The Effect of Platelet-Rich Fibrin on Post-Operative Inflammatory Sequelae after Mandibular Third Molar Surgery: A Randomised Controlled Trial

Correspondence: *Njokanma AR*
E-mail: *njokanmaazukaraphael@gmail.com*

Njokanma AR, Fatusi OA*, Ogundipe OK*, Adejobi AF*, Kuye OF**.*
**Department of Oral and Maxillofacial Surgery, Obafemi Awolowo University / Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife, Nigeria*
***Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Lagos State University College of Medicine, Ikeja, Lagos state, Nigeria*

Key words: Platelet-rich fibrin, post-operative inflammatory sequelae, mandibular third molar surgery.

ABSTRACT

Background: Surgical extraction of impacted mandibular third molars is commonly associated with post-operative inflammatory sequelae, causing distress to patients and affecting their quality of life. This study aimed to investigate the effect of using autologous platelet-rich fibrin (PRF) on post-operative pain, facial swelling, and trismus, following mandibular third molar surgery.

Methods: In this single-blinded prospective randomised controlled trial, 90 participants aged 18-35 years were enrolled. Eligible patients underwent surgical extraction of impacted mandibular third molars under local anesthesia between October 2017 and June 2018 at the Oral and Maxillofacial Surgery clinic of Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife, Nigeria. Participants were randomly assigned to either the PRF group or the non-PRF group. PRF was placed in the extraction socket of participants of the PRF group, while blood clot was allowed to form in the extraction sockets of the non-PRF group. Post-operative pain, facial swelling, and trismus were measured as outcomes. Longitudinal data analysis using generalised estimating equations was employed, adjusting for confounding factors, with statistical significance set at $p < 0.05$.

Results: The PRF group exhibited significantly lower pain scores (3.02 mm; $p < 0.001$), reduced facial swelling (0.55%; $p = 0.01$), and decreased trismus (4.52%; $p = 0.05$) compared to the non-PRF group.

Conclusion: Placement of PRF in the extraction socket following mandibular third molar surgery resulted in decreased post-operative pain, facial swelling, and trismus. These findings suggest that PRF may have a beneficial impact on the inflammatory outcomes of third molar surgeries.

INTRODUCTION

Surgical removal of impacted mandibular third molars (M3), commonly referred to as wisdom teeth, is a frequent procedure in the field of oral and maxillofacial surgery. Impacted M3s, those failing to fully erupt into the oral cavity by the age of 21 years,[1] pose a significant clinical challenge due to their potential to cause pain, infection, and other complications.[2,3]Their surgical removal is among the most prevalent interventions carried out by oral and maxillofacial surgeons, reflecting the high prevalence of impacted M3s worldwide. For instance, prevalence rates range from 1.1% in rural Nigeria to 10.7% in urban Nigeria,[4]17.5% in the United States of America[5], and a striking 56.8% in Turkey[6].

While surgical extraction of impacted M3s is necessary for many patients, it often leads to post-operative inflammatory sequelae, including pain, facial swelling, and trismus. These physiological responses are not only sources of discomfort but also contribute to a decline in the immediate post-operative quality of life[7]. In some cases, patients may even require days off from work due to the severity of these sequelae[8].

Platelet rich fibrin (PRF), an autologous second-generation platelet concentrate, has gained attention for its potential to enhance soft tissue healing and modulate the inflammatory process[9-15]. This is achieved through the sustained release of growth factors that are encapsulated within the fibrin network of PRF[16]. Key growth factors, such as platelet-derived growth factor (PDGF), transforming growth factor (TGF), and insulin-like growth factor (IGF), play pivotal roles in the healing mechanisms facilitated by PRF[16].

Nonetheless, existing studies present conflicting results, contributing to an ongoing debate regarding the impact of PRF on post-operative outcomes[17]. While some studies suggest positive effects, such as reduced pain, swelling, and trismus following PRF application during impacted M3 surgery[17,18], others have reported null or inconclusive findings[19-22]. These disparities may arise from varying methodologies, limited sample sizes, and the lack of consideration for potential confounding variables. Another study may be necessary to support or refute current evidence on the effects of PRF in modulating M3 post-surgical inflammation.

Furthermore, the majority of studies conducted on PRF have focused on Caucasian populations, with limited racial diversity. Moreover, previous research often overlooked the potential influence of confounders, such as age, sex, indication for extraction, impaction type, Pederson's difficulty index, and associated pathology. These factors could contribute to the inconclusive nature of the findings and underscore the need for a comprehensive investigation.

The primary objective of the present study was to ascertain the effect of autologous platelet rich fibrin (PRF) on post-operative pain, while secondary objectives included its effects on post-operative facial swelling, and trismus, following surgical extraction of impacted M3s. To achieve this, a randomised controlled trial was designed to rigorously evaluate the null hypothesis that PRF does not impact post-operative pain, facial swelling, and trismus. By addressing the limitations of prior research and considering a wide range of potential confounding variables, this study aims to provide a more robust and comprehensive understanding of the potential benefits of PRF in improving patient outcomes following impacted M3 surgery.

METHODOLOGY

Study Design: This study was designed as a single-blinded prospective randomised controlled trial to investigate the effect of autologous platelet rich fibrin (PRF) on post-operative pain, facial swelling, and trismus, following surgical extraction of impacted mandibular third molars (M3). The null hypothesis tested was that PRF would not affect these post-operative outcomes.

Ethical considerations: This study was conducted in accordance with the principles outlined in the Declaration of Helsinki. Ethical approval (Protocol number: ERC/2016/09/03) for this study was obtained from the Obafemi Awolowo University Teaching Hospital's Complex (OAUTHC), Ile-Ife, Nigeria.

Pilot Study: A pilot study involving ten (10) participants with impacted M3 indicated for surgical extraction, equally randomised into PRF and non-PRF groups, was conducted. Facial measurements and maximum interincisal distance were taken thrice (by the research assistant) for each participant and an intra-observer reliability test was performed on the facial measurements and maximum inter-incisal distance using the intra-class correlation coefficient. This yielded an intra-class correlation coefficient of 0.901 and 0.875 for facial measurements and maximum inter-incisal distance respectively. Since the pilot study only helped calibrate the research team and ensure accurate data collection methods, the data generated from it were not included in the results of this study.

Sample Size Determination: The calculated sample size for this study was 78 participants for the two groups combined, and with a 15% dropout rate considered, the final sample size was increased to 90 participants (45 in each group).

Participants: Ninety participants aged 18–35 years with impacted mandibular third molars, requiring surgical extraction under local anesthesia, were included. Patients with specific conditions, such as systemic diseases, anti-platelet or anticoagulant therapy, allergies, acute local infections, smoking, oral contraceptive use, visual impairment, pregnancy, lactation, jaw irradiation, and missing upper or lower central incisors or prosthetic replacements were excluded. All participants provided written informed consent.

Randomization: Participants were randomised into PRF and non-PRF groups using an online-generated sequence from www.graphpad.com. Each participant was assigned a randomisation code to conceal their identity during data collection.

Outcome Measures The primary outcome measure was post-operative pain. The secondary outcome variables included:

1. Trismus, determined by maximal inter-incisal mouth opening (MID) in millimetres.
2. Post-operative facial swelling, determined by facial measurements on the surgical side of the face in millimetres.

Data Collection Preoperatively and postoperatively, pain was assessed using the Visual Analogue Scale (VAS), a 100-millimeter horizontal line where 0mm and 100mm represented 'no pain' and 'most severe pain,' respectively. Participants marked their perceived pain level on the VAS line, and the score was measured in millimetres from the left end to the marked point.

Maximal inter-incisal mouth opening (MID) was measured using a digital vernier-caliper (Looyuan Stainless steel electronic digital vernier-caliper, Fig.1). The distance in millimetres between the maxillary and mandibular central incisal edges or a prosthetic equivalent was measured at maximal mouth opening.

Fig. 1: Measurement of maximal inter-incisal opening using a digital vernier-caliper

Facial measurements were conducted using a method adapted from Ustun et al[26]. Three-line measurements (AC, AD & BE) were taken on the surgical side of the face using five fixed reference points (A:the most posterior point in the middle of the tragus, B:the lateral canthus of the eye, C: the most lateral point on the commissure of the mouth, D:the soft tissue pogonion which is the most prominent point in the midline of the chin, and E:the most inferior point on the angle of the mandible). The sum of the three-line measurements constituted the facial measurement.

Fig. 2: Facial measurements conducted according to the method by Ustun et al[26].

Calculation of Trismus and Facial Swelling

Percentage trismus post-operatively was calculated using the formula described by Ustun et al[26]:

$$\frac{\text{postopMID - preopMID}}{\text{preopMID}} \times 100$$

Percentage facial swelling was quantified using the formula described by Cerqueira *et al*[27]:

$$\frac{\text{post op facial measurement - pre op facial measurement}}{\text{pre op facial measurement}} \times 100$$

Postoperative Assessments Post-operative evaluations of pain, facial swelling, and trismus were performed on days 1, 3, 5, 7, and 14 following tooth extraction.

Data Collection Procedures To ensure unbiased data collection, a research assistant (resident doctor in the Department of Oral and Maxillofacial Surgery) was calibrated during a pilot study and remained blinded to the participants' groups. This assistant conducted and recorded measurements for VAS scores, facial measurements, and MID for all participants pre- and post-operatively.

A standardised proforma was used to collect pre-operative and post-operative data. Visual Analogue Scale (VAS) scores for pain, maximal inter-incisal mouth opening (MID) measurements, and facial measurements were recorded. Pre-operative and post-operative assessments were conducted on post-operative days 1, 3, 5, 7, and 14.

Confounding Variables Considerations were made to control for possible confounders namely: age, sex, type of impaction, indication for surgery, duration of surgery, volume of local anaesthetic used, and tooth delivery method.

Platelet Rich Fibrin Preparation: PRF was prepared according to Choukroun's protocol using venous whole blood collected from participants in the PRF group as outlined by Dohan et al[28]. Blood collection was conducted in a side haematology laboratory established within the same Oral and Maxillofacial Surgery clinic. Prior to surgery, 10ml of venous whole blood was drawn from participants in the PRF group using a standardised aseptic venepuncture technique. The blood was immediately placed in a sterile glass-coated plastic vacutainer tube without anticoagulant. The tube was then centrifuged at 3,000 rpm for 10 minutes in an Axiom centrifuge (Model 800B, Axiom Medical Ltd, U.K). This resulted in a 3-layered component: a top layer of platelet-poor acellular plasma, a middle layer containing the platelet-rich fibrin clot, and a bottom layer with red blood cells.

One 10ml blood tube yielded a single PRF clot, suitable for filling one extraction socket, as reported in previous studies[22]. The platelet-poor plasma was extracted using a 5ml syringe and needle. The PRF fraction, including approximately 2mm of the red cell fraction, was collected using sterile tissue forceps (Fig.3). This step ensured the complete removal of all platelets, including those localised at the junction between PRF and red blood cells. The PRF was promptly inserted into the extraction socket within 45 minutes of its creation to maintain its quality[29].

Platelet Counting

The number of platelets in whole blood was counted manually for each participant in the PRF group[30]. The platelet count of the

acellular platelet poor plasma and red cell layer was also done to demonstrate the distribution of platelets in the three fractions after centrifugation of venous blood for preparation of PRF. All platelet counts were performed within two hours of sample collection by the second research assistant who is a medical laboratory scientist in the department of Haematology, OAUTHC, Ile-Ife.

Percentage Distribution of Platelets in Platelet Rich Fibrin (PRF).

The mean percentage of platelets contained in the PRF layer was determined by subtracting the mean percentage platelets contained in the platelet poor plasma (PPP) fraction and the red cell layer (RCL) from the mean percentage concentration of platelets in whole blood (100%).[31] Mean percentage concentration of platelets in PPP or RCL was determined by the formula:

$$\frac{\text{mean platelet count in PPP or RCL}}{\text{mean platelet count in whole blood}} \times 100$$

The mean percentage concentration of platelets in PRF was therefore calculated as:

100 - (mean %age platelet conc in PPP + mean %age platelet conc in RCL)

Fig. 3 PRF prepared according to Choukroun's protocol and collected with about 2mm of red cell fraction.

Surgical Procedure: All surgical extractions were performed using a standardised buccal guttering technique by one surgeon (AN). Subsequent to the surgical extraction, participants in the PRF group had autologous PRF introduced into the extraction socket (Fig.4), while blood clot only was allowed to form in the extraction sockets of individuals in the non-PRF group. The mucoperiosteal flap was sutured using 3.0 black silk sutures (Fig.5) in both groups. To achieve haemostasis, participants were advised to bite on a piece of gauze.

During the surgery, data were recorded, including the quantity of local anaesthetic administered in millilitres, and the duration of the surgical procedure in minutes. Comprehensive verbal and written post-operative instructions were provided to all participants. Standardised antibiotics (Amoxicillin Beecham capsule 500mg and metronidazole 400mg every 8 hours for 5 days) and analgesics (Ibuprofen capsule 400mg every 8 hours for 3 days) were uniformly prescribed. Participants were explicitly instructed not to take any additional medications.

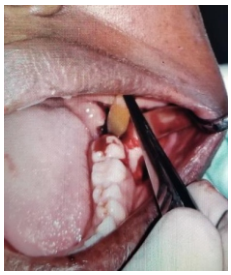

Fig. 4: PRF introduced into the extraction socket

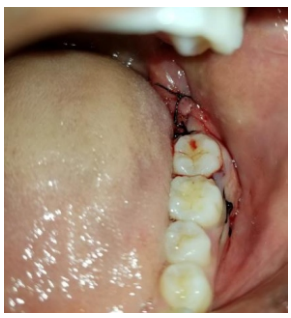

Fig. 5: During surgical extraction, the mucoperiosteal flap was sutured using 3/0 black silk sutures

Data Analysis: Data were analysed using IBM SPSS version 20. Statistical tests including Mann-Whitney U test, independent t-test, and longitudinal data analysis (using generalised estimating equation) were applied to assess differences between the PRF and non-PRF

groups in terms of pain, facial swelling, and trismus. Potential confounders such as age, sex, impaction type, surgery indication, duration, local anesthetic volume, and tooth delivery method were considered. Statistical significance was set at $p < 0.05$ based on a 95% confidence interval.

RESULTS

Participant Characteristics: Ninety participants were enrolled in the study, and no drop-outs were recorded (Fig.6). The participants had a mean age of 25.79 (SD=4.56)years, with ages ranging from 18 to 35 years. The two study groups, namely the PRF and non-PRF groups, exhibited no statistically significant differences in terms of age, sex distribution, indication for extraction, impaction type, Pederson's difficulty index, and associated pathology (p=0.407, 0.280, 0.514, 0.390, 0.063 respectively).

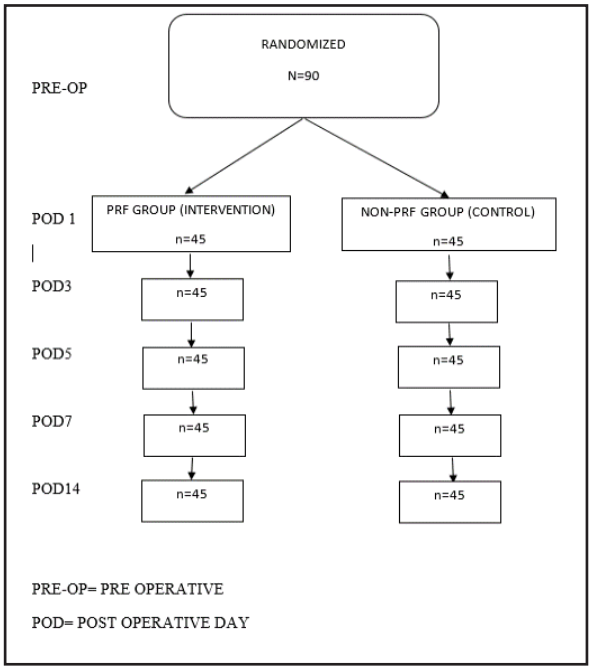

Fig. 6: Flow chart of participants in the PRF and non-PRF groups.

Platelet Count and Concentration in PRF: In the PRF group, the range of platelet count in whole blood was 157,000 to 400,000 cells/μl, with a mean platelet count of 278,177.78 ± 51,206.93 cells/μl. The platelet count in platelet poor plasma from the same group ranged from 5,000 to 8,000 cells/μl, with a mean count of 6,044.44 ± 998.99 cells/μl, constituting approximately 2.2% of the original count. Notably, no platelets ~~(0.0%)~~ were detected in the red cell layer. The platelet concentration in platelet rich fibrin was 97.8%.

Post-Operative Pain: The mean pain scores exhibited a peak on post-operative day 1 (POD1) and gradually decreased over subsequent days in both the PRF and non-PRF groups. Bivariate analysis demonstrated significantly lower mean pain scores in the PRF group on PODs 1, 3, 5, and 7, with no notable difference on day 14 (p = 0.002, 0.001, <0.001, <0.001, and 0.380, respectively, (Fig.7). Employing longitudinal analysis with the Generalised Estimating Equation (GEE) method to account for confounders, it was revealed that the PRF group consistently experienced significantly lower pain scores throughout the post-operative period up to POD14 ($p < 0.001$, Table 1).

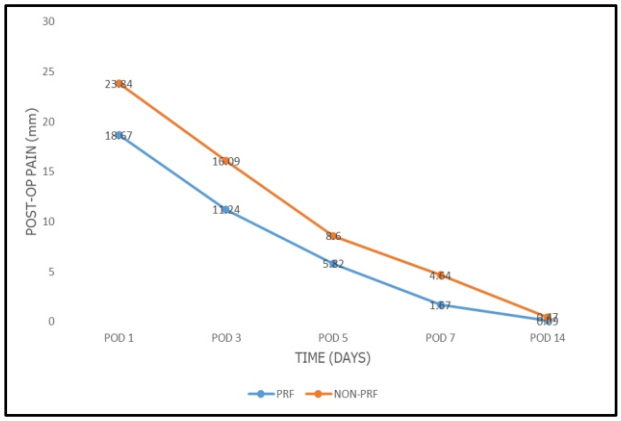

Fig.7: Comparison of the mean post-operative pain scores measured using the visual analogue scale in participants in the PRF and non-PRF groups

Table 1: Longitudinal data analysis of post-operative pain using Generalised estimating equation in participants in the PRF and non-PRF groups

Pain Score (mm).

	Coef.	SE	z	p value	95% CI LL	UL
Group						
Non-PRF*						
PRF	-3.02	1.06	-2.85	**<0.001**	-5.10	-0.94
Age	0.20	0.11	1.81	0.07	-0.02	0.41
Sex						
Female*						
Male	0.53	0.96	0.55	0.58	-1.35	2.40
Duration of surgery	0.03	0.07	0.45	0.65	-0.11	0.18
LA vol. used						
3.6ml*						
5.4ml	-1.07	1.25	-0.86	0.39	-3.52	1.38
Impaction type						
Mesioangular*						
Horizontal	-2.83	1.34	-2.11	**0.04**	-5.45	-0.20
Vertical	-0.31	1.26	-0.25	0.80	-2.78	2.15
Distoangular	-1.96	1.72	-1.14	0.25	-5.34	1.41
Tooth delivery method						
Ostectomy+elevation/forceps*						
Ostectomy+coronal section	0.95	1.33	0.72	0.47	-1.66	3.56
Complex extraction (root resection)	3.79	1.84	2.06	**0.04**	0.18	7.40
Indication for extraction						
Pericoronitis*						
Apical periodontitis	-0.01	1.10	-0.01	0.99	-2.16	2.14
Irreversible pulpitis	1.51	2.55	0.59	0.56	-3.49	6.50
Orthodontic reasons	-2.12	4.23	-0.50	0.62	-10.41	6.17
Constant	6.32	3.16	2.00	0.05	0.12	12.51

*Reference category

Facial Swelling: In the PRF group, the mean percentage of facial swelling was markedly lower on PODs 1, 3, 5, and 7 (p = 0.014, 0.022, 0.001, and 0.001, respectively), while no notable difference was observed on POD 14 (p = 0.603, Fig. 8). Employing longitudinal analysis with GEE while accounting for confounders, it was evident that participants in the PRF group consistently experienced 0.55% less facial swelling throughout the post-operative period up to POD14 (p = 0.01, Table 2).

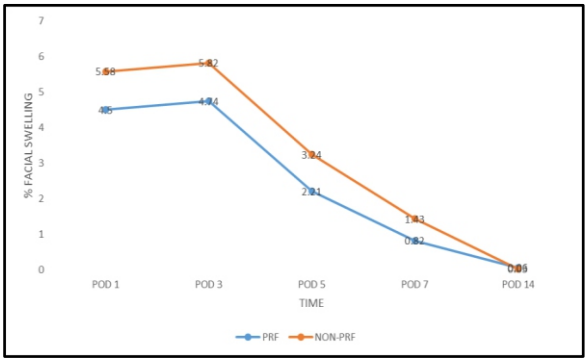

Fig.8: Comparison of the mean percentage post-operative facial swelling in participants in the PRF and non-PRF groups.

Table 2: Longitudinal data analysis of post-operative facial swelling using Generalised estimating equation in participants in the PRF and non-PRF groups Facial swelling (%).

	Coef.	SE	z	p value	95% CI LL	UL
Group						
Non-PRF*						
PRF	-0.55	0.22	-2.49	**0.01**	-0.99	-0.12
Age	0.03	0.02	1.31	0.19	-0.01	0.07
Sex						
Female*						
Male	-0.12	0.20	-0.61	0.54	-0.52	0.27
Duration of surgery	-0.02	0.02	-1.43	0.15	-0.05	0.01
LA vol. used						
3.6ml*						
5.4ml	0.01	0.26	0.04	0.97	-0.50	0.52
Impaction type						
Mesioangular*						
Horizontal	-0.42	0.28	-1.51	0.13	-0.97	0.13
Vertical	-0.22	0.26	-0.84	0.40	-0.74	0.30
Distoangular	-0.57	0.36	-1.57	0.12	-1.27	0.14
Tooth delivery method						
Ostectomy+elevation/forceps*						
Ostectomy + coronal section	0.99	0.28	3.57	**<0.001**	0.45	1.54
Complex extraction (root resection)	0.70	0.38	1.83	0.07	-0.05	1.46
Indication for extraction						
Pericoronitis*						
Apical periodontitis	-0.14	0.23	-0.59	0.56	-0.59	0.31
Irreversible pulpitis	-0.62	0.53	-1.16	0.25	-1.66	0.43
Orthodontic reasons	0.61	0.88	0.69	0.49	-1.12	2.34
Constant	2.63	0.66	3.98	0.00	1.34	3.93

*Reference category

Trismus: For participants in the PRF group, the mean percentage of trismus was notably lower on PODs 3 and 5 (p = 0.001, 0.004, respectively). The highest mean percentage of trismus in the PRF group occurred on POD 1, while the non-PRF group experienced the peak on POD 3 (Fig. 9). Through longitudinal data analysis with GEE while controlling for confounders, it was determined that the PRF group experienced 4.52% less trismus on average, though the difference was not statistically significant (p = 0.05, Table 3).

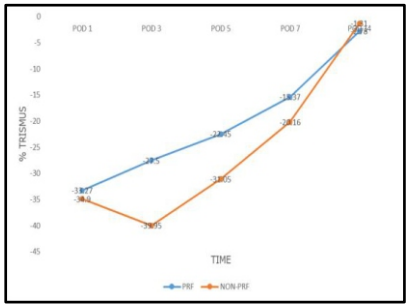

Fig. 9: Comparison of the mean post-operative percentage trismus in participants in the PRF and non-PRF groups.

Table 3: Longitudinal data analysis of post-operative trismus using Generalised estimating equation in participants in the PRF and non-PRF groups

Trismus (%).

	Coef.	SE	z	p	95% CI LL	UL
Group						
Non-PRF*						
PRF	-4.52	2.26	-2.00	0.05	-8.94	-0.09
Age	-0.09	0.23	-0.40	0.69	-0.55	0.36
Sex						
Female*						
Male	0.15	2.04	0.07	0.94	-3.85	4.14
Duration of surgery	0.00	0.16	0.00	1.00	-0.31	0.31
LA vol. used						
3.6ml*						
5.4ml	3.85	2.66	1.45	0.15	-1.36	9.07
Impaction type						
Mesioangular*						
Horizontal	2.01	2.85	0.71	0.48	-3.58	7.61
Vertical	-0.20	2.68	-0.07	0.94	-5.45	5.06
Distoangular	3.08	3.67	0.84	0.40	-4.11	10.27
Tooth delivery method						
Ostectomy+elevation/forceps*						
Ostectomy + coronal section	5.21	2.83	1.84	0.07	0.34	10.76
Complex extraction (root resection)	5.98	3.92	1.53	0.13	1.70	13.65
Indication for extraction						
Pericoronitis*						
Apical periodontitis	1.25	2.34	0.53	0.59	-3.33	5.83
Irreversible pulpitis	4.57	5.42	0.84	0.40	-6.06	15.20
Orthodontic reasons	3.14	9.00	0.35	0.73	-14.51	20.78
Constant	-19.57	6.73	-2.91	0.00	-32.76	-6.37

*Reference

DISCUSSION

Platelet rich fibrin (PRF) production results in the concentration of platelets rich in growth factors and cytokines within a fibrin clot[32]. The growth factors released from these platelets are known to promote the regulation of inflammation at surgical sites where PRF is applied. This regulatory effect is attributed to the retro-control action of cytokines entrapped in the fibrin matrix, released during the remodelling of the initial PRF structure[33]. Within PRF, several significant cellular mediators have been identified, including three pro-inflammatory cytokines (IL-1β, IL-6, and TNF-α), an anti-inflammatory cytokine (IL-4), and a key promoter of angiogenesis (VEGF). Notably, cytokines like IL-4 within PRF contribute to the retro-control modulation of inflammatory processes[33,34].

This study adopted a single-blinded randomised controlled clinical trial design, employing a relatively larger sample size than other comparable studies in the literature. A significant advantage of this study was the utilisation of longitudinal data analysis, which effectively accounted for potential confounders such as age, sex, impaction type, surgery indication, surgery duration, local anaesthetic volume, and tooth delivery method. These factors have been reported to influence the outcomes of mandibular third molar surgery[35].

The preparation and application of PRF from autologous blood collected from participants were demonstrated to be relatively straightforward, facilitating its placement into extraction sockets post-surgical extraction of impacted mandibular third molars. The adoption of Choukroun's standard protocol for PRF production ensured a high concentration of platelets, exceeding 97%. This platelet concentration was deemed adequate for modulating inflammation, in line with previous reports[29,31]. The PRF produced in this study exhibited a platelet concentration of 97.8%. Moreover, this study successfully utilised 10mls of venous blood for PRF production, ensuring that the PRF clot adequately filled extraction sockets, as also evidenced in previous research[22].

The primary outcome measure of this study was post-operative pain. The results highlighted significantly reduced pain scores in the PRF group compared to the non-PRF group. Mann-Whitney U test showed this difference to be statistically significant on post-operative days 1, 3, 5 and 7 implying reduced post-operative pain in extraction sockets filled with PRF compared to the non-PRF group. Longitudinal data analysis using the generalised estimating equation revealed that controlling for age, sex, type of impaction, indication for surgery, duration of surgery, volume of LA used, and tooth delivery method, the PRF group reported about 3.02 mm lower pain score than the non-PRF group over the pre-operative period to POD14 and this difference was statistically significant ($p < 0.001$; CI = 0.94 to 5.10 mm, Table 1). These findings echo similar reports in the literature, such as the study by Daugela et al[36], which also observed consistently lower pain scores in the PRF group across all post-operative assessment days. Additional studies, such as those conducted by Kumar et al[18] and Al-Hamed et al[17], supported the notion of diminished post-operative pain in PRF-treated groups. On the contrary, Singh et al[21], and Gulsen et al[20], reported no significant differences in mean post-operative pain scores between the PRF and non-PRF groups. This disparity in findings may be due to differences in their methodology and sample size.

Tissue trauma during M3 surgery causes spasm of the muscles of mastication and fluid accumulation in the interstitial area as a result of its transudation from injured blood vessels and fibrin obstruction of lymphatic drainage, causing facial swelling and trismus. The study findings showcased that the PRF group exhibited consistently lower mean percentages of post-operative facial swelling throughout the evaluation period. Significant differences were noted on multiple days, and after accounting for confounders, the PRF group experienced a significant reduction of 0.55% in facial swelling ($p = 0.01$, Table 2). These outcomes closely align with the studies conducted by Kumar et al[18] and Ozgul et al[37], which also reported significantly diminished facial swelling in PRF-treated groups. In contrast, the report from studies by Uyanik et al[22] and Gulsen et al[20] that PRF usage in impacted M3 surgery did not significantly reduce facial swelling may be attributed to variations in sample sizes.

Regarding trismus, the PRF group exhibited lower mean percentages of trismus on multiple post-operative days, with significant differences on PODs 3 and 5 (Fig. 9), indicating the potential of PRF to mitigate

post-operative trismus. Although both groups exhibited decreasing trismus over time, the non-PRF group exhibited a better mouth opening on POD 14, though the reason for this outcome remains uncertain. Longitudinal analysis controlling for confounders indicated a not significantly lower trismus of 4.52% in the PRF group throughout the evaluation period (p = 0.05; CI = 0.09 to 8.94%, Table 3). These findings are consistent with prior research reporting improved mouth opening following PRF application[18,22].

In conclusion, this study demonstrated the potential of autologous platelet rich fibrin (PRF) to reduce post-operative pain and facial swelling following surgical extraction of impacted mandibular third molars. PRF also reduced post-operative trismus, however, this did not attain statistical significance. By adopting a robust analytical approach, we were able to control for potential confounding variables that have previously been reported to influence surgical outcomes[33]. Our results corroborate previous research in the field and provide valuable insights into the clinical utility of PRF in improving patient comfort, thus, expediting post-operative recovery. Our findings suggest that PRF could be a valuable adjunct in reducing patient post-operative inflammatory sequelae after mandibular third molar surgery.

ACKNOWLEDGEMENTS

Mr. D.A Adegoke, a Medical Laboratory Scientist in the department of Haematology and Blood transfusion, Obafemi Awolowo University Teaching Hospitals complex, Ile-Ife, Nigeria who prepared the platelet rich fibrin and determined the platelet counts in this study.

REFERENCES

1. Miloro M, Ghali G, Larsen P, Waite P. Peterson's principles of oral and maxillofacial surgery: PMPH-USA; 2004.
2. Abdulal A, Nuamah I, Sackeyflo J, Hewlett S. Indications for surgical extraction of third molars: a hospital-base study in Accra, Ghana. International Journal of Medicine and Biomedical Research. 2015;3(3):155-160.
3. Diniz-Freitas M, Lago-Méndez L, Gude-Sampedro F, Somoza-Martin JM, Gándara-Rey JM, García-García A. Pederson scale fails to predict how difficult it will be to extract lower third molars. Br J Oral Maxillofac Surg. 2007;45(1):23-26.
4. Olasoji H, Odusanya S. Comparative study of third molar impaction in rural and urban areas of southwestern Nigeria. Odontostomatol Trop. 2000;23:25-28.
5. Dachi SF, Howell FV. A survey of 3,874 routine full-mouth radiographs: II. A study of impacted teeth. Oral Surg Oral Med Oral Pathol. 1961;14(10):1165-1169.
6. Celikoglu M, Miloglu O, Kazanci F. Frequency of agenesis, impaction, angulation, and related pathologic changes of third molar teeth in orthodontic patients. J Oral Maxillofac Surg. 2010;68(5):990-995.
7. Saheeb B, Obuekwe O. An audit of mandibular third molar surgery. Nigerian Journal of Surgical Research. 2004;3(2):66-74.
8. Obimakinde O, Fasola A, Arotiba J, Okoje V, Obiechina A. Comparative effect of tube drain on post operative inflammatory complications of impacted mandibular third molar surgery College Hospital, Ibadan, Nigeria.Niger Postgrad Med J. 2010;17(3):194-199.
9. Barone A, Marconcini S, Giacomelli L, Rispoli L, Calvo JL, Covani U. A

randomised clinical evaluation of ultrasound bone surgery versus traditional rotary instruments in lower third molar extraction. *J Oral Maxillofac Surg*. 2010;68(2):330-336.
10. Forouzanfar T, Sabelis A, Ausems S, Baart J, Van Der Waal I. Effect of ice compression on pain after mandibular third molar surgery: a single-blind, randomised controlled trial. Int J Oral Maxillofac Surg. 2008;37(9):824-830.
11. Koyuncu B, Zeytinoğlu M, Tetik A, Gomel M. Effect of tube drainage compared with conventional suturing on postoperative discomfort after extraction of impacted mandibular third molars. Br J Oral Maxillofac Surg. 2015;53(1):63-67.
12. LaureanoFilho JR, e SILVA EDdO, CAMARGO IB, GOUVEIA FM. The influence of cryotherapy on reduction of swelling, pain and trismus after third-molar extraction: a preliminary study. J Am Dent Assoc. 2005;136(6):774-778.
13. Olurotimi AO, Gbotolorun OM, Ibikunle AA, Emeka CI, Arotiba GT, Akinwande JA. A Comparative Clinical Evaluation of the Effect of Preoperative and Postoperative Antimicrobial Therapy on Postoperative Sequelae after Impacted Mandibular Third Molar Extraction. J Oral Maxillofac Res. 2014;5(2):1-6.
14. Sandhu A, Sandhu S, Kaur T. Comparison of two different flap designs in the surgical removal of bilateral impacted mandibular third molars. *Int J Oral Maxillofac Surg*. 2010;39(11):1091-1096.
15. Njokanma AR, Fatusi OA, Ogundipe OK, Arije OO, Akomolafe AG, Kuye OF. Does platelet-rich fibrin increase bone regeneration in mandibular third molar extraction sockets? J Korean Assoc Oral Maxillofac Surg. 2022;48(6):371-381.
16. Dohan DM, Choukroun J, Diss A, Dohan SL, Dohan AJ, Mouhyi J, et al. Platelet rich fibrin (PRF): a second-generation platelet concentrate. Part II: platelet-related biologic features. *Oral Surg Oral Med Oral Pathol Oral Radiol*. 2006;101(3):e45-e50.
17. Al-Hamed FS, Tawfik MA-M, Abdelfadil E. Clinical Effects of Platelet-rich Fibrin (PRF) following Surgical Extraction of Lower Third Molar. The Saudi Journal for Dental Research. 2016;8:19-25.
18. Kumar N, Prasad K, Ramanujam L, K R, Dexith J, Chauhan A. Evaluation of treatment outcome after impacted mandibular third molar surgery with the use of autologous platelet-rich fibrin: a randomised controlled clinical study. J Oral Maxillofac Surg. 2015 Jun;73(6):1042-1049.
19. Asutay F, Yolcu Ü, Geçör O, Acar AH, Öztürk SA, Malkoç S. An evaluation of effects of platelet-rich-fibrin on postoperative morbidities after lower third molar surgery. *Niger J ClinPract*. 2017 Dec;20(12):1531-1536.
20. Gulsen U, Senturk MF. Effect of platelet rich fibrin on edema and pain following third molar surgery: a split mouth control study. *BMC Oral Health*. 2017 Apr 24;17(1):79.
21. Singh A, Kohli M, Gupta N. Platelet rich fibrin: a novel approach for osseous regeneration. *J Maxillofac Oral Surg*. 2012;11(4):430-434.
22. Uyanık LO, Bilginaylar K, Etikan İ. Effects of platelet-rich fibrin and piezosurgery on impacted mandibular third molar surgery outcomes. Head & face medicine. 2015;11(1):1-7.
23. Araoye MO. Research methodology with statistics for health and social sciences. Ilorin: Nathadex Publisher. 2003 (9):115-120.
24. Eng J. Sample Size Estimation: How Many Individuals Should Be Studied? 1. Radiology. 2003;227(2):309-313.
25. Ogundipe OK, Ugboko VI, Owotade FJ. Can autologous platelet-rich plasma gel enhance healing after surgical extraction of mandibular third molars? J Oral Maxillofac Surg. 2011;69(9):2305-2310.

26. Üstün Y, Erdo an Ö, Esen E, Karsli ED. Comparison of the effects of 2 doses of methylprednisolone on pain, swelling, and trismus after third molar surgery. *Oral Surg Oral Med Oral Pathol Oral RadiolEndod*. 2003;96(5):535-539.
27. Cerqueira PRF, do EgitoVasconcelos BC, Bessa-Nogueira RV. Comparative study of the effect of a tube drain in impacted lower third molar surgery. J Oral Maxillofac Surg. 2004;62(1):57-61.
28. Dohan DM, Choukroun J, Diss A, Dohan SL, Dohan AJ, Mouhyi J, et al. Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part I: technological concepts and evolution. *Oral Surg Oral Med Oral Pathol Oral Radiol*. 2006;101(3):e37-e44.
29. Peck MT, Hiss D, Stephen L, Satti A, Majeed A. Platelet-Rich Fibrin (PRF)-The effect of storage time on platelet concentration. *S Afr Dent J*. 2015;70(10):448-451.
30. Cheesbrough M. District laboratory practice in tropical countries. Part 2. 2nd ed. New York: Cambridge university press; 2006.
31. Dohan Ehrenfest DM, Del Corso M, Diss A, Mouhyi J, Charrier J-B. Three-dimensional architecture and cell composition of a Choukroun's platelet-rich fibrin clot and membrane. Journal of periodontology. 2010;81(4):546-555.
32. Naik B, Karunakar P, Jayadev M, Marshal VR. Role of platelet rich fibrin in wound healing: A critical review. *J Conserv Dent*. 2013;16(4):284-293.
33. Dohan DM, Choukroun J, Diss A, Dohan SL, Dohan AJ, Mouhyi J, et al. Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part III: leucocyte activation: a new feature for platelet concentrates? Oral Surg Oral Med Oral Pathol Oral Radiol. 2006;101(3):e51-e55.
34. Masuki H, Okudera T, Watanebe T, Suzuki M, Nishiyama K, Okudera H, et al. Growth factor and pro-inflammatory cytokine contents in platelet-rich plasma (PRP), plasma rich in growth factors (PRGF), advanced platelet-rich fibrin (A-PRF), and concentrated growth factors (CGF).*Int J Implant Dent*. 2016 Dec;2(1):19.
35. Bello SA, Adeyemo WL, Bamgbose BO, Obi EV, Adeyinka AA. Effect of age, impaction types and operative time on inflammatory tissue reactions following lower third molar surgery. Head & face medicine. 2011;7(8):1-8.
36. Daugela P, Grimuta V, Sakavicius D, Jonaitis J, Juodzbalys G. Influence of leukocyte- and platelet-rich fibrin (L-PRF) on the outcomes of impacted mandibular third molar removal surgery: A split-mouth randomised clinical trial. Quintessence Int. 2018;49(5):377-388.
37. Ozgul O, Senses F, Er N, Tekin U, Tuz HH, Alkan A, et al. Efficacy of platelet rich fibrin in the reduction of the pain and swelling after impacted third molar surgery: randomisedmulticenter split-mouth clinical trial. *Head & face medicine*. 2015 Nov 26;11:37.