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To Compare Bone Formation in Terms of Mean-gray Scale Histographic Values After Surgical Removal of Bilaterally Impacted Mandibular Third Molars in Patients Treated with and without Simvastatin: A Split-mouth Randomized Controlled Trial

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ABSTRACT

Objectives: To compare bone formation in terms of mean-gray scale histographic values after surgical removal of bilaterally impacted mandibular third molars in patients treated with and without simvastatin.

Materials and Methods: This randomized, split mouth clinical study was conducted in the Oral and Maxillofacial Surgery Department, Dental Section, Allied Hospital, Faisalabad from November 2021 to May 2022. Thirty consecutive patients (30 extraction sockets per group) selected randomly, met the inclusion criteria and were included for study. Each patient underwent two surgical sessions, extracting one third molar during each session. The mouth was divided into study and control sides using the lottery method. After standard surgical removal, the study side received gel-foam soaked in simvastatin, while the control side received gel-foam soaked in normal saline. The study spanned 3 months, with bone formation assessment at the 12th post-operative week using digitalized intra-oral peri-apical radiographs and histogram analysis.

Results: A total of 30 patients participated in the study out of which 70% (n=21) cases categorized within 18-30 years age group whereas 30% (n=9) were aged between 31 to 35 years, with a mean age of 27.9 ± 4.33 years. Sixty percent (n=18) patients were male, while the remaining 40% (n=12) were female. Comparison of mean bone density on both sides showed a mean-gray scale histographic value of 107.83±3.99 on the study side and 97.40±4.42 on the control side, with a statistically significant *p*-value of 0.0001.

Conclusion: There is a significant difference in bone reformation in patients treated with simvastatin as compared to those without simvastatin application.

Keywords: Molars, Osteogenesis, Simvastatin

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INTRODUCTION

Third molars exhibit the highest rate of impaction, with a global prevalence of 24%.[1] Mandibular impaction is more prevalent, accounting for 58.5% of all cases,[2] with mesioangular impaction being the most common subtype (49.2%).[3] The highest number of impactions are seen between the ages of 25 and 45 years, with a female preponderance i.e., 56.6% of all cases.[4] The primary indications for the removal of mandibular third molar impaction are recurrent pericoronitis (62.9%), dental caries (11.7%), resorption of adjacent tooth (9.4%), periapical pathology (6.3%), diseases of the follicle including cysts and tumors (3.9%), tooth fracture (2.1%) and chronic periodontitis (1.8%).[5] Healing of the socket post-extraction occurs phase-wise and encompasses coagulation/hemostasis, inflammation, proliferation, and modelling/remodelling.[6] Improper healing leads to the formation of a dry socket, affecting 2.47% of all tooth extractions.[7]

Bone formation is based on the synthesis of newly formed matrices by specialized cells called osteoblasts, followed by mineralization. Growth factors, such as bone morphogenetic proteins (BMPs), play an essential role in inducing the differentiation of multipotent stem cells into cells with osteoblast-like characteristics.[8] Modalities thought to enhance bone formation after tooth extraction include platelet-rich plasma, platelet-rich fibrin, bone substitutions, collagen plugs with isocyanoacrylate sealing, dense polytetrafluoroethylene membranes, and allografts of freeze-dried bone in conjunction with collagen wound dressings, however, there is no clear consensus on which method is the most suitable and effective.[9,10] Simvastatin is a reversible inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, which is involved in conversion of HMG-CoA to mevalonate, an early rate limiting step in synthesis of cholesterol in liver.[11,12] It also upregulates the gene expression of bone morphogenetic protein-2 (BMP-2) and vascular endothelial growth factor (VEGF), promoting the differentiation of osteoblastic cells for new bone formation. Additionally, it inhibits bone resorption by down-regulating the expression of thrombin receptor activating peptide (TRAP) and cathepsin K, impeding the fusion of osteoclast precursors, thereby decreasing the number of active osteoclasts.[11,12] An important consideration is that this drug is relatively cheap and has a good safety profile.[13]

This study aims to investigate the efficacy of topically applied simvastatin in promoting bone formation subsequent to the surgical extraction of impacted mandibular third molars. The simplicity of its application and the potential for cost-effectiveness make simvastatin an appealing drug for expediting bone regeneration post-tooth extraction, However, its use has not been incorporated in clinical practice. Till date, no such study has been conducted in Pakistani population to the best of our knowledge. The outcomes of this study will provide useful data on the utilization of simvastatin for patients undergoing tooth extraction in our setups.

MATERIALS AND METHODS

This randomized, split-mouth clinical study was conducted in the Department of Oral and Maxillofacial Surgery, Dental Section, Allied Hospital, Faisalabad from November 2021 to May 2022. The Institutional Review Board granted ethical approval prior to the start of study [No.F.48-ERC/2020-21/PHRC/FMU/22]. A total of 30 eligible patients were enrolled based on predefined inclusion criteria, using non-probability consecutive sampling. The sample size calculation utilized the WHO sample size calculator for two means, with an anticipated population mean of 110.46, a test value of the population mean of 99.94, a pooled standard deviation of 5.73, and a study power of 90%. Inclusion criteria encompassed both male and female patients aged 18-35 years requiring bilateral extraction of mandibular third molars. Patients with medical conditions impacting bone metabolism such as osteoporosis, vitamin D deficiency, and/or parathyroid disease were excluded from the study. Additionally, teeth with radiographically evident extensive periapical changes (abscess, granuloma, or cyst formation), individuals on prolonged antibiotic or steroid therapy, those unwilling to commit to an extended follow-up period, pregnant women, smokers, and individuals with a history of drug or substance abuse were also excluded. All patients included in the research provided written informed consent for participation.

A comprehensive case history was obtained, together with standard haematological tests (complete blood counts, prothrombin time, international normalized ratio, activated partial thromboplastin time and viral serologies for screening) and intraoral periapical radiographs (IOPARs). In each patient, we split the mouth into two halves using the lottery method, with

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one half being the study side and the other being the control side. Extraction of the third molars was conducted in two separate surgical sessions. Under local anesthesia (lignocaine 2% with adrenaline 1:100,000) and aseptic measures, an envelope incision was made and full thickness mucoperiosteal flap was raised with a periosteal elevator to expose the bone. Bone was removed on the buccal and occlusal aspect with the help of a slow speed hand-piece and round surgical bur. The tooth was then sectioned with a straight fissure bur and delivered with a straight elevator. The socket was then irrigated with normal saline. Immediately after extraction, the sockets on the study side were filled with a gel foam soaked in a mixture of a crushed 10 mg simvastatin tablet and 2 ml normal saline, whereas the sockets on the control side were filled with gel-foam soaked in normal saline alone. A black braided silk 3-0 suture was used for surgical site closure. Patients were directed to exert gentle pressure on the gauze pack over the operated site for 30 minutes. For the initial postoperative week, chemical plaque control was performed using a 0.2% chlorhexidine gluconate solution, applied for one minute three times a day, starting twenty-four hours after the procedure along with amoxicillin clavulanate 625 mg and naproxen sodium 550 mg twice daily for three to five days. IOPARs were taken at the end of the twelfth week to measure bone density of both sides. The radiographs were acquired utilizing the paralleling technique to ensure reproducibility. Adobe Photoshop CS6 was used to analyze the IOPARs for gray-scale histographic values, which represent bone densities. Guided by the pre-operative radiographs, the extracted socket area was delineated using the Magnetic Lasso Tool to precisely measure the socket area and, subsequently, the Histogram Tool was used to measure the mean grayscale values of the extracted sockets, as shown in Figure 1.

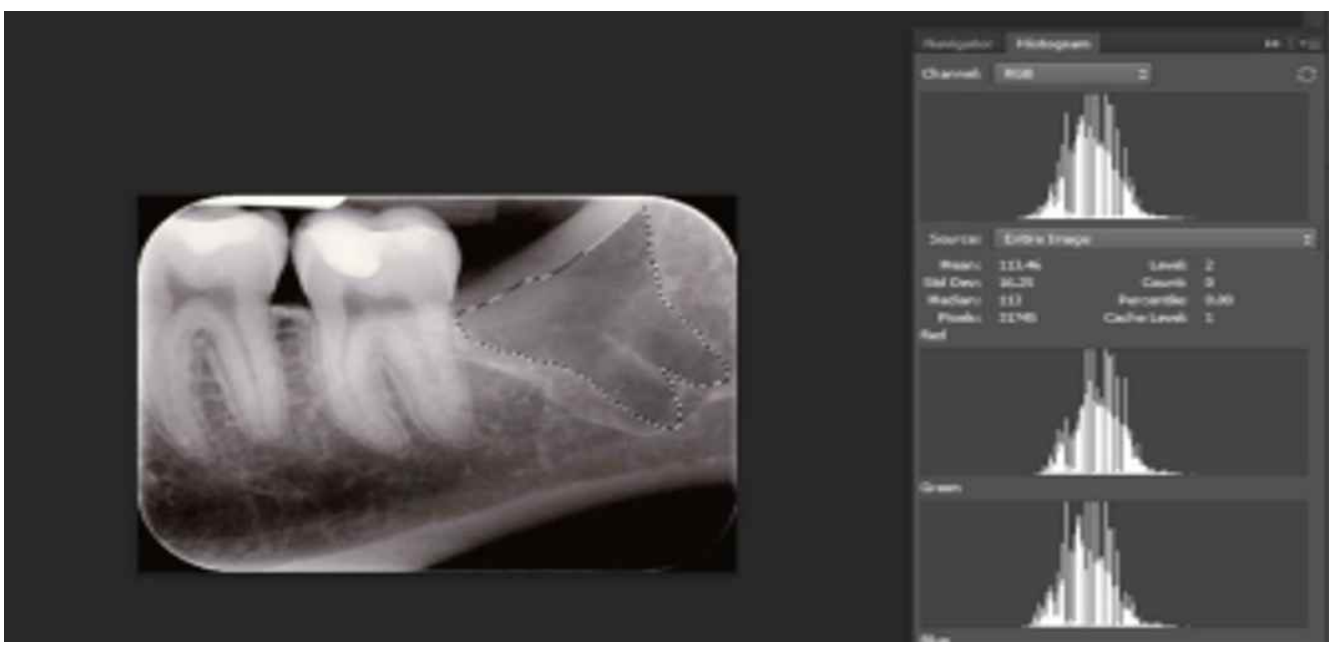

Figure 1: Assessment of digitalized intra-oral peri-apical radiograph using Adobe Photoshop CS6 for gray-scale histographic values. Dotted line points to a marked area being measured and the histogram shows the mean gray-scale value of the marked area

Data was analyzed using the Statistical Package for the Social Sciences version 23.0. Quantitative variables like age and the bone density measurement of the study and control socket at the 12th week were presented as means and standard deviations, and the independent sample *t*-test was applied for comparison. Qualitative variables like gender were calculated as frequencies and percentages. Confounding variables like age and gender were controlled through stratification. Post stratification independent samples *t*-test was applied and a *p*-value of ≤ 0.05 was taken as significant.

RESULTS

A total of 30 patients (30 mandibular extractions in each

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group) were enrolled in the study. The participants had a mean age of 27.9 ± 4.33 years, of whom 21 (70%) cases fell within the 18-30 years age group, while 9 (30%) were 31-35 years of age. Gender analysis showed that males were in majority in our study sample, accounting for 18 (60%) cases.

The mean bone density was 107.83 ± 3.99 in study group while it was 97.40 ± 4.42 in control arm, (p=0.0001), (Table I). The data was stratified for age, with the 18-30 years age group demonstrating a mean bone density of 108.14 ± 3.93 on the study side and 96.38 ± 4.01 on the control side, (p=0.0001). The 31-35 years age group showed a mean bone density of 107.11 ± 4.28 on study side and 99.78 ± 4.66 on the control side, (p=0.003). The data was also stratified for gender, it showed that male patients had a mean bone density of 107.78 ± 3.26 on the study side and 98.50 ± 4.15 in control arm, (p=0.0001). Similarly, females showed a mean bone density of 107.92 ± 5.05 in the study arm and 95.75 ± 4.47 in the control arm, (p=0.0001).

Table 1: Comparison of mean bone density on both sides (n=60)

Bone density	Study side (n=30)		Control side (n=30)		*p*-value
	Mean	SD	Mean	SD	0.001
	107.83	3.99	97.40	4.42	

DISCUSSION

The goal of modern surgery is to enhance clinical healing while minimizing invasiveness. Regenerative surgery has emerged to restore both hard and soft tissues.[14,15] Bone regeneration requires morphogenetic signals, scaffold matrices, and responsive host cells, as well as growth factors like BMPs, a subset of transforming growth factor-beta (TGF-β), which induce osteogenesis.[16] After tooth removal, various methods have been proposed to promote bone regeneration, however, the optimal method remains debated.[17,18] Non-invasive, cost-effective options like statins activate endogenous bone growth factors, and this study demonstrates that simvastatin may have a role in promoting bone formation post-surgical extraction of mandibular third molars.

Local application of simvastatin has an enhancing effect on bone formation post-tooth extraction, as demonstrated by the current study. Animal trials using simvastatin have demonstrated that the local application of this drug not only results in increased bone regeneration in rat models but also resulted in significant reduction in inflammation due to its anti-inflammatory properties.[19,20] Human clinical studies have explored the effectiveness of simvastatin in treating defects in periodontium, cystic defect in periapical region, extraction site sockets of premolar teeth, and osteoporotic bone disease in women.[21,22] Degala et al reported that there was a significant increase in the mean gray-scale histographic values within extraction sockets with the use of simvastatin as opposed to the use of a placebo, with consistent benefit seen at one, four, eight and twelve weeks post-extraction.[23] Harsha et al, in addition to noting an increase in bone regeneration based on mean gray-scale histographic values at one, four, eight and twelve weeks post-extraction, noted that the benefit in terms of bone regeneration was also visualised on cone-beam computed tomography when compared to placebo.[24] These results were in agreement with other studies on the subject such as Saifi et al and Gupta et al, both of whom compared simvastatin to placebo.[8,25]

It is pertinent to note here that not only does simvastatin demonstrate significant benefit versus placebo, but also maintains efficacy when compared to other modalities used to improve bone regeneration, both individually and in combination. Mathur et al compared simvastatin to platelet-rich fibrin for bone regeneration in extracted tooth sockets and noted that there was no difference between the two with regards to degree of bone regeneration, with both resulting in significantly increased regeneration.[26] Sezavar et al compared the use of simvastatin in combination with collagen versus collagen alone in sockets of extracted teeth and noted that while there was no difference in degree of bone regeneration between the two groups, the use of simvastatin was associated with higher levels of vital, amorphous, and trabecular bone, and lower proportions of dead and non-osteoblastic bone, indicating better healing.[27] Cruz et al compared simvastatin with polypropylene membranes versus polypropylene membranes alone in the same setting and noted that the

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former resulted in a significant reduction in dimensional changes in tooth sockets, post-extraction but had no effect on soft tissue healing or postoperative pain.[28] Conversely, Deshpande et al reported that while simvastatin was associated with an increase in bone regeneration in tooth sockets post-extraction, it was also associated with an increase in pain and swelling, at least within the first week of extraction, when compared to placebo.[29]

The current study was limited by its comparatively small sample size as well as it being conducted in a single-center, limiting its generalizability to the general population. Additionally, radiography with software assessment has limited utility in assessing early bone changes when compared to other modalities such as dual-energy x-ray absorptiometry, which may be used to perform more accurate assessments but are limited in their use by their expense and the increased dose of radiation. Lastly, invasive methods such as a biopsy with histological evaluation would be the most accurate modality for evaluating degree and type of bone healing, however, this was not done in our study due to practical, ethical and economic considerations. Future research should focus on comparing simvastatin to other modalities which enhance bone regeneration and compare cost-effectiveness of these methods.

CONCLUSION

The local application of simvastatin in promoting bone regeneration within the sockets of extracted mandibular third molars is efficacious. Notably, its affordability renders it a promising option for widespread use, particularly in resource-limited settings such as in Pakistan. By harnessing the therapeutic potential of simvastatin, we can significantly enhance post-extraction healing outcomes while addressing economic constraints, thus advancing dental care accessibility in developing countries.

DISCLAIMER

None to declare.

CONFLICT OF INTEREST

There is no conflict of interest among the authors.

ETHICAL STATEMENT

The ethical approval was provided by the Institutional Ethical Review Committee at Faisalabad Medical University [No.F.48-ERC/2020-21/PHRC/FMU/22].

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AUTHORS CONTRIBUTION

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Critical review of the manuscript: M.U. Malik

Approval of the final version of the manuscript to be published: R. Ashraf, M.U. Khalid, M.U. Malik, M.S. Ashraf, S. Hameed

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