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The Influence of Leukocytes and Platelet Rich Fibrin (L-PRF) on trismus, and Levels of Interleukin 6 (IL-6) in Saliva after Impacted Mandibular Third Molar Impaction Surgery

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The Influence of Leukocytes and Platelet Rich Fibrin (L-PRF) on trismus, and Levels of Interleukin 6 (IL-6) in Saliva after Impacted Mandibular Third Molar Impaction Surgery
Running title:influence of leukocyte and platelet rich fibrin (L-PRF) on trismus and salivary levels of IL-6 after surgical extraction of lower third molar.

ABSTRACT:
Aims: To assess the influence of L-PRF on trismus, and levels of salivary IL-6 after impacted lower third molar surgery. **Materials and Methods:** 30 patients were included in this study,aged (17-35) years who required surgical removal of an impacted mandibular third molar under local anesthesia. Patients were divided into two groups,the control group, and L-PRF. In the L-PRF group,after surgical extraction of the impacted lower third molar, an L-PRF clot was made from the patient's blood and centrifuged for 12 minutes at 2700 rpm. This clot was put in the socket, while the control group had no material applied to the socket. Preoperatively, on the second day after surgery, and the seventh day following surgery, mouth opening was measured. Preoperatively and on the 2ndday after tooth removal, the amount of IL-6 in the saliva was determined (using ELISA).**Results:** The results showed no significant differences of trismus at the two intervals when p-value ≤0.05, but there was a decrease in the mean of trismus in the L-PRF group was more than the control group.IL-6 level on the 2ndday after the operation, in the L-PRF group, was less than that in the control group, however; there was no significant difference between groups. **Conclusions:**The L-PRF decreases the trismus and the level of IL-6 in saliva but the difference was statistically not significant.

KEYWORDS: Impacted mandibular third molar surgery, trismus, L-PRF, salivary IL-6.

I. INTRODUCTION:

One of the most common procedures in oral and maxillofacial surgery is the extraction of impacted third molar (Ryalatet al., 2018). Because of adjacent teeth obstruction and bone, tissue embedding, impacted lower third molar (ILTM) extraction is more likely to have surgical problems than a regular tooth extraction. Pain, edema, local bleeding, and infection are all common surgical consequences **(**Yuan Zhang et al., 2021).As a result, various techniques to lessen the likelihood of these problems and increase tissue recovery have been devised. Implantation of leukocyte-platelet-rich fibrin (L–PRF) shortly after a tooth extraction is one of the current techniques. Previous research has shown that this autologous biomaterial is useful in minimizing postoperative problems in third molars.(Da Sliva 2021). This second-generation autologous blood-derived biomaterial has a dense fibrin mesh with higher platelet and leukocyte counts, as well as higher concentrations of important growth factors and cytokines that aid tissue repair, particularly in soft tissue healing. (Ghannati et al., 2014) (Kang et al. 2011).TGF–, Platelet-Derived Growth Factor (PDGF), Vascular Endothelial Growth Factor (VEGF), and basic Fibroblast Growth Factor (FGF–2) are among the important mediators produced by leukocytes and platelets in L–PRF membranes. The new autologous biomaterials have significant in vitro evidence of the continual creation and release of these mediators.(Kang et al,2011) (Dohan et al.2006) (Lourenco et al., 2018).Interleukin-6 (IL-6) is a cytokine that is both pro-inflammatory and anti-inflammatory. T cells and macrophages produce it to activate the immunological response. In addition, IL-6 is a precursor to tissue injury.(Singh et al., 2015). A biomarker is a quantifiable Nano-protein that is used to predict a biological state. With the wide research on oral cancer, emphasis has been laid on predictive biomarkers located in saliva. However, IL-6 among

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these biomarkers, it is known that acute inflammation raises the level of IL-6 (Sahibzada et al., 2017).

II. MATERIALS AND METHODS:

The Oral and Maxillofacial Surgery Department of the Dentistry College, Mosul University, Mosul, Iraq, conducted this clinical comparative investigation. The study included 30 patients, both male and female, aged 17 to 35, who required surgical removal of an impacted lower third molar under local anesthesia. This study examined patients with impacted third molars who had no history of systemic illnesses or medication hypersensitivity. Acute pericoronitis, smoking, pregnancy, alcoholism, and drug addiction were all ruled out. A patient who has been chosen should not take any anti-inflammatory medicines for at least three days before surgery. The operation lasted no more than one hour for all of the participants in our study. Each patient participating in the study delivered a written consent to sign.

Study Design:

The study involved 30 patients who were seeking surgery for an impacted lower third molar. They were divided into two groups: group 1 (control) who had the impacted lower third molar surgically removed without adding anything, and group 2 (L-PRF group) who had blood drawn, and L-PRF clot prepared, and the clot inserted in the surgical site before suturing. In two periods, unstimulated saliva was obtained from each subject (preoperative and 2nd day after operation).

Preoperative Assessment:

The maximum mouth opening, also known as trismus, was measured preoperatively and postoperatively at 24 hours and 7 days. The distance between the mesial incisal edge of the upper central incisor and the lower central incisors is used to determine the mouth's openness. The level of trismus will be the discrepancy between the readings.

Thirty participants with an impacted lower third molar were enrolled in the trial. They were split into two groups: group 1 (control) had the impacted lower third molar surgically removed without any additional treatment, and group 2 (L-PRF group) had blood collected, and L-PRF clot made, and the clot injected in the surgical site before suturing. Each subject's saliva was collected twice, once unstimulated and once stimulated (preoperative and 2nd day after operation).

Saliva Collection:

All patients had their saliva taken without being stimulated. Saliva was collected in the morning from 9-11 a.m. Patients were instructed to stop drinking and eating at least an hour before the collection and to rinse their mouths out. Saliva was collected by passive drooling for 5-10 minutes with the patient in a relaxed position, and approximately (1-3) ml of saliva was collected in a graduated plastic sterile container. The first sample was taken right before surgery, and the second sample was taken the next day following surgery. Each sample was centrifuged for 10 minutes at 3000 rpm to separate the clear supernatant, which was then transferred to Eppendorf's and frozen until analysis. Saliva samples are used to determine cytokine levels.

Blood Collection and L-PRF Preparation:

The entire venous blood was collected from the median cubital vein as part of the LPRF preparation technique. Before each procedure, the blood was collected in two 9 mL vacutainer glass tubes without anticoagulant and spun at 2700 rpm for 12 minutes. After centrifugation, three layers emerged: red blood cells at the bottom, straw-colored acellular plasma at the top, and the L-PRF clot in the center, which contained fibrin clot and platelets. The clot was extracted from the tube with sterile tweezers at the end of the surgery and placed on a piece of sterile gauze, ready for usage.

Surgical Procedure:

All surgical operations were conducted under similar conditions on all patientsto avoid operator-mediated errors, by the same right-handed surgeon. Anesthesia was achieved by blocking the inferior alveolar, lingual, and buccal nerves with two 1.8 ml cartridges of 2 percent lidocaine hydrochloride with epinephrine 1:80000. A triangular full-thickness buccal mucoperiosteal flap was used to get access to the lower second molar, starting at the retromolar pad area and proceeding anteriorly to the distal aspect of the lower second molar, with a releasing incision on the distobuccally side of the second molar. In the control group, after delivering the impacted tooth, the flap was restored to its original place and sutured. The L-PRF clot (which was manufactured before the procedure) was used in the second group, the L-PRF group.

Measurement of IL-6:

The salivary IL-6 was measured using the SALIMETRIX human salivary IL-6 ELISA Kit, which was based on Standard Sandwich-ELISA Assay Technology.

Statistical analysis:

The statistical analysis was carried out using a computer program (Sigma plot V12.0 / SYSTAT software). The data were reported as means standard error (SE) and evaluated using the

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t-test and paired t-test with a significant level of P 0.05. (Systat Software Inc. 2016).

III. RESULTS:

Thirty patients took part in the research and completed all of the measurements. The average age was 26.5, with a range of 26.5 to 26.5. (20-35), the study had a female participation rate of 73.10% and a male participation rate of 26.90 %. Patients were placed into two groups at random and equally (control and L-PRF). Each group had 15 individuals with impacted mandibular third molars from both sexes.The mean difference of trismus for each patient was obtained by subtracting the preoperative measurement of mouth opening from the postoperative reading at each postoperative day and averaging the results (1day and 7day). When the p-value is less than 0.05, the Trismus results within groups in the control group revealed that there were significant differences in all time intervals. Table 1 shows the results. While there were significant differences between preoperative and 2nd-day intervals in the L-PRF group, as well as between the 2nd day and 7day, there was no significant difference between preoperative and 7day when the p-value was 0.05. Table 2 displays the results. Comparing the two groups (control and L-PRF) the results showed no significant differences at the two intervals as shown in table3. IL-6 results: the mean was measured at two time intervals (preoperative and 2nd day after operation). In the controlgroup the mean preoperatively was 0.574 then increased 2nd day after the operation to 12.861, while in the L-PRF group, the mean results showed an increase from 0.57 preoperatively to 2.90 2ndday after the operation. Comparing the IL-6 results between two groups on the 2nd day. The results showed no significant difference between the two groups when the p-value ≤0.05 as shown in table 4.

IV. DISCUSSION:

Following the extraction of an impacted mandibular third molar t, patients frequently experience complications such as discomfort, trismus, edema, and alveolar osteitis (A.S) (9).Platelet-rich fibrin (PRF) appears to have the potential to reduce postoperative problems. It's a straightforward process that generates a second-generation platelet concentration (4). LPRF is made by spinning 10 ml of blood at 702 relative centrifugal force without utilizing any additives (RCF). Due to the presence of leukocytes, platelets, and various growth factors, the previous study has demonstrated that this autologous biomaterial is effective in reducing postoperative difficulties in third molars. PRF also secretes three major pro-inflammatory cytokines, IL-1b, IL-6, and TNF, which are secreted in large amounts during the first seven days (4, 10).L-PRF was utilized instead of regular PRF in this study to benefit from the presence of leukocytes, which play a key role in tissue healing and reducing problems (11). IL-6 is a crucial cytokine that leads to inflammation aggravation. It is rapid to respond to surgical trauma. IL-6 levels were measured in the current study for the reasons stated above.

In this investigation, IL-6 levels were assessed at two time periods (preoperative and 2nd day after surgery) since L-PRF may impact the expression of this pro-inflammatory cytokine.

In all groups, there was a statistically significant difference between preoperative and the second day after the operation, as well as the second day and the seventh day following the operation (control and L-PRF). This is explained by the fact that trismus increases after the surgery, peaks in the second or third day, and resolves by the seventh day. Moore et al. (Moore et al., 2005). The mean between groups was lower in the L-PRF group than in the control group, and the mouth opening in the L-PRF group was better than in the control group on the second day after the operation, but there was no statistically significant difference between groups.

(Gürler et al 2015) observed that L-PRF did not improve trismus after impacted mandibular third molar surgery and that the difference between the two groups (control and L-PRF) was not significant.

(Marouf, I., & Rejab, A. 2020) looked explored how PRF and hyaluronic acid (H.A) affected postoperative trismus, edema, and discomfort. They claimed that H.A was better at reducing swelling and pain when compared to PRF and control groups and that PRF was better than the control group in swelling and pain with a significant difference between groups. There was no significant difference in trismus when the threegroups were compared, but when looking at the mean, H. A was less.

The current study is also corroborated by the findings of (Asutay et al. 2016), who investigated whether the use of PRF in impacted lower third molar surgery reduces trismus. They discovered no statistical difference between groups at intervals of the 2nd and 7th day after surgery.

The PRF influenced the mean of mouth opening, although this effect was not statistically significant, according to (Afat et al 2018) and (Bilginaylar and Uyanik 2016).

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In contrast to our findings, (Trybek et al., 2021) discovered that using PRF can considerably reduce trismus when tested on the 2nd, 3rd, and 7th day following surgery.

Rezaei et al.(23) investigated the difference between salivary and serum IL-6 and IL-8 levels in patients with oral squamous cell carcinoma (OSCC) and discovered that salivary IL-6 and IL-8 levels were significantly higher than serum levels and that their detection in saliva was more beneficial than serum because saliva collection is a quick, painless, and non-invasive procedure. Because saliva is an excellent medium for detecting IL-6 levels, the levels of IL-6 were assessed in saliva to determine the local effects of L-PRF on the expression of IL-6 in the present study.

The mean of salivary IL-6 was higher on the next day of the operation in the current study, as expected, and there was a significant difference between preoperative and 2nd day after operation in the two groups (control and L-PRF). This finding could be explained by the fact that IL-6 is an acute pro-inflammatory mediator that is detected within 2 hours of surgery or trauma, peaks in 6 hours, and remains elevated for the first 24 hours after surgery or trauma. When compared to healthy controls, the IL-6 level can remain elevated for several days (Stensballe et al. 2009). As a result, we chose to assess the amount of IL-6 24 hours following surgery in this investigation.When comparing the two groups, the L-PRF had a lower mean of IL-6 than the control group, but the difference was not statistically significant.

The effects of PRF on odontoblastic development in human dental pulp cells treated with lipopolysaccharide was investigated in another work by (Kim et al 2017). The findings demonstrated that PRF treatment significantly reduced IL-6 production in human dental pulp cells activated by lipopolysaccharide and that PRF extract has both anti-inflammatory and odontoblastic differentiation stimulatory actions.

(Kartik et al 2021) examined the effects of A-PRF (advanced platelet-rich fibrin) alone and in combination with hyaluronic acid on diabetic foot to a control group, evaluating IL-6 and other growth factors. The results revealed that there was a significant difference between groups, with the level of IL-6 in the group of (H.A and A-PRF) having the lowest value among the three groups on the 3rd and 7th days while comparing the A-PRF and the control group in terms of IL-6, there was a decrease in IL-6 mean compared to control on the 3rd and 7th days, but it was still statistically not significant. This research-backed up the current research on the impact of PRF on the level of IL-6.

CONTROL M.OP

Duration	mean difference	$p \leq 0.05$
day 0-1	1.88± 0.2	0.001 *
day 0-7	0.55± 0.03	0.044*
day 1-7	1.32± 0.07	0.001 *

Table 1: Mean difference within the control group

L-PRF M.OP WITHIN GROUP

Duration	mean difference	p ≤ 0.05
day 0-1	1.35± 0.05	0.001 *
day 0-7	0.35± 0.02	0.346
day 1-7	1.01± 0.2	0.009 *

Table 2: Mean difference within L-PRF group

Compare between two groups

Time	mean difference	p ≤ 0.05
24hrs after operation	0.71±0.01	0.08 *
7days after operation	0.39± 0.01	0.24

Table 3: Mean difference between two groups

Compare between two groups

Time	Mean difference	p ≤ 0.05
24hrs after operation	9.96±	0.07 *

Table 4: Comparing mean difference of IL-6 between two groups

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