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IMPACT OF OZONE GEL, PRF & A-PRF ON PAIN AND TRISMUS DURING TISSUE HEALING AFTER EXTRACTION OF MANDIBULAR THIRD MOLARS

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ABSTRACT

Objective: This prospective study evaluated the impact of ozone gel, conventional PRF (C-PRF) and advanced PRF (A-PRF) on pain and trismus when applied after surgical extraction of impacted mandibular third molars.

Materials and Methods: This study included 48 patients with impacted mandibular third molars. They were randomly divided into 4 groups, group I (control); didn't receive any material in the socket, group II: sockets received ozone gel, group III: sockets received PRF while in group IV: sockets received A-PRF. Pain assessment using VAS, analgesics consumption, and maximum mouth opening were evaluated on day 1, day 3 and day 7 follow-up.

Results: Less values of VAS pain scores were recorded in group II in comparison with the control group on day 1, day 3 and day 7 with a statistically significant difference of (P=0.018), (P=0.044) and (P=0.015) respectively. The analgesics consumption showed a statistically significant difference in group II compared to group I (P=0.020) and (P=0.021) on day 1 and day 3 respectively, but day 7 was statistically insignificant (P=0.165). Maximum mouth opening was statistically significant (P=0.042) on day3 in group II when compared with group I.

Conclusion: The present study shows that ozone gel can be of paramount importance in minimizing post-operative symptoms as regard to pain and trismus following the extraction of impacted mandibular third molars and appears to improve patient's relief and quality of life after surgery.

KEYWORDS: Ozone gel, PRF, A-PRF, pain, trismus

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INTRODUCTION

Surgical extraction of impacted third molars is one of the most commonly accomplished oral surgical procedures. The reported frequencies of complications after this procedure range between 2.6 percent and 30.9 percent, so it requires accurate planning and surgical skills. Complications of such procedure range from minor pain and swelling post-operatively to permanent damage of the inferior alveolar nerve, mandibular fractures, and life-threatening infections, depending on the position of the tooth, bone density and the complexity of surgical extraction.[1,2] Undisturbed tissue healing, proper wound care and control of post-operative pain and trismus were the main issues of many studies to improve the patients' quality of life after this surgical procedure.[3]

Pharmacological therapy, alternative medicine, in addition to complementary protocols have been developed to control post-operative complications. Ozone therapy is considered as a modern, non-medicated alternative strategy with demonstrated antibacterial and anti-inflammatory properties. Ozone gas is a triatomic molecule with three oxygen atoms that is highly unstable thermodynamically and undergoes decomposition into pure oxygen with a half-life of 40 min at 20°C. When used as an antimicrobial agent, ozone proved to have 1.5 times more oxidation potentiality than chloride. It is capable of stimulating both blood circulation and the immune response which justifies its intervention in treatment of 260 different pathologies.[4]

Researches have proved its ability to modulate the cellular and humoral immunity as it activates macrophages and stimulates synthesis of biologically active substances that reduce inflammation and improve wound healing.[5] The use of ozone in the field of oral surgery is limited;[6] and up to our knowledge, articles regarding the use of ozone after impacted mandibular third molar surgery are scarce.

On the other hand, tissue engineers are saving no effort to develop newer biomaterials that enhance natural tissue healing, since it is the main stay in any surgical procedure. Efficiency in wound healing as well as biocompatibility are the main issue in any developing biomaterial, in addition to considering its cost effectiveness, its ease of production and availability to be widely used as a chairside biomaterial. The development of bioactive additives in surgery has evolved a great challenge in clinical research.[3] Platelet-rich fibrin (PRF) is one of the bioactive surgical additives that has become popular in tissue regeneration field.[7]

PRF is derived by centrifugation of autologous blood without addition of any anticoagulants. PRF contains platelet-rich concentrate and growth factors that favor healing and play a key role in micro-vascularization and cell migration in the socket post-extraction.[8] As a result, a homogeneous fibrin network is formed and acts as a three-dimensional organization that shows higher coherence than the natural fibrin clots.[9-13]

In addition to the platelets known role for hemostasis, they contain platelet-derived growth factors, two isomers of transforming growth factor-beta, vascular endothelial growth factor, and epithelial growth factor.[9] These growth factors increase cell mitosis, accumulate other cells that are responsible for recovery to the injury site, initiate the vascular growth, increase the production of collagen, and induce cell differentiation. Moreover, increasing platelets concentration at the injury site promotes healing process.[14-17]

Advanced platelet-rich fibrin (A-PRF) is prepared by centrifugation speed at 1500 rpm for 14 minutes, in contrast to the conventional platelet-rich fibrin which is prepared at 3000 rpm for 10 minutes. Few studies proved that increasing the centrifugation time with decreased rpm enhances the concentration of the platelets as well as the neutrophilic granulocytes in the clot, which in turn, influences the differentiation of host macrophages within the clot after implantation[18], and accelerates bone and soft tissue regeneration. As a result, the

inter-fibrous space increased, with higher cell count, more balanced distribution of regenerative cells in the clot and increased release of growth factors like TGF-b1, PDGF, EGF, and IGF.[19] Choukroun emphasized that A-PRF clot is softer than conventional PRF.[20] A-PRF has proved to accelerate both the proliferation and migration of the hematopoietic growth factors.[21]

This study was designed to compare the efficacy of ozone gel, conventional PRF and A-PRF in terms of pain and trismus when topically applied in sockets of surgically extracted mandibular third molars.

MATERIALS AND METHODS

This study was conducted on 48 patients seeking surgical extraction of impacted mandibular third molars. Patients were received at the outpatient clinic of Oral and Maxillofacial Surgery Department, Faculty of Dentistry, Mansoura University

The study followed the Declaration of Helsinki on medical protocol and ethics. It was approved by the Ethical Review Board of Mansoura University. All patients were informed about the nature of the study and signed a written consent with full rights to quit the study at any time.

The predictor variable was local application of ozone gel, PRF and A-PRF in the sockets of the surgically extracted mandibular third molars. The outcome variables were measuring pain analgesic consumption and trismus at days 1, 3 and 7 after the surgery.

Equipments

1. Centrifuge machine* (Spinplus Centrifuge: TC-SPINPLUS-6 Digital Desktop Centrifuge with 3074 RCF, 100-5000 rpm, LCD Display, Includes 15ML X 6 Rotor, Timer 1 5sec-99min) **(Fig. 1).**
2. Blood collection armamentarium.
3. Glass test tube (without anticoagulant)**.**

Fig. (1) The centrifuge machine.

Materials

1. **Ozone gel:** (Ozene; Premier Research Labs, lp, 3500-b Wadley pl, Austin, TX, USA. 78728), which is considered as a natural material composed of a suspension of medical grade ozone (a mixture of oxygen and ozone in the ratio of 0.25% and 99.75% respectively) and un-oxidized olive oil. **(Fig. 2)**

Fig. (2) Ozone gel

2. **PRF:** Under aseptic precautions, 10ml of venous blood was withdrawn from the antecubital vein of the patients and collected in a pre-sterilized test tube without an anticoagulant and immediately placed in a pre-programmed centrifuge at 3000 rpm (approximately 400 g) for 10 minutes in a table-top centrifuge to obtain PRF gel. After centrifugation, three isolated layers were defined as follows: the PRF layer at the top, white blood cells layer in the middle, and red blood cells at the bottom. The topmost PRF gel layer was retrieved from the test tube, squeezed on sterile saline-soaked gauze pieces to obtain the PRF membrane which was immediately placed in the extraction socket.[3, 22] **(Fig. 3)**

3. **A-PRF:** For preparing A-PRF, around 10 ml of venous blood sample was withdrawn from the patient's antecubital vein, then transferred to sterile glass test tubes without adding any anticoagulants and immediately centrifuged at 1500 rpm for 14 minutes at room temperature. Sterile tweezer was used to gently retrieve the clot. The RBCs fraction was removed in such a manner that the bottom of the fibrin-rich clot was not damaged, and then the later was inserted into the socket.[12, 23]

Patients' selection

The inclusion criteria included patients aged between 18-35 years of any gender with mandibular third molar impactions of moderate difficulty as per Pederson difficulty index.[24] Patients classified according to the American Society of Anesthesiologists classification as (ASA-I), who have good general health without any contraindication for minor oral surgery and/or local anesthesia were included in the study. Patients had to undergo a medical blood examination for hemoglobin, leukocyte count, platelet count, blood sugar, and serum creatinine so as to rule out any blood-related disorder.[7]

The exclusion criteria included patients with history of any relevant medical condition or chronic use of medications such as antihistamines, NSAID or steroids and antidepressants that may interfere with the subjective evaluation of their postoperative pain, history of platelet/ bleeding/ connective tissue disorders, patients on anticoagulants or immunosuppressant drugs, periodontally compromised patients, presence of pericoronitis, and those having deleterious habits (tobacco chewing, smokers and regular alcohol consumption), patients allergic toward ozone or penicillin or other drugs; status of pregnancy or lactation.[3, 9]

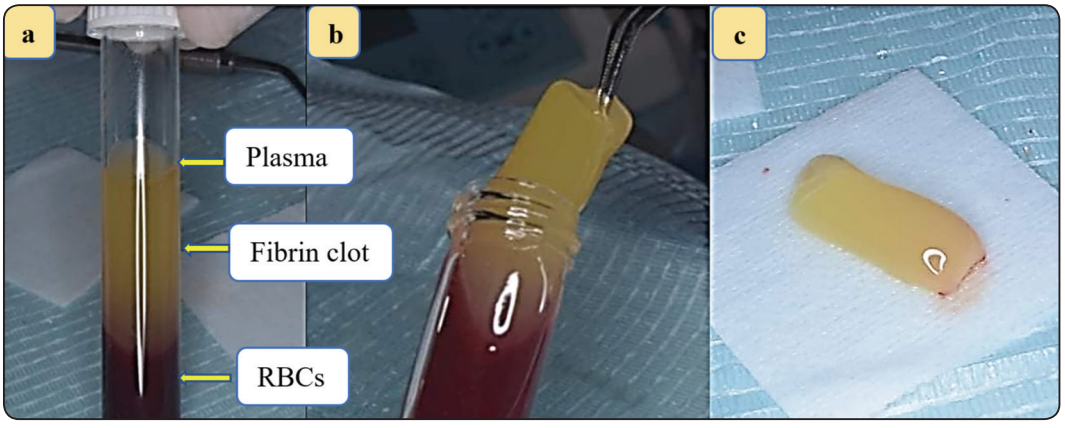

Fig. (3) PRF clot
a. The layers of the centrifuged blood.
b. PRF clot retrieved from the tube after blood centrifugation by a sterilized tweezer.
c. The RBCs fraction was removed in such a manner that the bottom of the fibrin-rich clot was not damaged

Groups Allocation

A total of 48 patients were randomly distributed by an independent viewer into 4 groups with 12 patients in each group **(Fig. 4)**, where the following were placed into the extraction sockets:

- **Group I:** No material was inserted in the socket after tooth extraction.
- **Group II:** Ozone gel.
- **Group III:** Conventional PRF clot.
- **Group IV:** Advanced-PRF clot.

Pre-operative phase:

All patients' data were recorded including; name, gender, age. A detailed history consisting of chief complaint, relevant past medical history, drug history/allergy, and personal history were recorded for every patient to rule out any systemic conditions.

Clinical examination included assessment of maximal mouth opening preoperatively. The maximal mouth opening was assessed and measured with a simple caliper. This was considered as the preoperative base line measurement of the maximal mouth opening. The maximal mouth opening was

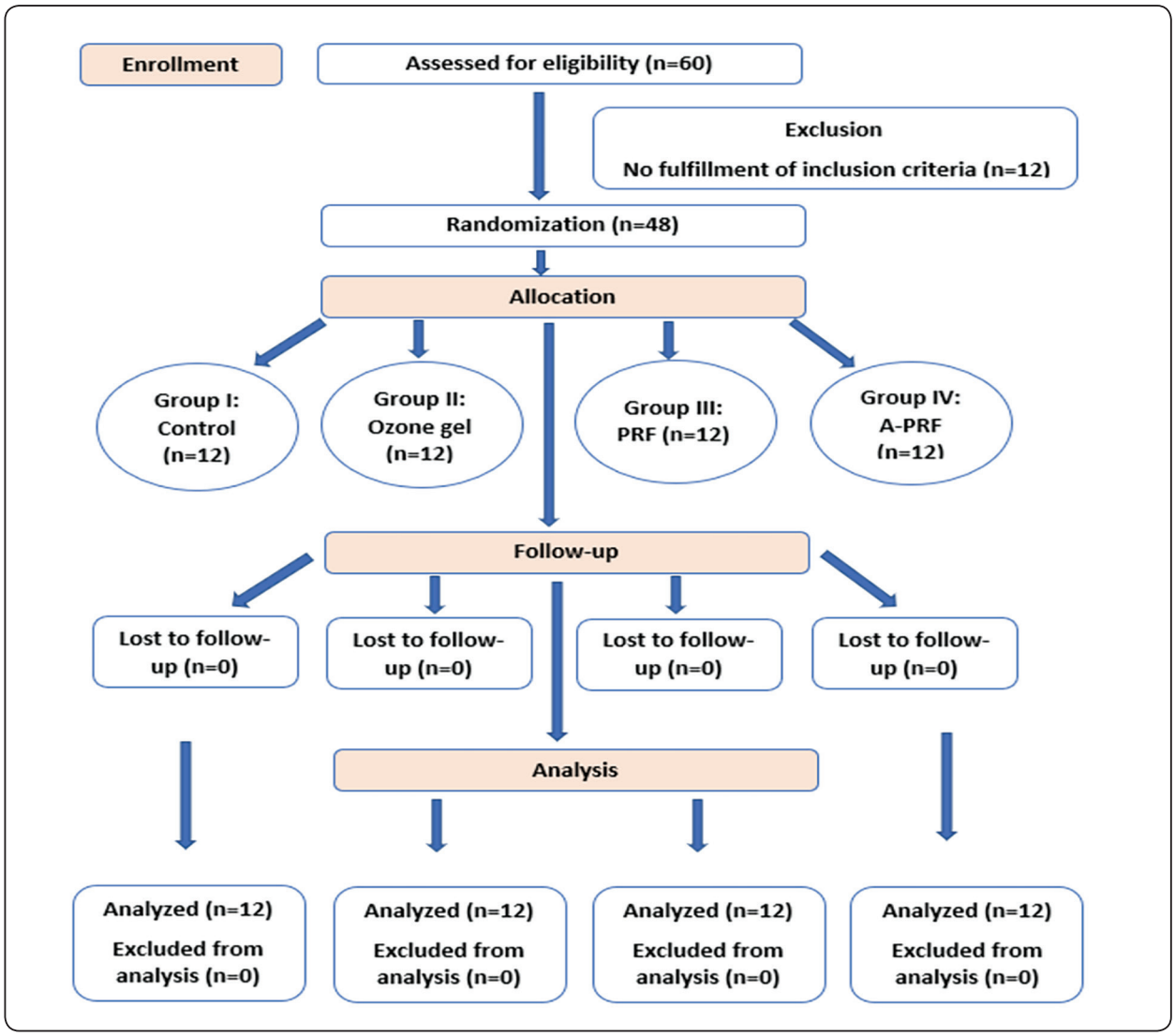

Fig. (4) Study design. (PRF: platelet rich fibrin, A-PRF: advanced platelet rich fibrin)

remeasured postoperatively at days 2, 5 and 7. All readings were recorded and analyzed statistically. Panoramic x-ray was used for evaluation of the position and morphology of the impacted tooth, surrounding bone, and its relation to the mandibular canal and adjacent tooth.

Patients were instructed firmly to follow proper oral hygiene. They also received periodontal scaling when needed, to reduce gingival inflammation. Antiseptic mouthwash rinse (Hexitol Chlorhexidine HCl 1.25%, by ADCO company Ltd, Alexandria, Egypt) was used for rinsing 30 seconds before the surgery, to decrease the risk of surgical field contamination. Prophylactic antibiotic of one gm amoxicillin + clavulanic acid was prescribed, one tablet the day before and another tablet two hours before surgery.

Surgical Procedure

All patients were operated by the same surgeon who was blinded to the group allocations until the end of the operation. Patients were not aware of their group assignments till the end of the study.

All groups followed surgical asepsis and a standardized surgical procedure. Complete anesthesia of site of interest was achieved by administration of local anesthesia (mepivacaine hydrochloride 2% and levonordephrine 1:20,000) as an inferior alveolar, lingual and buccal nerves block.

A three-sided mucoperiosteal flap was incised, with help of Ward's incision, using a bard parker blade #15 mounted on a bard parker handle. The incision was started just to the lingual side of the external oblique ridge of the ramus of the mandible at a distance of about 3⁄4 inch distally from the mandibular second molar. Then, it was directed anteriorly until it contacted the midpoint of the distal of the mandibular second molar. The incision was then continued buccally to just before the interproximal space between the first and second molar teeth. From there it was extended down toward the mucobuccal fold at a 45-degree angle. The full thickness mucoperiosteal flap was reflected using Molt periosteal elevator and retracted by Minnesota retractor.

Osteotomy was performed as minimal buccal bone guttering and tooth sectioning, if needed, was done by a high-speed micro-motor hand piece using straight fissure carbide burs under constant irrigation with saline to avoid over-heating of bone. Tooth was delivered and the bone edges were smoothened.

After tooth extraction, any debris or loose fragments were removed using tweezers and periapical curettes, bone margins were smoothened out with bone file. The socket was irrigated with sterile saline and bleeding was controlled with pressure gauze packs.

After thorough isolation and debridement, the socket was managed as follows:

- **Group I:** control group; no materials were added to the socket.
- **Group II:** received topical ozone. After careful isolation of the surgical field, the entire socket was filled with the ozone gel and a smear layer was applied on the vertical limb of the incision. The patients were instructed to apply topical ozone as a smear layer on the edges of the flap, using a scoop for 2 minutes, 3 times a day, for 3 days. [25]
- **Group III:** The socket was packed with sterile gauze until conventional PRF was received and inserted.
- **Group IV:** the same was done as group III, but A-PRF was placed instead of conventional PRF.

The socket was sutured with 3–0 braided black silk sutures and packed with sterile gauze on which the patient was asked to apply pressure.

Postoperative phase

All patients received postoperative instructions to bite down on the gauze pack that have been placed over the surgical area, making sure it remains in place almost for an hour. After this time, the pack should be removed and discarded.

Patients were instructed that if bleeding persists, to repeat the process. Rinsing or swishing was avoided for 24 hours after extraction. Ice packs were advised to be placed extra-orally where the surgery was performed to help reduce swelling.

After 24 hours, patients began gentle rinsing with an antiseptic mouthwash (0.12 % Chlorhexidine hydrochloride) (Hexitol® mouthwash: Arab Drug Company (ADCO), Cairo, Egypt) 3 times a day for 7 days. They were instructed to take soft foods for the first two days and maintain proper oral hygiene measures Each participant was maintained on antibiotic (Amoxicillin 875 mg &Clavulanic acid 125 mg) (Augmentin® 1 g Tablet by Galaxosmithkline co ltd, USA) every 12 hours for 5 days), Ibuprofen 400 mg (BRUFEN® 400 mg tablets: Abbott Egypt, Cairo, Egypt) was prescribed to be taken whenever needed (every 8 hrs. maximum 3 tablets per day).

Post extraction wound management varied for group II, where the patients were instructed to apply topical ozone as a smear layer on the edges of the flap, using a scoop for 2 minutes, 3 times a day, for 3 days.

Measurement of the outcomes

All participants were scheduled for follow-up on day 1, i.e. immediate post-operative day, 3rd day and 7th day postoperatively. All the sutures were removed on the 7th post-operative day. Pain, analgesic consumption, and maximum mouth opening (MMO) were evaluated by a single physician as follows:

- ***Evaluation of pain by VAS scale:***

Using Visual Analog Scale (VAS)[26], the patients were asked to score their pain level as a number between 0, indicating ''no pain'', and 10, indicating ''severe pain'' at day 1, day 3 and day 7 after surgery. Then, the pain values were compared. **(Fig. 5)**

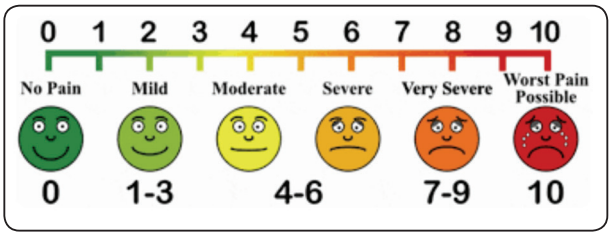

Fig. (5) VAS for pain assessment

- ***Evaluation of analgesic consumption:***

The patients were asked to record the number of tablets of analgesic consumed at day 1, day 3 and day 7.

- ***Evaluation of maximum mouth opening:***

Using calipers, the maximum mouth opening was measured in millimeters for each patient as the distance between the right maxillary and mandibular central incisors. It was measured preoperatively and then repeated on the 1st, 3rd and 7th days postoperatively. The trismus was evaluated by comparing the pre-operative and subsequent post-operative values.

Statistical Analysis

Statistical Package for Social Sciences (SPSS) version 25 was used for data analysis. Data with numerical values were summarized by means and standard deviations or medians and ranges. Data were explored for normality using Kolmogrov-Smirnov test and Shapiro-Wilk test. The one-way ANOVA was used for comparing between the 4 groups with respect to normally distributed numeric variables. The over-time comparison was done by

repeated measure ANOVA followed by Bonferroni post hoc test. Non normally distributed numeric variables were compared by Kruskal Wallis test. Friedman test was used for comparing over time regarding numeric variables (pain score), and pairwise difference was detected by the Wilcoxon rank test. Chi square (c2) test and Fisher's exact test were used, when appropriate, for analysis of differences of categorical variables. The Bonferroni method for multiple testing was used for adjustments of p value. All p-values are two-sided and P-value ≤0.05 was considered significant.

RESULTS

This study included 48 patients having 48 impacted mandibular third molars reported that were selected from the outpatient clinic of the Oral and Maxillofacial Surgery Department, Faculty of Dentistry, Mansoura University. The patients were grouped as follows: Group I; control (n = 12), Group II; ozone gel (n = 12), Group III; PRF (n=12) and Group IV; A-PRF (n=12). Random selection of patients reporting with age range from 18 to 35 years. The age and sex distribution of patients involved in this study are mentioned in **Table 1 and Fig. 6**.

TABLE (1) Descriptive statistics, one way ANOVA and Chi square tests for the demographic data of the tested groups

	Group I (Control)	Group II (Ozone)	Group III (PRF)	Group IV (A-PRF)	p value
	(n=12)	(n=12)	(n=12)	(n=12)	
Age (yrs.)					
Mean± SD	26.4±4.8	25.8±4.7	25.8±5.4	26.4±4.1	0.976
Range	18-33	18-33	18-35	21-34	
Sex					
Male	6(50.0)	6(50.0)	7(58.3)	5(41.7)	0.881
Female	6(50.0)	6(50.0)	5(41.7)	7(58.3)	

SD: Standard deviation, P≤ 0.05 is significant

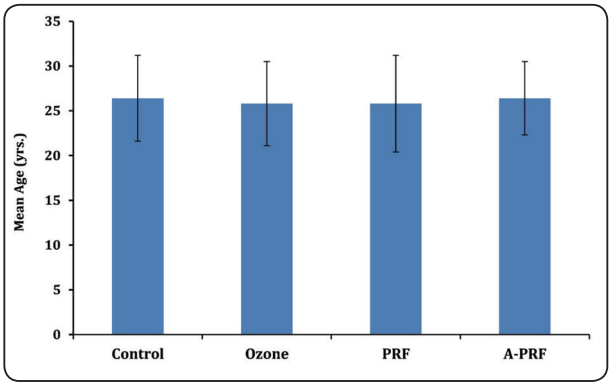

Fig. (6) Demographic data of the tested groups

Evaluation of pain by VAS scale:

Pain assessment showed less pain scores on Ozone gel side as compared to control group at day 1, day 3 and day 7 with statistically significant difference of (P=0.018), (P=0.044) and (P=0.015) respectively. Comparing change over time in each group, there was statistically significant difference in each time point as shown in **Table 2 and Fig. 7.**

TABLE (2): VAS score of the tested groups and overtime

VAS score	G. I (Control), n=12	G. II (Ozone), n=12	G. III (PRF), n=12	G. IV (A-PRF), n=12	
	Median (Range)	Median (Range)	Median (Range)	Median (Range)	p value1
Day 1	8(7-9) [a]	6(5-9) [a]	8(5-9)	8(3-9)	**0.018**
Day 3	5(4-6) [b]	4(2-5) [b]	5(2-8)	5(2-8)	**0.044**
Day 7	3(1-4) [c]	1(1-2) [c]	1(0-10)	2(0-7)	**0.015**
P value 2	<0.001	<0.001	<0.001	<0.001	

P≤ 0.05 is significant, P value 1: comparing 2 groups at each time point by Kruskal Wallis test, p value 2: comparing overtime in each group by Friedman test, small case letters are statistically significant

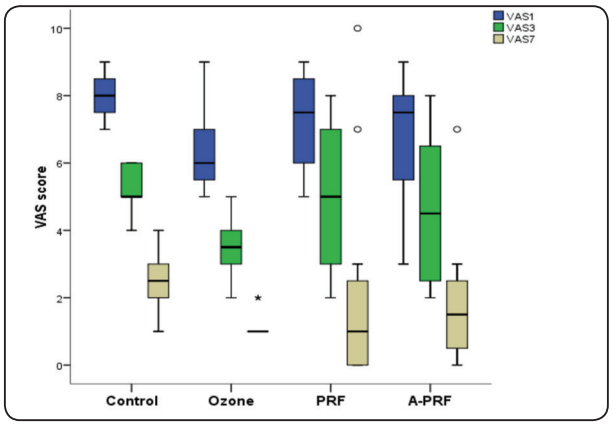

Fig. (7) **VAS score of the tested groups and overtime**

Evaluation of analgesic consumption:

Data collected by subjective questioning about analgesics consumed by the patients showed statistically significant difference on Ozone gel side as compared to control group; (P=0.020) and (P=0.021) at day 1 and day 3 respectively, indicating towards the analgesic properties of Ozone gel. However, data for day 7 did not show statistically significant difference (P=0.165). Comparing change over time in each group, there was statistically significant difference in each time point as shown in **Table 3 and Fig. 8.**

TABLE (3) Analgesics requirements of the tested groups and overtime.

Analgesics	G. I (Control), n=12	G. II (Ozone), n=12	G. III (PRF), n=12	G. IV (A-PRF), n=12	p value 1
	Median (Range)	Median (Range)	Median (Range)	Median(Range)	
Day 1	1400(800-1600) [a]	800(800-1200) [a]	1200(800-1600)	1200(800-1600)	**0.020**
Day 3	1200(400-1600) [b]	400(400-1200) [b]	800(400-1600)	800(0-1200)	**0.021**
Day 7	800(0-1600)	0(0-800)	400(0-1600)	400(0-1600)	0.165
P value 2	<0.001	<0.001	<0.001	<0.001	

P≤ 0.05 is significant, P value 1: comparing 2 groups at each time point by Kruskal Wallis test, p value 2: comparing overtime in each group by Friedman test, small case letters are statistically significant, All p values were adjusted for multiple comparisons by Bonferroni adjustment method.

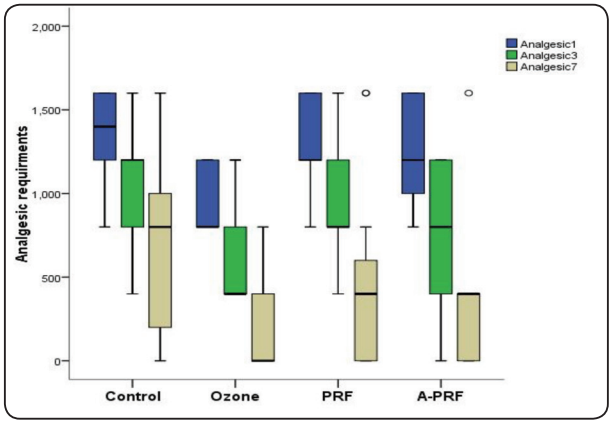

Fig. (8) **Analgesics requirements of the tested groups and overtime.**

Evaluation of maximum mouth opening:

The difference between groups was statistically non-significant preoperatively and throughout the whole observation periods at days 1 and 7. However, statistically significant difference (P=0.042) was recorded at day 3 towards the Ozone group (mean mouth opening was 33.3 mm with standard deviation 3.6 mm) as compared to the control group (mean mou9h opening was 28.8 mm with standard deviation 4.7 mm) as shown in **Table 4 and Fig. 8.**

TABLE (4) One way ANOVA and Repeated measure ANOVA for the Mouth opening of the tested groups and overtime

Mouth opening at	G. I (Control),n=12	G. II (Ozone), n=12	G. III (PRF), n=12	G. IV (A-PRF),n=12	
	Mean± SD	Mean± SD	Mean± SD	Mean± SD	p value1
Day 0	44.3±5.2	44.2±5.1 ^{B}	44.2±5.1	40.8±3.9 ^{B}	0.221
Day 1	35.1±5.2	38.6±4.7	37.3±6	35.9±4	0.352
Day 3	28.8±4.7 ^{a}	33.3±3.6 ^{a}	32.8±4.6	32.8±3.9	**0.042**
Day 7	42.1±4.9	43.9±5.2 ^{B}	43±4.8	40.7±4 ^{B}	0.390
P value 2	<0.001	<0.001	<0.001	<0.001	

SD: Standard deviation, P≤ 0.05 is significant, p value 1 : comparing 2 groups at each time point by one way ANOVA, p value 2: comparing overtime in each group by RM-ANOVA, small case letters are statistically significant , upper case letters are statistically not significant

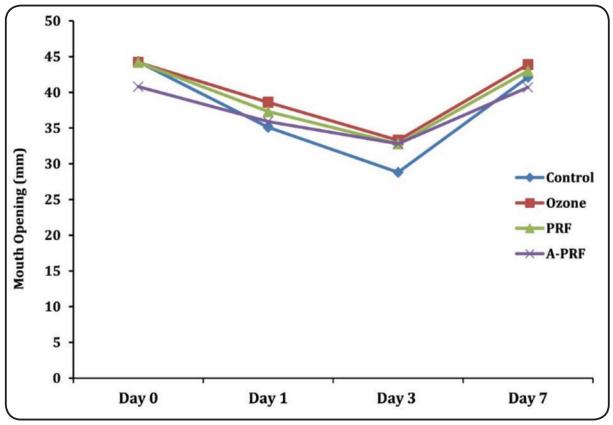

Fig. (9) Maximum mouth opening of the tested groups and overtime.

DISCUSSION

Postoperative discomfort is one of the main concerns for patients to get surgical extraction of an impacted tooth. The release of various inflammatory mediators post-operatively increases the vascular changes and peripheral oedema and results in local tissue alterations with subsequent pain and trismus. It is worth mentioning that the degree of these symptoms varies from one patient to another according to the position of impaction.[7,27]

In the present study, only **participants with similar difficulty index were selected** (as per Pederson difficulty index)[24] so as to minimize the variables and bias among the participants. Pre-operative examination and the surgical procedure were done by the **same operator** to avoid differences among different surgeons' skills, which may influence the results. Also, **age distributions** of the participants were homogeneous among the groups.

VAS was selected for pain assessment in this study, since it is a widely used, straightforward and easily applied method with proven reliability and sensitivity after oral surgical procedures.[28] Non-steroidal anti-inflammatory drugs and corticosteroids are most commonly used in post-surgical period for management of post-operative complications. In this study, **Ibuprofen** was prescribed for all participants, since it is a commonly used anti-inflammatory drug.[29]

This study followed **Choukroun's protocol for the fabrication of the PRF gel** since this method is simple, less sensitive, less time consuming and does not indicate use of chemical additives.[30] However, speedy blood collection and immediate centrifugation is mandatory to obtain a successful clot.

Commercial ozone has been supplied in various forms such as gas, aqueous solution and gel for topical therapeutic purposes. Kazancioglu et al[31]

applied ozone extra-orally on the face after third molars extraction and recorded successful results regarding wound healing. For the present study, **gel form was selected** since it can be easily applied by the patient and contains higher concentration of ozone molecules that are stable for longer duration.[32] Using ozone gel eliminates the need of complex armamentarium for its production and storage. Moreover, aqueous ozone has proved to conserve the biological characteristics of cells better than gaseous ozone.[3]

In the present study, **pain evaluation using VAS and analgesic consumption** were considered as subjective primary outcome measurements that may vary based on individual patient perception. While the **assessment of trismus** was considered as a secondary outcome that compares among the groups.

Post-operative pain is common consequence of any surgery. It is a multi-factorial result of release of pain mediators from the injured tissues, in addition to the patient's physiological threshold and anxiety level.[34, 35] The present study results showed gradual pain decrease over the days following the surgical extraction which can be attributed to biosafety, surgeon's experience, adherence of the patients to the post-operative instructions.

As regard to pain, it was clear from the results that the **ozone therapy group** has lower records on VAS scale all over the observation periods than the other groups and this was statistically proved significant. The explanation is that the ozonized gel contains stable ozonide, when comes in contact with the wound (body temperature), decomposes to reactive active ozone forming a protective layer over the surgical site that covers the exposed nerve endings, thereby significantly reduces pain.[36]

The considerably smaller number of analgesics needed by the ozone group in our clinical trial on the 1st and 3rd days with statistically significant difference, clearly confirms the analgesic role of ozone gel which was applied for 3 days only in this study group.

This result coincided with Sivalingam VP et al[5] in their split mouth study on patients who required extraction of bilateral impacted mandibular third molars. The patients were instructed to apply ozonized gel to the surgical wound for 2 min, twice daily for 3 days. Significant reduction of post-operative pain as well as trismus was recorded. In another study, Kazancioglu HO et al [31] concluded that the ozone group recorded significantly less degree of pain and number of consumed analgesic tablets.

Researches recorded that post-operative **oedema** reaches its peak within 48-72 hours after surgery,[37, 38] while **trismus** usually reaches its peak on the 2nd post-operative day and then gradually resolves till the end of the first week.[39] A significant reduction of maximum mouth opening was noted in the current study after 24 hours of the operation, and gradually resolved by the 7th day. This trismus can be related to multiple penetrations of the needle during local anesthesia administration, injured muscle fibers, reflection and retraction of the flaps and edematous changes of the tissues.

Concerning trismus, it was clear from the results of this study that the ozone therapy group has highest mean value of maximum mouth opening all over the observation periods than the other groups. Our results showed no significant difference between all groups except for the ozone group on day 3 which can be attributed to the application of ozone after extraction for 3 days. Ozone acts by modulating both the cellular and humoral immunities, through activating macrophages and stimulating synthesis of biologically active substances respectively. This remarkably reduces inflammation and improves wound healing, which consequently results in better results of trismus in ozone group.[4] However, the non-significant decrease in trismus for both groups III and IV can be related to the increase in the concentration of white blood cells along with growth factors in C-PRF and A-PRF.

The results of this study disagreed with those of Kazancioglu HO et al [31] whose study revealed

non-significant impact of ozone in decreasing edema and trismus after third molars extraction on day 3 postoperatively, while significant differences were recorded on day one and day 7 which agrees with our results. The disagreement between the results may be attributed to the extra-oral route of ozone administration used by Kazancioglu HO et al.[31] unlike the present study. Considering that the topical application of ozone gel coincided with the peak of inflammation on day 3, this can explain the significant reduction of post-operative oedema and the resultant trismus in the ozone group on day 3.

PRF is a natural fibrin-based biomaterial, which aids in micro-vascularization and wound healing.[8] The growth factors found in PRF have four key functions including "angiogenesis, immune control, circulating stem cells trapping, and epithelialization".[40]

Although our clinical observations revealed marked decrease in swelling, pain and trismus **conventional PRF sites** showed, a statistically non-significant difference was recorded for **pain reduction, analgesic consumption and maximum mouth opening** in both Group III compared to the control group on the 1st, 3rd and 7th days after surgery. In another study comprised of 30 patients, Asutay et al.[41] emphasized non- significant difference between the PRF group and the control group due to improvement of pain values in all groups recorded by a Likert-type VAS. **This comes in agreement with many other studies that noted no appreciable difference on extraction sites when conventional PRF was applied as compared to regular extraction sites**. [42-49]

On the other hand, many studies reported that PRF usage decreased pain values significantly.[28,50-53] In a randomized controlled clinical study that included 31 patients, Kumar et al.[51] applied autologous platelet-rich fibrin after impacted mandibular third molar surgery and noted significant reduction of pain values on the first follow-up day. Their pain assessment was done by a Likert type VAS as required by Pasqualini et al.[52] In another split-mouth study conducted on 20 participants, Singh et al.[53] reported that PRF application decreased pain on a Likert-type VAS in the 1st, 3rd and 7th days after surgery. Nevertheless, this finding has proved to be non-significance statistically. They evaluated pain with a line-type VAS. Additionally, Uyanık et al.[28] inserted PRF in the sockets of extracted third molars in a clinical study of 20 patients and found significant decrease of pain values on a Likert-type VAS on the 1st, 2nd, 3rd and 7th days after surgery.

On the contrary to the results of the present study, Gupta N. et al.[7] have found significant **co-relation between improvement of pain and trismus and the use of A-PRF**. Their results are explained by the released growth factors as well as the inflammatory mediators by A-PRF which starts from the 1st day and continues till the 8th -10th day. However, the disagreement with our results can be attributed to the difference in the study designs, sample sizes, assessment methods and follow-up intervals among the studies.

CONCLUSION

Taking into account that ozone is a non-drug therapy with no side effects, the present study shows that ozone gel can be of paramount importance in minimizing post-operative symptoms as regard to pain as well as trismus when applied after the surgical extraction of impacted mandibular third molars and appears to play a significant role to patients' relief and quality of life after the surgery.

RECOMMENDATIONS

Further studies with longer evaluation period, larger sample size and different study designs are required to get more affirmative and conclusive results about the topical effect of conventional PRF and A-PRF in the sockets of mandibular third molars.

ABBREVIATIONS

PRF: Platelet rich fibrin.

A-PRF: Advanced platelet rich fibrin.

VAS: Visual analogue scale.

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