Evaluation and Comparison of Histologic Changes in Extraction Sites Grafted with Simvastatin mixed with Nanobone

Original Article

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ABSTRACT

Objective: The objective of the present study was to compare the effect of mixing simvastatin with Nanobone graft on the healing process of extraction sockets regarding to the histological parameter.

Materials and Methods: In a prospective randomized clinical study, ten patients (study group, Group II) treated by simvastatin mixed with Nanobone after tooth extraction. The other ten patients (Control group, Group I) received Nanobone only. Three months after tooth extraction and socket preservation, histological biopsies were taken at the time of implant placement. The biopsies were evaluated in the terms of the histological parameter for the identification of vascularization and bone metabolism factors.

Results: The use of simvastatin combination results in slightly higher values of mineralized area of newly formed bone andnumerous well differentiated capillary vascularization.

Conclusion: Simvastatin and Nanobone combination showed improvement in socket preservation.

Key Words: Simvastatin Nanobone, Socket preservation.

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INTRODUCTION

Dental implant has been used in the rehabilitation of completely and partially edentulous patients[1]. The good aesthetic and successful functional restoration of an implant depend on its optimal placement, which influenced by implant height and buccolingual position as well as by the dimensions of the alveolar ridge[2]. The alveolar ridge undergo significant changes following tooth extraction[3], alveolar ridge resorption observes following tooth extraction[4].

An average amount of alveolar bone loss was 5 to 7 mm within the first year of postextraction, an two third of this bone resorption occurred during the first three months[5]. The most relevant alteration occured in the buccal plate of the alveolar ridge where the bone is thin[6]. Tan *et al*. 2012 reported that, during the first six months of postextraction there were 11 % to 22 % of the vertical bone loss and 29 % to 63 % of the horizontal bone loss without socket preservation technique[4].

Alveolar ridge resorption and remodeling after tooth removal is a natural healing phenomenon, which can negatively affect the placement of the implant[7]. The remaining alveolar ridge should be preserved in order to meet the contemporary requirements of the prosthetically guided implant placement. Different socket preservation techniques have been used to counteract the alveolar ridge resorptions following tooth extraction. The socket preservation techniques ranging from flapless atraumatic tooth extraction[8 and 9], immediate implant placement[10], socket preservation by using bone grafting materials with and without barrier membranes[8 and 9].

Animal and human studies showed the beneficial effect of socket preservation after tooth extraction[3]. Bone autograft, allografts, xenografts and most recently growth factors have been evaluated with varying degrees of success to preserve the alveolar ridge dimensions following tooth extraction. Nanotechnology has been used for periodontal tissue regeneration. Several studies demonstrated the significalt effect of nanoscale geometry and topography on the cell differentiation and regeneration[11]. Nanobone (Artoss Co, Germany) consisted of synthetic nano crystalline hydroxyapatite and silica fabricated in sol/gel process. The silica gel stimulate the formation of collagen and bone[6]. Nanobone increases osteoblasts proliferation better than the deproteinized bovine bone mineral[12]. The inflammatory reaction is less in Nanobone graft than β-tricalcium phosphate graft[13]. Simvastatin is used for the treatment of arteriosclerosis and hyperlipidemia. In addition, simvastatin exerts antiinflammatory and immunomodulatory actions. Simvastatin modulate the formation of the bone by increasing the bone morphogenetic protein-2[14]. It has also been suggested that simvastatin directly affect osteoclasts because statins exert their effects

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by inhibiting the mevalonate pathway[15]. Statins was able to prevent alveolar bone loss and enhance new bone formation[16].

Alveolar bone resorption reduction after tooth extraction by using completely absorbed graft materials is promising concept. We hypothesized that combination therapy of statin and Nanobone may improve the healing quality of the extraction sockets better than when it used separately.

MATERIALS AND METHODS

Trial design:

The presented study was designed to examine the effect of a combination therapy of statin with Nanobone on the postextraction sockets healing process prepared for dental implant placement in a prospective controlled randomized clinical trial. There were no changes in the trial design after commencement.

Participants:

The study performed in the Department of Oral Medicine and periodontology, Faculty of Dentistry, South Valley University from January 2015 to May 2019. Before participation all the study patients were informed about the study and signed written informed consent.

Grouping and randomization:

20 consecutive patients with at least one lower first premolar tooth indicated for extraction and replacement by implant were enrolled in this study. Block randomization was performed using blocks of four (BBAA, BAAB, BABA, ABBA, ABAB, AABB) that allocated individuals into control and test groups. The randomization sequence was generated by a statistician who was not involved in the care of patients. Extraction sockets of 10 patients received Nanobone (Artoss GmbH, Rostock, Germany) and considered as a control group (I) while the other 10 extraction sockets received a combination therapy of Nanobone and statin (Corvast 80 mg, Egyphar, Egypt) and considered as a test group (II).

Inclusion criteria:

1. Patients need extraction of lower first premolar (right or left).
2. Age range between 18 to 35 years.
3. Presence of periapical radiographic changes related to the tooth to be extracted.
4. Clinically the probing depth less than 3 mm.
5. Technique of extraction is conventional intraalveolar forceps method under local anesthesia.

The exclusion criteria:

1. Patients with systemic diseases affecting bone metabolism.
2. Large periapical radiographic changes related to the tooth to be extracted, in the form of abscess, granuloma or cyst.
3. Tooth need trans-alveolar extraction.

The Surgical Procedures:

All operations were done by the same surgeon. On the day of tooth extraction the interventions included local anesthesia (2 % xylocaine with 1:100,000 epinephrine), atraumatic extraction of the teeth following complete mobilization then curettage done for the extraction socket. Intrasulcular incision done extending along the study tooth to the neighboring teeth. Buccal and lingual full-thickness flaps were elevated that did not extend beyond the mucogingival junction. Atraumatic tooth extraction was then carefully performed by using periotome and the appropriate dental forceps to minimize surgical trauma for the surrounding hard tissue and the socket walls. At this point, extraction socket was filled with Nanobone granules in group I while in group II, the extraction socket was filled with a combined therapy of simvastatin and Nanobone. Interrupted sutures were used to reposition the flap over the augmented socket in all cases (Figure 1). Patients were instructed to apply pressure on the gauze pack over the operation site for a period of thirty minutes. Plaque control was advised using 0.2 % chlorhexidine gluconate mouthwash for the first postoperative week, 1 minutes / three times a day. 500 mg amoxicillin antibiotics and 400 mg ibuprofen were prescribed three times a day for five days.

Surgical reentry for implant placement:

Regular follow up was done for all patients. Any arising complications at the first postoperative week such as pain, dry socket, pus discharge or swelling were recorded. Periapical x ray was taken immediately after extraction and at six months postoperatively. After Three months, the sites were reentered for implant placement. A full thickness mucoperiosteal flap was elevated to expose the area of socket preservation. By using a trephine drill with diameter od 2.3 mm (Komet Inc., Lemgo, Germany), a minimum depth of 7 mm core bone biopsy has been taken from the center of the site. After the harvesting of the sample of the bone, the preparation of the implant osteotomy site was completed at the same site and an implant was placed (Impla, schuetz dental group, Germany) according to the recommended surgical protocol by manufacturer, then the mucoperiosteal flap was closed with interrupted sutures (Figure 2).

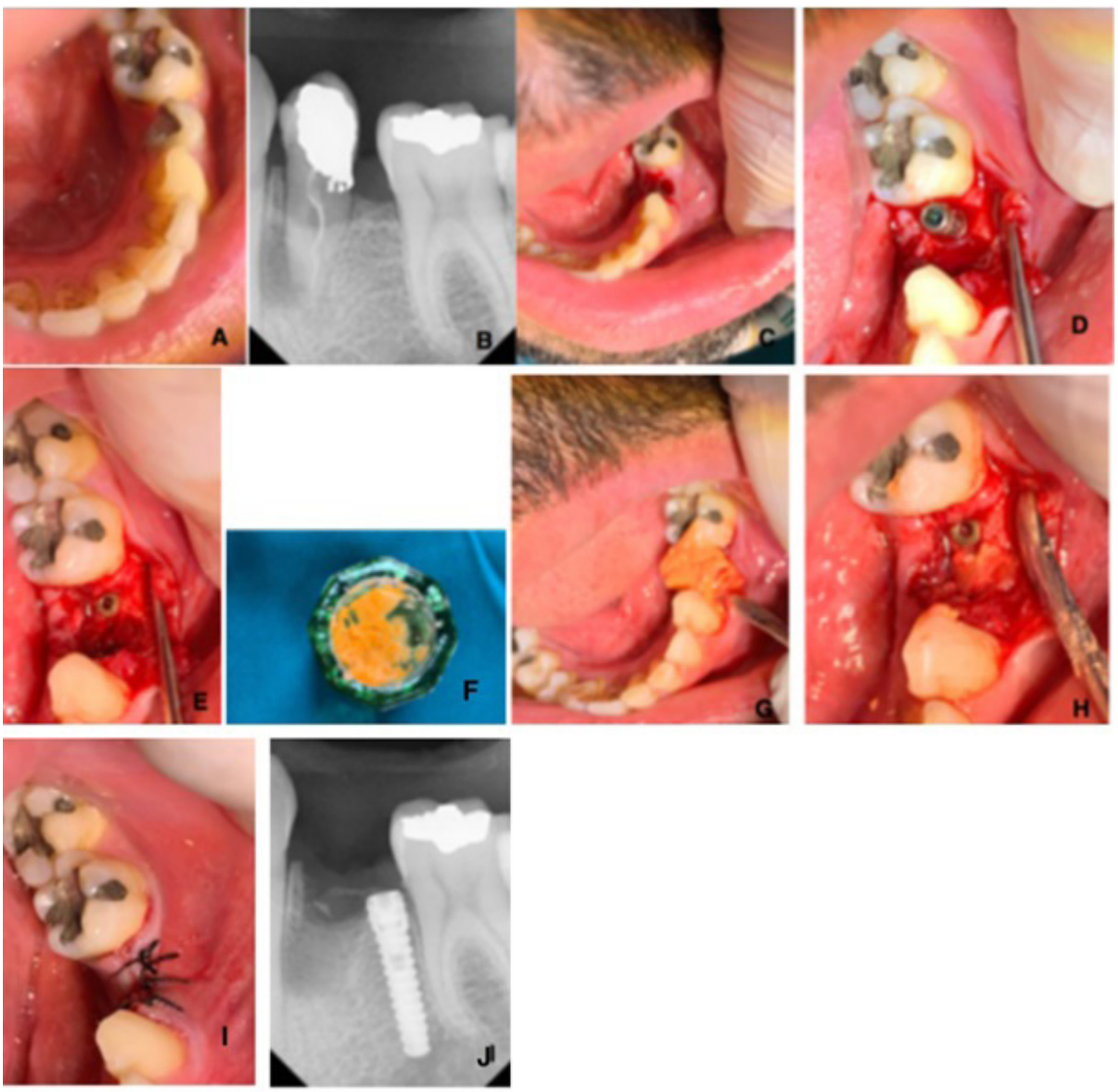

Figure 1: Showing socket preservation using nano bone mixed with Simvastatin:

A: Lower left first premolar indicated for extraction. B: Preoperative periapical X ray film. C, D and E: Tooth extraction and flap preparation. F: Nano bone mixed with simvastatin. G and D: Placemen of nano bone and simvastatin in the socket. J: Postoperative periapical X ray film.

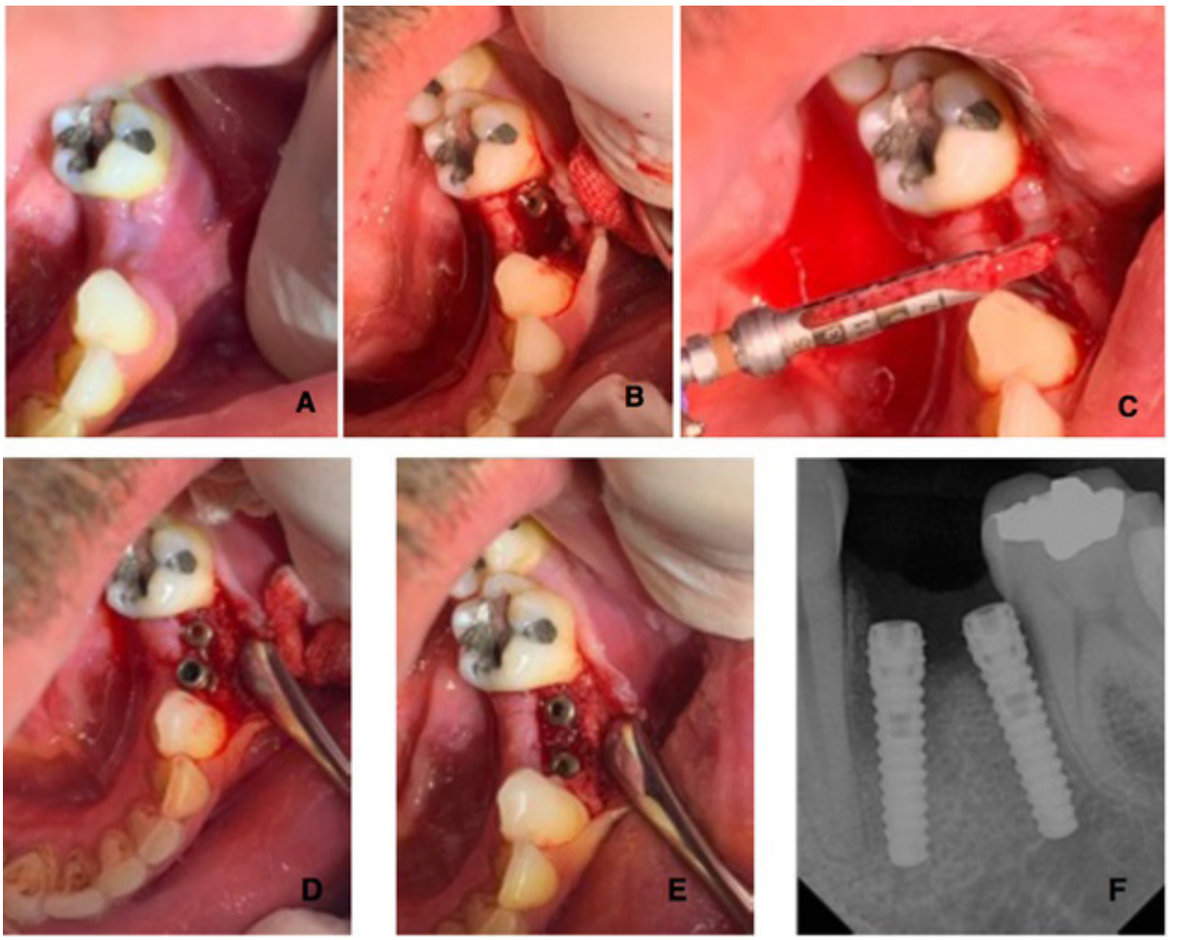

Figure 2: Showing surgical reentry for implant placement: ridge three months after socket preservation (A), drilling for implant site preparation and obtaining bone core biopsy (B and C), implant placement in augmented socket (0 and), postoperative periapical Xray (F).

Histological Evaluation:

The biopsies were stored in 10 % buffered formalin, decalcified in EDTA then processed for hematoxylin-eosin and Masson's trichrome stains. Each specimen was evaluated histologically.

Histological evaluation:

According to the basis of established scoring methods in histology and pathology, the qualitative and semiquantitative evaluation of the histological sections was done[17] and also according to the applied methods in the similar studies[18 and 19]. Blind evaluation done by two professional investigators in three different sections of the serial sections. The interested representative regions were localized in the section center in apically, coronally or laterally. The stained slides with Masson's trichrome investigated using an Olympus microscope with half photo adaptor. Digital images were captured by a ToupView digital camera with objective lens for a magnification of X4. 7 images with resolution of 300 dpi from each samples of the both group were digitally analyzed with Fiji Image processing software. The parameters assessed were the total tissue area, including unmineralized bone or osteoid area, granulation tissue and remaining bone substitute[18].

RESULTS

According to the study clinical protocol all patients were treated. All patients completed the study and there were no postoperative complications. 20 histological samples were evaluated histologically for bone metabolism and vascularization factors identification. Mineralized area of newly formed bone were observed by microscopic analysis at x100 magnification and were scattered in all the Simvastatin mixed with Nanobone group. In Simvastatin mixed with Nanobone group specimens numerous well differentiated capillary vascularization were demonstrated. In all sections of Simvastatin mixed with Nanobone group, there was no evidence of acute or chronic inflammatory infiltrate. In all samples of the Nanobone

group, inflammatory cell infiltration within the new bone, primarily mononuclear cells such as lymphocytes and macrophage were seen. The inflammatory cells were considered as indicative of a significant inflammatory or immune response (Figure 3). According to the descriptive data, the use of a combination material seems to result in slightly higher values.

the protocol of delayed immediate implant placement. It is important to keep in mind that some grafts after four months of healing are expected to completely resorbed[22], this consistent with the present study which designed to evaluate the effect of mixing Simvastatin with Nanobone three months after extraction and socket preservation. The signs of an inflammatory reaction in both group were

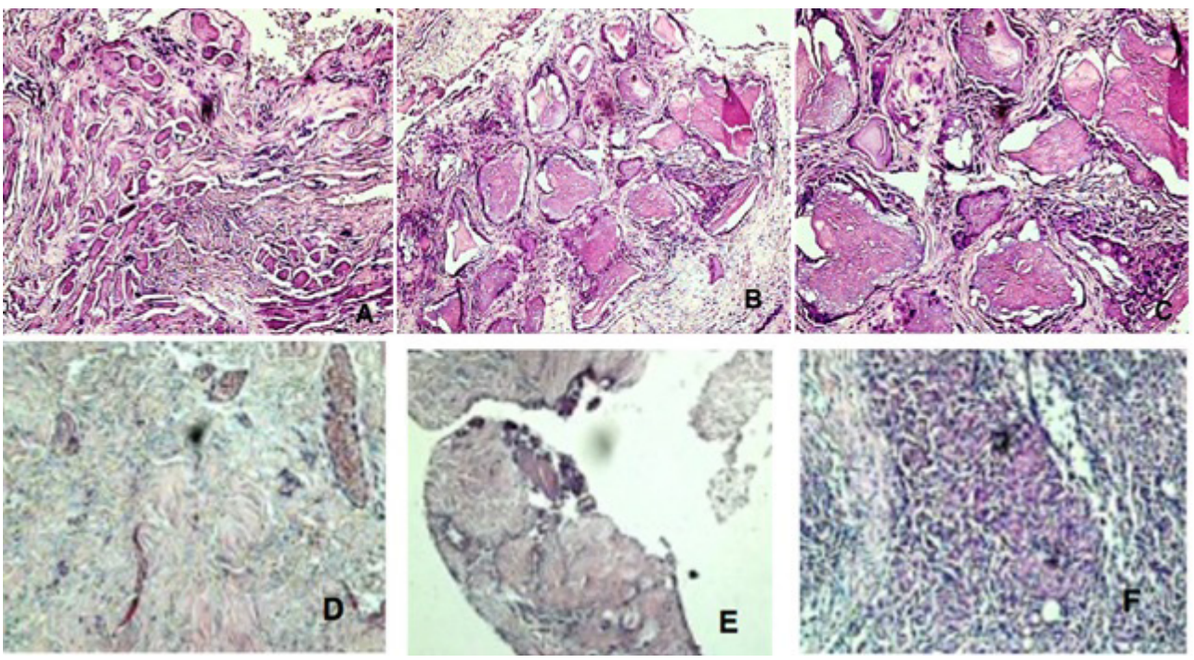

Figure 3: Showing Histological results.

A, B and C: H and E-stained sections for test group showing new bone formation. D, H and E-stained section for test group showing numerous blood vessels formations. E: H and E-stained section for control group showing remanent of Nano bone graft. F: H and E-stained section for control group showing numerous inf amatory cells.

DISCUSSION

In today's competitive world, research scholars are never satisfied and are always on the hunt for new techniques. The emergence of statin group as a potent anti-inflammatory[20] and osteoblastic differentiation[21], the present study focusing on the regenerative effect of Simvastatin and mixing it with Nanobone to study the combination effects. In this study, the patients indicated for tooth extraction and placement of the implant, the patients were treated with atraumatic extraction and socket preservation with Simvastatin mixed with Nanobone in group II while in control group the socket filled with Nanobone alone. Implant placement was performed Three months following ridge preservation, and during implant placement procedures a core bone biopsy obtained and used for histological analysis. The histological effects of the socket preservation measures were mostly examined three months postextraction. This was in the line with detected histologically. In some specimens of group 2 and all specimens of group 1 showed small cellular infiltrations, this may be due to weak inflammatory reactions which may considered to be bone substitute healing process this results was consistent with Schmidt-Bleek K *et al*. 2012[23]. The bone healing in both group was similar to osteoconductive process as shown histologically by the presence of membranaceous osteogenesis around the graft material, remodeling of newly formed bone from fibrous into mature lamellar bone tissue and bone graft degradation by osteoclastic activity.

The histological results of this study showed that there were no significant differences between the two groups which indicate a similar healing process or osteogenic activity of both groups, this was consistent with the results of the study done on human healing of different allograft materials[24] and the result of an animal study Hawthorne AC *et al*. 2013. No pathological alteration like necrosis or

abnormal tissue like cartilage were seen in all sections of both groups, this was inconsistent with the study of Spin-Neto R *et al*. 2015 who showed the presence of necrotic areas and increased graft resorption[25].

There were some limitations in the current study. First, the histological results influenced by patients selection, the new bone formation in patients who have periodontal disease take more time and less predictable than the patients without periodontitis[26]. Patients age is another influence on postextraction bone healing which are delayed in old patients[27]. Other influences like extraction socket size, location and patients features such as gender and patient habits[5]. Moreover, due to small study sample, further studies with considering these influences and comparison of simvastatin with the established biologic agents would be needed to fully delineate the utility of simvastatin in socket preservation.

The results of this study suggest that socket preservation, when done by using simultaneously with simvastatin and Nanobone, showed positive correlations with new bone formation. Both groups showed significant improvements in new bone formation. Therefore, the present results confirm that Simvastatin combined Nanobone bone graft, placed in extraction socket site, promotes bone formation in socket sites, thereby reducing bone resorption.

CONCLUSION

This clinical trial demonstrated that Simvastatin mixed with Nanobone implies synergetic effects, amplifying their task as a regenerative material in the socket preservation.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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