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ORIGINAL ARTICLE

Autologous Growth Factor-Rich Concentrate (GFC) Injection in Non-union of Fractures: A Quasi-experimental Study

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Abstract

Introduction Non-union fractures represent a significant challenge in orthopedic practice, contributing to considerable morbidity and socioeconomic burden. Traditional treatments, such as autologous bone grafting, are effective but have limitations, including donor-site morbidity and limited tissue availability. Autologous peripheral blood-derived orthobiologics, including growth factor-rich concentrate (GFC), have emerged as a minimally invasive alternative, leveraging the body's natural healing mechanisms by concentrating and applying growth factors directly to the fracture site. This study evaluates the safety and efficacy of GFC injections in the treatment of non-union fractures.

Materials and Methods This quasi-experimental study included 17 patients with non-union fractures of various long bones, treated under fluoroscopic guidance with three doses of 5 mL GFC injections, administered 2 weeks apart at the non-union site. Demographic data, injury characteristics, and comorbid conditions were recorded. Growth factor levels were quantified via enzyme-linked immunosorbent assay (ELISA), and statistical analyses were conducted to explore associations between the amount of growth factors and treatment outcomes. Radiographic assessments and bony callus appearance were evaluated at the baseline and at 1-, 3-, and 6-month follow-up post-last injection.

Results No adverse effects were reported throughout the duration of the study. The majority of patients (82.4%) showed significant improvement, evidenced by enhanced bony callus formation and reduced non-union signs. No significant correlation was found between the specific growth factor levels and the clinical outcomes of non-union of fractures. However, the presence of comorbid conditions significantly influenced treatment efficacy, underscoring the importance of patient selection in clinical practice.

Conclusion Administration of GFC injection is safe and potentially efficacious for the treatment of non-union fractures, offering an alternative to traditional surgical interventions. These results laid the foundation for prospective, adequately powered, randomized and non-randomized clinical studies with longer follow-up to further establish the efficacy of GFC in patients with non-union fractures. Moreover, formulation protocols need to be optimized while considering patient-specific variables, to ensure reproducibility and repeatability of outcomes from these studies.

Keywords Regenerative medicine · Growth factors · Growth factor-rich concentrate · Growth factor concentrate · GFC · Non-union fractures · Autologous peripheral blood-derived orthobiologics

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Introduction

Non-union fractures pose a significant challenge for orthopedic surgeons worldwide. Non-union affects 7–10% of surgically treated long bone fractures in the United States (US), with a marginal cost of $33,000–$45, 000 per year, with reoperation costs adding to $16,000–$34,0000 a year [1]. In the United Kingdom, the average cost of long bone non-union treatment is £29,204 per patient [2]. In 2019, the global prevalence of fractures soared to 445 million cases, a significant 70.1% increase from 1990, despite a decline in age-standardized rates, highlighting the effects of an aging population and the greater incidence of fractures with advancing age; that year also recorded 178 million new fractures and 25.8 million years lived with disability (YLDs), emphasizing the growing challenge of non-union in fractures, particularly in the lower leg and markedly impacting older age groups [3, 4]. Furthermore, non-unions are a considerable source of morbidity and negatively impact patient's overall quality of life due to the need for additional hospitalization, uncomfortable surgical treatments, and a recovery period that can last between 4 and 8 months, assuming no further complications arise during treatment and rehabilitation [5–7].

The US Food and Drug Administration (FDA) defines a long bone non-union as a type of fracture that does not heal within the initial 9-month period following an injury and exhibits no sign of healing for at least 3 consecutive months [8]. However, there is currently no standardized method to define a non-union, thereby enabling instances of fractures to be categorized as non-union beyond the specified parameters [9, 10]. The radiological classification by Weber and Cech, defined in the 70 s, is the most frequently used classification system that divides non-unions into hypertrophic, oligotrophic, and atrophic [11]. Hypertrophic non-unions are considered to be hypervascular and with preserved biological healing potential, oligotrophic non-unions are considered to be and with minimal amounts of callus in fracture zone, and atrophic pseudoarthrosis is considered to be avascular and therefore with a compromised biological status [12–14].

The diagnosis of non-union is contingent upon several factors, including the location of injury, since healing times vary based on the type and location of the bone [15]. Currently, the use of autologous cancellous bone grafts is considered the "gold standard" treatment for non-union fractures [16–19]. This treatment approach involves transplanting bone from another part of the body to the non-union site to serve as a scaffold on which new bone can grow. Despite its efficacy, researchers are exploring alternative therapies and treatment options due to the limited supply and donor-site morbidity associated with autologous grafts.

Regenerative medicine involves use of biologics derived from both autologous and allogenic sources. Autologous sources include orthobiologics derived from peripheral blood called autologous peripheral blood-derived orthobiologics (APBOs) including platelet-rich plasma (PRP) [20], platelet lysate (PL) [21], autologous conditioned serum (ACS) [22], gold-induced cytokines (GOLDIC) [23], plasma rich in growth factors (PRGF) [24], growth factor concentrate (GFC) [25], autologous protein solution (APS) [26], platelet-rich fibrin (PRF) [27], hyperacute serum (HAS) [28], and autologous conditioned plasma (ACP) [29]. Allogenic sources include platelet-rich plasma, bone marrow, adipose tissue, perinatal tissue (amniotic fluid and/or membrane, umbilical cord, Wharton's jelly), etc. [30–33]. There is limited literature investigating the safety and efficacy of autologous peripheral blood-derived formulations for the treatment of non-union fractures. Here, we utilized a growth factor-rich concentrate (GFC), as a potential alternative and evaluated its safety and effectiveness in accelerating the healing of non-union of fractures. GFC is a leukocyte-rich PRP formulation rich in growth factors, depleted in RBCs. We hypothesize that administration of GFC is safe and can augment healing of non-union fractures.

Materials and Methods

After obtaining institutional ethics clearance, a quasi-experimental study was initiated with 17 cases of radiologically diagnosed non-union of various long bone fractures. An informed and written consent was obtained from all participants of the study. The cases were done by a single surgeon in a single tertiary care hospital.

Study Participants

Patients with radiologically diagnosed non-union of long bone fractures, age more than 18 years, and who gave consent for augmentation of non-union of long bone fractures with autologous GFC injections were included in the study. Patients with a platelet count of less than 1,50,000 per mm^3 of blood, age less than 18 years, on medicines known to influence platelet function, local or systemic infections, pseudoarthrosis, pathological fractures, old and/or neglected fractures, fractures with implant failure, and open fractures were excluded from the study.

Technique of GFC Injection

Twenty milliliters of whole blood were obtained by venepuncture, and 5 mL each was transferred into four acid citrate dextrose anticoagulant tubes [ACD-A tubes] (BD Vacutainer ACD-A 6 mL 13 × 100 mm). All the

tests were done in the same setting at our center. The collected sample was centrifuged by two spins in a non-refrigerated centrifuge machine (Remi 8c)—a "soft" spin (at 900 rpm for 10 min) initially, followed by a "hard" spin (at 1800 rpm for 10 min). Following the first spin, three layers (red blood cells (RBCs), buffy coat, and plasma) were formed from whole blood. For the second spin, the sample was separated and transferred into sterile plain tubes (6 mL 13 × 100 mm). Using the buffy coat method (leukocyte-rich platelet-rich plasma): the supernatant plasma was collected along with the whole buffy coat layer using a 5 mL disposable syringe with a 24G needle and subjected to a second hard spin, where upper two-thirds form platelet-poor plasma which were discarded and lower one-thirds form platelet-rich plasma which were subjected for growth factors, namely transforming growth factor-beta (TGF-β), vascular endothelial growth factor (VEGF), platelet-derived growth factor-AB (PDGF-AB), fibroblast growth factor (FGF), and epidermal growth factor (EGF) estimation by enzyme-linked immunosorbent assay (ELISA) [EMP Microplate reader model M201] as per the manufacturer's instructions (Fig. 1).

Study Protocol and Follow-Up

All the participants ($n = 17$) received three doses of 5 mL GFC injections under fluoroscopic guidance, where each injection was administered at the interval of 2 weeks apart at the non-union site. All the participants were followed up for three-cortical bony union (Warden's score) as evidenced by callus formation in the plain radiograph at the baseline and at the end of 1-, 3-, and 6-month follow-up.

Statistical Analysis

Data were meticulously entered and processed using SPSS Version 25.0 (IBM Corp., Armonk, NY, USA) alongside R statistical software (R Foundation for Statistical Computing, Vienna, Austria). To provide a comprehensive summary of demographic details, injury characteristics, and growth factor concentrations among participants, descriptive statistics were employed. For continuous variables, means and standard deviations (SD) were calculated to ascertain distribution patterns. Outcome analysis pivoted on categorizing the effects of GFC injection treatment into 'Good' and 'Bad', according to predetermined benchmarks, with their frequencies and percentages charted to assess treatment

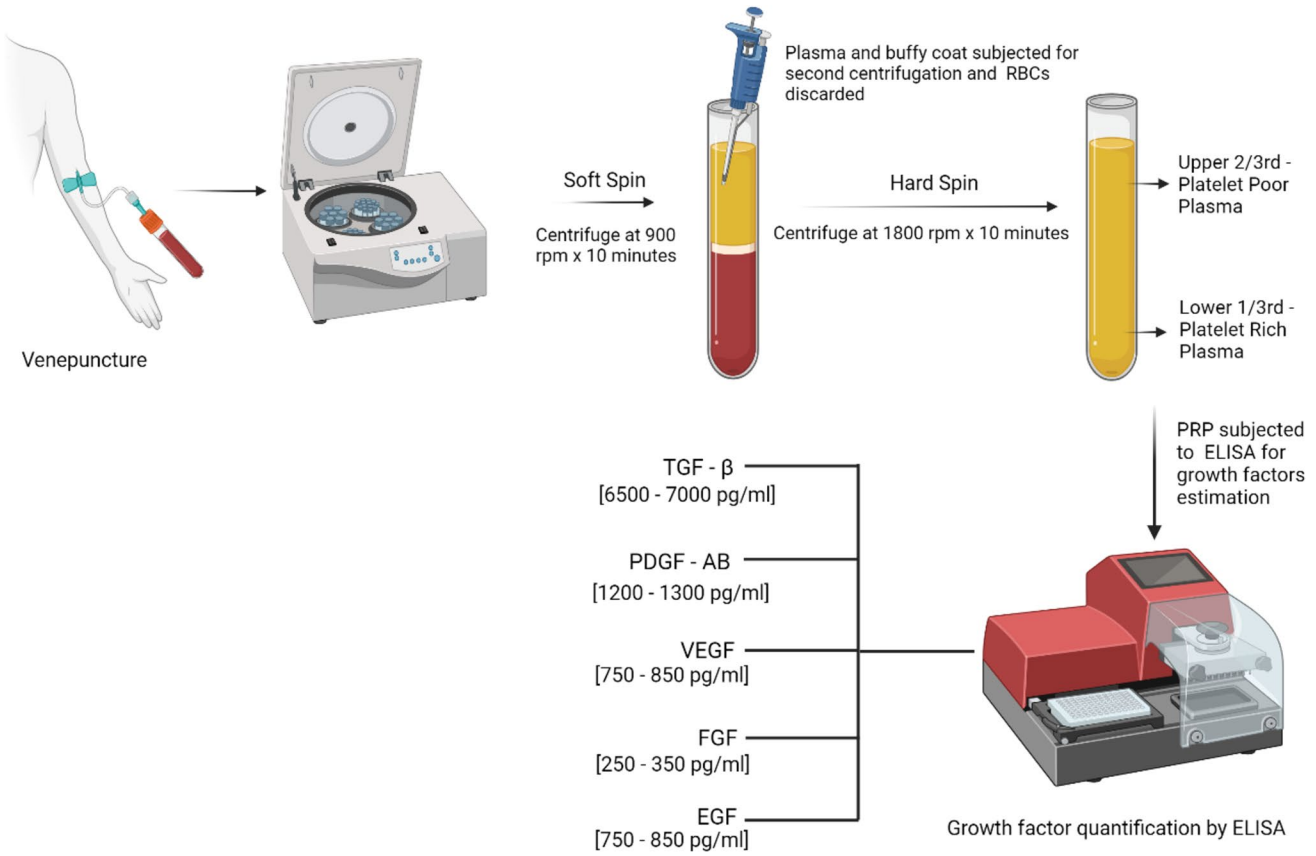


Fig. 1 Schematic representation of preparation of autologous growth factor concentrate (GFC) injection

efficacy. In the scope of growth factor analysis—focusing on TGF-β, VEGF, PDGF-AB, EGF, and FGF, both *T* tests and the Mann–Whitney U test were applied contingent on the normality of data distribution. The study adhered to a significance level of $p < 0.05$ across all statistical tests, with outcomes yielding a *p* value below this threshold deemed statistically significant.

Results

The demographic details of the study participants are tabulated in Table 1. The mean age of the study participants is 37.8 ± 12.4 years [range: 19–65 years] where males (52.9%) outnumbered females (47.1%). Out of 17 participants, 35.3% had road traffic accident, 29.4% had fall from height, 29.4% had sports injuries, and 5.9% had trauma secondary to assault. The injury pattern and characteristics are discussed in Table 2. The mean ± SD months since fracture at the time of presentation was 7.5 ± 1.0 months [range: 6 to 9 months] (Table 2). The primary fixation of plating has outnumbered

Table 1 Demographic details of the study participants ($n = 17$)

Demographic details		Data/no of cases (%)
Age	Mean ± SD (years)	37.8 ± 12.4
	Range	19–65 years
Sex	Males	9 (52.9%)
	Females	8 (47.1%)
Mode of injury	Road traffic accident	6 (35.3%)
	Fall from height	5 (29.4%)
	Sports injuries	5 (29.4%)
	Trauma secondary to assault	1 (5.9%)
Smoking consumption	Yes	7 (41.2%)
	No	10 (58.8%)
Alcohol consumption	Yes	8 (47.1%)
	No	9 (52.9%)
Co-morbidities	None	11 (64.7%)
	Diabetes mellitus	3 (17.6%)
	Hypertension	2 (11.8%)
	Diabetes mellitus + hypertension	1 (5.9%)

Table 2 Injury characteristics of the study participants ($n = 17$)

Injury characteristics		Data/no of cases (%)
Causes of non-union	Inadequate stabilization	6 (35.3%)
	Patient-related factors	6 (35.3%)
	Infection	5 (29.4%)
Months since fracture at the time of presentation	Mean ± SD (months)	7.5 ± 1.0
	Range	6–9 months
Site of non-union of fracture	Femur	5 (29.4%)
	Humerus	3 (17.7%)
	Tibia	3 (17.7%)
	Both bone forearm (radius + ulna)	3 (17.7%)
	Both bone leg (tibia + fibula)	2 (11.7%)
	Ulna	1 (5.8%)
Primary treatment	Plating	7 (41.2%)
	Nailing	5 (29.4%)
	Conservative management	5 (29.4%)
GFC treatment outcomes	Good	14 (82.4%)
	Poor	3 (17.6%)

nailing and conservative management in the development of non-union of fractures (Table 2). The Chi-square test suggested a significant association between comorbid conditions (diabetes mellitus, hypertension, or both) and treatment outcomes with GFC injections for non-union of fractures with a Chi-square statistic of 10.745 and p value of 0.013.

GFC Characterization

The characterization of whole blood and GFC is presented in Table 3. In GFC samples, the [mean ± SD] growth factors estimation was performed (at each injection visit) via ELISA for TGF-β1 [6,981.64 ± 48.74322 pg/mL], VEGF1 [757.2041 ± 14.80228 pg/mL], PDGF-AB1 [1,246.947 ± 15.67529 pg/mL], EGF1 [347.8296 ± 16.00078 pg/mL], and FGF1 [808.9589 ± 13.74634 pg/mL]; TGF-β2 [6,987.183 ± 61.62529 pg/mL], VEGF2 [757.8111 ± 13.12725 pg/mL], PDGF-AB2 [1,261.926 ± 13.32983 pg/mL], EGF2 [333.9293 ± 13.50167 pg/mL], and FGF2 [843.7851 ± 9.291601 pg/mL]; and TGF-β3 [6,555.875 ± 46.43769 pg/mL], VEGF3 [812.6632 ± 12.99214 pg/mL], PDGF-AB3 [1,210.602 ± 17.26581 pg/mL], EGF3 [293.0526 ± 16.48482 pg/mL], and FGF3 [769.984 ± 13.66251 pg/mL]. The characterization of growth factors among study participants in every follow-up is tabulated in the supplementary file.

Treatment Outcomes

Upon examination of treatment outcomes in relation to growth factor quantities [TGF- β, $p = 0.67$; VEGF, $p = 0.42$; PDGF-AB, $p = 0.30$; EGF, p; and FGF = 0.47, $p = 0.25$], no statistically significant correlation ($p > 0.05$) was observed between the two variables. A total of 82.4% of patients had good outcome with 3 doses of GFC injections where each injection was administered 2 weeks apart in the non-union site (Figs. 2, 3, 4, 5, 6 and Table 2), whereas 17.6% of patients had poor outcome where they underwent bone grafting and secondary plating at the end of 6th month follow-up (Table 2). The mean Warden's score improved significantly at 3- and 6-month follow-up compared to the baseline ($p < 0.001$) (Table 4 and Fig. 7). No adverse effects were reported throughout the duration of the study.

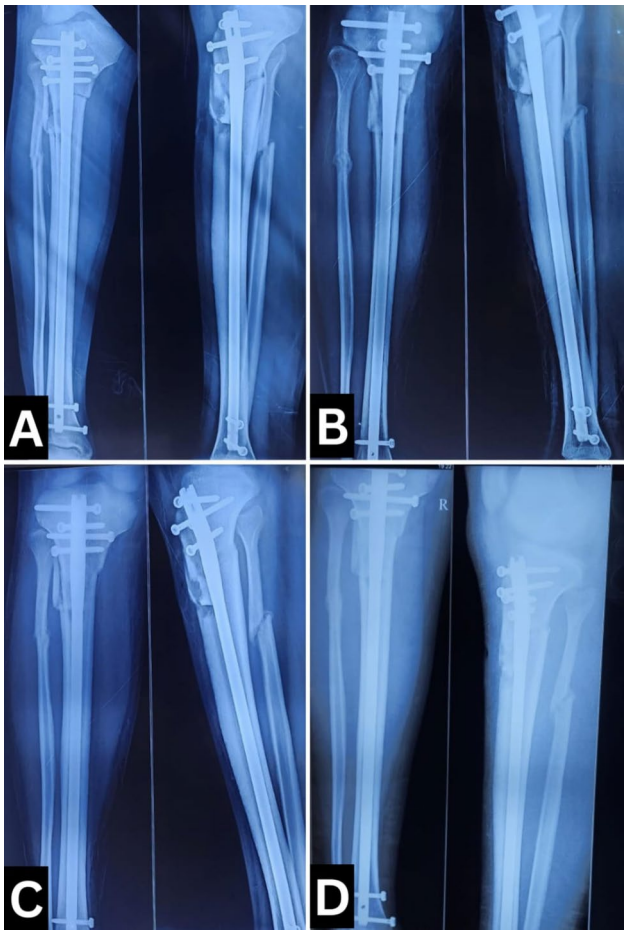


Fig. 2 Plain radiographs of right leg—anteroposterior and lateral view with intramedullary nailing of tibia **A** showing 10-month-old non-union of fracture at proximal 1/3rd of tibia and fibula, **B** at the end of 1st month follow-up, **C** at the end of 3rd month follow-up, and **D** at the end of 6th month follow-up demonstrating satisfactory union of fracture following GFC injections

Table 3 Characterization of whole blood and GFC

Parameters	Whole blood (mean ± SD)	Growth factor-rich concentrate (mean ± SD)
Platelet	$335.33 \pm 65.68 \times 10^3$ cells per μL	$1835.33 \pm 65.68 \times 10^3$ cells per μL
RBCs	$5.54 \pm 1.24 \times 10^6$ cells per μL	$1.03 \pm 0.17 \times 10^6$ cells per μL
Total leucocyte counts	4,051 ± 120.2 cells per μL	20,584 ± 287.5 cells per μL
Neutrophils	2,118 ± 40.1 cells per μL	11,065 ± 89.4 cells per μL
Lymphocytes	974 ± 89.7 cells per μL	5,931 ± 172.5 cells per μL
External activator	No	No

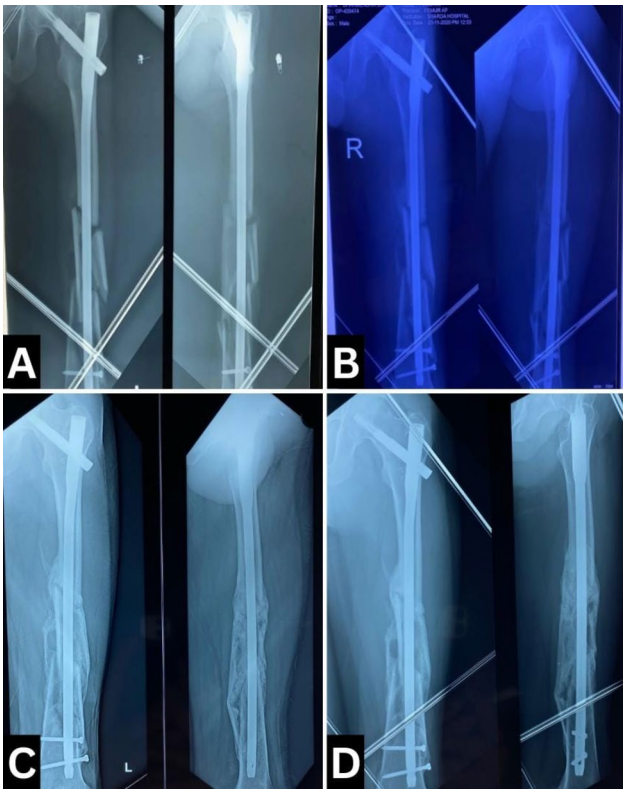


Fig. 3 Plain radiographs of right thigh—anteroposterior and lateral view with intramedullary nailing of femur (PFN-A2) **A** showing 9-month-old non-union of fracture at middle 1/3rd of femur with a butterfly fragment, **B** at the end of 1st month follow-up, **C** at the end of 3rd month follow-up, and **D** at the end of 6th month follow-up demonstrating satisfactory union of fracture following GFC injection

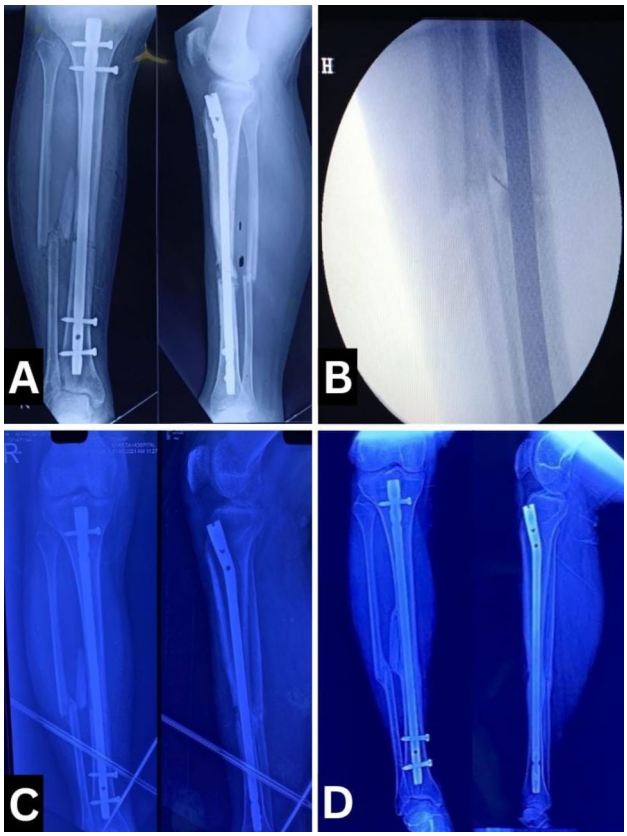


Fig. 4 Plain radiographs of right leg—anteroposterior and lateral view with intramedullary nailing of tibia **A** showing 12-month-old non-union of fracture at middle 1/3rd of tibia and fibula, **B** at the end of 1st month follow-up, **C** at the end of 3rd month follow-up, and **D** at the end of 6th month follow-up demonstrating satisfactory union of fracture following GFC injections

Discussion

Non-union fractures pose a significant challenge for orthopedic surgeons and leads to significant burden on healthcare systems across the globe. Traditional management strategies often require invasive procedures that carry substantial risks and limitations. The use of autologous peripheral blood-derived orthobiologics, including GFC, for the treatment of non-union fractures represents a promising intersection between regenerative medicine and orthopedic surgery.

GFC, an RBC-depleted, LR PRP formulation harnesses the natural healing pathways by delivering a high concentration of growth factors directly to the injury site. These growth factors, including TGF-β, VEGF, PDGF-AB, FGF, IGF-1, and EGF, are pivotal in mediating various processes essential for bone repair, such as cell proliferation, angiogenesis, chemotaxis, and differentiation [34–37]. The therapeutic benefits of GFC, attributed to these mechanisms, encompass not only the acceleration of bone healing but also the promotion of angiogenesis, adipogenesis, and collagen formation [38].

The current study demonstrated that administration of GFC is safe, and the majority of patients (82.4%) experienced significant improvements following GFC injections. This finding is aligned with the therapeutic premise of GFC, offering a less invasive and potentially more accessible treatment option for non-union fractures. However, while the outcomes are promising, they necessitate contextualization within the broader landscape of fracture healing research.

Previous studies have extensively explored the use of biologics, including platelet-rich plasma (PRP) and bone marrow aspirate (BMA), in bone healing. A network meta-analysis highlighted that combinations of BMA with PRP or autologous cancellous bone showed potential benefits, though the results were tempered by concerns over study heterogeneity and potential biases [39]. Similarly, systematic reviews involving use of PRP have documented a range of outcomes, from encouraging preclinical results to more mixed findings in clinical settings [40]. These varied outcomes underscore the complexities of bone healing and the multifaceted roles of different growth factors.

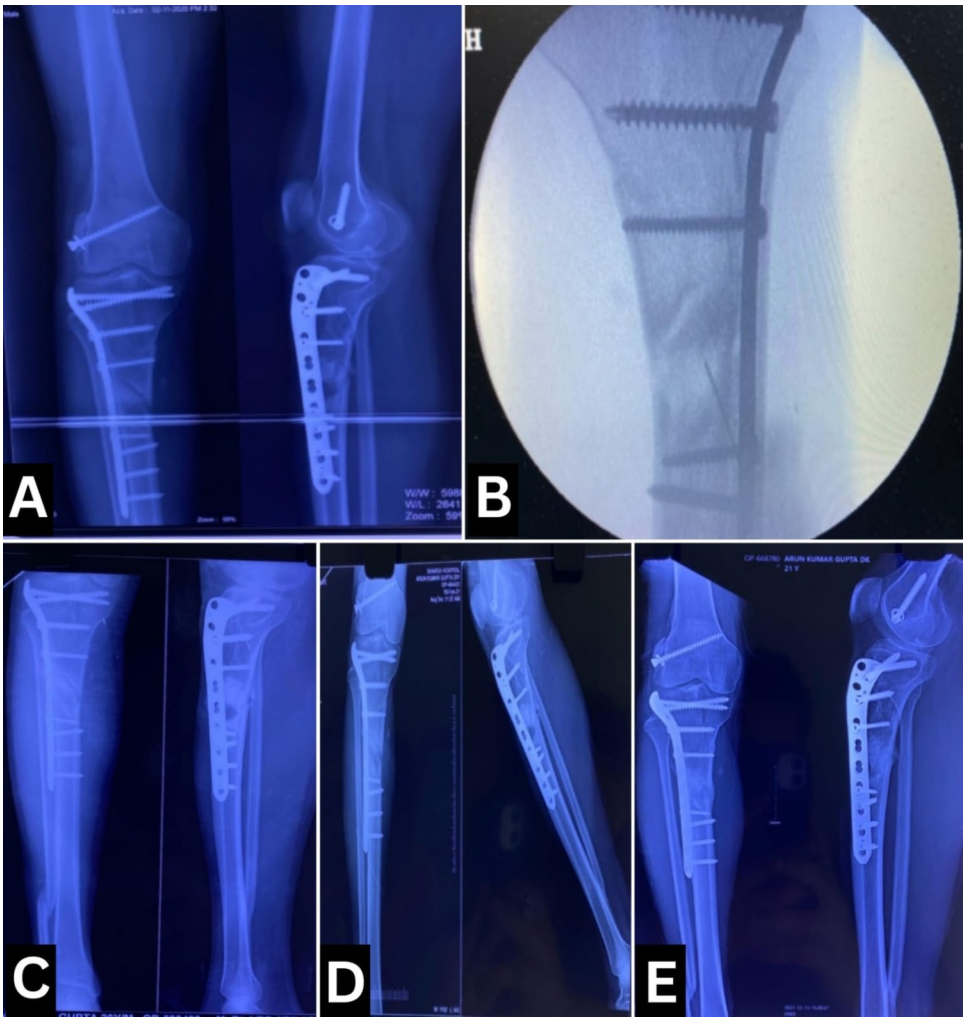


Fig. 5 Plain radiographs of right knee—anteroposterior and lateral view with interfragmentary screw at distal end of the femur and plate at proximal 1/3rd of the tibia in situ **A** showing 7-month-old non-union of fracture at proximal 1/3rd of tibia, **B** and **C** at the end of 1st month follow-up, **D** at the end of 3rd month follow-up, and **E** at the end of 6th month follow-up demonstrating satisfactory union of fracture following GFC injections

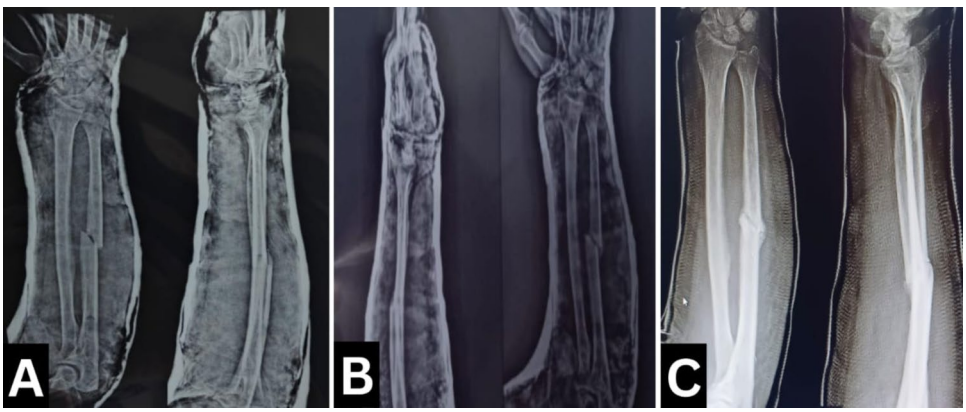


Fig. 6 Plain radiographs of right forearm—anteroposterior and lateral view **A** showing 3-month-old non-union of fracture at middle 1/3rd of ulna with above elbow slab in-situ, **B** at the end of 1st month follow-up, **C** at the end of 6th month follow-up demonstrating satisfactory union of fracture following GFC injections

Despite the promising results, the therapeutic application of GFC extends beyond non-union fractures to other orthopedic conditions such as osteoarthritis, tendinopathies, and various soft tissue injuries [25, 41–45]. The broadening scope of GFC's applicability underscores its potential as a versatile therapeutic tool in musculoskeletal medicine. Nevertheless, this expansion also necessitates a cautious approach, considering the heterogeneity in preparation techniques, activation methods, and dosing protocols observed across studies [25, 46–48].

The preparation and delivery of GFC remain areas of considerable variability and debate as the volume of GFC delivered varies significantly between studies. The total dose is usually given to a patient as a single dose but may also

Table 4 Mean Warden's score among the study participants ($n=14$)

Time scale	Mean Warden's score	ANOVA statistic (F)	p value
Baseline	0.92	2.45	1.321
At the end of 1st month follow-up	1.52	1.54	0.534
At the end of 3rd month follow-up	3.52	8.87	<0.001*
At the end of 6th month follow-up	3.65	9.56	<0.001*

*Represents statistically significant ($p<0.05$) compared to the baseline

be divided into multiple injections over consecutive weeks, which cause differing levels of efficacy between methods of application as injecting a single dose in comparison to the same dose being divided over several weeks will impact the rate of bone regeneration [2]. Differences in centrifugation rates, activation methods, and injection volumes across studies introduce challenges in standardizing treatment protocols [49, 50]. For instance, the use of different centrifugation forces and activators, such as thrombin, calcium chloride, or calcium gluconate, can significantly impact the concentration of platelets and leukocytes, and thereby the efficacy of the resulting GFC product. The study's adoption of the dual-spin method and the natural activation of GFC through hard spin represent steps toward standardization, yet further research is needed to establish optimal protocols that maximize therapeutic outcomes.

The dependence of our study on a quasi-experimental framework and a limited participant cohort underlines the imperative for additional investigations. The lack of a control group and restricted applicability of our results underscore the exploratory essence of our work. Moreover, the absence of significant correlations between certain growth factor levels and therapeutic outcomes intimates the intricate involvement of biological processes in the GFC-mediated bone regeneration. This intricacy might be attributed to the small scale of the study or the brevity of the follow-up period—factors that should indeed be acknowledged as constraints of our research. The consensus of APBOs for gap non-union of fractures have not been determined. Various variables such as weight bearing status, immobilization status, exercise modalities, and the type of fixation are not analyzed. This complexity underscores the need for more exhaustive studies, notably randomized controlled trials (RCTs), to thoroughly understand the effectiveness and mechanisms underlying GFC use in treating non-union fractures. Given that only a fraction of the known growth factors were analyzed, future research should expand the assay spectrum to include a broader range of growth factors.

The potential for GFC to enhance bone healing when used alone or in combination with other treatments, such as mesenchymal stem cells (MSCs), internal fixation, or autologous bone graft, has been demonstrated in clinical practice [51]. However, the evidence base is predominantly constituted of case series and preliminary studies, which are limited by their observational nature and lack of control groups [50]. This evidentiary gap underscores the urgency for conducting prospective RCTs aimed at providing high-level evidence on the effectiveness and mechanistic basis of GFC in the management of non-union fractures.

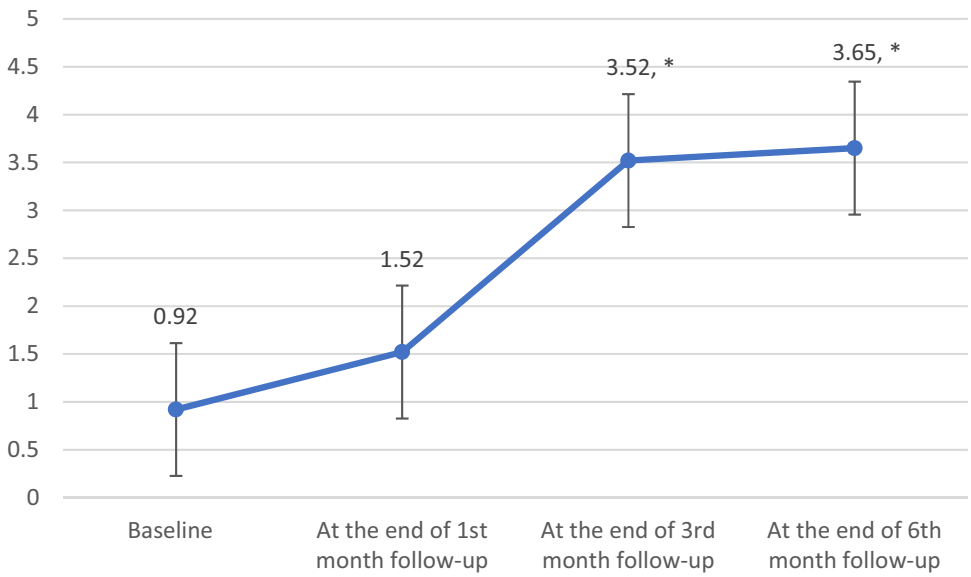


Fig. 7 Graph representing the mean Warden's score in various time-points. *represents statistically significant ($p<0.05$) compared to the baseline

Conclusion

The study presents compelling evidence supporting the safety and potential efficacy of autologous growth factor-rich concentrate (GFC) injections in treating non-union fractures. These findings highlight the promise of GFC as a minimally invasive alternative to traditional surgical interventions, underscoring the importance of leveraging the body's innate healing mechanisms. While the majority of patients demonstrated significant improvements, the study also emphasizes the need for careful patient selection, given the influence of comorbid conditions on treatment outcomes. The absence of a significant correlation between specific growth factor levels and clinical outcomes suggests the complexity of the biological processes involved in fracture healing. This research lays the groundwork for future studies, calling for prospective, randomized controlled trials to establish GFC's efficacy more definitively and optimize treatment protocols. The study underscores the evolving landscape of regenerative medicine in orthopedics, offering a new avenue for enhancing patient care in the management of challenging non-union fractures.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s43465-024-01278-1.

Authors Contributions KJ and AG conceptualized the study. KJ performed the procedures. KJ, MJ, and NJ wrote the initial manuscript draft. AG reviewed and edited the manuscript draft and supervised the study. All authors have read and agreed to the published version of the manuscript.

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Data Availability All relevant data are contained within the article.

Declarations

conflict of interest The authors declare that they have no conflict of interest.

Ethical standard statement This article does not contain any studies with human or animal subjects performed by the any of the authors.

Informed consent For this type of study informed consent is not required.

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