

Effect of Autologous Fibrin Gel and Platelet Rich Plasma Activated by Ozone Versus Those Activated by Calcium Chloride on Wound Healing and Prevention of Infection in High Risk Cesarean Sections: Randomized Controlled Study

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ABSTRACT

Background: Cesarean delivery, one of the most common major surgical procedures performed worldwide used for 15% of births around the world and it continues to increase in frequency. It is an important contributor to surgical site complications such as infection, hematoma and dehiscence. Platelet Rich Plasma (PRP) is a volume fraction of blood having a high concentration of platelets above the baseline. This product is a rich source of growth factors. PRP can be activated by CaCl_2 or medical Ozone. The induction of growth factors found in PRP by ozone can support and potentiate their action.

Aim: The purpose of this study is to compare the effect of application of autologous Fibrin Gel and Platelet Rich Plasma (PRP) activated by medical Ozone (Ozonated PRP) versus those activated by CaCl_2 on wound healing and prevention of infection in high risk cesarean sections.

Design: 90 high risk pregnant females undergoing cesarean section were selected randomly according to inclusion criteria and divided into 3 groups each contains 30 patients. Group Ca received Autologous PRP and Fibrin gel activated by CaCl_2 at the wound site. Group O received Autologous PRP and Fibrin gel activated by Medical Ozone at the wound site while group P (control) did not receive any of them. All patients were examined for wound complications and healing progress on day 1, day 7 and day 21 postoperative by visual analogue scale (VAS), REEDA scale and Vancouver Scar System (VSS).

Results: the results revealed statistically significance decrease in pain by VAS in group Ozone compared to group P on day 7 and 21. For REEDA also group O showed significant decrease compared to group Ca on day 1 and 7 while compared to group P was also decreased on day 1, 7 and 21. Group Ca was significantly decreased compared to group P on day 7 and 21 only. Regarding VSS both group O and group Ca were significantly decreased compared to group P on all days.

Conclusion: this study gives evidence that applying autologous platelet rich plasma (PRP) promote wound healing and when activated by Ozone gives better results and helps prevention of infection in cesarean section of high risk patients.

Keywords: Cesarean section, PRP, Ozone.

INTRODUCTION

Cesarean section accounts for a substantial proportion of total deliveries. In the United States, the rate has risen steadily in the last half century, peaking at 31% in 2006. At the same time, the overall rate of post-CS complications is decreasing owing to use of antibiotic prophylaxis and regional anesthesia. Nevertheless, as compared to vaginal deliveries, CS is still associated with a higher risk of immediate and long-term maternal morbidity and mortality than vaginal delivery^[1].

Abdominal wound infection following cesarean section is a commonly encountered complication. Its incidence ranges between 3%

and 15% and depends on factors such as antibiotic prophylaxis, length of labor, duration of internal monitoring and duration of rupture of membranes^[2].

Surgical wounds heal through a sequence of several distinct physiological and biological events that include hemostasis, inflammation, proliferation, epithelialization, fibroplasia, and maturation. This process is initiated immediately upon injury. A platelet plaque develops which is composed of platelets and fibrin, and the platelets release granules containing multiple growth factors and thromboxane A_2 , the latter of which acts as a potent vasoconstrictor^[3]. Platelets are small discoid blood cells. The

average platelet count ranges from 1.5 to 3.0×10^5 /mL of circulating blood, and the in vivo half-life time of platelets is about 7 days. Normally, in the resting state, platelets are nonthrombogenic and require a trigger before they become a potent and an active player in hemostasis and wound healing^[4].

PRP is a volume fraction of blood having a high concentration of platelets above baseline that markedly improves the adhesive properties and the process of wound healing^[5]. After application of PRP, the tissue-healing substances are released. The high concentration of platelets at the wound site accelerates the healing process and protects the wound against the infection^[6].

PRP has been used for more than a decade in injectable or gel form, and many studies have demonstrated that PRP stimulates regeneration of the soft tissues (fat, skin, and mucosa) as well as the hard tissues (tendons and bones)^[7].

Ozone (O_3), a gas discovered in the mid-nineteenth century, is a molecule consisting of three atoms of oxygen in a dynamically unstable structure. The gas is colorless, acrid in odor and explosive in liquid or solid form. It has a half-life of 40 min at 20°C and about 140 min at 0°C^[8].

Two unpublished non randomized controlled trials demonstrated that the patients with caesarian sections treated with ozone had less infectious complications compared to controls^[9].

The beneficial effects of O_3 on wound healing might be assumed to be due to decreased bacterial infection, ameliorated impaired dermal wound healing or increased oxygen tension by O_3 exposure in the wound area. It was reported that O_3 exposure is associated with activation of transcription factor NF- κ B; this is important to regulate inflammatory responses and the entire process of wound healing^[10].

Exposure of PRP to O_2/O_3 stimulates the release of different growth factors. PRP treatments O_2/O_3 exposure releases significant ($p < 0.05$) quantities of other platelet growth factors, and this pool of factors is thought to participate in the regeneration of tissues^[11].

Aim of the study:

The purpose of this study is to compare the effect of application of autologous Fibrin Gel and Platelet Rich Plasma (PRP) activated by medical Ozone (Ozonated PRP) versus those

activated by $CaCl_2$ on wound healing and prevention of infection in high risk cesarean sections.

PATIENTS AND METHODS

All procedures were conducted in accordance with guiding principles for care of patients and were approved by Ain Shams University Ethics Board.

Selection of Subjects

Ninety high risk pregnant women undergoing cesarean section

were recruited from Labor ward in Ain Shams University Maternity Hospital who agreed to participate in our study provided that they suffer from one or more of the following conditions: body mass index (BMI) $> 30 \text{ kg/m}^2$, premature Rupture of Membrane (PROM), previous cesarean wound infection or dehiscence, diabetes or gestational diabetes, chorioamionitis, twin pregnancy, polyhydraminous.

Exclusion criteria: hepatitis, hemoglobin (Hb) $< 9 \text{ mmol/L}$, coagulation disorders, platelet levels < 150000 / microliter of blood. They were randomly divided into 3 groups.

Study Procedure

- **Preoperative Data:** History taking to identify the high risk patients included
- **PRP preparation:** In the operating room before the start of each procedure, approximately 50 cc of whole blood was drawn from each patient into a 60ml sterile syringe containing 2.5 ml citrate 3.8 % in the ratio of 20:1 (volume : volume) for anticoagulation. Another 10 cc of blood was drawn in another syringe without adding anticoagulant for preparation of autologous Thrombin. The first blood sample was immediately centrifuged at 704G first spin for 3 min. Following 15 minutes of centrifugation, second spin of plasma portion of blood at 1252G, 5 mL of PRP and 15 ml of Platelet Poor Plasma (PPP) was obtained. The second syringe is added to a tube containing special gel without anticoagulant and left to form full clot then centrifuged for 10 min at 1252G to separate the red blood cells down and serum containing thrombin up. The serum is drawn in a syringe to be mixed with syringe containing PPP obtained from the first syringe

in subcutaneous tissue at the wound site to form gel.

- **PRP Activation:**

- i) In Group Ozone:

- Platelet Rich Plasma (PRP) was treated by volume of 80 μ l mixture of O₃ generated by Longivity EX120 Desktop Medical Ozone Generator for activation and sterilization.

- Platelet Poor Plasma (PPP) which is rich in fibrin and clotting factors was treated with Ozone same as PRP.

- ii) In Group Cacl:

- PRP was treated by 1ml 10% CaCl₂ for activation.

- PPP was treated same way as PRP.

In both groups PRP was injected in the subcutaneous tissue at the wound site by using a sterile syringe while PPP mixed with mixture of CaCl₂ and autologous thrombin was applied directly on the subcutaneous tissue by special applicator to form gel within 30 seconds.

- **In Group control:**

Did not receive neither PRP injection nor fibrin gel.

For all participants, continuous or simple interrupted sutures were taken in the subcutaneous tissue and the skin was closed with subcuticular Proline according to the procedures of routine care and they received the routine antibiotic dose intraoperative and postoperative.

All of the procedures were conducted in an operation room with the purpose of safe guarding sterilization.

After skin closure, a wound dressing with a compressed bandage was applied.

- **Postoperative Data**

The patients were examined by the physician on day one, day seven and three weeks after the procedure.

Pain was evaluated by the visual analog scoring system (VAS). The wound healing was evaluated by using the Vancouver scar scale (VSS) and the edema ecchymosed discharge approximation (REEDA) scale.

The scores obtained were compared for the three groups to evaluate the results of the study.

- **Outcomes**

- i) **Primary Outcomes:**

- They measured by the REEDA scale and VSS for assessing the changes in wound healing.

REEDA as a descriptive scale has 4 points in a categorical score that measures 5 items of healing: redness (hyperemia), edema, ecchymosis, discharge, and approximation of the wound edges (coaptation). Each item is rated on a scale of 0 to 3, and total scores may range from 0 to 15. A lower score indicates better healing.

VSS was used to detect formation of keloids or hypertrophic scars. It assesses 4 subjective variables: vascularity, height/thickness, pliability, and pigmentation within a possible range of 0 - 14 for the total score.

- ii) **Secondary Outcomes:** Measured by VAS.

VAS assesses pain via a continuous measurement instrument that is operationally comprised of a horizontal line, anchored at each end by verbal descriptors such as no pain and the worst pain imaginable. The subject is asked to indicate a spot on the scale that best represents her degree of pain. The score is determined by measuring the distance (mm) between the no pain anchor to the point that the patient marks, providing a range of scores from 0 -10. A higher score indicates greater pain intensity.

- **Ethical Consideration**

All procedures were done after taking consent from the patients and with privacy. The study was approved by the Ethics Board of Ain Shams University.

- **Statistical Analysis**

Data were analyzed using Stata® version 14 (StataCorp LLC, College Station, TX, USA). Normally distributed numerical data were presented as mean \pm SD, and skewed data as median and interquartile range. Categorical data were presented as number and percentage.

- **RESULTS**

Recruitment took place between October 2016 and April 2017 in the labor ward in Ain Shams University Maternity Hospital. A total number of 200 women were assessed for eligibility for this study. 110 women were excluded as they haven't met the inclusion criteria and eventually 90 women were randomized in the study. They were divided into 3 groups each containing 30 women.

As regarding the VAS score compared on day 1, day 7 and day 21 between the three groups, there were some differences. On day 1 and day 7 the group O showed statistically significant difference versus group P on the same days where VAS was 8 for day 1 and 0 for day 7 in

group O lower than 9 and 1 for group P. On day 21 this significance disappeared and there was no difference between the three groups. Group Ca showed no significance on day 1, 7 and 21 as shown in Table 1.

Table 1. VAS score in the three study groups

Variable	Group Ozone (n=30)	Group Cacl (n=30)	Group control (n=30)	P-value¶
VAS day 1	8 (7-8)+	8 (7-9)	9 (8-9)	.13
VAS day 7	0 (0-0)+	0 (0-1)	1 (0-3)	<.001
VAS day 21	0 (0-0)	0 (0-0)	0 (0-1)	<.001

Data as median (interquartile range).

¶Kruskal-Wallis test.

+Statistically significant difference versus Group P (p-value <.0167).

Regarding REEDA score compared between the three groups on day 1, day 7 and day 21, there was statistically significant difference between group Ozone and Group Cacl (p-value <.0167), where on day 1 and day 7 the score in Ozone group was lower than group Cacl, while on day 21 there was no significance between them. As for comparing the score between group Ozone and group Cacl versus group P there was statistically significant difference on day 1, day 7 and day 21 for group Ozone and day 7 and day 21 for group Cacl. (p-value <.0167). Table 2.

Table 2. REEDA score in the three study groups

Variable	Group Ozone (n=30)	Group Cacl (n=30)	Group control (n=30)	P-value¶
VAS day 1	3 (2-5)+‡	5 (3-6)	5 (5-8)	<.001
VAS day 7	1 (1-3)+‡	3 (1-4)‡	5 (3-7)	<.001
VAS day 21	0 (0-0)‡	0 (0-1)‡	2 (0-2)	<.001

Data as median (interquartile range).

¶Kruskal-Wallis test.

+Statistically significant difference versus Group Cacl (p-value <.0167).

‡ Statistically significant difference versus Group control (p-value <.0167).

As for VSS score there was no significance between group Ozone versus group Cacl while there was statistically significance between group Ozone and group Cacl on day 1, day 7 and day 21 versus group control. (p-value <.0167) Table 3.

Table 3. VSS score in the three study groups

Variable	Group Ozone (n=30)	Group Cacl (n=30)	Group control (n=30)	P-value¶
VAS day 1	3 (3-4)+	4 (3-5)+	6 (5-7)	<.001
VAS day 7	2 (1-3)+	3 (1-4)+	5 (3-6)	<.001
VAS day 21	0 (0-0)+	0 (0-0)+	1 (0-3)	<.001

Data as median (interquartile range).

¶Kruskal-Wallis test.

+Statistically significant difference versus Group control (p-value <.0167).

There was no wound infection occurred in group Ozone, two cases of infection occurred in group Cacl and three cases in group control but this didn't make a statically significance between the three groups. Table 4.

Table 4. Incidence of wound infection in the three study groups

Variable	Group Ozone (n=30)	Group Cacl (n=30)	Group control (n=30)	P-value¶
Wound infection	0 (0.0%)	2 (6.7%)	3 (10.0%)	0.363

Data are number (%).

¶Fisher's exact test.

DISCUSSION

Cesarean delivery, one of the most common surgical procedures performed worldwide. Its rate is about 15% of births around the world and is increasing in frequency^[12].

There are common risk factors that lead to complications related to cesarean sections especially wound complications such as: gestational diabetes mellitus, history of previous cesarean deliveries, extremes of maternal weight, underlying medical illness like pregestational diabetes mellitus, anemia and immunocompromised conditions^[13].

Although prophylactic antibiotics is the only way guarding against postoperative infection, but some studies have shown significant reduction of total postoperative maternal infectious febrile morbidity rate after CS by the use of prophylactic antibiotics, while others did not find such association^[14].

Platelet Rich Plasma (PRP) is a volume of blood having high concentration of platelets that markedly improves the adhesive properties and the process of wound healing^[5].

After application of PRP, the tissue-healing substances are released. The high concentration of platelets at the wound site accelerates the healing process and protects the wound against the infection^[6].

Autologous fibrin gel acts like the last steps in the coagulation cycle with the conversion of fibrinogen to fibrin with the help of thrombin and calcium, helping cross-link the fibrin into a stable clot^[15].

Ozone therapy has been used since the 1800s. During the First World War (1914-18) doctors familiar with O₃'s antibacterial properties and with few other medical resources available to

them applied it topically to infected wounds and found O₃ not only remedied infection, but also had hemodynamic and anti-inflammatory properties^[16].

Exposure of PRP to O₂/O₃ stimulates the release of different growth factors as fibrin growth factor (FGF) which has an essential role in the differentiation of stem cells and is a potent in vitro mitogen for capillary endothelial cells, stimulating angiogenesis in vivo, and may participate in tissue repair. This pool of factors is thought to participate in the regeneration of tissues^[11].

This study confirmed that Ozonated PRP showed better outcome than PRP activated by CaCl₂ as regards pain, wound healing and prevention of infection and at the same time the outcome of application of PRP is better than non-application especially in high risk women undergoing cesarean section.

These results may be due the fact of presence of a very high concentration of platelets and growth factors in PRP which helps dramatically in wound healing especially when applied locally at the wound site. Also being autologous (prepared from the patient herself) guard against transmission of diseases and this was one of the most important steps in this study to prepare not only autologous PRP but also autologous thrombin which forms gel with fibrin (PPP) when applied in subcutaneous tissue at the wound site which acts as a tissue sealant.

This was for using PRP in general, as for using also Ozonated PRP added a significant advantage to applying PRP alone theoretically and practically from the results of this study and this was due to the fact of Ozone being almost 100% bactericidal so prevent infection and even

the resistant organisms for antibiotics also acts as immunomodifier and helps in doubling the concentration of platelets and growth factors released from PRP as mentioned and proved by past studies. All these advantages promote wound healing and prevent post-operative wound complications as dehiscence and infection.

The previous results came in agreement with the study of **Tehrani et al.**^[17] which was the first prospective, randomized controlled trial evaluating the efficacy of autologous PRP in cesarean section, and it has concluded that PRP has positive effects on wound healing and pain reduction in high-risk patients undergoing cesarean section.

Carter et al.^[18] conducted a systematic review and meta-analysis on the use of Platelet Rich Plasma Gel on wound healing. The primary outcome assessed was complete healing. In both chronic and acute wound studies, complete wound closure was more likely in wounds treated with PRP therapy. Similar partial healing and wound area/volume reduction outcomes were noted more frequently with the PRP-treated wounds. That meta-analysis and other systematic reviews showed PRP had sufficient efficacy to stimulate healing installed wounds. Infection and exudate were evaluated in many acute primary closure wound studies because both can delay healing. Overall, infection rates appear to be lower in PRP-treated subjects. Pain outcomes appeared mixed with some studies reporting no difference between treatments and others showing significantly statistical differences favoring PRP treatment. Eventually on the basis of the 10 years of research, the results of that systematic review and meta-analysis suggested that PRP therapy can positively impact wound healing and associated factors such as pain and infection in both chronic and acute cutaneous wounds.

Regarding the studies done to determine the effect of Ozone therapy and came in agreement with this study a clinical review was done by **Elvis et al.**^[19] who found that Ozone was effectively used as an antibacterial agent to treat oral infections caused by *Actinomyces naeslundii*, *Lactobacilli casei* and *Streptococcus mutans*.

A case report done by **Titiksha et al.**^[20] on the use of ozone in healing failed pectoralis major myocutaneous advancement flap, they concluded that there was significant amount of healing with 47-50% reduction in the wound size seen after 7 weeks (36 sessions) of ozone therapy. The infection subsided considerably according to the WBC and swab cultures. There was also significant amount of wound healing seen as formation of healthy granulation tissue, healing evident at the wound edges with decrease in the amount of purulent discharge. They attributed this to the fact that, ozone acts by activating the immunocompetent cells and induction of biological antioxidants and radical scavengers^[21].

Ozone also improves circulation, oxygenates hemoglobin and kills parasites, acts as a bactericidal as well as a veridical. Thus ozone helps in debridement of the dead tissue as well as provides O₂ and antioxidants stimulation to help facilitate healing. At the same time the bactericidal and veridical property leads to destruction of the fat cell membrane of the organisms leading to their death and resolution of infection^[22].

In 2010 **Re et al.**^[23] conducted a study on the role of Ozone/Oxygen mixture in fibroblast growth factor activation and found that the concentration of FGF was increased by approximately 580-700 percent after 2h incubation of PRP with O₂/O₃ compared to the basal concentration 1.636±0.969 pg/mL. which plays an important role in wound healing.

So the present study confirmed past evidence and added more evidence in comparing ozonated PRP versus non ozonated PRP and their effect on wound healing and prevention of infection in high risk women undergoing cesarean section.

CONCLUSION

This study gives evidence that applying autologous platelet rich plasma (PRP) promote wound healing and when activated by Ozone gives better results and helps prevention of infection in cesarean section of high risk patients.

RECOMMENDATIONS

It is recommended to do further studies on broader scale of patients to evaluate the results

more accurately. Also it is recommended that high risk patients for developing wound complications and infection to use Ozonated PRP or at least CaCl₂ activated PRP during procedure of cesarean section.

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