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Salivary α -Amylase as a Non-Invasive Biomarker of PRF-Enhanced Wound Healing After Third Molar Surgery - A Randomized Controlled Study

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ABSTRACT

Introduction: Salivary biomarkers may facilitate noninvasive monitoring of early postoperative responses after mandibular third molar surgery. This study aimed to compare salivary α -amylase activity between the platelet-rich fibrin (PRF) and non-PRF groups after mandibular third molar surgery and to describe the postoperative temporal pattern of salivary α -amylase during early wound healing.

Materials and Methods: This single-center, parallel-group randomized trial included 28 patients (aged 18-60 years) who underwent mandibular third molar surgery at the Dental Hospital of Hasanuddin University, Makassar, Indonesia. The patients were divided into two groups: those receiving PRF and those without PRF. Salivary α -amylase activity was measured on postoperative days 0, 3, and 7. Statistical analyses were performed to evaluate the results.

Results: In this study, we found increased mean α -amylase activity in the PRF group at any given time interval compared to the non-PRF group, which was not statistically significant. Within-group comparisons revealed no significant changes at any time interval.

Discussion: PRF is rich in bioactive mediators and has potential benefits in reducing postoperative pain and improving wound healing. Salivary α -amylase cannot be considered a reliable inflammatory biomarker for PRF-enhanced wound healing in this study. The limitations of this study include the small sample size and incomplete randomization reporting.

Keywords: Salivary biomarker, α -amylase, platelet-rich fibrin, third molar surgery, wound healing

INTRODUCTION

Mandibular third molar surgery is the most frequent procedures in oral and maxillofacial surgery.^{1,2} The procedure followed by pain, acute inflammation, swelling, trismus, root fracture, temporomandibular joint trauma, and displacement tooth into adjacent anatomical spaces.^{3,4} Postoperative swelling commonly decreases on the 7th day after third molar surgery.⁵ Preoperative assessment was performed clinically and radiographically using orthopantomography (OPG) and computed tomography (CT) imaging.^{6,7}

Saliva is a powerful diagnostic tool that contains proteins, hormones, antibodies, enzymes, and cytokines that reflect local and systemic biological processes. It can be used to monitor and diagnose diseases.⁸

Salivary components can be designated as biomarkers with the potential to reflect the status of various

pathologies beyond the borders of oral health.⁹ Platelet-rich fibrin (PRF) is an autologous platelet concentrate that contains regenerative biomolecules and produces minimal inflammatory reactions when applied to surgical wounds.¹⁰ PRF contains bioactive mediators that promote soft and hard tissue healing by releasing growth factors and has been increasingly used in regenerative dental procedures.¹¹ Evidence from systematic reviews suggests the potential benefits of autologous platelet concentrates in reducing postoperative pain, swelling, alveolar osteitis, and promoting soft tissue healing after mandibular third molar surgery, although heterogeneity across studies remains substantial.^{12–15} Evidence regarding salivary α -amylase as a biomarker of postoperative inflammatory response after receiving PRF following mandibular third molar surgery is limited. Salivary α -amylase has been explored as a non-invasive pain indicator, monitors stress levels and also inflammatory responses. after mandibular third molar surgery, but remains limited and inconsistent.^{16–21} This study aimed to compare salivary α -amylase activity between the PRF and non-PRF groups after mandibular third molar surgery and to describe the postoperative pattern of salivary α -amylase during early wound healing. The research question was: Does socket application of PRF after mandibular third molar surgery alter salivary α -amylase activity compared with no PRF during the first postoperative week?

MATERIAL AND METHODS

Patient and public involvement

No formal patient or public involvement was reported in the design, conduct, interpretation, or reporting of this study.

Trial design and setting

This Randomized controlled study design with two parallel groups followed the CONSORT 2025 guidelines²² (Figure 1). This study was performed in the Department of Oral and Maxillofacial Surgery, Dental Hospital of Hasanuddin University, Makassar, Indonesia. Salivary α -amylase analyses was performed at the HumRC Laboratory, Faculty of Medicine, Hasanuddin University. Data will be collected on postoperative days 0, 3, and 7 from May to November 2024.

Eligibility criteria

Eligible participants were aged 18–60 years with class I-II A and B impacted third molars (Pell and Gregory's classification). Participants had no systemic diseases, blood disorders, immune disorders, or tumors. Patients who refused surgery, did not complete examinations, were unable to return for evaluation, were on anti-inflammatory drugs, had poor oral hygiene, were smokers or alcoholics, were pregnant or lactating, or had abscesses or any pathology related to the tooth to be extracted were excluded.

Intervention and comparator

All participants received a local anesthetic blockade of 2% lidocaine HCl with 1:80,000 epinephrine administered for the inferior alveolar, lingual, and buccal nerves. A triangular incision was made in the ramus, and the mucoperiosteal flap was elevated. Bone removal was performed using tungsten carbide burs with a surgical handpiece. Tooth luxation and extraction were followed by socket curettage, saline irrigation, and 4.0 black silk simple interrupted suturing.

In the intervention group, 10 mL of venous blood was collected into a 4 dry glass sterile tube without anticoagulant and centrifuged at 2700 rpm for 12 min. The freshly prepared autologous PRF was placed on a gauze to be compressed to form a thin fibrin membrane and was placed in the extracted socket.¹⁷ The PRF clot was placed in the surgical socket before the suturing. In the comparator group, the same surgical procedure was performed without PRF placement.

Outcomes

The primary outcome was unstimulated salivary α -amylase activity measured on postoperative days 0, 3, and 7, and compared between the PRF and non-PRF groups. The measurement variable was salivary α -amylase activity, and the analysis metric was the between-group difference at different time intervals, supplemented by descriptive group means and standard deviations. The method of aggregation was the mean and standard deviation for the reported tables. Additional within-group analyses were performed to describe the changes in salivary α -amylase activity over time within each group across the same observation days.

Harms

This study did not report any detailed adverse events or harm related to the use of Platelet-Rich Fibrin (PRF) in mandibular third molar surgery.

Sample size

The sample size was estimated using a sample size formula²³ with a confidence level of 95%. The calculation was based on the expected standard deviation of salivary α -amylase concentrations and the anticipated difference in the means between the two groups (PRF and non-PRF). The minimum sample size was 14 for each group, resulting in a final target sample size of 28.

Randomization, allocation concealment, and implementation

This study was reported as a randomized clinical study. the method used for sequence generation, any restrictions such as blocking or stratification, the allocation concealment mechanism, and whether personnel enrolling participants and assigning interventions had access to the allocation sequence. No masking procedure was documented for the participants, care providers, outcome assessors, or data analysts. Because PRF placement was an intraoperative intervention, blinding the surgeon may not have been feasible.

Statistical methods

Descriptive statistics were performed using the Mann-Whitney test. Normality of the data was assessed using the Kolmogorov-Smirnov test. Kruskal-Wallis test was utilized to compare salivary α -amylase activity at different time intervals followed by wilcoxon signed-rank test. The significance level was set at $p < 0.05$ and considered statistically significant for all tests.

RESULTS

Participant flow and recruitment

Of the 28 patients in the study, 20 females and 8 males with a mean 25 years of age were assessed for eligibility and enrolled in the study. All eligible participants were randomly separated into two groups consisting of 14 participants each: group A (PRF) and group B (non-PRF). Recruitment will be conducted at the Dental Hospital of Hasanuddin University, Makassar, Indonesia, from May to November 2024. Follow-up and salivary α -amylase assessments were performed on the 0th day, 3th day, and 7th days.

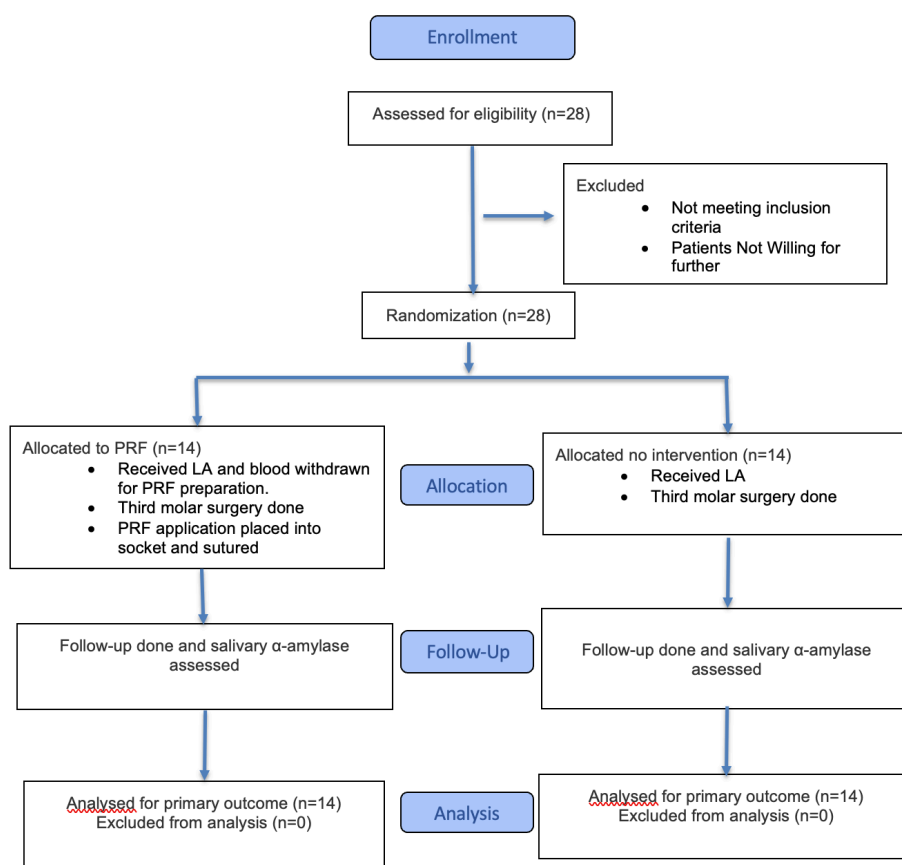


Fig 1. CONSORT 2025 Flow Diagram.²² Hopewell S, Chan AW, Collins GS, Hróbjartsson A, Moher D, Schulz KF, et al. CONSORT 2025 Statement: updated guidelines for reporting randomized trials. *BMJ*. 2025;388:e081123. doi:10.1136/bmj-2024-081123.

Intervention and comparator delivery

The intervention involved the application of Platelet-Rich Fibrin (PRF) in the surgical socket after mandibular third molar extraction. The comparator group underwent the same surgical procedure, but No PRF was applied. Salivary α -amylase levels were assessed on postoperative days 0th day, 3th day, and 7th day.

Baseline data

The baseline demographic and clinical characteristics are shown in Table 1.

Table 1. Demographic and clinical characteristics

Characteristic	PRF (n = 14)	Non-PRF (n = 14)	Total (n = 28)
Age, years; n (%)			
15–24	5 (35.7)	6 (42.9)	11 (39.3)
25–59	9 (64.3)	7 (50.0)	16 (57.1)
60–99	0 (0.0)	1 (7.1)	1 (3.6)
Gender, n (%)			
Men	3 (21.4)	4 (28.6)	7 (25.0)
Women	11 (78.6)	10 (71.4)	21 (75.0)

Tooth, n (%)				
38		5 (35.7)	7 (50.0)	12 (42.9)
48		9 (64.3)	7 (50.0)	16 (57.1)
Diagnosis, n (%)				
Impacted tooth	38 class II A mesioangular	1 (7.1)	1 (7.1)	2 (7.1)
Impacted tooth	38 class II A vertical	2 (14.3)	4 (28.6)	6 (21.4)
Impacted tooth	38 class II A horizontal	2 (14.3)	2 (14.3)	4 (14.3)
Impacted tooth	48 class II A mesioangular	4 (28.6)	3 (21.4)	7 (25.0)
Impacted tooth	48 class II A vertical	0 (0.0)	1 (7.1)	1 (3.6)
Impacted tooth	48 class II A horizontal	5 (35.7)	3 (21.4)	8 (28.6)

Primary Outcome: Between-Group Comparison

In table 2. showed higher mean salivary α -amylase levels in the PRF group than in the non-PRF group on postoperative 0th day, 3th day, and 7th day. However, Mann–Whitney comparisons were statistically significant (all $p > 0.05$). The number of participants analyzed and the number with available data at any given time interval were 14 per group, according to the study report.

Table 2. Salivary α -amylase activity at each postoperative time point

Day / Group	Mean	Standard deviation (SD)	p-value
Day 0 – PRF	131.76	368.91	0.138
Day 0 – Non-PRF	33.28	34.66	
Day 3 – PRF	128.88	370.63	0.896
Day 3 – Non-PRF	29.82	26.88	
Day 7 – PRF	49.31	61.46	0.295
Day 7 – Non-PRF	18.98	10.70	

As shown in Table 3, within-group comparisons across postoperative days were not statistically significant in either group (Kruskal–Wallis test: PRF $p = 0.519$; non-PRF $p = 0.366$). The pairwise Wilcoxon test (Table 4) showed no significant differences between the observation times in either group.

Table 3. Salivary α -amylase activity across postoperative observation

Group	Day	Mean	Standard deviation (SD)	p-value
PRF	Day 0	131.76	368.91	0.519
	Day 3	128.88	370.63	
	Day 7	49.31	61.46	
Non-PRF	Day 0	33.28	34.66	0.366
	Day 3	29.82	26.88	
	Day 7	18.98	10.70	

Table 4. Salivary α -amylase activity comparison (wilcoxon test)

Group	Intervention	Day 0	Day 3	Day 7
PRF	Day 0		0.407	0.254
	Day 3			0.934

	Day 7		
Non PRF	Day 0	0.927	0.198
	Day 3		0.251
	Day 7		

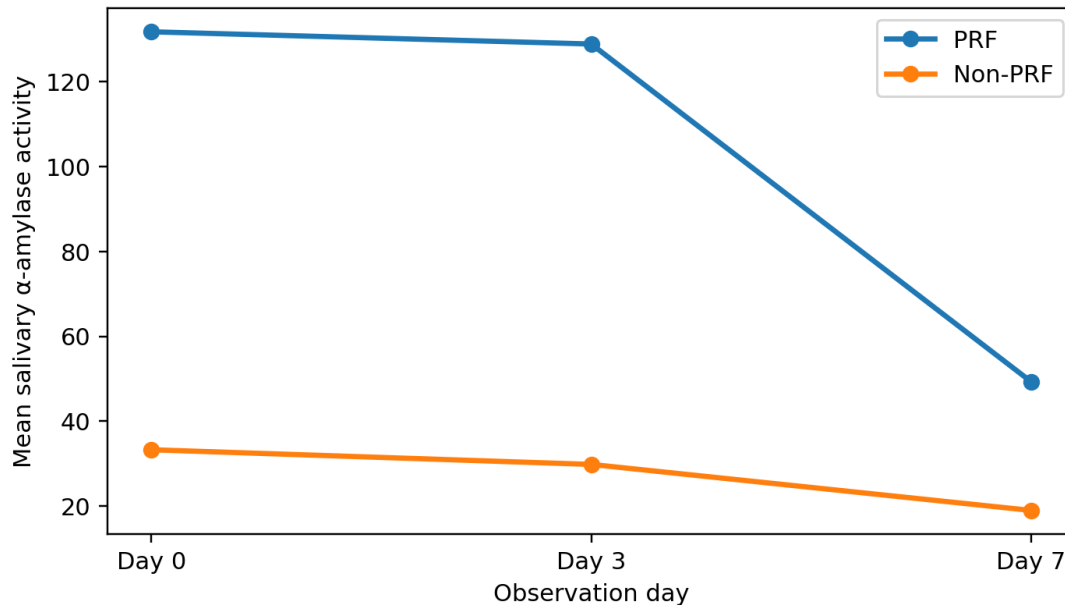


Fig 2. Mean salivary α -amylase activity on postoperative days in both groups.

Both groups showed a downward trend in mean salivary α -amylase activity at any given time interval (fig. 2). Although the PRF group remained descriptively higher than the non-PRF group throughout the follow-up, the overall temporal pattern suggested a decline rather than a sustained postoperative increase in either group.

Harms and ancillary analyses

No procedure-related harm or unintended events were prespecified or systematically collected in the available manuscript materials. The ancillary analyses were exploratory and did not change the primary interpretation.

DISCUSSION

In our study, platelet-rich fibrin (PRF) showed higher mean salivary α -amylase activity than the non-PRF group on postoperative 0th day, 3th day, and 7th day. However, the differences between the groups were not statistically significant at any interval point. Therefore, the findings do not provide sufficient evidence to conclude that PRF significantly alters salivary α -amylase activity after mandibular third molar surgery. Salivary α -amylase cannot yet be considered a reliable stand alone marker of inflammatory response or wound healing after PRF application in mandibular third molar surgery. Although PRF is a biologically plausible regenerative material, this study did not demonstrate a statistically significant difference in salivary α -amylase activity between patients who received PRF and those without PRF.

PRF may improve and have potential value in postoperative wound healing and can reduce postoperative complications, such as pain, edema, and trismus.^{18,19,21} However, its effect could not be confirmed using salivary α -amylase activity alone in this sample. Interpretation should remain cautious because the study involved a relatively small number of participants (14 participants in each group), and the biomarker response showed high variability.

The results showed a decline in mean α -amylase activity from day 0 to day 7 in both groups. This may reflect the resolution of postoperative stress, pain, and inflammatory responses during early healing. However, the wide variability in the Platelet Rich Fibrin (PRF) group, especially on the 0th and 3rd days, suggests substantial inter-individual heterogeneity and limits the precision of effect estimates.

These findings are broadly consistent with previous reports describing inconsistent or nonsignificant relationships between salivary α -amylase levels and postoperative outcomes after mandibular third molar surgery. Surin et al. described postoperative changes in salivary α -amylase after mandibular third molar surgery, with day-7 values not meaningfully differing between clinical groups.²⁴

Several factors influenced the results, including the patient's systemic condition, oral hygiene, medication, psychological stress, cooperation after treatment, and the operator or surgical technique.²⁵ PRF is expected to influence tissue healing through complex growth factor and inflammatory pathways. Future studies should consider combining salivary α -amylase levels with clinical outcomes, such as pain, swelling, trismus, soft tissue healing, and other biochemical markers. This interpretation is closely associated with postoperative inflammatory changes and pain-related outcomes, while an accurate preoperative assessment remains important for estimating the surgical difficulty in impacted mandibular third molars.²⁶

The limitations of this study were the small sample size and the wide variability in α -amylase values, which reduced the precision of the treatment effect. Non-significant results should not be interpreted as PRF and non-PRF treatments being equivalent, reducing their specificity as markers of wound healing. The study was conducted at a single center with specific patient demographics, which limits the generalizability of the results to other settings or populations. The lack of blinding and repeated analyses at any given time interval may introduce bias and increase the risk of chance findings in the study.

PRF was associated with descriptively higher salivary α -amylase levels after mandibular third molar odontectomy, but the between-group and within-group differences were not statistically significant. Salivary α -amylase cannot be recommended as a reliable inflammatory biomarker of PRF-enhanced wound healing in this study. Future studies should include prospective registration, a public protocol, an adequate sample size, blinded outcome assessment, and additional clinical or biochemical endpoints.

Data Availability Statement

All data used to generate the results presented in this manuscript will be made available upon reasonable request from the corresponding author.

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Declaration of competing interest

The authors declare that they have no competing financial interests or personal relationships that could have influenced the work reported in this study.

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CRediT authorship contribution statement

Faizal Manrapi: Writing– original draft, Visualization, Resources, Methodology, Investigation. **Abul Fauzi:** Writing – review and editing, Writing – original draft, Validation, Supervision, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. **Nurwahida:** Writing– review and editing, Validation, Methodology, Investigation. **Andi Tajrin:** Writing– review & editing, Supervision. **Hasanuddin:** Writing–review and editing, Visualization, Supervision.

Ethical Clearance

The study was evaluated and approved by the Research Ethics Committee of the Faculty of Dentistry, Hasanuddin University (No. 0015/PL.09/KEPK FKG-RSGM UNHAS/2024). All participants provided written informed consent before enrolment, and all procedures were conducted in accordance with the applicable ethical standards and the principles of the Declaration of Helsinki for research involving human subjects.

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