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ORIGINAL ARTICLE

Juxta-epiphyseal fractures at the base of the proximal phalanx of the finger in pediatric patients

Burak Kuşcu, MD[1], Kaan Gürbüz, MD[2]

[1]Department of Orthopedics and Traumatology, Special Bandırma Royal Hospital, Balıkesir, Türkiye
[2]Department of Orthopedics and Traumatology, Kayseri Kızılay Hospital, Kayseri, Türkiye

ABSTRACT

Objectives: This study aims to evaluate the mid-term clinical and functional outcomes of juxta-epiphyseal fractures of the proximal phalanx in pediatric patients.

Patients and methods: Between July 2013 and December 2023, a total of 23 pediatric patients treated for juxta-epiphyseal fractures of the proximal phalanx were retrospectively analyzed. Functional outcomes were assessed using four subtests of the Jebsen-Taylor Hand Function Test. To compare the Jebsen-Taylor Hand Function Test, a control group of healthy children (n = 23) from the outpatient clinic was formed. Patients were treated either with closed reduction and splinting or with closed reduction followed by Kirschner wire (K-wire) fixation according to fracture displacement and stability. Radiographs were evaluated for residual deformity and malunion using Campbell's lines.

Results: Of a total of 23 patients, 13 were male and 10 were female with a mean age of 7.24 ± 2.21 (range, 6 to 13) years. The fifth digit was the most commonly affected (n = 14), followed by the fourth (n = 5), middle (n = 2), and thumb (n = 2). In 15 patients, the injury occurred on the non-dominant hand. No malunions were detected. One patient demonstrated a pseudo-claw deformity. Hand function tests revealed statistically significant delays in patients with dominant-hand injuries compared to healthy controls ($p < 0.05$). Seven patients reported a change in hand dominance after injury.

Conclusion: Our study results suggest that appropriate reduction techniques, including closed or K-wire-assisted fixation, offer favorable outcomes with minimal complications. However, functional impairment can be more notable, when the dominant hand is involved. Taken together, these findings emphasize the need for early intervention, close follow-up, and consideration of hand dominance during recovery planning.

Keywords: Campbell's line, closed reduction, fracture, Juxta-epiphyseal, Kirschner wire fixation, pediatric, phalanx.

The hand is the most commonly injured anatomical region in the pediatric age range.[1] While the phalanx is the most frequently injured area of the hand, the proximal part is the most commonly injured localization.[2] Proximal phalanx injuries are most common in the little finger, following the thumb.[3] Although toddlers and preschool-aged children are more likely to sustain a phalangeal fracture at home due to crush and laceration, the incidence of phalangeal fractures is highest in children aged 10 to 14 years, which coincides with the time when most of them begin playing contact sports without their guardians' supervision.[3,4] In prepubertal children, bone growth in the hand occurs via the physeal plate located at the proximal end of the phalanges.[4] The phalangeal epiphyses remain open until about 16.5 and 14.5 years of age in males and females, respectively, corresponding to the peak incidence of proximal phalanx fractures.[1-7]

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Correspondence: Burak Kuşcu, MD. Özel Bandırma Royal Hastanesi, Ortopedi ve Travmatoloji Kliniği, 10200 Bandırma, Balıkesir, Türkiye.

E-mail: dr.burakkuscu@hotmail.com

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Most proximal phalangeal fractures are Salter-Harris type II fractures. Juxta-epiphyseal fracture is that the fracture line is entirely through the metaphysis, 1 to 2 mm distal to the growth plate.[8] Juxta-epiphyseal fractures of the proximal

phalanx base are metaphyseal fractures located approximately 1 to 2 mm distal to an intact physis. On unilateral plain radiographs they typically appear as a small metaphyseal wedge or step-off adjacent to the growth plate, and may easily be misinterpreted as Salter-Harris type II injuries if these subtle metaphyseal changes are overlooked. Immobilization is used to treat minimally or non-displaced juxta-epiphyseal proximal phalanx fractures, with favorable clinical outcomes expected. On the other hand, displaced fractures or iatrogenic fractures caused by multiple fracture reduction attempts can cause physeal arrest, resulting in a deformity that easily compromises children's hand functions.[9] Closed reduction of displaced juxta-epiphyseal proximal phalangeal fracture should be the first line of therapy. The periosteum that surrounds pediatric hand bones is thick and highly vascularized, and it can become entrapped at the fracture site, preventing adequate reduction and resulting in multiple fracture reduction attempts performed by surgeons.[10] Open reduction may be required occasionally to avoid multiple fracture reduction attempts and the possibility of deformity occurrence.[2]

Despite the high prevalence of proximal phalanx fractures in prepubertal children and the potential for disastrous clinical outcomes if treated carelessly and/or inadequately, the literature on the clinical mid-term outcomes of any treatment option is scarce. In the present study, we aimed to evaluate the mid-term clinical and functional outcomes of juxta-epiphyseal fractures of the proximal phalanx base in pediatric patients.

PATIENTS AND METHODS

This multi-center, retrospective study was conducted at Special Bandırma Royal Hospital and Kayseri Kızılay Hospital, Department of Orthopedics and Traumatology between July 2013 and December 2023. Both centers are pediatric level III trauma centers. All pediatric patients with phalanx fractures treated in these centers were screened. The study included patients who suffered from juxta-epiphyseal phalanx fractures. Patients with a combined and/or multiple phalangeal fracture and juxta-epiphyseal phalanx fracture and those who previously underwent surgical or conservative treatment were excluded from the study. In addition, patients whose legal guardians refused to participate in the trial, who had missing data, who were lost to follow-up, or who had a postoperative follow-up time of less than one year were excluded. Of 93 patients initially screened, 23 who met the eligibility criteria and completed the one-year follow-up required for functional evaluation were recruited (Figure 1). Twenty-three of the 93 patients aged 7 years or older who came in for their last checkup were assessed using four parameters of the Jebsen-Taylor Hand Function Test.[11] These parameters were as follows: (1) the period it took the first child to turn five cards on the table; (2) participants were instructed to pick up two coins, two paper clips, and two bottle caps from the table and place them into a nearby box on the table; (3) the time it took to stack four standard wooden cubes (5 × 5 × 5 cm), and (4) the time it took to pick up five objects on a plate with a spoon and put them in a box to test eating ability. To compare the Jebsen-Taylor Hand Function Test, a control group of healthy children (n = 23) from the outpatient clinic was formed with informed consent. Four subtests from the original seven-item Jebsen-Taylor Hand Function Test were selected based on their age-appropriateness, standardizability, and feasibility in a pediatric clinical setting. Tasks requiring advanced cognitive or strength-based performance, such as handwriting and lifting large objects, were excluded. A written informed consent was obtained from the parents and/or legal guardians of the patients. The study protocol was approved by the Necmettin Erbakan University Non-Drug and Non-Medical Device Research Ethics Committee (Date: 17.03.2023, No.: 2023/4249). The study was conducted in accordance with the principles of the Declaration of Helsinki.

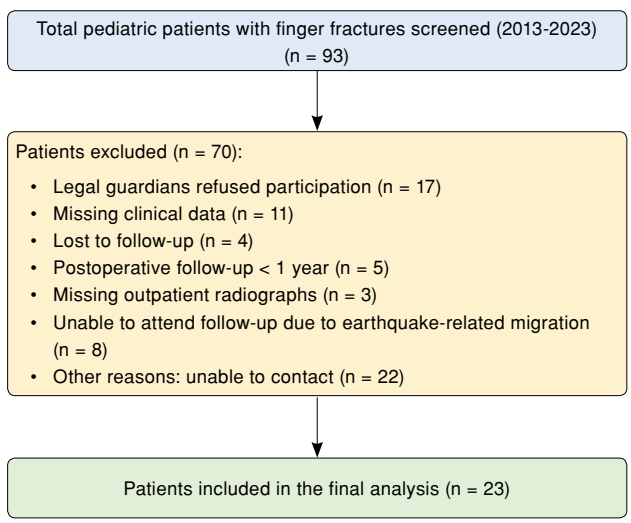


FIGURE 1. Flowchart of patient inclusion and exclusion process.

Demographic and clinical data, including details of their first and last clinical examinations and radiography at diagnosis, as well as at the end of the first-year radiographs were recorded. Cause of trauma, comorbidities at the time of first admission to the hospital, type of reduction used, and incidence of postoperative clinical infection were also noted.

Closed reduction and splinting were preferred for fractures with < 15° angulation, < 2 mm displacement, intact periosteal sleeve, and stability after manual reduction. The Kirschner wire (K-wire) fixation was indicated when displacement exceeded 2 mm, when rotational or angular instability persisted after reduction, or when a periosteal sleeve interposition prevented anatomic alignment.[8,10]

For unstable or inadequately reduced fractures, a single 0.9-1.0 mm K-wire was introduced retrograde through the proximal interphalangeal (PIP) joint, passed across the fracture site, and advanced into the flexed metacarpophalangeal (MCP) joint under fluoroscopic guidance. The MCP joint was flexed 70° to 80° during wire advancement to avoid iatrogenic injury to the metacarpal head physis. After confirming reduction and stability, the wire was externalized proximally and removed at Week 3.

A circumferential cast was not applied after K-wire fixation. Instead, a protective splint and bulky dressing were used, as the wire provided sufficient internal stabilization. This approach allowed soft-tissue rest without increasing the risk of displacement, and no loss of reduction or physeal injury occurred in any patient.

All percutaneous K-wire fixations were performed within 24 to 48 h after injury, once soft-tissue swelling allowed safe wire insertion.

Any decreased range of motion or deformity was assessed at the final follow-up. Malunion was evaluated using Campbell's lines[12] on standard radiographs. If the Campbell's line did not pass through the metacarpal head center, malunion was recorded (Figure 2).

Statistical analysis

Statistical analysis was performed using IBM SPSS version 22.0 software (IBM Corp., Armonk, NY, USA). The normality of distribution of the variables was checked using the Kolmogorov-Smirnov test. Continuous variables were presented in mean ± standard deviation (SD) or median (min-max), while categorical variables presented in number and frequency. T tests were performed on all constant variables with Gaussian distributions. The Mann-Whitney U test was used for continuous variables with non-Gaussian distributions. The Pearson chi-square tests were used to determine the significance of ranking variables. A p value of < 0.05 was considered statistically significant.

RESULTS

Of a total of 23 patients, 13 were male and 10 were female with a mean age of 7.24 ± 2.21 (range, 6 to 13) years. The fifth digit was the most commonly affected (n = 14), followed by the fourth (n = 5), middle (n = 2), and thumb (n = 2). In 15 patients, the injury occurred on the non-dominant hand. Dominance data were available for 16 patients; of these, seven reported a shift in hand preference after injury. All patients suffered from a juxta-epiphyseal phalanx fracture, which is the most common cause of subsequent falls on the same level. None of the patients had comorbidities at the time of their first hospital admission. The injured digits were all laterally deviated (Table I).

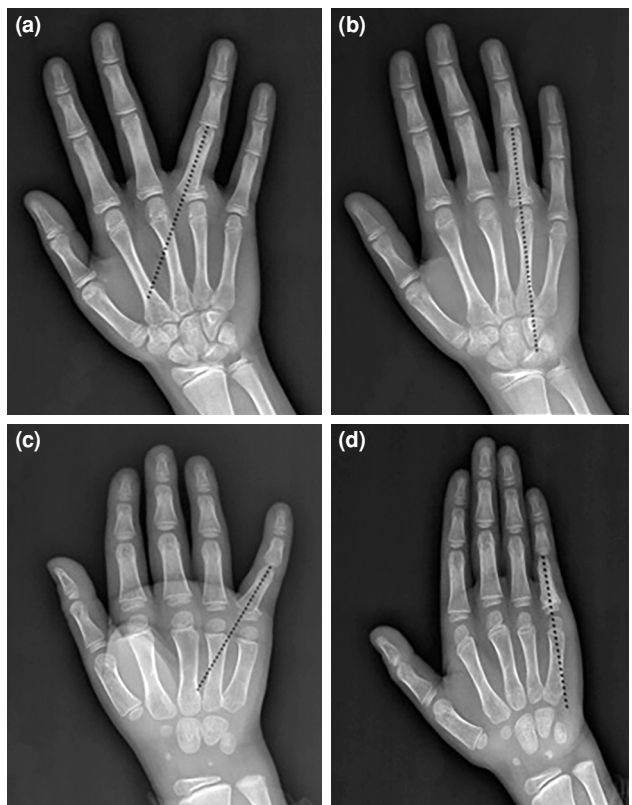


FIGURE 2. (a, b) Dominant right-hand fourth digit: preoperative X-ray with Campell's line and postoperative X-ray with Campell's line. **(c, d)** Non-dominant right-hand fifth digit: preoperative X-ray with Campell's line and postoperative X-ray with Campell's line.

TABLE I
Demographic characteristics and injury distribution of the patients

Patient	Age (year)	Sex	Side	Finger involved
1	6	M	R	5th
2	7	F	L	5th
3	8	M	R	4th
4	6	F	R	5th
5	9	M	L	5th
6	7	M	R	5th
7	8	F	L	4th
8	10	M	R	3rd
9	6	F	L	2nd
10	11	M	R	5th
11	13	F	R	Thumb
12	7	M	L	5th
13	9	F	R	4th
14	6	M	L	5th
15	8	F	R	5th
16	10	M	R	Thumb
17	7	M	L	4th
18	12	F	R	5th
19	6	M	L	5th
20	9	F	R	4th
21	6	M	L	5th
22	8	F	R	5th
23	7	M	L	5th

Among the 23 patients, 11 were managed with closed reduction and splinting and 12 were treated with K-wire fixation. All fractures were successfully reduced using closed methods, and no case required open reduction. Patients treated with closed reduction and splinting (n = 11) showed full radiographic union by final follow-up, with restoration of alignment and near-complete range of motion in all cases. Stable fractures with minimal displacement responded well to closed reduction alone, whereas unstable fractures achieved equally successful outcomes following K-wire stabilization. Following reduction, the finger was taped at the interphalangeal joint to the adjacent uninjured finger, and an ulnar gutter splint was worn for three weeks. Patients treated with percutaneous K-wire fixation (n = 12) similarly achieved complete union, full motion, and no hardware-related complications. No significant differences were found between the two groups in terms of final alignment, range of motion, or functional recovery ($p > 0.05$).

Ten of 23 fractures and two closed reductions were fixed with a single K-wire introduced through the PIP joint and passed across the fracture and the flexed MCP joint under the C-arm scope. The wire was pulled proximately from the metacarpal head to free the PIP joint. This method of K-wire introduction was used, as oblique K-wires inserted into the base of the fractured proximal phalanx tended to displace the fracture. The K-wire was



FIGURE 3. The periosteal sleeve was seen on the fifth digit of the proximal juxta-epiphyseal fracture. The enlarged figure on the right side shows the sleeve with an arrow.

TABLE II
The complications encountered and their management

Digits	n	Management	Complication and management	n
First digit	2			
Second digit	1			
Third digit	0	Closed reduction via pencil technique fixed with taping		
Fourth digit	3			
Fifth digit	6		Residual deformity, closed reduction, and K-wire fixation	2
First digit	0			
Second digit	0			
Third digit	1	Closed reduction via pencil technique fixed with K-wire		
Fourth digit	2		Mal-alignment	1
Fifth digit	8		Mal-position, open reduction, and K-wire fixation	1

K-wire, Kirschner wire; Residual deformity, persistent mild angular deviation < 10°; Malalignment, radiographic deviation > 10° after reduction; Malposition, inadequate initial alignment corrected during procedure.

removed after three weeks, and no splints were used.

Radiographs taken at the time of first admission to the hospital revealed that the fracture line ran entirely through the injured digit metaphysis, about 2 mm below the growth plate. The proximal metaphyseal fragment had a triangular shape on the side of the deviation. A subtle white line was occasionally observed extending from the triangular fragment to the distal fragment, indicating the presence of an intact periosteal sleeve. This periosteal sleeve was present in all fifth-digit fractures (Figure 3) and one in each fourth- and middle-digit fracture.

Table II summarizes the complications encountered and their management. Residual deformity was defined as a mild persistent angular deviation (< 10°) without functional impairment. Malalignment referred to coronal or sagittal plane deviation > 10° observed radiographically after reduction but not requiring re-intervention. Malposition indicated inadequate anatomic alignment during the initial reduction attempt that was subsequently corrected before fixation. One patient had mild residual deformity that improved with therapy, one had early malalignment that remodeled during follow-up, and one case demonstrated malposition that was corrected intraoperatively. No cases resulted in long-term functional limitation.

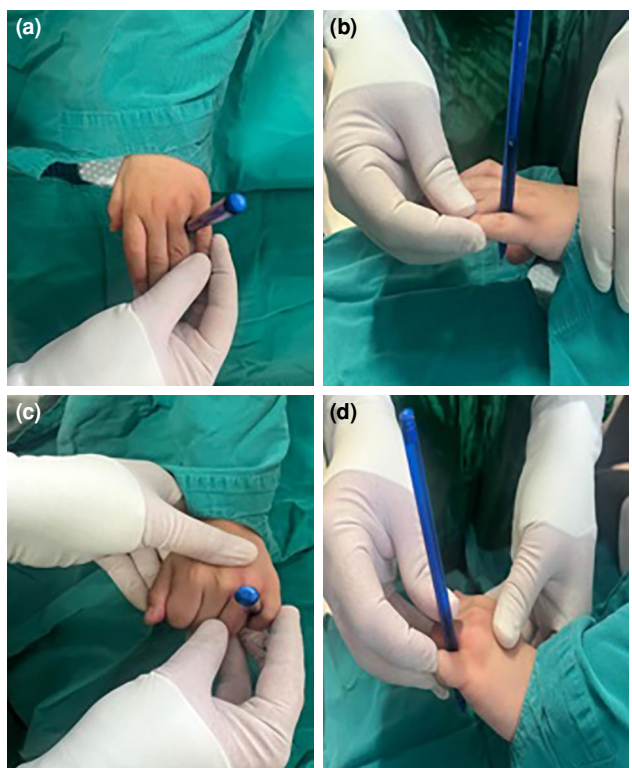


FIGURE 4. Sample case for the closed reduced via pencil technique. **(a)** Anterior, posterior clinical view of the placement of the pencil. **(b)** Lateral clinical view of the placement of pencil. **(c)** Anterior, posterior clinical view of the flexion of the MCP joint with the maneuver of proximal phalanx of the fifth-digit adduction. **(d)** Lateral oblique clinical view of the flexion of the MCP joint with the maneuver of the proximal phalanx of the fifth-digit adduction.
MCP, metacarpophalangeal.

No malunion was detected in any of the 23 patients based on Campbell's line[12] assessment. One patient showed a pseudo-claw deformity, characterized by hyperextension at the MCP joint and flexion at the PIP joint during extension. At the final follow-up, all 11 patients treated with closed reduction and splinting and all 12 patients treated with closed reduction and percutaneous K-wire fixation demonstrated a full range of motion without permanent deformity, except for one patient who developed a transient pseudo-claw deformity (Figure 4). One patient exhibited a pseudo-claw deformity, characterized by hyperextension at the MCP joint and flexion at the PIP joint during attempted finger extension. During finger extension, hyperextension ranged from 17° to 142° at the MCP joint, whereas flexion ranged from 20° to 140° at the PIP joint. Unaffected hand fingers in this case lacked floppy joints and camptodactyly. Furthermore, 12 patients treated with closed reduction under the C-arm scope and fixed with K-wire demonstrated a full range of motion with no deformity.

Pseudo-claw deformity in the affected patient was attributed to a transient imbalance between the intrinsic musculature and the extrinsic extensor mechanism, likely resulting from angular deviation and periosteal sleeve injury at the time of trauma. This led to MCP hyperextension and PIP flexion during attempted finger extension. The deformity gradually improved with supervised hand therapy and did not lead to any persistent functional limitation.

TABLE III
Comparison of fractured and healthy individuals' hand functions test with four Jebsen-Taylor test parameters

	Fractured digit patient	Healthy individuals	
	Mean ± SD	Mean ± SD	*p*
Card turning			
Right	11.36 ± 4.42	5.10 ± 0.22	0.034
Left	18.14 ± 3.18	5.25 ± 0.20	0.030
Picking up six…			
Right	9 ± 2.24	7.21 ± 0.39	0.034
Left	15 ± 5.04	7.47 ± 0.17	0.027
Stacking wooden blocks…			
Right	11 ± 4.71	3.44 ± 0.15	0.047
Left	17 ± 3.24	3.50 ± 0.47	0.042
Simulating feeding			
Right	13 ± 2.44	9.52 ± 0.18	0.015
Left	15 ± 4.70	12.40 ± 0.54	0.024

SD, standard deviation.

TABLE IV
Comparison of four Jebsen-Taylor test parameters according to dominant and non-dominant sides of participants in each group

	Card turning	Picking	Stacking	Simulating feeding	
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	*p*
Fracture digit patients (n = 22)					
Dominant hand	11.24 ± 3.24*	7 ± 0.19	10 ± 2.41**	11.24 ± 3.44***	< 0.005
Non-dominant hand	15.17 ± 1.04	13 ± 0.47	14 ± 1.24	14 ± 2.41	> 0.05
Healthy individuals (n = 22)					
Dominant hand	5.04 ± 0.17	6.47 ± 1.21	3.23 ± 0.04	9.24 ± 1.14	< 0.05
Non-dominant hand	5.69 ± 1.27	7.13 ± 0.47	4.17 ± 1.21	11.14 ± 3.21	< 0.05

SD, standard deviation; * Statistically significant difference in card turning test between fractured dominant hand and healthy dominant hand; ** Statistically significant difference in stacking test between fractured dominant hand and healthy dominant hand; *** Statistically significant difference in simulating feeding test between fractured dominant hand and healthy dominant hand.

Campbell's lines confirmed the absence of malunion in all cases during and after the first-year follow-up. Similar to the complication of malunion, no infections associated with the K-wire were discovered.

On the most recent one-year follow-up radiographs, no physeal line irregularity, narrowing, or bridge formation was observed in any patient, and the physes were symmetric compared with the contralateral side.

All 23 patients were of school age at their most recent outpatient follow-up examination, conducted at least one year postoperatively. Table III compares the results of the Jebsen-Taylor Hand Function Test (four parameters) between the 23 patients and a healthy control group. Table IV presents a comparison of hand function test results between the two treatment groups (closed reduction and splinting versus closed reduction with K-wire fixation), with assessments performed on both the dominant and non-dominant hands.

DISCUSSION

In the present study, we evaluated the mid-term clinical and functional outcomes of juxta-epiphyseal fractures of the proximal phalanx in pediatric patients. Our study results showed that most fractures occurred in the fifth digit, with ulnar deviation being a common presentation. The treatment strategy varied, with closed reduction using the pencil technique and K-wire fixation being the primary methods. The results were favorable in terms of range of motion and deformity, with few complications encountered during the follow-up period. These findings suggest that juxta-epiphyseal fractures of the proximal phalanx in pediatric patients can be effectively managed with both closed reduction techniques and K-wire fixation, with favorable functional and radiological outcomes.

Pseudo-claw deformity, although uncommon, is a recognized consequence of juxta-epiphyseal fractures. It usually results from a temporary imbalance between intrinsic muscles and the extrinsic extensor mechanism following periosteal sleeve injury or initial angular deformity. This imbalance produces MCP hyperextension with compensatory PIP flexion during attempted finger extension. In the present study, the deformity occurred in one patient and gradually resolved with targeted hand therapy. No long-term functional limitation was observed. This finding aligns with previous reports suggesting that pseudo-claw deformity is typically transient and rarely progresses to a structural deformity when appropriate rehabilitation is provided.

In our series, closed reduction and splinting were preferred for fractures with $< 15°$ of angulation, < 2 mm displacement, an intact periosteal sleeve, and stability after manipulation. These criteria are consistent with established recommendations for managing minimally displaced juxta-epiphyseal fractures. Conversely, K-wire fixation was indicated in the presence of > 2 mm displacement, recurrent angulation or rotation after attempted closed reduction, or suspected periosteal sleeve interposition. These findings mirror the observations of Al-Qattan[8] and Abzug et al,[10] who emphasized early stabilization in unstable fracture patterns to prevent malunion or secondary deformity.

Patients treated with closed reduction demonstrated excellent functional outcomes, with full range of motion in all but one case and no radiographic malunion at final follow-up. The K-wire fixation also produced uniformly favorable outcomes, with all patients achieving complete union, full motion, and no infection or hardware-related complications. When compared using Jebsen–Taylor functional benchmarks, both treatment groups showed reliable recovery, although minor transient delays were more common when the dominant hand was involved. These results underline that both treatment modalities are effective when selected according to fracture stability and displacement criteria.

Compared to the existing literature, our study findings provide valuable insights into managing pediatric phalanx fractures. Liu et al.[3] highlighted the importance of juxta-epiphyseal fractures in children and adolescents, emphasizing the need for appropriate reduction techniques to avoid deformities. In the present study, we used the pencil technique and K-wire fixation, consistent with the recommendations for stable and displaced fractures, ensuring adequate reduction and stabilization.[13] Dover and Kiely[14] conducted a systematic review to compare the incidence of infection in operative and non-operative fracture management, emphasizing the importance of infection prevention strategies in pediatric fractures. The present study's lack of infection-related complications supports the efficacy of the chosen treatment modalities in reducing postoperative risks. In Al-Qattan's study,[8] one of 34 patients who had a severely displaced fourth finger fracture underwent open reduction

and K-wire fixation, as the fracture could not be closed. We did not observe such a phenomenon in our series; nevertheless, surgeons should remain aware of this possibility and proceed with open reduction when an acceptable closed reduction cannot be achieved.

In the current study, the Jebsen–Taylor test was used to assess hand function, which provides valuable information about functional outcomes after treatment. Similar assessments have been conducted in previous studies, such as the work by Sığırtmaç and Öksüz,[11] which emphasized the importance of functional evaluations in hand injuries requiring closed reduction. The comparison of hand function test results with healthy controls and between treatment groups improves our understanding of functional recovery after phalanx fractures.

The absence of malunions and deformities in the present study is consistent with Guerrero et al.[15] and Baumgartner et al.,[16] who reported low complication rates in plate fixation of phalanx fractures. The meticulous reduction techniques in the present study most likely contributed to the positive results observed during follow-up assessments.

Identifying changes in the dominant hand following trauma in some patients is consistent with the findings of Gurbuz et al.,[17] who investigated the impact of hand injuries on hand dominance and function. Understanding such changes is critical for tailoring rehabilitation strategies to maximize functional recovery in pediatric hand-injured patients. The observed shift in hand dominance following injury in some patients underscores the functional impact of juxta-epiphyseal fractures, particularly when the dominant hand is involved. This may have influenced the results of the hand function tests and highlights the importance of early and accurate functional rehabilitation.

The use of Campbell's lines to confirm malunion and the lack of such complications in this study are consistent with Zhi et al.'s[18] recommendations for assessing fracture alignment and union in pediatric hand fractures. Adherence to established guidelines for radiographic evaluation helps with accurate diagnosis and treatment monitoring.

Of note, the study's focus on school-age patients is consistent with previous reports documenting a high incidence of hand fractures in this population.[19] Emphasizing functional outcomes and hand function in this age group is particularly important for minimizing disruption to daily activities and academic performance. While our study provides valuable insights into the clinical outcomes of the intervention, several limitations should be acknowledged that may impact the validity and generalizability of our findings. First, the relatively small sample size of 23 participants raises concerns regarding statistical power and the ability to detect meaningful differences between groups. As noted by Luoto et al.,[20] a small sample size can limit the detection of significant effects and may not adequately represent the broader population, thereby affecting the external validity of the results. Furthermore, the male-to-female ratio in our sample was not balanced, which could introduce bias and limit the applicability of our findings across different sexes. This imbalance may restrict the generalizability of our results to the target population, as highlighted by Susukida et al.,[21] who emphasized that selective recruitment criteria in clinical trials could lead to findings that did not adequately represent the characteristics of the broader patient population. Additionally, the strict enrollment criteria employed in our study may have further narrowed the participant pool, potentially excluding individuals who could benefit from the intervention. This limitation echoes the concerns raised by Maas et al.[22] regarding the impact of recruitment strategies on the generalizability of clinical trial findings. Future research should aim to include a larger and more diverse sample to enhance the external validity of the results and to ensure that the findings are applicable to a wider range of patients.

Despite the limited sample size, this fracture pattern is rare, and the number of cases with complete one-year follow-up is inherently restricted. Similar sample sizes have been reported in previous studies investigating rare pediatric phalangeal fractures.[8,13,18] In addition, although no radiographic evidence of growth disturbance was detected at one-year follow-up, longer-term monitoring until skeletal maturity would provide additional information on late physeal complications and represents an important direction for future research. Given the rarity of juxta-epiphyseal proximal phalanx fractures and the functional implications associated with inadequate treatment, we believe that our findings contribute additional clinical and functional insight to the limited pediatric hand trauma literature.

In conclusion, our study results suggest that appropriate reduction techniques, including closed

or K-wire-assisted fixation, offer favorable outcomes with minimal complications. However, functional impairment can be more notable, when the dominant hand is involved. Taken together, these findings emphasize the need for early intervention, close follow-up, and consideration of hand dominance during recovery planning. Further multi-center, large-scale, prospective studies are needed to confirm these findings and to better define optimal management strategies for juxta-epiphyseal fractures of the proximal phalanx in pediatric patients.

Data Sharing Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions: B.K., K.G.: Conception, design of the work, the acquisition, analysis, prepared the figures, wrote the main manuscript, drafted the work. All authors read and approved the final manuscript.

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