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Comparative Study Between The Efficacy of Ozone Gel and Hyaluronic Acid on Bone Healing After Enucleation of Mandibular Odontogenic Cysts

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ABSTRACT

Aim: The aim of this study was to evaluate the clinical and radiographic effect of both Ozone gel and Hyaluronic acid after enucleation of mandibular odontogenic cysts. **Subjects and methods:** 24 patients complained of an odontogenic cystic lesion in mandible classified into three groups, Group I (Ozone group): Included 8 patients with odontogenic cysts treated by Enucleation followed by Ozone gel application in the surgical cavity. Group II (HA group): Included 8 patients with odontogenic cysts treated by Enucleation followed by Hyaluronic acid application in the surgical cavity. Group III (Control group): Included 8 patients with odontogenic cysts treated by Enucleation only. Postoperative one-month panoramic view, 3, 6 cone-beam C.T as the radiographic follow-up. Facial edema, pain, and trismus were the clinical parameters at 1,3 and 7 days. **Results:** The study showed a significant increase in the bone density of both group I (ozone), II (Hyaluronic acid) than the control group comparing the percentage of bone density to the contralateral bone, it was for group I (Ozone group) 45.8%, 69% at follow up of 3,6, months. In group II (HA) it was for 51.9%, 72% at follow up of 3, 6, months. It was for group III (control group) 32.6%, 56.1% at follow up of 3, 6, 9 months as there was high statistical significance between-group I, II in comparing with the group I with P-value = 0.04,0.05.**.Conclusion:** Both ozone gel and Hyaluronic acid can be considered to enhance bone regeneration after cystic enucleation due to their specific antimicrobial, anti-inflammatory properties, and increasing angiogenesis in the post-surgical cavities.

KEYWORDS

Hyaluronic Acid, Ozone gel, odontogenic mandibular cysts, enucleation, antimicrobial.

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INTRODUCTION

Over 90% of maxillofacial cysts are odontogenic in origin that is considered to be the most common group lesion that affects the maxilla and mandible. Many odontogenic cysts are asymptomatic and may be found accidentally during radiographic examination. **Kramer**[(1)] defined the cyst as a pathological cavity containing fluid, semifluid or gaseous material. In the maxillofacial area, odontogenic cysts are lined by epithelium except for solitary bone cysts and aneurysmal bone cysts [(2)]

From the most common types of odontogenic cysts in incidence in the mandible and maxilla, the odontogenic radicular and odontogenic keratocyst tumors, are of high majority types prevalence[3]. Treatment of odontogenic cysts as the principle of enucleation procedure, tend to complete removal of the cystic lining to prevent their recurrence [4].

The resulted surgical cavity after cystic enucleation can be left untreated, waiting for spontaneous bone regeneration. New bone formation or regeneration occurs from the formed blood clot spontaneously or be the effect of adjacent bone walls that are still covered by the oral mucosa. It is well known that if the lingual and buccal bone were removed during enucleation, the cystic cavity will not completely heal as there will be a chance for fibrous tissue formation instead of bone regeneration[5,6].

Different bone remodeling initiating material may be grafted in the surgical defect after enucleation that aid in the process of bone formation, however, is still now no evidence to support any specific material that indicated [7].

Ozone is an allotropic form of oxygen consisting of 3 oxygen atoms with a molecular weight of 47.9 g/mol. Ozone therapy is still used as an effective adjuvant for surgical treatment of various lesions due to its antimicrobial activities, immunostimulation, and promotion of vascularities[8-10].

However the use of ozone in oral and maxillofacial surgery was still limited but favorable outcome results were achieved in such as unhealed ulcer, infection, post-surgical impaction. Ozone is available in numerous forms such as gas, water, and gel to be used in parental and topical routes[11].

Hyaluronic acid (HA) is the simplest glycosaminoglycan polysaccharide composed of repeating disaccharide units and glucuronic acid. HA is characterized by its biophysical properties and its features of fungistatic, anti-inflammatory, anti edematous, osteoinductive characters[12,13].

HA exerts its role in wound healing by creating temporary structures during the early stage of wound healing and stimulating trigger cell proliferation. Therefore HA is used in oral wound healing and increasing the leucocytes and fibroblast proliferation [14]

PATIENT AND METHODS

24 patients complained of an odontogenic cystic lesion in the mandible referred to the Oral &Maxillofacial surgery department, Faculty of Dental Medicine and Oral Surgery, Zagazig University. The selected time of the study was from the period of Feb 2019 – Oct 2021 on the age range 10 to 40 years old. All the patients were informed about the protocol of the study, steps of work, the follow-up, the future complications associated, and signed written consent of acceptance of the study. The study theater was the Zagazig University, Faculty of Medicine Hospital, and the study has followed the declaration of Helsinki of medical protocol and ethics.

Inclusive criteria

- Patients suffered from extended odontogenic cyst more than 1 cm in diameter with an age range from 10 to 40 years old.
- Mandibular in location.
- All the patients are fit healthy for the surgical procedure

Exclusive Criteria:

- Patients are unable to fit the surgical procedure as uncontrolled diabetic, history of chemotherapy or radiotherapy, hematological disorders, osteoporosis, and patients under systemic corticosteroids that will affect bone metabolism.

Patient Grouping:

24 patients were divided into 3 equal groups as follows:

Group I (Ozone group): Included 8 patients with odontogenic cysts treated by Enucleation followed by Ozone gel application in the surgical cavity.

Group II (HA group): Included 8 patients with odontogenic cysts treated by Enucleation followed by Hyaluronic acid application in the surgical cavity.

Group III (Control group): Included 8 patients with odontogenic cysts treated by Enucleation only.

Grafted materials:

- **For group I:** Ozone gel: Pur O3**,** *saturates olive and hemp oils with activated oxygen O3 to form cleansing oxygen-rich paste manufactured by Pur O3 LLC, USA.* **Fig (2)**
- **For group II***:* Hyaluronic acid*, Hylgan as Hyaluronic acid sodium salt, manufactured by Fidia Farmaceutici S.P.A- Abano Terme, Italy***. Fig (7)**

A-Preoperative Clinical Assessment:

- Including intraoral examination of the related area, teeth affected adjacent anatomical structure, presence of infection due to the extension of the cysts.
- Aspiration of the cystic contents for cytology analysis.
- Endodontic treatment of indicated teeth was performed before the surgical procedure.

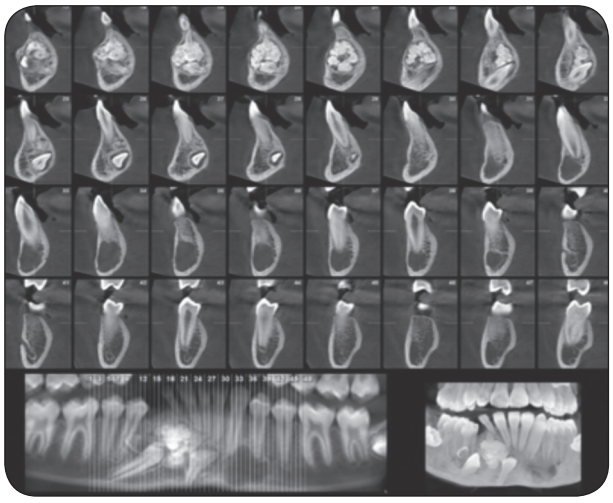


Fig. (1) Preoperative CBCT of GP I

Fig. (2) Puro3 Ozone gel for GPI

B- Preoperative radiographic assessment:

Preoperative Cone Beam Computerized Tomograph CBCT were required to detect the extension of the cystic lesion to the anatomical structure as maxillary, floor of the orbit, and inferior alveolar canal as Fig (1,8).

C- Operative procedure:

- All the surgical procedures were operated under general anesthesia in the Faculty of Medicine Hospital, Zagazig University under complete aseptic condition.
- A full-thickness mucoperiosteal flap was performed depending on the extension of the affected area.
- After reflection of the designed flap, the cystic lesions widow obtained by removal of unhealthy bone or through the access with surgical bur and sterile saline irrigation.
- The cystic lining was gently separated from the underlying bone surface to complete the enucleation procedure with associated origin as odontoma or impacted tooth in case of dentigerous cystic lesions.
- The bony edges after cystic enucleation were smoothed with a bone file and the cystic cavity was washed by normal sterile saline solution.

- Sterile gauze packs were placed into the surgical cavity to act as dry the area before applicate the study drug of each group.
- For group I after the previous steps the post cystic cavity is filled or grafted with ozone gel as **Fig(3-6).**
- While in group II the post cystic cavity is grafted with HA as in **fig (9-12)** while in group III the post cystic cavity is left without any graft material.
- The mucoperiosteal flap was then repositioned to its original place and sutured with 3/0 vicryle suture material.

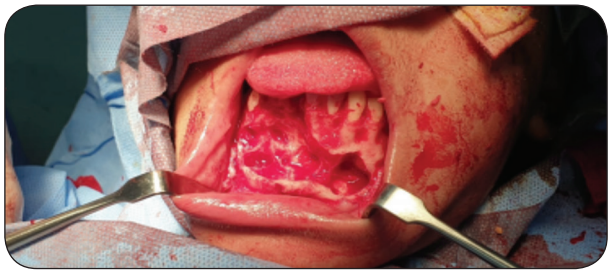
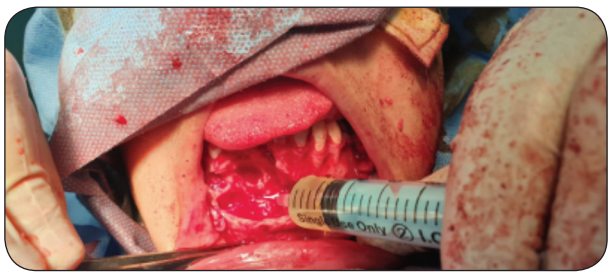


Fig (3) intraoperative surgical cavity after enucleation GPI

Fig (7) Hylgan as Hyaluronic Acid substitute GPII



Fig(4) Ozone gel application in surgical cavity for GP

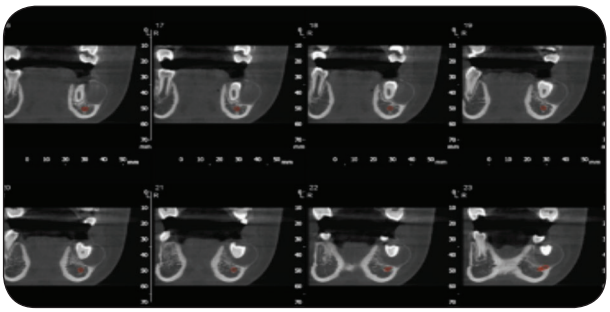
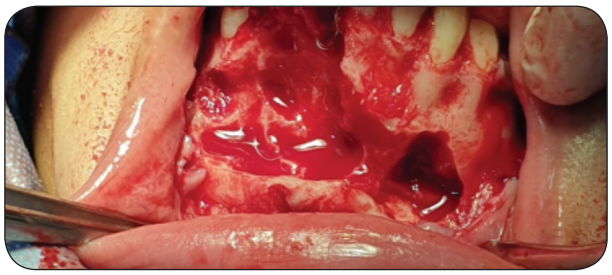
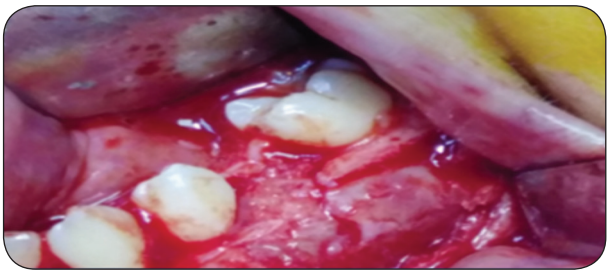


Fig (8) Preoperative CBCT for GPII



Fig(5) Surgical cavity with Ozone gel GPI



Fig(9) Intra-operative before cystic Enucleation GP II

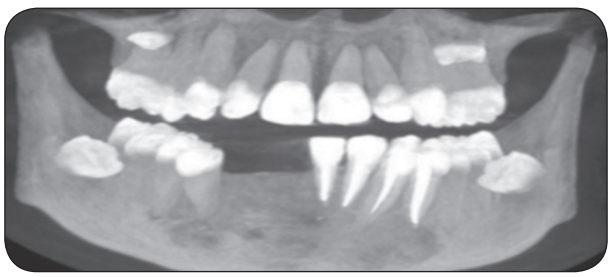


Fig. (6) One month postoperative Panoramic view GPL

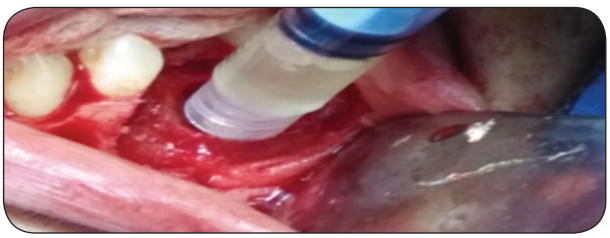
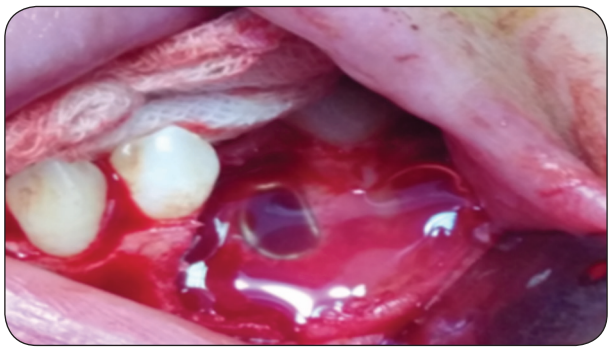
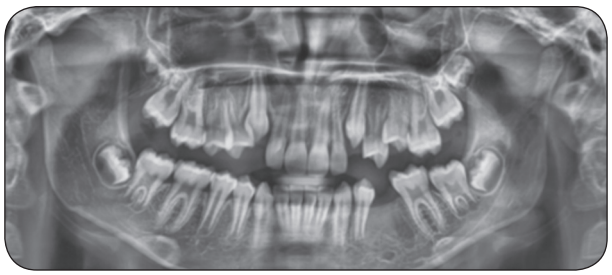


Fig (10) Application of Hyaluronic acid for GPII



Fig(11) Surgical cavity filled with HA for GPII



Fig(12) Postoperative one month panoramic view

Postoperative care:

- Gauze packs were placed and patients were instructed for cold application on the day of surgery after the patient in house care. The patients were instructed to avoid smoking and hot drinks during the first 24 hours of surgery. Patients were instructed to a soft diet and maintain oral hygiene by tooth brushing during the first week of surgery. All patients in the 3 groups were subjected to the following drugs after the surgery:

a. Amoxicillin with Clavulanic acid (Augmentin*) is available as 1 gm tablets every 12 h for 7 days.

b. Metronidazole ((Flagyl**)) available as 500 mg tablet every 8 h for 7 days.

c. Ibuprofen (Brufen***) available as 400mg tablets as required.

d. Chlorhexidine antiseptic mouth wash (Hexitol**** available as 15 ml of 0.12 % Chlorhexidine mouth wash twice daily for 7 days postoperatively.

Postoperative assessment:

Postoperative clinical assessment :

Clinical evaluation on 1st 3rd,7th day of surgery was performed to measure the parameters of :

A- **Postoperative edema:** The patients were instructed to be in an upright position with centric teeth occlusion, the 4 points were determined as Tragus, corner of the mouth, Gonion, and External canthus of the eye 3 lines are outlined starting from the corner of the mouth to each peripheral points and the mean value was calculated as follows:

B- **Trismus:** The amount of maximum mouth opening (MMO) is the parameter to evaluate the post-operative Trismus.

C- **Pain**: By using the Visual Analog Scale (VAS) as the patients were instructed to mark the degree of perceived pain on A 10- cm Horizontal line demarcated from 0 (no pain) to 10 (worth pain).

Postoperative radiographic assessment:

1. Postoperative Radiographic assessment at the follow-up of one-month panoramic view, **fig (5, 11).**

2. CBCT 3, 6 months to evaluate the bone densities of grafted areas in CT cuts by Hounsfield units for the 3 groups. Three areas were determined in each cut of CT to measure the bone density then the mean value was obtained of each group at the follow-up of 3 and 6 months fig **(13).** In the same manner, the mean of the bone density of contralateral normal bone was performed at the follow-up of 3 and 6month.

3. Postoperative 3 D after 6 months **fig (14).**

* Augmentin is produced by medical union pharmaceuticals, Abu Sultan, Ismailia under license from the GlaxoSmithKline group of companies.

** Flagyl is produced by Sanofi-Aventis Egypt s.a.e. under the license of Sanofi-Aventis France.

*** Brufen is manufactured by Kahira Pharma & Chemical Ind. Co. under license of Abbott Laboratories.

**** Hexitol produced by The Arab Drug Company, Cairo, A.R.E.

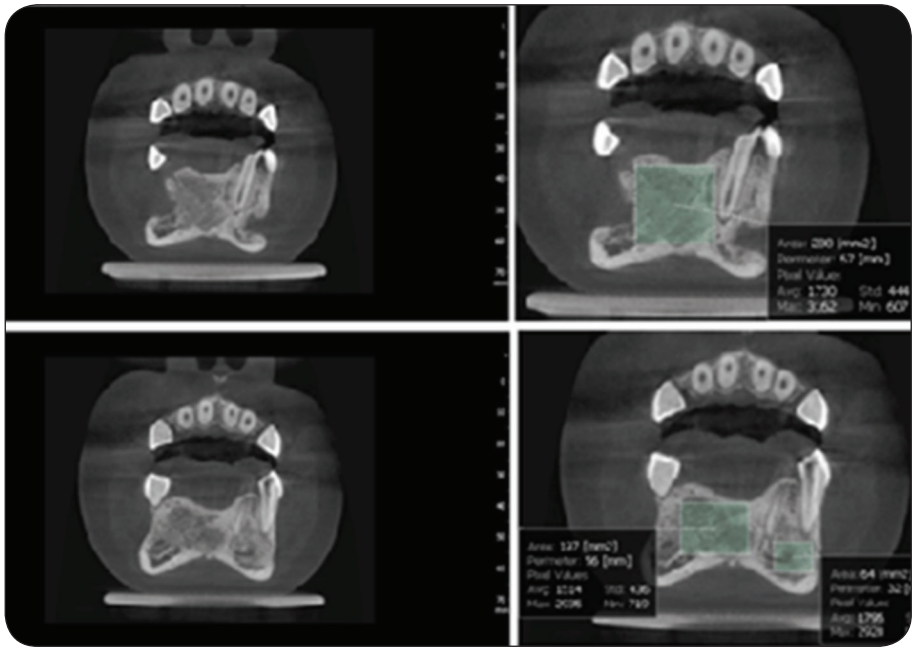


Fig. (13) Postoperative follow up CBCT with measurement of Bone Density at follow up 3,6 For GP I, II, III

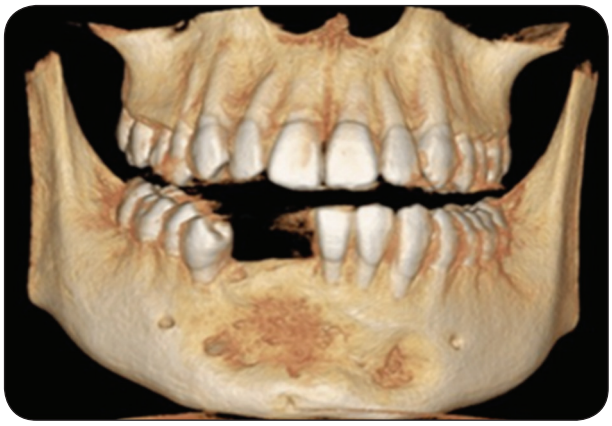


Fig. (14) Postoperative 3D reconstruction

Statistical analysis

- Statistical package of social science (SPSS program): was used for data analysis.
- Number and percent were used to describe qualitative data.
- For normally distributed data, continuous variables were described as mean ±SD(standard deviation).
- Collected data were statistically analyzed using an independent T-test to compare each study group with the control group at the follow-up time

RESULTS

This study was conducted in Oral & Maxillofacial surgery Faculty of Dental Medicine &Oral Surgery, Hospital of Faculty of Medicine, Zagazig University.

This study was evaluated 24 patients who suffered from,

A- Postoperative edema:

The mean of facial contour was measured for each group at follow-up of 1st,3rd,7th days postoperative and compared with the preoperative baselines measurements. The patients were instructed to be in the upright position with centric teeth occlusion, the 4 points were determined as Tragus, corner of the mouth, Gonion, and External canthus of the eye 3 lines are outlined starting from the corner of the mouth to each peripheral point and the mean value was calculated as follows:

- 1st day postoperative: There was a significant difference between the 3 groups in comparing with the preoperative baselines measurements,

as the baseline was 10.4±0.7 Cm^2 and 10.8± 0.5 Cm^2 for Group I (Ozone), 10.6± 0.8 Cm^2 for Group II (HA), 10.6± 0.7 Cm^2 for Group III (Control) with (P-value ≤ 0.05).

- 3st day postoperative: There was a significant increase in the mean of facial contour that explained the maximum value of post-operative edema in all groups. while in the study of this value in the same time of each group, There was a significant difference between group I, II in comparing with the preoperative baselines measurements, than in group I . As the mean of each group as follows: 11.8± 1.5 Cm^2 for Group I (Ozone), 11.7± 0.9 Cm^2 for Group II (HA), 16.2± 0.7 Cm^2 for Group III (Control) (P-value = 0.0001).

- 7th day postoperative: There significant decrease in postoperative edema on the 7th day for all groups at this time .while there was no significant difference between the 3 groups in comparing with the preoperative baselines measurements, as 10.8± 0.5 Cm^2 for Group I (Ozone), 10.4± 0.8 Cm^2 for Group II (HA), 10.6± 0.7 Cm^2 for Group III (Control) with (P-value ≤ 0.05) as no significant difference.

B- Postoperative Trismus:

The maximum mouth opening (MMO) was measured as the interincisal distance for each patient at the follow-up of 1st, 3rd, 7th postoperative days and recorded. On comparing the 3 groups than baseline preoperative as 3.1±0.05 and the value of MMO of each group were:

- 1st day postoperative: There was no significant difference between the 3 groups in comparing with the preoperative baselines measurements, as the baseline was3.1±0.05, 2.63.1±0.05 for Group I (Ozone), 2.7± 0.8 for Group II (HA), 2.7± 0.7 for Group III (Control) with (P-value ≤ 0.05) as no significant difference.

- 3rd day postoperative: There was no significant difference between the 3 groups in comparing with the preoperative baselines measurements, as the baseline was 3.1±0.05, 2.8.1±0.05 for Group I (Ozone), 2.7± 0.8 for Group II (HA), 2.8± 0.7 for Group III (Control) with (P-value ≤ 0.05) as no significant difference.

- 7 day postoperative: There was no significant difference between the 3 groups in comparing with the preoperative baselines measurements, as the baseline was 3.1±0.05, 3.1±0.05 for Group I (Ozone), 3.4± 0.8 for Group II (HA), 3.6± 0.7 for Group III (Control) with (P-value ≤ 0.05) as no significant difference.

C-Postoperative Pain:

In group III as a control group, the mean VAS of patients was 87.04 ± 3.55 on the 1st day of surgery. There was a highly significant decrease after one day post-operatively, where the mean VAS was 88.90 ± 6.59 (P-value = 0.001).

Similarly, there was a highly significant decrease after the 3rd and 7th days of surgery where the mean VAS was 89.60±9.30 (P-value = 0.0001) and 56.48± 7.1 (P-value = 0.0001).

On comparing the 3 groups, there were statistically significant differences in VAS scores of pain at 1st, 3rd,7th postoperative days. On the day of surgery, the mean pain score was significantly decreased in a group (I), (II) compared to group (III), as it was 71 ± 3.1 in the group (I), 84.9 ± 3.50 in the group (II) and 98.9 ± 3.10 in the group (III) (P-value = 0.0001). After one, three, and seven days of surgery, pain score was significantly lower in groups I, II compared to group III.

D- Bone Density:

The mean of bone densities of newly formed bone in the surgical cavities after cystic enucleation and application of the Ozone gel (GP I) and HA(GPII) were measured at the follow-up time of 3 and 6 months post-operative with CBCT in HU:

1. For group (I) Ozone group: It was recorded as follows, 145.9 HU at 3 months, 197.4 HU at 6 months.

2. For group (II) HA group: 155.2 HU at 3 months, 198.4 HU at 6 month

3. For group (III) control: 123.7 HU at 3 months, 173.4 HU at 6 months.

On comparing group (I, II) with a group (III) there was a highly significant(P-value = 0.0001).

By comparing the percentage of bone density to the contralateral bone, it was for group I (Ozone group) 45.8%, 69% at follow up of 3,6, months. In group II (HA) it was for 51.9%, 72% at follow up of 3, 6, months. It was for group III (control group) 32.6%, 56.1% at follow up of 3, 6, 9 months as there was high statistical significant between-group I, II in comparing with the group I with P-value = 0.04,0.05.

DISCUSSION

This study aimed to evaluate the effect of both Ozone gel and Hyaluronic acid on bone remodeling after mandibular odontogenic cysts enucleation compared with the control group. The clinical parameters were postoperative pain, trismus, and edema. While the radiographic assessment was a thorough evaluation of bone densities of each group of patients and compared with that of the control group.

Our study was in agreement with ***Gupta and Mansi 2012***[15]***& Sechi 2001***[16] who assess the local application of ozone in form of a gel. We tend to use gel application as a source of both Ozone and HA as from point of view, the gel form becomes stable for a long duration with sustained release of the active material once applicated within the surgical cavity to be clinically beneficial.

Our study within the same protocol of clinical parameters as swelling, pain, and edema to be with acceptance of the study by ***Varun et al 2017***[17] who investigate the effect of ozone gel after surgical removal of the impacted third molar as the parameters were the same as our study.

During our study follow up we estimated after one month by panoramic radiograph as an overall outline to detect the surgical cavity and related area after one month. This in agreement with ***Kwon et al 2020***[18] who was used a panoramic view to evaluate the volumetric changes after cystic enucleation.

In our study during the follow-up period of our study at 3, 6 months there was a CBCT to estimate the extension of the cystic cavity and detect the bone densities, and comparing each cut with the contralateral area overall the 3 groups. it was found that for the group (I) It was recorded as follows, 145.9 HU at 3 months, 197.4 HU at 6 months, for the group (II) 155.2 HU at 3 months, 198.4 HU at 6 months, for the group (III) 123.7 HU at 3 months, 173.4 HU at 6 months. These results explain the effective action of both ozone and HA in increasing the densities of newly formed ossification islands compared with the control group. This is in agreement with ***Lacin N et al***[19] who explained the superior effect of ozone gel as bone remodeling as increasing the angiogenesis and osteogenesis in bone remodeling.

Our study by comparing the percentage of bone density to the contralateral bone was for group I (Ozone group) 45.8%, 69% at follow up of 3,6, months. In group II (HA) it was for 51.9%, 72% at follow up of 3, 6, months. It was for group III (control group) 32.6%, 56.1% at follow up of 3, 6, 9 months as there was high statistical significance between group I, II in comparing with the group I with P-value = 0.04,0.05. This is with the agreement of ***Mohamed et al, 2015***[20] who used ozone gel experimentally in mandibular bony defects and they explained the high ratio increasing the bone density and vascularization in the study group than in control. In addition, our study was in agreement with other authors ***Agrillo et al 2007***[21] who study

the effect of topical use of ozone on post-extraction socket healing in 18 patients with bisphosphonates.

Our study was in agree with ***Holmes 2002***[22], that reported that effect of ozone as anti-inflammatory and antimicrobial improvement in periodontal diseases to enhance bone formation. Also in our results both group I, II was of high bone density as this accepted the study by ***MS Matar 2016***[23] who explained the best result during osseointegration of implant as the action of study group by ozone than the control group.

Our study was in agreement with ***Yazan et al*,2018**[24] who considered Hyaluronic acid to be one of the important glycosaminoglycans in the extracellular matrix and is synthesized by synoviocytes, fibroblasts, and chondrocytes. It is involved in cell proliferation and migration and is a mechanism for tissue repair they aimed to investigate its effects in gel form on the Osseointegration of dental implants. During Osseointegration, the woven bone that formed during the first stage of healing adapts to the loading forces and is converted to lamella bone, which consists of parallel fibers.

CONCLUSION

Both ozone gel and Hyaluronic acid can be considered to enhance bone regeneration after cystic enucleation due to their specific antimicrobial, anti-inflammatory properties, and increasing angiogenesis in the post-surgical cavities.

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دراسة مقارنة بين فعالية جل الأوزون وحمض الهيالورونيك في التئام العظام بعد استئصال كيسات الفك السفلي السنية

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الملخص :

الهدف: هدفت هذه الدراسة إلى تقييم التأثير الإكلينيكي والشعاعي لكل من هلام الأوزون وحمض الهيالورونيك بعد استئصال كيسات الفك السفلي السنية.

المواد والأساليب: اشتكى 24 مريضًا من آفة كيسية سنية المنشأ في الفك السفلي مصنفة إلى ثلاث مجموعات. المجموعة الأولى (مجموعة الأوزون): تضم 8 مرضى يعانون من أكياس سنية تم علاجهم بالقص متبوعًا بتطبيق هلام الأوزون في التجويف الجراحي. المجموعة الثانية (مجموعة HA): ضمت 8 مرضى يعانون من أكياس سنية تم علاجهم بالاستئصال متبوعًا بتطبيق حمض الهيالورونيك في التجويف الجراحي. المجموعة الثالثة (المجموعة الضابطة): تضم 8 مرضى يعانون من أكياس سنية تم علاجهم بالاستئصال فقط. منظر بانورامي بعد الجراحة لمدة شهر واحد ، 3 ، 6 شعاع مخروطي CT كمتابعة شعاعية. كانت الوذمة في الوجه والألم والتشقق هي العوامل السريرية في الأيام 1,3 و 7

النتائج: أظهرت الدراسة زيادة معنوية في كثافة العظام لكلا المجموعتين الأولى (الأوزون) والثانية (حمض الهيالورونيك) مقارنة بالمجموعة الضابطة مقارنة نسبة كثافة العظام بالعظم المقابل. وكانت للمجموعة الأولى (مجموعة الأوزون) 45.8٪ ، 69٪ عند متابعة 3,6 شهر. في المجموعة الثانية (HA) كانت 51.9٪ ، 72٪ في متابعة 3 ,6, أشهر. كانت للمجموعة الثالثة (المجموعة الضابطة) 32.6٪ ، 56.1٪ عند متابعة 3 ، 6 ، 9 أشهر حيث كانت هناك دلالة إحصائية عالية بين المجموعة الأولى والثانية في المقارنة مع المجموعة الأولى بقيمة P = 0.04,0.05

الخلاصة: يمكن اعتبار كل من جل الأوزون وحمض الهيالورونيك لتعزيز تجديد العظام بعد استئصال الكيس بسبب خصائصهما المضادة للميكروبات والمضادة للالتهابات وزيادة تكوين الأوعية في تجاويف ما بعد الجراحة

الكلمات المفتاحية: حمض الهيالورونيك ، جل الأوزون ، كيسات الفك السفلي سنية المنشأ , كنس , مضاد بكتيرى.