

EFFECT OF XENOGRAFT- OZONATED GEL MIXTURE ON ALVEOLAR BONE HEALING FOLLOWING REMOVAL OF IMPACTED THIRD MOLAR: A RANDOMIZED CONTROLLED CLINICAL TRIAL

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ABSTRACT

Objectives: This study aimed to evaluate the clinical and radiographic outcomes of mixing the xenograft with ozonated gel and their application in the extraction socket following the surgical removal of impacted mandibular third molar.

Patients & Methods: The study was conducted on sixty patients with asymptomatic bilateral impacted mandibular third molars. The molar on one side was extracted and xenograft (Bio-oss® Geistlich Pharma AG, Switzerland) mixed with ozone gel was applied in the extraction socket (study side); the molar on the other side was extracted two weeks later and xenograft alone was placed in the extraction socket (control side).

Results: Pain, maximal incisal opening and swelling were significantly improved in the study group when compared to the control group. Values of bone density was higher in the study group throughout the follow-up period.

Conclusion: Incorporation of ozone gel within the xenograft material plays an important role in increasing the alveolar bone density, enhancement of postoperative pain, swelling, range of mouth opening and gives better wound healing.

KEY WORDS: Ozone gel, Third molar, Pain, Trismus, Swelling, Bio-Oss graft.

INTRODUCTION

Removal of impacted lower third molar is considered as the most common surgical procedure carried out in the oral and maxillofacial Clinics.⁽¹⁾ The surgical removal of the impacted mandibular third molar is usually associated with pain, swelling and inability to open the mouth. Those symptoms

reach the maximum intensity between the third to fifth hours postoperatively for the pain and 24 to 48 hours postoperatively for the swelling and then, they gradually diminished until the 7th day postoperatively.^(2,3)

In addition to those symptoms, there is a considerable risk of developing periodontal defects

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on the distal aspect of mandibular second molars after mandibular third molar removal. Kan and his colleagues in 2002⁽⁴⁾ published a study that observed the periodontal conditions distal to mandibular second molars 6-36 months after routine surgical extraction of adjacent impacted third molars. They stated that 67% of patients exhibiting Periodontal Pocket Depth (PPD) ≥ 5 mm and 23% exhibiting PPD ≥ 7 mm and they observed a mean recession of 0.8 ± 1.0 mm along the distal aspect of the second molar.

Once the periodontal defect and intrabony defect developed, they became difficult to treat through the traditional root planning, or simple local curettage and debridement⁽⁵⁾ Therefore, it is beneficial to take into consideration that bone grafting of the resultant defects immediately following the surgical removal of impacted mandibular third molars as this will maintain the periodontal health through preserving the alveolar bone height⁽⁴⁾ Various studies with different materials have been used for prevention and management of this common complications.⁽⁶⁻⁸⁾

Among these materials used to fill the socket of the mandibular third molar was Bio-Oss® xenograft material which presented in the market as a resorbable anorganic porous bovine hydroxyapatite scaffold. The Bio-Oss® xenograft material had been used to repair the periodontal and maxillofacial osseous defects. It is degraded slowly in the body which considered as advantage as it reduces the bone loss within the defect that results from the remodeling after the augmentation procedures.⁽⁹⁻¹²⁾

Moreover, many methods have been used to stimulate bone healing either with chemical or physical stimuli. The use of physical stimuli varies from ultrasounds, electromagnetic fields, low level laser therapy and more recently ozone therapy.^(13,14)

In the past decade the ozone therapy had been widely used in dentistry. It has been used in treatment of early carious lesions, sterilization of cavities, root canals, and periodontal pockets, enhancing epithelial wound healing such as ulcerations and

herpetic lesions and as a rinse for avulsed teeth or as a denture cleaner.⁽¹⁵⁻¹⁷⁾

In oral and Maxillofacial Surgery, the ozone therapy used during the surgical intervention as well as post-surgery as a topical disinfectant and healing agent to promote hemostasis, enhance the local oxygen supply, and inhibit bacterial growth.^(18,19)

The published papers regarding the ozone therapy in oral and maxillofacial surgery consider mainly the antimicrobial effect of the ozone.⁽¹⁷⁻¹⁹⁾ There is a lack of adequate evidence regarding effects of ozone therapy on bone healing and more specifically on the healing of extraction sockets. Therefore, the aim of this study was to evaluate the clinical and radiographic outcomes of mixing Bio-Oss® xenograft material with ozonated gel on extraction socket following the surgical extraction of impacted mandibular third molar.

PATIENTS AND METHODS

Participants

Sixty healthy patients require removal of bilateral impacted mandibular third molar participated in this study. Their age ranged from 18 to 35 years. The participants were recruited from Outpatient clinics of Oral and Maxillofacial Surgery Department, Faculty of Dentistry, Alexandria University.

Patient selection was based on certain inclusion and exclusion criteria. The inclusion criteria include age > 18 years, with no systemic disease(s), and bilateral impacted mandibular third molar class II position B on Pell- Gregory classification. On the other hand, the exclusion criteria include any patients that had any contraindication to ozone therapy, local infection, tobacco use, oral contraceptive, pregnancy, lactation in addition to patients who had taken analgesics or anti-inflammatories for 1 week prior to enrollment in the study.

Completed medical and dental history was taken for all patient and the position of the impacted teeth was evaluated by panoramic films. Patients were

fully informed about the treatment procedures, follow-up examinations and complications of surgical procedures and ozone treatment. Informed consent was obtained from each patient prior to participation in the study. The study was approved by the ethics committee of Faculty of Dentistry, Alexandria University.

Study Design

The study was carried out as a randomized controlled clinical trial, split mouth design. Randomly, by the use of predefined computer-generated randomization table, molar on one side was allocated to receive xenograft (Bio-oss® Geistlich Pharma AG, Switzerland) mixed with ozone gel in the extraction socket (study side). The molar on the other side was extracted 2 weeks later and bone graft alone was applied to extraction socket (control group). Patients were blinded to which side was ozonated and which was control.

All Patients were subjected to a standardized surgical protocol by the same surgeon and another operator carried out the postoperative measurements.

Ozone gel

The ozone gel was obtained by bubbling of 25 μ / ml O₃ gas through pure olive oil for 2 days until olive oil transforms from greenish colored liquid status to the whitish gel status. This procedure was performed by the longevity Ext 120 ozone generator (Longevity Extra120, Longevity Co., Canada)

Surgical procedures

All surgeries were performed under complete aseptic conditions. Before surgery, the patient's mouth was rinsed with a chlorhexidine digluconate solution 0.2% for 2 minutes. Surgery was carried out under local anesthesia consisting of 2% lidocaine hydrochloride with 1: 80,000 adrenaline (Lignospan Special, Septodont, UK). An extended buccal mucoperiosteal flap was elevated providing access to the impacted third molar. Osteotomy was achieved under continuous sterile saline solution

irrigation. When necessary, sectioning of crown and roots was performed with a fissure bur. After tooth extraction, the alveolus was inspected, curetted, and irrigated with 0.9% sterile saline solution. Thereafter Bio-oss® was mixed either with ozone gel (study side) or saline only (control side) and carefully packed in the extraction socket. The three-sided mucoperiosteal flap was repositioned and sutured with 3-0 black silk suture.

Postoperatively, the patients were instructed to apply ice bags 20 min/hour for the next 6 hours to the surgical side, eat soft cold diet by the aid of a straw and to avoid hot diet and fluid on the day of surgery. Postoperative antibiotic of 875 mg Amoxicillin and 125 mg Clavulanic acid (Augmentin 1 gm, Smithline Beecham Pharmaceutical Co., Bentford, England) twice a day for five days post-operatively and non-steroidal anti- inflammatory drug in the form of diclofenac potassium 50 mg (Cataflam 50mg tablets, Novartis Pharma AG, Basle, Switzerland) three times daily and chlorhexidine gluconate solution (Hexitol mouth wash, the Arab Drug Co., Cairo, Egypt) as a mouth rinse for a period of five days were prescribed to the patients. Sutures were removed after 7 days postoperatively.

Postoperative Evaluation

All the patients were evaluated on the 1st, 3rd, 5th and 7th postoperative days for pain, preoperative and postoperative mouth opening, and degree of postoperative swelling. Also the bone density within the extraction socket was evaluated.

Pain was assessed using a visual analogue scale (VAS) of 10 units in combination with a graphic rating scale.⁽²⁰⁾ On the VAS, the left most end represented the absence of pain (score 0) and the right most end indicated the most severe pain (score 10). Preoperative and postoperative mouth opening was evaluated by measurement of the maximal distance between the cutting edge of the right maxillary and right mandibular central incisors with a caliper⁽²¹⁾. The degree of postoperative swelling was measured (cm) using a tape measure,

from the tragus to the corner of the mouth.⁽²²⁾ The preoperative measurements were considered as the baseline values and the extent of the swelling was determined on postoperative days 1, 3, 5, and 7. The difference between each postoperative evaluation and the baseline value indicated the facial swelling for that day.

The bone density within the extraction socket was measured using software of digora (DIGORA® for Windows 2.7 software, The Dental Imaging Company Ltd Digora, USA.). For this purpose, Patients received panoramic radiograph immediate, 1st, 3rd and 6th months postoperatively.

During examination of the patients, each patients was precisely aligned at the X-ray machine using laser alignment beams, hence guaranteeing positioning all the patients at the same position.

Statistical analysis of the data

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. Qualitative data were described using number and percent. Student t-test was used to compare two groups for normally distributed quantitative variables while Paired t-test was assessed for comparison between different periods. For abnormally distributed quantitative variables Mann Whitney test was used for comparing each two groups, while Wilcoxon signed ranks test was assessed for comparison between different periods. Significance of the obtained results was judged at the 5% level.

RESULTS

This study was conducted on sixty (60) patients with bilateral impacted mandibular third molar. Twenty-nine patients were males (48.3%) and thirty-one were females (51.7%) their mean $\pm$ SD age was 23.33 ± 4.70 (range 18-35).

The surgical procedure was performed for both sides without any complication in all patients. The mean operation time (from the start of the incision

to the end of suturing) was 22 ± 9 min for the study side while in the control side, the mean operation time was 23 ± 11 min ($p < 0.001$).

The patients were evaluated and followed up for pain, swelling, maximum mouth opening for intervals of 1st, 3rd, 5th and 7th day postoperatively. In addition to those clinical parameters, the patients were also evaluated and followed up radiographically for bone density on intervals of 1st, 3rd and 6th months postoperatively.

Pain

The pain scores recorded their highest value on the first day postoperative in both groups and it drops significantly by the 7th day postoperative. The pain score recorded a significantly lower value on the study side than that of the control side at all intervals of evaluation ($p < 0.001$). (Table1)

Swelling

The postoperative swelling occurred on both control and study sides. The study side showed significantly less facial swelling than the control side all over the follow up period (Table 2). Also the results showed that in the study side the swelling resolved on the 7th day postoperative while in the control side the patients still had residual swelling that did not resolved completely by the end of the first postsurgical week.

Maximum interincisal opening

The Maximum interincisal opening was restricted on both sides. Maximum interincisal opening showed lowest value in the 1st day postoperative in both groups and improved significantly by the 7th day postoperative. When comparing both sides, trismus on the study side was significantly less than that on the control side. (Table3)

Bone Density

Within the study side, the mean bone density value increased continuously through the 1st, 3rd and 6th

months postoperative. The increase in bone density was statistically significant throughout the different follow up periods. In the control side, the mean bone density value was also increased throughout the fol-

low up period. The improvement in the bone density was greater in the study side than in the control side. The difference between two sides showed a statistically significant relation ($P < 0.001$) (Table 4).

TABLE (1) Comparison between the two studied groups according to pain score

	Pain Score			
	1 st Day	3 rd Day	5 th Day	7 th Day
Study (n=60)				
Mean $\pm$ SD.	5.05 $\pm$ 0.87	2.92 $\pm$ 0.87	1.53 $\pm$ 0.75	0.27 $\pm$ 0.45
P₁		<0.001*	<0.001*	<0.001*
Control (n=60)				
Mean $\pm$ SD.	6.80 $\pm$ 0.73	4.55 $\pm$ 0.72	2.22 $\pm$ 0.67	0.88 $\pm$ 0.49
P₁		<0.001*	<0.001*	<0.001*
P₂	<0.001*	<0.001*	<0.001*	<0.001*

p₁: p value for Wilcoxon signed ranks test for comparing between 1st day and each other periods

p₂: p value for Mann Whitney test for comparing between the two studied groups

**: Statistically significant at $p \leq 0.05$*

TABLE (2) Comparison between the two studied groups according to swelling

	Swelling				
	Pre-operative	1 st Day	3 rd Day	5 th Day	7 th Day
Study (n = 60)					
Mean $\pm$ SD.	12.82 $\pm$ 1.21	16.38 $\pm$ 1.30	15.20 $\pm$ 1.16	14.18 $\pm$ 1.27	13.18 $\pm$ 1.21
P₁		<0.001*	<0.001*	<0.001*	<0.001*
Control (n = 60)					
Mean $\pm$ SD.	12.82 $\pm$ 1.21	19.70 $\pm$ 1.28	17.62 $\pm$ 1.01	15.72 $\pm$ 0.83	14.13 $\pm$ 1.05
P₁		<0.001*	<0.001*	<0.001*	<0.001*
P₂	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*

p₁: p value for Paired t-test for comparing between Preoperative and each other periods

p₂: p value for Student t-test for comparing between the two studied groups

**: Statistically significant at $p \leq 0.05$*

TABLE (3) Comparison between the two studied groups according to maximal incisal opening

	Mouth opening				
	Pre-operative	1 st Day	3 rd Day	5 th Day	7 th Day
Study (n = 60)					
Mean ± SD.	41.58 ± 1.24	31.12 ± 1.42	34.78 ± 1.54	38.15 ± 1.44	41.58 ± 1.24
P₁		<0.001*	<0.001*	<0.001*	<0.001*
Control (n = 60)					
Mean ± SD.	41.58 ± 1.24	25.73 ± 1.81	31.20 ± 1.68	34.82 ± 1.72	38.33 ± 1.42
P₁		<0.001*	<0.001*	<0.001*	<0.001*
P₂	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*

p₁: p value for Paired t-test for comparing between Preoperative and each other periods

p₂: p value for Student t-test for comparing between the two studied groups

**: Statistically significant at $p \leq 0.05$*

TABLE (4) Comparison between the two studied groups according to bone density

	Bone density		
	1 st Month	3 rd Months	6 th Months
Study (n = 60)			
Mean ± SD.	659.57 ± 25.70	782.47 ± 25.04	914.58 ± 41.59
P₁		<0.001*	<0.001*
Control (n = 60)			
Mean ± SD.	539.0 ± 49.87	647.22 ± 47.32	795.15 ± 34.37
P₁		<0.001*	<0.001*
P₂	<0.001*	<0.001*	<0.001*

p₁: p value for Paired t-test for comparing between Preoperative and each other periods

p₂: p value for Student t-test for comparing between the two studied groups

**: Statistically significant at $p \leq 0.05$*

DISCUSSION

The removal of the impacted mandibular third molar is usually associated with pain, swelling and trismus as postoperative sequelae following surgery. Moreover, there is a risk of developing a periodontal defect on the distal surface of mandibular second

molar. All of those symptoms affect the quality of the patient's life after the surgical procedure.^(3-5, 23)

Many methods had been used to decrease the postoperative symptoms and improve the healing of the surgical sites. The present study aimed to investigate the effect of the ozone therapy mixed

with Xenograft on the bone healing after surgical removal of mandibular third molar.

The use of ozone therapy in oral and maxillofacial surgery has proposed because of its antimicrobial, disinfectant and healing properties. Many studies had suggested that the ozone has a positive effect on oxygen metabolism, cell energy, the immune-modulatory property, antioxidant defense system and microcirculation.⁽²⁴⁾ Based on these finding many authors suggest the use of the ozone therapy to enhance the bone haling in sites where the bone healing could be compromised such as in cases of patients treated with bisphosphonates.⁽²⁵⁾

In the current study, the ozone therapy has been used to improve the bone healing of the socket in addition to reduce the postoperative symptoms such as the pain, trismus and swelling based on the properties of the ozone therapy.

There are several forms used to deliver the ozone to the operation site such as gaseous form, dissolve in water or the gel form. In the current study, the ozonated olive oil was selected as it provides long stay in the oral cavity, adequate drug penetration, high efficacy and acceptability.⁽²⁶⁾

Split- mouth design was chosen to conduct the current study to avoid any possible bias in allocating individuals in control and study groups. The current study was conducted on patients with bilateral third molar which are symmetrical in position and degree of difficulty. Furthermore, single surgeon preformed all surgical procedures and another surgeon collect the data in the follow-up period in order to remove any possible differences that could resulted from different surgeon's skills.

In the present study, the pain was evaluated using the visual analog scale on intervals of 1st, 3rd, 5th and 7th day postoperative. The patients experience significantly less pains at the study side than that on the control side. This results were in agreement with the results of Kazancioglus et al (2014)⁽²⁷⁾ who conducted a study to evaluate the effect of the ozone therapy on pain after the third molar surgery.

In addition to the effect of ozone therapy on pain, the trismus and swelling also affected by the addition of the ozone gel to the extraction socket. The results of this current study showed a positive effect of ozone on the trismus which decreased significantly in the study side that received ozone gel. Also the facial swelling had reduced and resolved more rapidly in the study side that received ozone gel when compared to the control side. Those results differ from the results that were presented by Kazancioglus et al⁽²⁷⁾ which showed that the ozone had no effect on swelling and trismus. This could be explained by the difference of the method by which the ozone was applied. In the current study, the ozone was applied in a form of gel which ensure longer exposure of the surgical field to the effect of ozone.⁽²⁶⁾

Grafting the extraction socket with Bio-oss Xenograft had been tested widely in the literature.⁽⁹⁻¹¹⁾ Regarding the bone density within the surgical site, the results of present study showed a statistically significant improvement in the bone density in the ozonated side. Those results were in agreement with Kazancioglu et al⁽²⁴⁾ who studied the effect of ozone and laser on bone healing in calvarial defects and concluded that ozone therapy had a positive effect on the bone healing more than the low level laser therapy.

Moreover, in a recent study performed in 2016 by Lektemur et al⁽²⁸⁾ who studied the effect of ozone therapy on bone healing in diabetic rat calvarial defects treated with xenograft also concluded that gaseous ozone application accelerates xenograft resorption and enhanced bone regeneration, especially, in early stages of bone healing in diabetes mellitus.

Another study by Erdemci et al (2014)⁽²⁹⁾ investigated the effects of systemic and topical ozone applications on alveolar bone healing following tooth extraction. The study concluded that postoperative long-term systemic ozone application can accelerate alveolar bone healing following extraction.

Also, our results run parallel with a study conducted by Elhadary et al ⁽³⁰⁾ on the effect of ozonated oil on osseointegration of dental implant, they concluded that topically applied ozonated oil may influence bone density and the quality of osseointegration around dental implants. In contrast, Matsumura et al ⁽³¹⁾ reported that ozone does not have a major impact on stimulation of gingival cells for osteoblastic activity in regeneration of periodontium around implants.

CONCLUSIONS

Within the limitations of the current study, it may be concluded that the incorporation of ozone gel within the graft material plays an important role in increasing the alveolar bone density, improvement of postoperative pain, swelling, range of mouth opening and gives better wound healing. However, additional studies are required to clarify the effects of different ozone applications on new bone formation. In addition, studies focusing on the effects of ozone application on bone healing in patients treated with radiotherapy or receiving bisphosphonates are also necessary.

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