by Mann–Whitney and Wilcoxon tests using SPSS software ($P \leq 0.05$).
Results: Two months after extraction, dimensional changes in height ($P = 0.07$), width of the alveolar socket ($P = 0.381$), and keratinized gingival thickness ($P = 1$) showed no significant difference between the two groups. In both the groups, the keratinized gingival width increased significantly, but there was no significant difference between the two groups ($P = 0.347$). The percentage of lamellar bone formation in the test group was about 28% higher than that of the control group ($P = 0.098$), and the thickness of trabeculae in both the groups was similar ($P = 0.78$).
Conclusion: Although 1.2% ATV gel increased lamellar bone formation and reduced dimensional changes in postextraction sockets, the differences were not significant compared with the control group.

Key Words: Alveolar ridge augmentation, atorvastatin, osteogenesis, tooth socket

INTRODUCTION

Following tooth extraction, the physiologic remodeling process of soft and hard tissues results in alveolar ridge dimensional changes in terms of height and width, which vary according to the alveolar socket, mucosal thickness, metabolic factors, and functional forces. Dimensional changes of the alveolar ridge were reported for horizontal bone resorption in the range of 63%–29% and vertical bone resorption in the range of 22%–11% within 6 months after tooth extraction.[1] The pattern of resorption is more significant in the first 3–6 months and less

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intense during the remainder of life.[2,3] Approximately 50% of the alveolar ridge thickness decreases within 6–12 months after tooth extraction.[4] Tissue loss after extraction is physiological and one of the unintended consequences of tooth loss. Changes in the dimensions of the alveolar ridge make it difficult to place a conventional bridge or implant, and in severe cases, implant placement is problematic[5] and requires complex treatments such as bone grafting, which result in increased treatment costs and multiple treatment sessions.[6,7] Many techniques have been proposed for ridge preservation, but until now, there is still no consensus concerning which technique or biomaterial is most suitable for this purpose.[8,9] In this way, more evidence regarding methods and biomaterials is essential to make the procedures reproducible and more predictable.

Statins are drugs first used in patients with hypercholesterolemia to lower cholesterol by inhibiting coenzyme 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase.[10] They have also been studied because of their anabolic effects on bone tissue. Statins increase the expression of bone morphogenetic protein-2 (BMP2) and vascular endothelial growth factor, which significantly impact bone regeneration therapy.[11,12] The use of statins, due to their effect on bone cells, can improve the quality and quantity of bone after tooth extraction and prevent further bone resorption. The role of atorvastatin (ATV) in osteogenesis has been recently highlighted in some studies, and it has played an important role in maintaining the dimension of the alveolar ridge.[13] Topical use of simvastatin causes bone formation in extracted teeth and is an effective method for rapid bone regeneration after tooth extraction.[12] Randomized clinical trials have evaluated statin gel for the treatment of periodontal bone defects,[14-17] and systematic reviews[18] have shown favorable results for bone regeneration in infrabony defects.

Thus, this study aimed to evaluate alveolar bone dimensional changes and bone trabecular thickness in postextraction sockets filled with 1.2% ATV gel covered with a collagen membrane.

MATERIALS AND METHODS

Study design

This split-mouth, double-blind randomized clinical trial was conducted in accordance with the Helsinki Declaration at the Department of Periodontology, Isfahan University of Medical Sciences. The methodological workflow is illustrated in Figure 1. The Ethics Committee of Isfahan University approved the research under registration number IR.MUI.MED.1398.107. This study was registered in the Iranian Registry of Clinical Trials under the number IRCT20110109005570N11. Written informed consent was signed by all patients, and participation was voluntary.

Outcomes measures

The primary outcomes were vertical and horizontal dimensional changes (bone loss) in postextraction sockets between the test and control groups. The secondary outcomes were changes in trabecular thickness, and the tertiary outcomes were the width and thickness of keratinized gingiva.

Selection criteria

The inclusion criteria for patient selection were as follows: at least 18 years of age, requiring single-root tooth extraction, and presenting a minimum of 10 mm vertical bone height without invading adjacent critical structures. The exclusion criteria included smokers, pregnant patients, systemic disease, periapical or acute periodontal infections, bone wall fracture at the time of tooth extraction, and statin use for hypercholesterolemia.

Sample size, randomization, and blinding

The sample size was calculated to be 15 patients assuming $\alpha = 0.05$, $\beta = 20\%$, and a study power of 80%. Clinical examination was performed to select individuals with at least two single-root teeth requiring extraction in different quadrants and not adjacent to each other. In this split-mouth clinical trial, in each patient, one dental socket was selected as the case and the other socket as the control. Randomization for selecting the socket in each patient was done by sealed envelope. The envelopes were numbered, and each socket was assigned a number. The pathologist, patients, and data analysts were blinded to the allocated arm.

Production of 1.2% atorvastatin gel

The ATV gel was synthesized by mixing 1.2% ATV powder (Amin Pharmaceutical Company, Isfahan, Iran) with 30% pistachio resin and 2% oleo-gum-resin. It was then mixed with polyethylene glycol and gelation materials to become injectable. Oleo-gum has disinfecting properties and solidifies in the presence of blood, filling the socket. It can also serve as a matrix for ATV and enable its sustained release

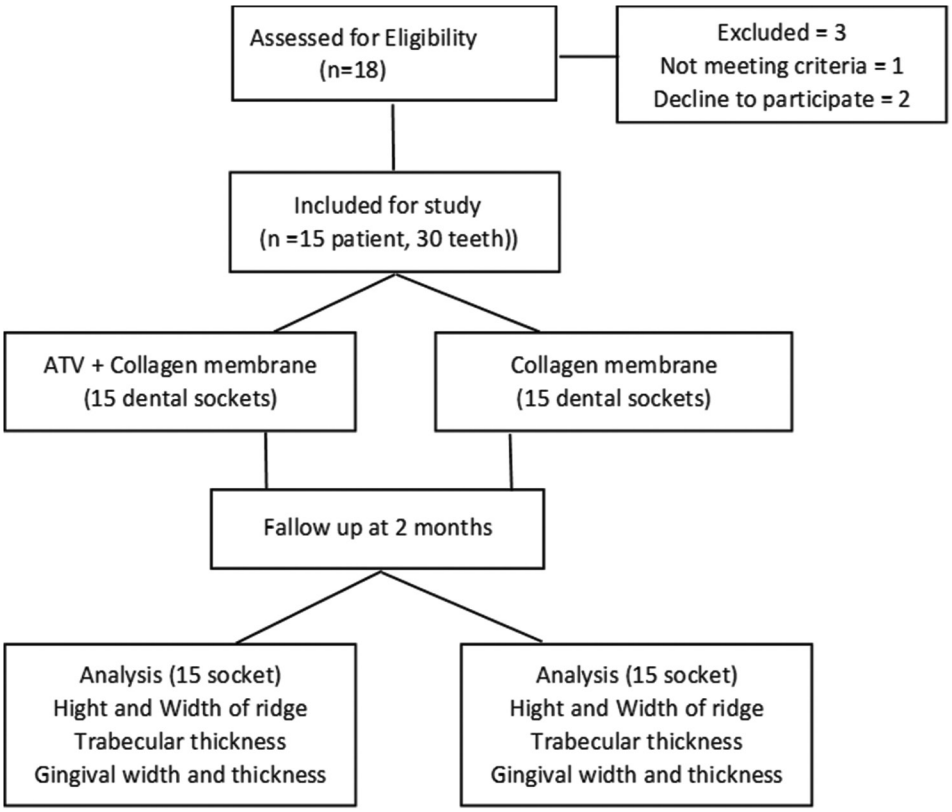

Figure 1: Flow diagram of the study.

within 6 weeks. The homogeneity of the formulation was assessed macroscopically after 48 h (presence of palpable folliculated particles and also in terms of color and transparency) and microscopically after 24 and 48 h, 1 week, and 1 and 3 months to ensure homogeneity. The pH of the compound was also measured at 48 h to 3 months after synthesis. Stability against centrifugal force, thermal stability, freezing and melting temperatures, rheological properties, viscosity, dispersion, microbial contamination, and the release rate of ATV were all evaluated using respective methods.

Surgical procedures

The same surgeon (N.H.) conducted all surgical procedures. After signing informed consent forms, all patients underwent oral and dental examination and received the required periodontal treatments to optimize oral conditions for wound healing. Patients received oral prophylaxis 1 week preoperatively and were instructed to rinse with 0.12% chlorhexidine mouthwash for 1 min twice daily. For the surgical procedure, local anesthesia (lidocaine 2%, Aburaihan Pharmaceutical Co., Tehran, Iran) was first administered, and atraumatic tooth extraction was performed using a fine periotome and forceps. The width (KGW) and thickness (KGT) of keratinized gingiva were measured with a periodontal probe (Williams Probe, Hu-Friedy) perpendicular to the bone before flap elevation. The sockets were thoroughly curetted, irrigated with sterile saline, and inspected for perforations, fenestration, or dehiscence. Thereafter, the mucoperiosteal flap was reflected 5 mm beyond the alveolar crest on the buccal side, and a titanium pin was fixed 4 mm apical to the crest of the ridge. The height of available bone was measured from the crest of the edentulous ridge to the opposing landmark (titanium pin) with a periodontal probe. The titanium pin served as a reference for assessing the vertical dimension of bone and as a guide for bone core harvesting. The width of available bone was measured between the facial and lingual plates at the crest of the alveolar socket using a caliper. Then, 0.5 ml of ATV gel was randomly chosen to fill the sockets and subsequently covered with a collagen membrane (Collprotect membrane, Botiss). In the control socket, only the resorbable membrane was placed. The membrane was first hydrated in sterile saline for 5 min and trimmed to completely cover the extraction socket, extending 3 mm beyond it. The flap was then repositioned.

No attempt was made to cover the socket with the flap. In case of membrane exposure into the oral cavity, it was left exposed. Horizontal cross-mattress resorbable sutures were used to stabilize and fix the membrane. Patients were prescribed 500 mg amoxicillin three times daily for 10 days, as well as analgesics and 0.12% chlorhexidine twice daily for 4 weeks. Sutures were removed 2 weeks after surgery. Patients were recalled for implant placement after 2 months. Following administration of local anesthesia, all clinical parameters were measured as described earlier. KGW and KGT were measured with a probe. A mucoperiosteal flap was elevated, alveolar width was measured with a caliper at the crest of the ridge, and bone height from the crest of the ridge to the titanium pin was measured with a periodontal probe.

Bone core harvest

A trephine with a 3 mm internal diameter and 6 mm length was used to obtain a core sample of bone. Harvested bone cores were immediately placed in 10% neutral buffered formalin. The osteotomy was then widened for implant placement according to standard protocol. Patients were followed according to standard clinical protocols until completion of implant restoration.

Bone core analysis

The biopsy specimens were decalcified in 5% nitric acid for 1 week, sectioned, and the margins marked with India ink. They were subsequently transferred into a tissue processor, immersed in 10% formalin, and subjected to ascending concentrations of alcohol (60%, 70%, 80%, 90%, and 100%). They were immersed in xylol and embedded in paraffin blocks after 13–17 h. The paraffin blocks were sectioned into 3.5-µm slices by a microtome, deparaffinized in xylol, dehydrated in descending concentrations of alcohol, and stained with hematoxylin and eosin. Slides were inspected under a light microscope (Nikon E400, Japan) at magnifications of ×100 and ×400 to determine the percentage of osteogenesis (woven, cancellous, and lamellar bone). The thickness of bone trabeculae was measured by histomorphometric analysis. All measurements were made by a single expert pathologist.

In histological studies, the percentage of bone formation (woven, spongy, and lamellar) and, in histomorphometry studies, bone trabecular thickness was measured.

Interim analyses and stopping guidelines

No interim analyses were performed, and no stopping guidelines were established.

RESULTS

In this split-mouth clinical trial, 15 patients (11 men and 4 women) with an average age of 41.33 years underwent surgical extraction of two single-root teeth in two adjacent quadrants.

Primary outcome: Ridge preservation

Alveolar ridge dimensions (height: from the crest of the edentulous ridge to the opposing landmark [titanium pin] and width: between the facial and lingual plates at the crest of the alveolar ridge) were measured immediately after extraction and before implant placement. No statistical differences between the case and control groups were noted [Tables 1 and 2].

Secondary outcome: Histologic analysis

The percentage of lamellar bone formation and trabecular thickness in the experimental group was greater than in the control group, but the differences were not significant [Table 3, Figures 2 and 3].

Table 1: Comparison of the relative height of bone before and after of intervention

Relative height of crest to pin (mm)	Test group	Control group	Significant level*
Baseline	4.21 ±./54	4.13 ±./61	1/00
After 2 months	3.73 ±./88	3.13 ±./74	0/087
Significant level**	0/0003	0/0001	

*Mann–Whitney test; **Wilcoxon test

Table 2: Comparison of width of ridge before and after of intervention

Ridge width (mm)	Test group	Control group	Significant level*
Base line	6.46±1.06	6.56±0.99	0.896
After 2 months	5.66±1.39	5.20±1.26	0.038
Significant level**	0.006	0.001	

*Mann–Whitney test; **Wilcoxon test

Table 3: Mean histological indices measured in both groups after 2 months

	Test group	Control group	Significance level*
Percent of lamellar bone formation	50/51±36.77	22.50±17.98	0.098
Percent of woven bone formation	7.11±52.25	7.45±53.25	0.584
Trabecular thickness	2.58±3.30	2.54±3.40	0.782

*Mann–Whitney test

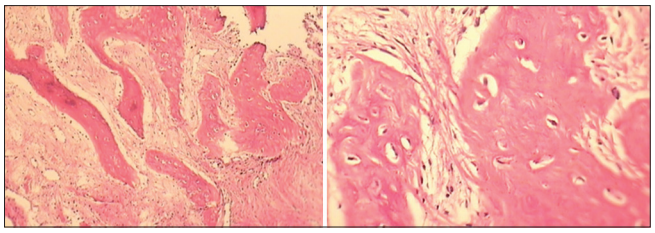

Figure 2: Lamellar, woven, and cancellous bone formation after 2 months. Test group (×100 and ×400).

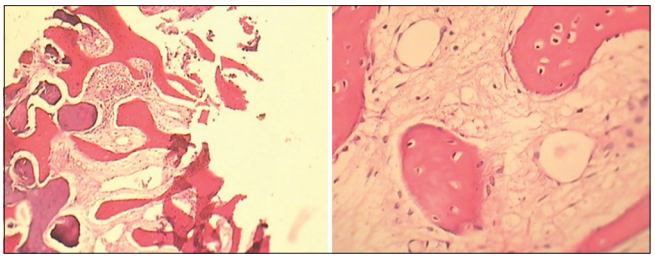

Figure 3: Lamellar, woven, and cancellous bone formation after 2 months. Control group (×100 and ×400).

Tertiary outcome: Soft tissue changes

Evaluation of keratinized gingival width after 2 months showed that in the experimental group, the mean keratinized gingival width increased from 5.33 ± 1.45 to 7.33 ± 1.83 mm (P = 0.000). In the control group, the mean keratinized gingival width increased from 5.53 ± 1.40 to 7.46 ± 1.45 mm 2 months after the intervention (P = 0.000). In both the groups, the mean keratinized gingival thickness increased after 2 months, but there was no significant difference between the two groups (P = 0.347).

DISCUSSION

This study aimed to evaluate the efficacy of ATV gel with a collagen membrane for ridge preservation after tooth extraction. The results showed that ATV resulted in less ridge dimensional change following tooth extraction; however, the difference between the two groups was statistically nonsignificant.

Some studies reported a significant reduction of alveolar ridge changes following ridge preservation methods. However, most of these studies used bone graft materials.[19,20] The lack of agreement between our results and those studies may be related to this point. No graft material was used in this study; we only used ATV gel. The lack of a grafting material may have resulted in the absence of a scaffolding effect.

Statins have anabolic actions. Statins promote osteoblasts to synthesize BMP2, a growth factor that causes osteoblasts to differentiate, proliferate, mature, and form new bone *in vitro* and *in vivo*, as well as inhibit adipocyte differentiation. Simvastatin and ATV have been shown to reduce alveolar bone loss. ATV is known to inhibit osteoclastic bone resorption and is proposed to have osteostimulative properties.[21-23] These studies favor statins as an effective material for bone regeneration. In the present study, ATV was chosen as it has been recognized as a safe and low-priced drug. This study showed that the application of ATV can enhance bone formation in extraction sockets, though not significantly, and our study is in accordance with Bertl *et al*.,[23] Sezavar *et al*.,[24] Jenabian *et al*.,[25] and Yaghobee *et al*.[26]

Our results are not in agreement with some studies, which may be attributed to several factors. One may be variability in follow-up times. Our assessment was done after 2 months, compared to 6 months in the studies of Shirke *et al*.,[27] Martande *et al*.,[28] and Pradeep *et al*.[29] It has been shown that alveolar ridge remodeling begins after tooth extraction and continues for several months, with the majority of changes occurring in the first 3 months.[2] Therefore, the 2-month healing period was selected for assessment of the quality and quantity of clinical and histologic changes of bone and soft tissue.

Most studies have employed simvastatin, while few experimental studies used ATV. It is important to highlight that ATV was used because it has fewer side effects than simvastatin and superior kinetics.[30] Therefore, new generations of statins have been investigated in recent studies. There have been very few studies reporting the use of 1.2% ATV for alveolar ridge preservation.

Different assessment methods may influence the results. Shirke *et al*.[27] evaluated the clinical efficacy of 1.2% ATV in bone defects using cone-beam computed tomography at baseline and 6 months later and reported significantly greater bone fill. In this study, histologic findings showed more lamellar bone formation in the test group, but woven bone and trabecular thickness in both the groups were similar, and all findings were not significant.

Martande *et al*.[28] reported greater bone defect reduction following platelet-rich fibrin combined with 1.2% ATV gel. This may be due to the benefit of the synergistic effect of various regenerative materials.

Although there are no exactly similar studies, consistent reports in this field have shown comparable results. Sezavar *et al*.[24] investigated the application of simvastatin in bone regeneration of the alveolar ridge after tooth extraction. The study concluded that normal bone was found in both the groups. Although the vital and trabecular bone in the simvastatin group was higher than in the other group, the results were not statistically significant.

The present study did not observe a significant effect of ATV on soft-tissue healing. The width and thickness of keratinized soft tissue in both the groups were similar. This finding is in agreement with Cruz *et al*.[31]

A small sample size and the lack of a control group with no intervention were among the limitations of this study. Future studies with a larger sample size and a no-intervention control group are recommended to obtain more reliable results.

CONCLUSION

Although the differences between the test and control groups were nonsignificant regarding alveolar ridge dimensions, the quantity of newly formed lamellar bone was greater in the ATV gel group compared with the control. Further research in this area is needed to clarify the role of statins in alveolar ridge preservation.

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Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or non-financial in this article.

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