



Effect of local application of leukocyte and platelet-rich fibrin (L-PRF) on postoperative swelling and salivary IL-6 levels after surgical removal of impacted mandibular third molars

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Abstract

Aims: To measure the effects of leukocyte and platelet-rich fibrin (L-PRF) on swelling and saliva levels of interleukin-6 (IL-6) at different intervals after surgical removal of the impacted lower third molar. **Materials and Methods:** The study included 30 patients aged between (17-35) years who needed surgical removal of an impacted lower third molar under local anesthesia. Patients were divided into two groups, L-PRF, and control groups. In the L-PRF group, the L-PRF was prepared from the patient's blood, and the blood was centrifuged for 12 minutes at 2700 rpm. The clot was inserted in the socket after surgical removal of the impacted lower wisdom teeth, while in the control group, no material was inserted inside the socket. Facial swelling was assessed preoperatively, 2nd, and 7th days after the operation. Saliva level of IL-6 was measured (using ELISA) preoperatively and 7th day after the operation. **Results:** Regarding swelling within groups, in the L-PRF group, swelling showed a significant difference, while in the control group, there was no statistically significance difference. Between groups, there was no statistical difference in swelling between the preoperative and 2nd day postoperatively, and between 2nd and 7th day after the operation. Regarding IL-6 levels on the 7th postoperative day, there were no statistically significant differences between both groups. **Conclusions:** L-PRF decreases swelling following surgery within the group, but differences in swelling and levels of IL-6 in saliva among groups were not statistically significant; after surgical removal of the impacted mandibular third molar.

تأثير التطبيق المحلي لليوكوسيت والفيبرين الغني بالصفائح الدموية (L-PRF) على تورم ما بعد الجراحة ومستويات IL-6 اللعابية بعد الإزالة الجراحية للأضراس الثالثة الفك السفلية المتأثرة

المخلص

الأهداف: قياس تأثير الفيبرين الغني بالكريات البيض و الصفائح الدموية (L-PRF) على التورم ومستويات اللعاب من الإنترلوكين 6 (IL-6) على فترات مختلفة بعد الإزالة الجراحية لضرس العقل السفلي. **المواد وطرائق العمل:** اشتملت الدراسة على 30 مريضاً تتراوح أعمارهم بين (17-35) سنة احتاجوا إلى إزالة جراحية لضرس العقل السفلي تحت التخدير الموضعي. تم تقسيم المرضى إلى مجموعتين، مجموعة الفيبرين الغني بالكريات البيض و المجموعة الضابطة. تم تحضير الفيبرين الغني بالكريات البيض و الصفائح الدموية من دم المريض نفسه وتم طرد الدم لمدة 12 دقيقة عند 2700 دورة في الدقيقة. تم إدخال هذه الفيبرين الغني بالكريات البيض و الصفائح الدموية في تجويف السن بعد الإزالة الجراحية لأسنان العقل السفلية، بينما في المجموعة الضابطة لم يتم وضع أي مادة داخل تجويف السن. تم تقييم تورم الوجه قبل الجراحة وفي اليوم الثاني والسابع بعد العملية و قياس مستوى الإنترلوكين 6 في اللعاب باستخدام ELISA قبل الجراحة وفي اليوم السابع بعد العملية. **النتائج:** فيما يتعلق بالتورم داخل المجموعات، في مجموعة الفيبرين الغني بالكريات البيض و الصفائح الدموية، أظهر التورم فرقاً معنوياً، بينما في المجموعة الضابطة، لم يكن هناك فرق معتمد به إحصائياً. بين المجموعات، لم يكن هناك فرق إحصائي في التورم بين اليومين قبل الجراحة و اليوم الثاني بعد الجراحة، وبين اليوم الثاني والسابع بعد العملية. فيما يتعلق بمستويات IL-6 اللعابية في اليوم السابع بعد الجراحة، لم تكن هناك فروق ذات دلالة إحصائية بين المجموعتين. **الاستنتاجات:** الفيبرين الغني بالكريات البيض و الصفائح الدموية يقلل التورم داخل المجموعة، ولكن الاختلافات في التورم ومستويات الإنترلوكين 6 في اللعاب بين المجموعات لم تكن ذات دلالة إحصائية، بعد الإزالة الجراحية لضرس الثالث من الفك السفلي.

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INTRODUCTION

Surgical removal of the impacted lower third molar is usually associated with pain, swelling, trismus, and other problems ⁽¹⁾. Various ways to decrease complications after wisdom teeth operations would help the surgeon and the patients in the management of impacted lower third molars ⁽²⁾. The complication rates related to third molar removal may vary between 2.6 -30.9 percent ⁽³⁾. It is assumed that surgical trauma causes complications such as pain, edema, and trismus depending on the inflammatory process ⁽⁴⁾. Different techniques have been used to support patients' social activities. Among these are non-steroid anti-inflammatory drugs (NSAIDs), steroids, laser treatment, and ultrasound. Platelet-rich fibrin (PRF) appears to be promising in decreasing postoperative complications. It is a second-generation platelet concentration and is generated by a simple protocol ⁽¹⁾. The L-PRF is simply prepared without using additives via one spin of 10 ml of blood at 702 relative centrifugal force (RCF). The dense L-PRF polymerization network delivers growth factors slowly over 7 to 14 days post-operative wound healing longer than other platelet concentrates. ⁽⁵⁾. Scientifically, platelets deliver growth factors and mediators (cytokines) required in angiogenesis ⁽⁶⁾. Interleukin-6 is a cytokine synthesized in reaction to triggers such as infection and traumatic wounds by a diversity of cells such as macrophages,

neutrophils fibroblasts, keratinocytes, and endothelial cells ⁽⁷⁾. Saliva is a unique oral fluid; it may be used to present clinical statistics about patients' quick increase. Within the last few years, saliva has been used as a diagnostic medium, and this can be performed by measuring antibodies, protein concentration, and flow of saliva ⁽⁸⁾.

MATERIALS AND METHODS

This clinical comparative study was accomplished at the Department of Oral and Maxillofacial Surgery, College of Dentistry, University of Mosul, Mosul, Iraq. Approval of the study was from the scientific ethical committee under the number UoM.Dent/ H.12/ 21.

The study comprised 30 patients aged between (17-35) years, from both genders, who needed surgical removal of an impacted lower third molar under local anesthesia. Patients with impacted third molars were included in this study, with no history of systemic diseases, or hypersensitivity to any drug. Any patient with acute pericoronitis, smoking, pregnancy, alcohol, or drug addiction was excluded. A patient who was selected should not be taking any anti-inflammatory drugs at least 3 days before the operation. The operation in all patients in this study was not to extend for more than one hour. Each patient delivered informed written consent, which should be signed by the patient who participated in the study.

Study Design

The study was performed on 30 patients seeking impacted lower third molar

surgery, divided into 2 groups, group 1 (control) who had undergone surgical removal of the impacted lower third molar without adding anything, and group 2 (L-PRF group) in which blood was collected and L-PRF clot prepared and inserted in the surgical site and sutured in place. Unstimulated saliva was collected from each patient in two intervals (preoperative and 7th day after operation).

Preoperative Assessment:

Facial swelling was assessed by four facial measurements by a flexible length measurement tape to approximate millimeter; these measurements included the following: Tragus- midline (pogonion), (A- D), Gonion-pogonion, (E- D), Tragus-canthus of mouth (A-C), and Gonion-lateral canthus (B -E). Figure (1) ⁽⁹⁾.

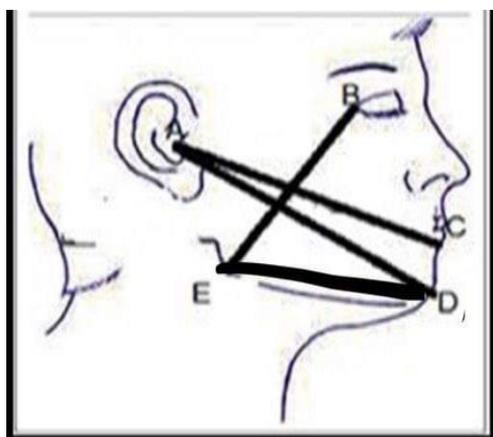


Figure 1: Swelling measurement points (A: Tragus of the ear, B: Lateral canthus of the eye, C: Commissure or corner of the mouth, D: Pogonion, E: Gonion).

Saliva Collection

Unstimulated saliva was collected from all patients. Saliva was collected in the morning from (9-11 am). The patients were told to stop drinking and eating at least an

hour before saliva collection and to rinse their mouths out. The saliva was collected by passive drooling for (5-10) minutes with the patient seated in a relaxed position, and about (1-3) ml of saliva was collected in a graduated plastic sterile container. Two samples of saliva were collected as follows: 1st sample just before the surgery, and the 2nd sample on the 7th day after the surgery. Each sample was centrifuged at 3000 rpm for 10 minutes, the clear supernatant was separated, transferred to Eppendorf tubes, and stored frozen until analysis ⁽¹⁰⁾. To determine cytokine, saliva samples were stored at -20C. Salivary IL-6 was assessed by using a Human enzyme-linked immunosorbent assay (ELISA) kit for IL6 (SALEMITRICS, USA).

Blood Collection and L-PRF Preparation

The protocol for LPRF preparation was done by the collection of whole venous blood from the median cubital vein. The blood was collected before each surgery into two vacutainer glass tubes of 9 ml without anticoagulant and centrifuged immediately at 2700 rpm for 12 minutes ⁽¹¹⁾. After centrifugation, three layers were formed: red blood cells at the bottom, upper straw-colored acellular plasma, and the middle part containing the fibrin clot and platelets, which is the L-PRF clot. At the end of the surgery, the clot was removed from the tube with sterile tweezers and laid on a piece of sterile gauze, here it is ready for use.

Surgical Procedure

All surgical procedures were performed under similar conditions on all patients by the same right-handed surgeon to avoid operator-mediated errors. Anesthesia was performed using (two cartridges) 1.8 ml cartridges of 2% lidocaine hydrochloride with epinephrine 1:80000 by a block of the inferior alveolar and lingual nerves and buccal nerve. The surgical access was done routinely through a triangular full-thickness buccal mucoperiosteal flap starting from the retromolar pad area proceeding anteriorly to the distal aspect of the lower second molar and releasing incision on the distobuccal side of the second molar. After delivering the impacted tooth, in the control group, the flap was then returned to its initial position and sutured. In the second group, the L-PRF group, the L-PRF clot, (which was prepared before the operation) was applied in the socket before suturing and secured in place by suturing.

Measurement of IL-6

SALIMETRIX human salivary IL-6 ELISA Kit was used to measure the salivary IL-6 and this was based on Standard Sandwich-ELISA Assay Technology.

Statistical analysis

A computer package (Sigma plot V12.0 / SYSTAT software) was used to conduct the statistical analysis. Data were presented as means \pm SE (standard error) and were analyzed using t-test and paired t-test with

a significant level set on $P \leq 0.05$ (Systat Software Inc. 2016).

RESULTS

Thirty patients were enrolled in the study and all completed the measurements. Age selection was 20-35 years with a mean age of (26.5 \pm 0.80). Gender distribution was 73.10% females and 26.90% males. Patients were distributed randomly and equally divided into two groups (control and L-PRF groups). Each group consisted of 15 patients of both sexes with unilateral impacted mandibular third molars. Facial swelling was assessed as postoperative readings minus preoperative measurements taking the average of 5 measurements were then taken for patients over days of the study. The mean swelling measurement increased on the 2nd day after the operation then the swelling decreased on the 7th day of the operation, in both groups. Comparing within groups between preoperative and 2nd day postoperative and between 2nd day and 7th day after the operation, and between preoperative and 7th day, in the control group, there was no significant difference in all time intervals as shown in Table (1).

Table 1: mean differences within the control group.

Duration	Cont. Mean Difference	$p \leq 0.05$
day 0-1	1.32 \pm 0.02	0.182
day 0-7	0.07 \pm 0.01	0.478
day 1-7	1.25 \pm 0.8	0.939

Swelling results comparing within-group II revealed that there were significant differences when comparing between preoperative and 2nd day postoperatively and when comparing between 2nd day and 7th day yet no significant difference between preoperative and 7th day after the operation as shown in Table (2).

Table 2: mean differences within the L-PRF group.

Duration	L-PRF Mean Difference	p ≤ 0.05
day 0-1	0.83± 0.04	0.001 *
day 0-7	0.02± 0.001	0.753
day 1-7	0.82± 0.03	0.002 *

When comparing swelling measurement results between two different groups at two-time intervals (2nd day and 7th day), the results showed no significant differences between the two groups at the two intervals (2nd day and 7th day) as shown in Table (3).

Table 3: comparing swelling mean differences between groups at two-time intervals (2nd day, 7th day of operation).

Time	Mean Difference between Two Groups	p ≤ 0.05
24hrs after operation	1.35± 0.2	0.32
7days after operation	0.92±0.02	0.43

For IL-6, mean results for group I at times before the operation and 7th day after the operation, the mean preoperatively was

0.574 which increased to 3.439 on the 7th postoperative day as shown in Table (4).

Table 4: IL-6 mean difference within the control group.

Duration	Mean difference	p ≤ 0.05
day 0-7	2.87± 0.4	0.308

In group II, mean results showed a slight increase from 0.57 preoperatively to 0.71 after 7 days of the operation, as shown in Table (5).

Table 5: IL-6 mean difference within the L-PRF group.

Duration	Mean difference L-PRF	p ≤ 0.05
day 0-7	0.13± 0.04	0.267

A paired sample T-test was used to compare the two groups.—Comparing between time intervals; preoperative and 7th day in both groups, the results revealed no significant difference between preoperative and 7th day after the operation. The independent sample T-test was used to compare the IL-6 results between two groups at 7 days. The results showed no significant difference between the two groups as shown in Table (6).

Table 6: IL-6 mean difference between groups.

Time	Mean difference	p ≤ 0.05
7days after operation	2.73±	0.32

DISCUSSION

Patients frequently experience complications following the extraction of an impacted mandibular third molar tooth, including but not limited to discomfort, trismus, edema, and alveolar osteitis (A.S)⁽¹²⁾. Implantation of leukocyte-platelet-rich fibrin (L-PRF) shortly after a tooth extraction is one of the current techniques to decrease inflammation and postoperative complications and optimize healing. Previous research has shown that this autologous biomaterial is useful in minimizing postoperative problems in third molars, due to the presence of leukocytes, platelets, and numerous growth factors. These growth factors are released in high quantities during the first 7 days, also PRF secretes three important pro-inflammatory cytokines IL-1b, IL-6, and TNF^(2, 11). In the current study, L-PRF was used instead of standard PRF to have the added advantage of the presence of leukocytes, which have an important role in tissue healing and minimizing complications⁽¹³⁾. Interleukin-6 is an essential cytokine that contributes to the exacerbation of inflammation. It reacts quickly to surgical trauma and for the aforementioned reasons, IL-6 levels were assessed in the current research. In this study, in addition to the use of L-PRF to decrease swelling, IL-6 levels were measured at two-time intervals (preoperative, and 7th day of operation), as L-PRF might affect the expression of this pro-inflammatory cytokine. An objective method was used to measure facial swelling

which was done using a measuring tape preoperatively, 2nd, and at 7th days. In this method, the sum of particular measurements was taken as baseline data for that side. The difference between the postoperative measurements and the baseline data is the facial swelling for that day^(14, 15). Facial swelling in the control group at three intervals of time showed no significant difference between the preoperative, 2nd day, and the 7th day. Whereas in the L-PRF group, there was a significant difference between preoperative and the 2nd day after the operation and between the 2nd day and 7th day postoperative and the swelling subsided in the 7th day with no significance between preoperative and 7th day after the operation. The time course for facial swelling findings revealed in this study is consistent with other studies demonstrating comparable results that peaked at day 1 or 2 postoperatively and then gradually diminished at day 7⁽¹⁶⁾. When comparing the two groups, the mean of swelling in the control group was higher than the L-PRF group but the difference was statistically not significant. In a similar study, Gürler et al⁽¹⁷⁾, evaluated the effect of L-PRF on the postoperative complications occurring after impacted lower third molar surgery; conducted on 40 patients and concluded that there was no significant difference between the control and L-PRF group regarding swelling and trismus and pain. Gülşen, U., and Şentürk, M. F.⁽¹⁸⁾; agreed with our study. They studied the effect of

PRF on postoperative edema after surgical removal of the impacted mandibular third molar—and disclosed no significant difference found between groups. Trybek, G. et al ⁽¹⁹⁾, also found that the application of PRF in the socket after impacted mandibular third molar surgical removal did not significantly affect the swelling intensity when measured at 6 hours, 1, and 3 days after surgery. In a study by Bilginaylar and Uyanik ⁽²⁰⁾, the results showed no significant difference between the PRF and other groups when the swelling was evaluated at 1, 3, and 7 days after the surgery. The above researchers used a measuring tape to assess the swelling in both vertical and horizontal dimensions which is considered cheap, simple, and effective. Asutay *et al.*, ⁽²¹⁾—used a 3D imaging system to assess the swelling after lower third molar impaction surgery with similar results to our study.

In contrast, several researchers like Afat *et al* ⁽²²⁾; Daugela *et al.*, ⁽²³⁾, Kumar *et al* ⁽²⁴⁾, and Ozgul *et al* ⁽⁴⁾ used PRF to evaluate the postoperative complications after mandibular third molar impaction surgery and found that there was a significant reduction in swelling. In a study by Rezaei *et al.*, ⁽²⁵⁾ who evaluated the difference between the levels of saliva and serum IL-6 and IL-8 in patients with oral squamous cell carcinoma, they found that salivary IL-6 and IL-8 were significantly higher than serum levels, and their detection in saliva was more beneficial than in serum, as the collection of saliva is a fast,

easy and non-invasive procedure. In this study, the levels of IL-6 were measured in saliva to determine the local effects of L-PRF on the expression of IL-6, since saliva is a good medium for detection of the levels of IL-6.

In the current study and when comparing between groups, the mean of IL-6 in the L-PRF group was lower than in the control group yet not statistically significant. Kim *et al* ⁽²⁶⁾ investigated the effects of PRF on odontoblastic differentiation in human dental pulp cells treated with lipopolysaccharide. Their findings revealed that PRF treatment dramatically reduced the lipopolysaccharide-stimulated production of IL-6 in human dental pulp cells and that PRF extracts both anti-inflammatory and odontoblastic differentiation stimulatory effects. Kartik *et al.*, ⁽²⁷⁾ studied the effect of A-PRF (advanced platelet-rich fibrin) alone, and in combination with hyaluronic acid on diabetic foot, and compared them with the control group; the comparison was by measuring IL-6 and some growth factors. The results showed that there was a significant difference among groups and the level of IL-6 in the group of (H.A and A-PRF) was the lowest value among the three groups on the 3rd and 7th day, while comparing the A-PRF and the control group regarding IL-6 there was a decrease in IL-6 mean compared to control on the 3rd and 7th day, but still statistically not significant. This study agreed with the

current study regarding the effect of PRF on the level of IL-6 after 7 days.

CONCLUSIONS

Regarding postoperative swelling, in the L-PRF group, statistical analysis showed no significant differences within groups between the preoperative and 2nd day after the operation and between the 2nd and 7th day postoperatively. However, among groups, the results were not statistically significant. Among groups, descriptively, L-PRF decreased the salivary IL-6 but this decrease was statistically not significant after lower third molar impaction surgery.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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