Study Protocol with Statistical Analysis Plan (SAP)

Title: Evaluation of the Postoperative Effects of Horizontal Platelet-Rich Fibrin after Impacted Third Molar Extraction: A Randomized Controlled Clinical Study

NCT Number: NCT06244797

Last update/approval date: December 28, 2023 (Ethics Committee and Ministry of Health Approval)

1. Background and Rationale

Impacted mandibular third molars are frequently removed due to complications such as pericoronitis, cyst formation, or damage to adjacent teeth. Postoperative sequelae include pain, swelling, and trismus. Platelet-rich fibrin (PRF) is a second-generation platelet concentrate with regenerative and anti-inflammatory potential. Horizontal PRF (H-PRF), produced via horizontal centrifugation, yields higher concentrations of platelets and leukocytes compared with leukocyte PRF (L-PRF). No randomized controlled trial has directly compared H-PRF and L-PRF in mandibular third molar surgery.

2. Study Objectives

Primary Objective: To evaluate whether H-PRF reduces postoperative swelling compared with L-PRF and control.
Secondary Objectives: To compare effects on pain, trismus, soft tissue healing, analgesic consumption, and quality of life (QoL).

3. Study Design

Design: Prospective, randomized, controlled, double-blind clinical trial.
Setting: Tokat Gaziosmanpaşa University, Faculty of Dentistry, Department of Oral and Maxillofacial Surgery.
Duration: Recruitment and follow-up between January and March 2024.

4. Study Population

Inclusion Criteria:
- Patients aged 18–40 years
- Indication for extraction of mucosally retained mandibular third molars
- ASA I classification
- Signed written informed consent

Exclusion Criteria:
- Systemic disease
- Allergy to penicillin or local anesthetics
- Previous radiotherapy to head and neck
- Requirement for antibiotic prophylaxis
- Presence of pericoronitis
- Inability to attend follow-up visits

5. Randomization and Blinding

Randomization performed via sequentially numbered, opaque sealed envelopes prepared by an independent nurse.
Allocation ratio: 1:1:1 (H-PRF, L-PRF, Control).
Patients and outcome assessor blinded.
Operating surgeon aware of allocation.

6. Interventions
H-PRF Group: 10 mL venous blood centrifuged horizontally (2200 rpm, 8 min) and applied to socket.
L-PRF Group: 10 mL venous blood centrifuged via fixed-angle centrifugation (2700 rpm, 12 min).
Control Group: Standard extraction without PRF application.
All sockets closed with 3-0 silk sutures.
Standard postoperative medication: dexketoprofen trometamol (25 mg as needed), chlorhexidine mouth rinse (0.12%, twice daily for 7 days). No prophylactic antibiotics prescribed.

7. Outcomes
Primary Outcome: Swelling (linear facial measurements), assessed pre-op, Day 2, Day 7.
Secondary Outcomes:
- Pain (VAS, 0–100 mm), Days 1–7
- Trismus (maximum interincisal distance), baseline, Day 2, Day 7
- Soft tissue healing (Landry index), Days 2 and 7
- Analgesic consumption (daily record)
- QoL (Majid's questionnaire), Days 4 and 7

8. Statistical Analysis Plan (SAP)
Software: IBM SPSS v23
Normality: Shapiro-Wilk test
Categorical variables: Chi-square test
Continuous variables: One-way ANOVA (normal distribution) or Kruskal-Wallis test (non-normal)
Repeated measures: Repeated measures ANOVA with Bonferroni post hoc (normal) or Friedman with Dunn's post hoc (non-normal)
Baseline imbalance: ANCOVA with baseline covariates (for swelling)
Effect sizes: Mean differences with 95% CI, standardized effect sizes reported
Significance level: $p < 0.05$
Missing data: No missing data expected (all 75 randomized completed)

9. Ethical Considerations
Approved by Tokat Gaziosmanpaşa University Clinical Research Ethics Committee (Approval No: 23-KAEK 146).
Additional authorization: Ministry of Health (Decision No: E-57955685-000-224940348).
Registered at ClinicalTrials.gov (NCT06244797).
Written informed consent obtained from all participants.

10. Data Handling

Data anonymized and stored securely.

Analyses conducted according to pre-specified protocol.

Data available upon reasonable request from corresponding author.

INFORMED VOLUNTARY CONSENT FORM

Researcher/Physician's Statement

We are planning to conduct a scientific study to improve the post-extraction period of patients after the extraction of impacted wisdom teeth. The name of the planned study is "Investigation of the Effects of H-PRF (Horizontal Platelet-Rich Fibrin) on the Post-Extraction Period of Impacted Third Molar Tooth: Randomized Controlled Clinical Trial".We invite you to this study, which will be applied to patients diagnosed with an extraction indication for impacted wisdom teeth and/or clinically followed up due to pericoronitis, since your medical condition meets these conditions. However, it should be noted immediately that participation in the study or not is based on voluntary basis. You should make the decision to participate in this scientific study completely with your own free will. You cannot be suggested or pressured by anyone while making this decision.

Before you make your decision, we would like to inform you about the scientific study in question and the procedures that will be performed if you accept to participate in this study. If you want to participate in this scientific research after reading and understanding this information, please sign the form. Information about the scientific study.

GENERAL INFORMATION ABOUT THE PROCESS Teeth that are partially or completely impacted in soft or bone tissue (e.g. wisdom teeth) are frequently infected and may cause serious systemic disorders over time, as well as pathologies in neighboring teeth, soft tissues, and bone tissue, and may require extraction. For this procedure, a small incision is made in the soft tissue under local anesthesia, and a small amount of bone tissue is removed to remove the tooth. Teeth that do not come out in this way may need to be divided. After the tooth is extracted, whether it is infected or not, its follicle (growth sac) is removed. The area is washed with plenty of serum, stitched, and a tampon is placed. The stitches should be removed after 1 (one) week. During this one-week period, the patient is given a number of medications (antibiotics + mouthwash = to control infection and keep the wound clean, Analgesic = to control pain). After the extraction of impacted teeth, temporary or permanent loss of sensation may occur in the lips and/or tongue if they are closely related to the vascular-nerve bundle passing under the tooth. It may not be possible to see and predict this situation in advance with classical radiography methods. In cases of suspicion, your doctor may request a CT scan.

The reason you have been invited to the research is that you are an individual between the ages of 18-40, without any chronic illness, diagnosed with impacted wisdom tooth extraction. This research will be carried out in collaboration with the Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Gaziosmanpaşa University.

The PRF to be investigated in this research is Platelet-rich fibrin (PRF), a second-generation platelet concentrate produced with a simplified protocol. PRF consists of a polymerized fibrin matrix in an atetramolecular structure, the inclusion of platelets, leukocytes and cytokines, and the presence of stem cells in circulation.

The aim is to reveal the situation of this structure, which is so important for the human organism, on human anatomy.

In this project designed for this purpose, the effects of this structure on the healing role in the human body will be investigated and the results will be evaluated.

- Estimated Procedure Duration: 15 min to 30 min:
- Risks and Complications of the Procedure:
- Swelling, redness, pain, soreness in the extraction area, redness and cracking due to tension in the corners of the mouth
- After the extraction, a local infection of the area called Alveolitis may occur and may cause pain that lasts more than a week. In this case, your doctor will intervene with the necessary dressings.
- Development of infection and late healing of the wound
- Trismus: Restriction in opening the mouth due to inflammation or swelling.
- Bleeding: Severe bleeding is not common.

However, bleeding in the form of leakage can continue for a few hours, rarely for a day or two. • In order to obtain PRF, blood must be taken from the patient. Among the medical side effects, the most important side effect due to needle insertion is hematoma and it is the most frequently developing complication. Hematoma can initiate thrombophlebitis or infection. Causes of hematoma are; damage to the vein during insertion, not applying pressure while withdrawing the needle or catheter in the vein, and tying the tourniquet too tightly during vein insertion. Blood collection is usually

done from the arm or hand. Other complications that may occur during or after blood collection are: dizziness, headache, arm numbness, fainting due to fear of needles, pain in the arm due to the procedure.

IMPORTANT FEATURES OF THE DRUGS TO BE USED
Local Anesthesia; It can be briefly described as the temporary blocking of a certain area of the nerves that transmit sensation in the human body with anesthetic substances (lidocaine, mepivicaine, etc.). The duration of loss of sensation resulting from local anesthesia used in dentistry varies between 1-4 hours, depending on the anesthetic substance used, the area where the anesthesia is applied, and the anatomical structure of the person. Complications such as facial paralysis (temporary facial paralysis), emphysema (swelling on the face), hematoma (redness, bruising on the face) may occur as a result of anesthesia. These conditions are temporary and there is no need to worry.

LIFESTYLE RECOMMENDATIONS CRITICAL TO THE PATIENT'S HEALTH
In the post-extraction period; 1- Regular use of prescribed medications is required. 2- The operation area should be kept clean, the area should not be traumatized and the operation area should not be attempted to be seen. 3- Oral hygiene should be taken care of in the postoperative period and the habit of brushing teeth should be continued routinely. 4- Very hot foods should not be consumed in the first few days after the extraction. 5- If smoking is involved, smoking should be stopped for a week. 6- Consumption of granular foods should be avoided for a week after the extraction.

Groups:

Control: Patient group in which no material is placed in the extraction socket after the impacted tooth extraction. There will be 25 patients in this group.

L-PRF (Leukocyte-platelet rich fibrin): Patient group in which L-PRF will be placed in the extraction socket after the impacted tooth extraction. There will be 25 patients in this group.

H-PRF (horizontal platelet rich fibrin): Patient group in which H-PRF will be placed in the extraction socket after the impacted tooth extraction. There will be 25 patients in this group.

Randomization: Patients will be divided into three groups before the operation: control group, PRF group and H-PRF group. The closed envelope method will be applied during the process of assigning patients to the groups. Sealed envelopes of the same color will be used for each group and the patient will not know which group they are assigned to, but the physician will know which group the patient is assigned to.

SURGICAL PROCEDURES

Before the surgical procedure begins, blood will be drawn from the patient. The blood taken will be processed for centrifugation and then the surgery will begin. Surgery will be performed under local anesthesia with articaine 2 ml 4% and epinephrine 1:100,000. All patients will be operated on by the same surgeon. After local anesthesia is provided, an incision is made and a full thickness mucoperiosteal flap will be lifted and the area will be exposed. The soft tissues surrounding the impacted tooth with mucosal retention will be curetted and the tooth extraction will begin. After the tooth is extracted, the surgical area will be washed with sterile 0.9% saline and PRF material will be placed. Primary closure will be performed with 3.0 silk suture. All patients will be given postoperative instructions by the surgeon. Patients will be prescribed an antibiotic (amoxicillin 650 mg po, t.i.d. for 3 days; patients with a history of allergy to penicillin will be prescribed clindamycin 300 mg po, t.i.d. for 3 days), an NSAID (preferably ibuprofen, at least 12 hours apart as needed), and a mouthwash (0.12% chlorhexidine mouthwash, t.i.d. for 7 days).

Will blood be taken from you for the study?*

Yes. The blood will be placed in the drawing socket to speed up the healing of the area.

Situations to be known within the scope of the study and rules that researchers and volunteers must follow

If you participate in the study;
1. You will not be charged any fee.
2. You will not be paid any additional payment for participating in the study.
3. Great care and respect will be shown for the confidentiality of your information, which must remain between you and the physician.
4. Your personal information will be protected with great sensitivity during the use of the research results for educational and scientific purposes.
5. The researchers are responsible for any health-related or other negativities that may occur during the study.
6. You may withdraw from the study at any stage of the study in which you are participating as a volunteer. However, it is important to inform the researchers of this situation before leaving.
7. If you do not accept to participate in the study, there will be no change in your treatment and clinical follow-ups, and your disease will be treated with the same care and attention as always.

Participant (Volunteer) / Patient's Declaration

Esteemed Dr. Lecturer Esengül ŞEN stated that a research would be conducted with the Department of Oral and Maxillofacial Surgery of Tokat Gaziosmanpaşa University Faculty of Dentistry and conveyed the above information regarding this research to me. After this information, I was invited to such a research as a "participant".

If I participate in this research, it has been clearly and concisely stated that the confidentiality of my information, which should remain between me and the doctor, will be treated with great care and respect during this research, and that my personal information will be meticulously protected during the use of the research results for educational and scientific purposes. I do not assume any financial responsibility for the expenses incurred for the research. It has been clearly and definitely stated that no fee will be requested from me and no payment will be made to me.

It has been stated that I have the right to withdraw from the research without giving any reason during the implementation of the project. However, I am also aware that it would be appropriate to notify the researchers in advance that I will withdraw from the research in order not to put them in a difficult situation. In addition, I may be excluded from the research by the researcher provided that no harm is done to my medical condition.

The researchers are responsible for any negative health conditions that may arise during the research process, whether directly or indirectly, and I will not be under any financial burden. I am not obliged to participate in this research and I may choose not to participate. I have not encountered any coercive behavior regarding my participation in the research. I also know that if I refuse to participate, this will not harm my medical care or my relationship with the physician. I have understood all the explanations given to me in detail. After a certain period of self-reflection, I have made the decision to take part in this research project as a “participant” (volunteer) of my own free will. I accept this invitation with great pleasure and voluntariness.

"I have been informed about the procedure to be performed and I understand the procedure to be performed." (This section will be filled in by the patient's relative.……
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DATE:

Participant (Volunteer)
ID :
Address :
Phone :
Signature :

Researcher Interviewing Participant (Volunteer)
Name, Surname, Title :
Address :
Phone :
Signature :

(A copy of this form with all pages signed will be given to the participant)