

Relationship between serum β -catenin mRNA expression and femoral fracture healing after head trauma: an experimental rat study

 Ender Gümüőođlu,¹  Volkan Öztuna,²  Zeynel Mert Asfurođlu,¹  Hatice Oruç Demirbađ,³
 Savaş Aktaş,³  Mehmet Tuđhan Kızıltuđ,⁴  Mehmet Emin Erdal⁵

¹Department of Orthopedics and Traumatology, Mersin University, Faculty of Medicine, Mersin-Türkiye

²Department of Orthopedics and Traumatology, VM Medical Park Mersin Hospital, Mersin-Türkiye

³Department of Histology and Embryology, Mersin University, Faculty of Medicine, Mersin-Türkiye

⁴Department of Pediatrics, Dr. von Hauner Children Hospital, Munich-Germany; Ludwig-Maximilians-Universität München, Munich-Germany

⁵Department of Medical Biology and Genetics, Mersin University, Faculty of Medicine, Mersin-Türkiye

ABSTRACT

BACKGROUND: Fracture healing may be influenced by concomitant traumatic brain injury (TBI). Both clinical and experimental studies have reported accelerated union and enhanced callus formation in the presence of TBI. The Wnt/ β -catenin signaling pathway is thought to play a role in this process; however, the relationship between serum β -catenin mRNA relative expression and fracture healing in the context of TBI remains unclear.

METHODS: Thirty-six female Wistar albino rats were randomly assigned to four groups: control, TBI only, femoral fracture only, and combined TBI + femoral fracture. Radiographic healing was evaluated using the Radiographic Union Scale for Tibial fractures (RUST) at weeks 3 and 6. Serum β -catenin mRNA relative expression was quantified by real-time polymerase chain reaction at baseline (week 0) and during follow-up (weeks 3 and 6). Histological analysis was performed at week 6.

RESULTS: Radiographic evaluation demonstrated progressive healing in all fracture groups, with significantly higher RUST scores in the TBI + fracture group compared to the fracture-only group at both time points ($p < 0.05$). Serum β -catenin mRNA relative expression decreased significantly over time in both fracture groups, whereas no significant temporal changes were observed in the control or isolated TBI groups. Because this decline occurred in both fracture groups, it did not indicate a TBI-specific molecular effect. Histological analysis showed a tendency toward more mature osseous callus formation in the TBI + fracture group; however, these differences were not statistically significant.

CONCLUSION: Concomitant TBI was associated with enhanced radiographic fracture healing and showed a non-significant trend toward more advanced osseous callus formation. The observed decline in serum β -catenin mRNA relative expression in the fracture groups suggests phase-dependent regulation of Wnt/ β -catenin-related activity during repair. However, serum β -catenin mRNA represents an indirect systemic marker and does not establish a mechanistic, TBI-specific pathway. These findings highlight the complex systemic influence of TBI on skeletal repair and support further mechanistic studies—particularly those incorporating fracture-site (local) analyses—to clarify the biological pathways underlying the observed radiographic association.

Keywords: Callus formation; experimental rat model; fracture healing; traumatic brain injury; Wnt/ β -catenin signaling.

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Address for correspondence: Zeynel Mert Asfurođlu

Department of Orthopedics and Traumatology, Mersin University, Faculty of Medicine, Mersin, Türkiye

E-mail: z.mert.asfuroglu@gmail.com

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INTRODUCTION

Fracture healing is a complex biological process involving a highly coordinated cascade of cellular and molecular events that restore bone integrity and mechanical strength.^[1,2] This intricate repair mechanism is central to orthopedic traumatology given the high global incidence of bone fractures and the clinical challenges that arise in cases of delayed union or nonunion.^[3] A striking clinical observation that has attracted increasing attention is the phenomenon of accelerated fracture healing in patients who sustain traumatic brain injury (TBI).^[4] Evidence from both clinical and preclinical studies supports this association, demonstrating enhanced callus formation, earlier bridging, and a shorter time to union in the presence of concomitant head trauma.^[5,6] This accelerated healing response, often described as “TBI-induced osteogenesis,” has been reported across different fracture types and injury severities, suggesting a systemic influence of head injury on skeletal repair.^[7] For example, studies of femoral shaft fractures indicate that more than one-third of patients present with associated head or neck injuries, underscoring the clinical relevance of this phenomenon.^[8] Proposed mechanisms include neuroendocrine alterations, the release of specific humoral factors, and the systemic dissemination of extracellular vesicles following TBI.^[5-7,9]

Accumulating evidence implicates the Wnt/ β -catenin signaling pathway as a key pathway that may contribute to this enhanced osteogenic response.^[10-14] β -catenin, the central effector of this pathway, plays a crucial role in multiple phases of fracture healing, including the early differentiation of mesenchymal stem cells into osteoblasts and chondrocytes, subsequent bone formation, and the later remodeling of the callus.^[10,12] Although the role of β -catenin in bone metabolism and fracture repair is well established, studies directly examining the relationship between serum β -catenin mRNA relative expression and fracture healing outcomes in the context of TBI remain scarce. This gap represents a critical area of investigation because clarifying the dynamics of serum β -catenin mRNA relative expression could provide valuable insight into the systemic regulation of bone repair after TBI. Therefore, the present experimental rat study aimed to investigate this relationship in greater depth by examining femoral fracture healing in the presence of concomitant head trauma. To achieve this, we conducted a comprehensive evaluation integrating radiological, histological, and serum-based molecular analyses.

We hypothesized that concomitant traumatic brain injury would accelerate femoral fracture healing radiographically, promote more mature osseous callus formation histologically, and alter systemic β -catenin dynamics, as reflected by temporal changes in serum β -catenin mRNA relative expression.

The primary research question was whether fracture healing differs between isolated femoral fracture and combined femoral fracture with TBI. Secondary questions addressed

whether serum β -catenin mRNA relative expression changes over time within each group and whether these changes correspond with radiological and histological findings.

MATERIALS AND METHODS

This prospective, randomized, controlled experimental animal study was conducted at the Mersin University Experimental Animal Research Laboratory and was approved by the Mersin University Rectorate Animal Experiments Local Ethics Committee (Date: 25.03.2019, Decision no: 15). A preliminary pilot study was performed to identify potential challenges, including the feasibility of the surgical procedures, the adequacy of the instruments, potential issues related to animal housing, and expected animal survival throughout the study period.

Animals

The study included 36 female Wistar albino rats, 24 weeks old and weighing 200–250 g. The animals were acclimatized for two weeks before the experiment. During this period, they were maintained on standard rodent chow and tap water at a constant room temperature of 22°C under a 12-hour light/dark cycle. Each rat was housed individually in a cage until sacrifice.

Experimental Design

The rats were randomly assigned to four groups. Group 1 served as the control group (no trauma), Group 2 was subjected to head trauma only, Group 3 underwent surgically induced femoral fracture, and Group 4 was subjected to both head trauma and surgically induced femoral fracture. In Groups 3 and 4, standardized anteroposterior and lateral radiographs of the fractured femurs were obtained during follow-up, and femoral specimens were harvested for histological evaluation at the end of the study. Blood samples for serum β -catenin mRNA analysis were collected at baseline (week 0), week 3, and week 6. All animals were sacrificed at the end of week 6.

Of the initial 36 rats, four were excluded from the final analysis. In Group 3 (femoral fracture), one rat developed pseudoarthrosis. In Group 4 (head trauma + femoral fracture), one rat died during the induction of head trauma and two developed pseudoarthrosis. Consequently, the final analysis included 32 rats (Group 1: n=6; Group 2: n=10; Group 3: n=9; Group 4: n=7).

Anesthesia and Surgical Procedure

All animals were anesthetized via intraperitoneal injection of xylazine 2% (10 mg/kg; Xylazin Bio®, Bioveta, Czech Republic) and ketamine 10% (80 mg/kg; Ketazol®, Richter Pharma, Austria). Adequate anesthesia was confirmed by the absence of whisker and pedal withdrawal reflexes.

Femoral fractures were created using a modified version of the method described by Bonnarens and Einhorn.^[15] A medial

parapatellar approach was performed, and the intramedullary canal was reamed using a 20-gauge needle. A 0.45-mm Kirschner wire was inserted for stabilization, after which a fracture was created at the mid-diaphysis using bone scissors. The fracture configuration was standardized as a simple mid-diaphyseal fracture produced under direct visualization. Using bone scissors, fractures were consistently created as transverse or short oblique patterns. Fracture alignment and configuration were confirmed intraoperatively by macroscopic inspection; comminution was not intended in this model.

Head trauma was induced using a modified experimental mild TBI model.^[16] To prevent depressed fractures, a steel disc (2 cm in diameter and 2 mm thick) was fixed between the coronal and lambdoid sutures. A 300-g weight was then dropped from a height of 1 m onto the disc to produce closed head trauma.

For postoperative analgesia, paracetamol (Calpol™ Suspension 120 mg/5 mL; GlaxoSmithKline, Türkiye) was added to the drinking water during the first postoperative day. Wounds were treated with topical oxytetracycline HCl (Neo-Caf® Aerosol Spray; Intervet, Türkiye). All rats were monitored daily for feeding behavior, mobility, and signs of pain.

Radiographic Evaluation

At weeks 3 and 6, anteroposterior and lateral radiographs of the fractured femurs (Groups 3 and 4) were obtained. Radiographs were acquired using a standardized protocol with consistent limb positioning (true anteroposterior and lateral views) and fixed imaging parameters throughout the study. Fracture healing was assessed independently by two blinded orthopedic surgeons using the RUST (Radiographic Union Score for Tibial fractures) scoring system.^[17] This system evaluates healing at four cortices—medial and lateral cortices on one projection, and anterior and posterior cortices on the other. Each cortex is scored from 1 to 3 points: 1 indicates a visible fracture line without callus, 2 indicates a visible fracture line with callus formation, and 3 indicates bridging callus with no visible fracture line. The total score ranges from 4 to 12. The mean score of the two observers was calculated and recorded as the final score. To minimize bias, radiographs were anonymized and coded, and images were evaluated independently in randomized order without access to group allocation or time-point information. Scoring was repeated in separate sessions to reduce recall bias. Pseudoarthrosis was defined radiographically as persistence of a visible fracture line without bridging callus and absence of interval progression between weeks 3 and 6 on both anteroposterior and lateral views, despite intramedullary stabilization.

Serum β -Catenin mRNA Relative Expression

Blood samples were collected from the jugular vein at predefined time points and processed to obtain serum. For RNA isolation, approximately 200–300 μ L of serum was transferred into 1.5-mL microcentrifuge tubes, mixed with 500 μ L Ribozol, vortexed, and incubated for 15 minutes. Subsequent-

ly, 200 μ L chloroform:isoamyl alcohol (24:1), pre-cooled to 4°C, was added, and the samples were centrifuged at 14,000 rpm for 10 minutes at 4°C. The aqueous phase was transferred to new tubes, and RNA was precipitated with 500 μ L isopropanol for 10 minutes at room temperature, followed by centrifugation at 14,000 rpm for 10 minutes at 4°C. The RNA pellet was washed with 1 mL of cold 80% ethanol and centrifuged again at 14,000 rpm for 10 minutes at 4°C. After air-drying for 10–15 minutes, the pellets were resuspended in 50 μ L RNase/DNase-free water, briefly vortexed, incubated for 10 minutes at room temperature, and stored at –20°C until complementary DNA (cDNA) synthesis. Complementary DNA was synthesized using RevertAid RT reagents with oligo d(T)18 primers (37°C for 60 minutes, followed by 95°C for 5 minutes and a hold at 4°C). For each sample, 5 μ L RNA was added to 50 μ L of the prepared RT reaction mixture, resulting in a final volume of 100 μ L. A pooled RNA sample from control rats served as the calibrator. Quantitative real-time polymerase chain reaction (PCR) was performed on an ABI Prism 7500 platform using the comparative Ct ($\Delta\Delta$ Ct) method to estimate relative serum-derived Ctnnb1 (β -catenin) mRNA expression normalized to Actb. Primer and hydrolysis probe sets were designed from rat reference sequences (Actb: NM_031144.3; Ctnnb1: NM_053357.2) and synthesized commercially; the sequences are provided in the manuscript. Each reaction contained 12.5 μ L 2 \times master mix, primers at a final concentration of 900 nM, probes at 200 nM, 2.5 μ L cDNA (~30 ng), and 5 μ L distilled water. Cycling conditions consisted of 50°C for two minutes and 95°C for 12 minutes, followed by 50 cycles of 95°C for 15 seconds and 60°C for one minute. Data acquisition and analysis were performed using ABI 7500 system software (SDS v2.0.6; Applied Biosystems). Actb was selected as the reference gene based on its common use in rat quantitative polymerase chain reaction (qPCR) assays; however, its stability in serum was not formally validated in the present study. Therefore, the reverse transcription quantitative polymerase chain reaction (RT-qPCR) results should be interpreted as reflecting relative transcript dynamics rather than absolute quantification of circulating β -catenin protein.

Histological Analysis

At the end of week 6, fractured femurs were harvested following sacrifice. The specimens were fixed in 10% formaldehyde solution and decalcified in ethylenediaminetetraacetic acid. After decalcification, the tissues were embedded in paraffin, sectioned, and stained with hematoxylin–eosin and Masson's trichrome for histological evaluation. Histological assessment and histomorphometric measurements were performed by an assessor blinded to group allocation. For each specimen, representative sections through the fracture region were analyzed using a standardized region-of-interest approach. The proportions of osseous, cartilaginous, and fibrous tissue were quantified using predefined measurement criteria applied consistently across all samples. Final values were recorded for each specimen prior to statistical analy-

sis. Fracture healing was further graded using the histological scoring system described by Huo et al.^[18]

Statistical Analysis

All data were analyzed using SPSS software version 25.0 (IBM Corp., Armonk, NY, USA). No a priori sample size or power analysis was performed. Descriptive statistics were expressed as mean \pm standard deviation for normally distributed data and as median (interquartile range) for non-normally distributed data. The Shapiro–Wilk test was used to assess data normality. For comparisons among more than two groups, one-way analysis of variance (ANOVA) was applied to parametric data, followed by Tukey's post hoc test. For non-parametric data, the Kruskal–Wallis test was used, with Dunn's test for pairwise comparisons. Within-group comparisons over time were performed using repeated-measures ANOVA or the Friedman test, as appropriate. Interobserver reliability for radiographic scoring was assessed using intraclass correlation coefficients (ICC). A p-value <0.05 was considered statistically significant.

RESULTS

Radiological Findings

Interobserver reliability analysis demonstrated excellent agreement for RUST scoring in both groups at weeks 3 and 6 (ICC=0.904–0.937). According to the RUST system, both groups exhibited progressive fracture healing from week 3 to week 6. At week 3, the median union score in Group 3 (isolated femoral fracture) was 7.5 (range, 4.0–8.5), whereas Group 4 (concomitant head trauma + femoral fracture) showed a significantly higher median score of 8.0 (range, 8.0–11.5) ($p=0.008$). By week 6, Group 3 reached a median score of 9.0 (range, 7.0–10.5), while Group 4 demonstrated more advanced healing with a median score of 11.0 (range, 9.0–11.5) ($p=0.006$). Within-group analyses also showed significant temporal progression. Group 3 improved from week 3 to week 6 ($p=0.012$), and Group 4 demonstrated a significant increase over the same period ($p=0.047$). Overall, these findings indicate that radiographic fracture healing was more advanced in the presence of concomitant head trauma.

Table 1. Relative serum β -catenin mRNA expression measured by reverse transcription quantitative polymerase chain reaction (RT-qPCR) and temporal changes within groups

Group	Week 0 (Mean \pm SD)	Week 3 (Mean \pm SD)	Week 6 (Mean \pm SD)	p (within group)
Group 1 (n=6)	1.34 \pm 0.13	1.34 \pm 0.13	1.18 \pm 0.08	0.144
Group 2 (n=10)	1.33 \pm 0.12	1.28 \pm 0.20	1.19 \pm 0.20	0.089
Group 3 (n=9)	1.37 \pm 0.18	1.17 \pm 0.19	1.09 \pm 0.09	0.005
Group 4 (n=7)	1.36 \pm 0.13	1.16 \pm 0.13	1.11 \pm 0.11	<0.001

SD: Standard deviation. Overall comparison (group \times time interaction): $p=0.261$.

Table 2. Histomorphometric analysis of callus composition and Huo scores in Group 3 (femoral fracture) and Group 4 (head trauma + femoral fracture)

Parameter	Group 3 (n=9)	Group 4 (n=7)	p-value
	Median (Min–Max)	Median (Min–Max)	
Histology – Bone callus			
Callus area (mm ²)	0.48 (0.29–0.83)	0.72 (0.26–0.83)	0.347
Callus area (%)	37.03 (22.59–64.6)	55.9 (20.11–62.19)	0.347
Histology – Cartilaginous callus			
Callus area (mm ²)	0.041 (0–0.35)	0.024 (0–0.36)	0.670
Callus area (%)	3.18 (0–27.17)	1.86 (0–27.8)	0.670
Histology – Fibrous callus			
Callus area (mm ²)	0.015 (0–0.19)	0 (0–0.59)	0.639
Callus area (%)	1.16 (0–14.36)	0 (0–45.89)	0.639
Histology – Huo score	8 (6–9)	9 (6–9)	0.999

Serum β -Catenin mRNA Relative Expression (RT-qPCR Findings)

RT-qPCR results of serum β -catenin mRNA relative expression are summarized in Table 1. No significant temporal changes were observed in Group 1 (control) or Group 2 (head trauma) ($p>0.05$). In contrast, both Group 3 (femoral fracture) and Group 4 (head trauma + femoral fracture) exhibited a significant decline in serum β -catenin mRNA relative expression from baseline to week 6 ($p=0.005$ and $p<0.001$, respectively). The group \times time interaction was not statistically significant ($p=0.261$). Overall, these findings indicate that serum β -catenin mRNA relative expression decreased over time, particularly in groups with fractures.

Histological Findings

Histomorphometric evaluation revealed no statistically significant differences between the isolated femoral fracture group and the concomitant head trauma + femoral fracture group (Table 2). Although the head trauma group showed a tendency toward a greater proportion of osseous callus and slightly higher Huo scores compared with the isolated fracture group, these differences did not reach statistical significance ($p>0.05$). Similarly, the proportions of cartilage and fibrous tissue within the callus were comparable between the groups. Overall, the histological analyses suggested a trend toward enhanced bone formation in the presence of concomitant head trauma, although the differences were not statistically significant (Figs. 1, 2).

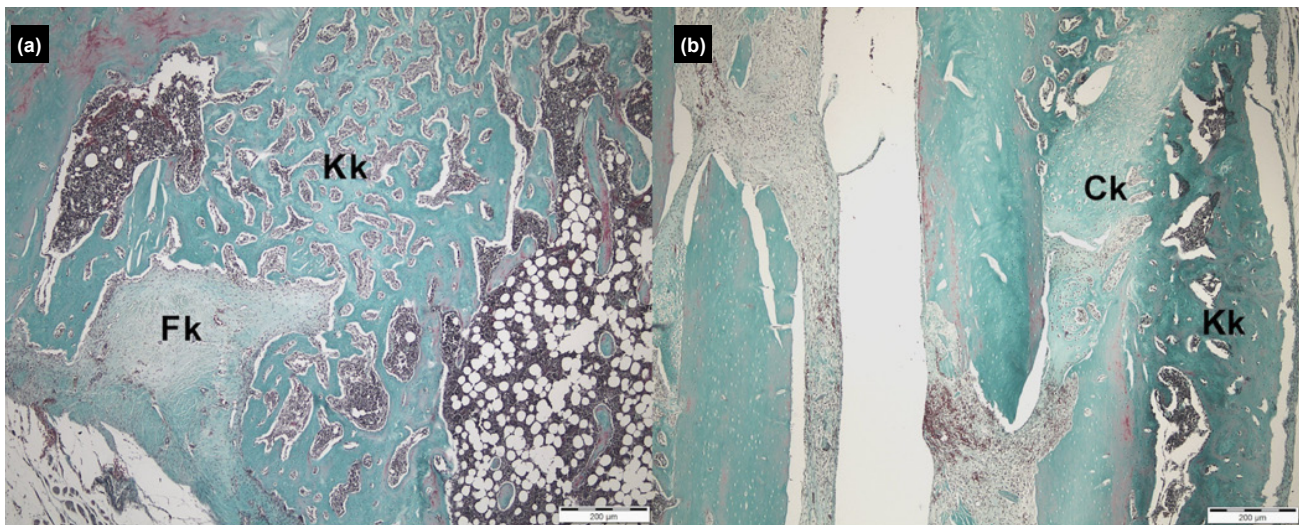


Figure 1. Histological sections stained with Masson's trichrome (magnification $\times 40$). (a) Representative section from Group 3 demonstrating abundant bone callus (Kk) formation with a smaller proportion of fibrous callus (Fk). (b) Representative section from Group 4 showing prominent bone callus (Kk) together with a lesser amount of cartilaginous callus (Ck).

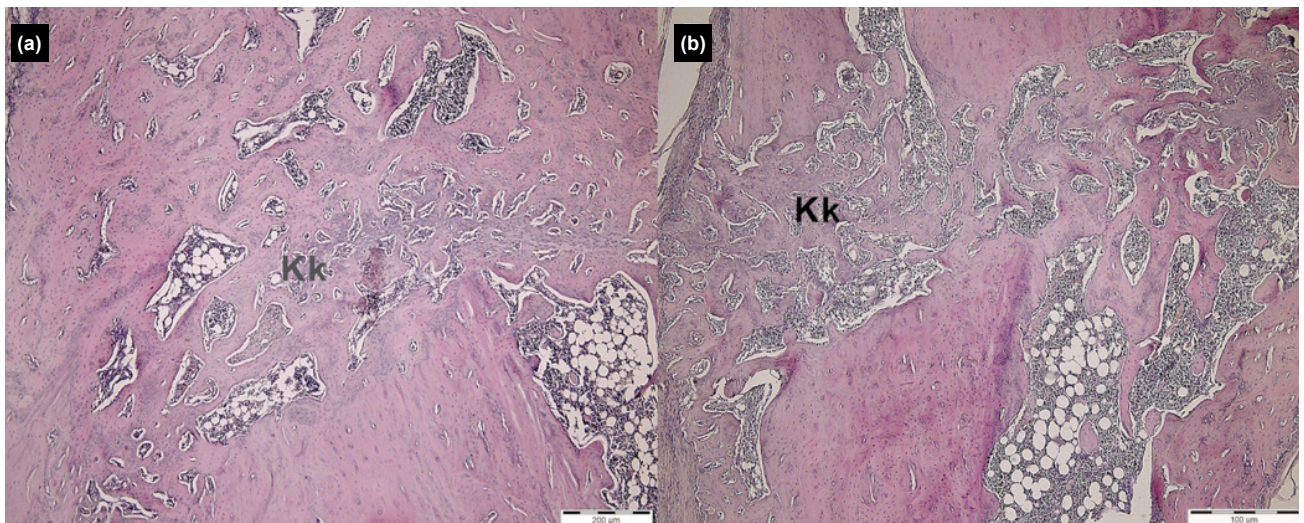


Figure 2. Histological sections stained with hematoxylin and eosin (magnification $\times 40$). (a) Representative section from Group 3 showing bone callus (Kk). (b) Representative section from Group 4 also demonstrating bone callus (Kk).

DISCUSSION

In this experimental study, we examined the impact of concomitant head trauma on femoral fracture healing using radiological, histological, and serum-based molecular assessments. Our primary endpoint was radiographic healing, and RUST scores were consistently higher in the fracture + TBI group than in the fracture-only group at weeks 3 and 6, supporting an association between concomitant TBI and accelerated radiographic healing in this model. Secondary endpoints provided more limited support. Histological evaluation demonstrated only a non-significant trend toward more mature osseous callus formation at a single terminal time point, while serum β -catenin mRNA relative expression declined over time in the fracture groups without a significant group \times time interaction. Accordingly, these serum transcript dynamics should be interpreted as an indirect systemic readout and do not establish a TBI-specific mechanistic pathway. Taken together, these findings support a clear radiographic association while underscoring the need for future studies incorporating longitudinal tissue-level and fracture-site molecular analyses to clarify the underlying biological mechanisms.

Radiological evaluation showed that the concomitant TBI group demonstrated significantly higher RUST scores at both weeks 3 and 6 compared with the isolated fracture group, consistent with the well-documented phenomenon of “TBI-induced osteogenesis.” Although the RUST scoring system was originally developed for tibial fractures, its validity has also been demonstrated for femoral fractures in clinical studies, where it provides reliable predictive value for union.^[19] Our findings are consistent with previous clinical reports describing more exuberant callus formation and accelerated radiographic healing in patients with concomitant TBI.^[5,20,21] Similarly, a recent comprehensive review reported that, across both clinical and experimental studies, TBI consistently shortens the radiological time to union.^[7] Collectively, these observations reinforce the concept that TBI significantly accelerates early radiographic fracture healing, in agreement with our findings.

β -catenin functions primarily as an intracellular effector of canonical Wnt signaling; therefore, serum-based mRNA measurements should be interpreted as an indirect systemic readout rather than as a circulating signaling mediator. At the molecular level, our study demonstrated that serum β -catenin mRNA relative expression declined significantly over time in the fracture groups, whereas no significant temporal changes were observed in the control or isolated TBI groups. Importantly, the group \times time interaction between the fracture-only and fracture + TBI groups was not significant, suggesting that this decline was primarily associated with the presence of fracture rather than with concomitant brain injury (Table 1). These findings are consistent with previous reports highlighting the phase-dependent regulation of Wnt/ β -catenin signaling during bone repair. Chen et al.^[12] demonstrated that excessive β -catenin activity may impair early chondrogenesis,

whereas appropriate levels are required for ossification; sustained activation may also hinder the remodeling phase. Consistent with these findings, Bao et al.^[10] reported that optimal—rather than excessive— β -catenin activity during the remodeling phase is essential for maintaining bone quality and mechanical stability. Reviews have further emphasized that Wnt/ β -catenin must remain within a physiological range, as both insufficient and excessive signaling may compromise repair.^[11,13] Human tissue studies have likewise demonstrated strong Wnt activation in fracture callus but reduced or dysregulated signaling in nonunion tissue.^[14] Taken together, these observations suggest that the temporal downregulation of β -catenin observed in our fracture groups may represent a physiological adaptation that supports appropriate callus maturation. At the same time, they highlight that systemic serum β -catenin mRNA relative expression may not fully reflect the localized regulatory activity occurring within fracture tissue.

In the present study, Group 4 (head trauma + femoral fracture) demonstrated a greater proportion and volume of osseous (mature) callus than Group 3 (femoral fracture alone), although this difference did not reach statistical significance (Table 2). In contrast, several experimental studies have reported significant histological differences indicating enhanced callus formation in the presence of TBI.^[22-24] The absence of statistical significance in our data may be attributable to factors such as the relatively small sample size, the assessment of histology at a single time point (week 6), and the inherent biological variability of fracture healing in animal models. Despite these limitations, the observed trend toward more mature callus in the TBI group is biologically plausible and consistent with recent studies. For example, Yang et al.^[22] reported that exosomes released following TBI stimulate osteoblast proliferation and differentiation, while Xia et al.^[25] identified neuron-derived, miRNA-enriched vesicles that enhance osteogenesis, both of which may represent potential mechanisms underlying increased callus ossification. Taken together, although not statistically significant, our findings are consistent with the broader body of evidence suggesting that concomitant TBI may promote the formation of more mature callus during fracture repair.

In our study, serum β -catenin mRNA relative expression did not increase over time; instead, a significant decline was observed in the fracture groups. This finding may reflect phase-specific regulation of the Wnt/ β -catenin pathway and underscores that systemic serum measurements may not fully capture local activity within the fracture callus. Accordingly, the serum β -catenin mRNA expression findings should be interpreted as associative and cannot be considered evidence that systemic β -catenin signaling mediates the enhanced radiographic healing observed with concomitant TBI. Previous studies have demonstrated that β -catenin plays a central role in fracture repair at the tissue level, exerting stage-dependent effects on chondrogenesis, ossification, and remodeling.^[10,12,14] Therefore, the decrease in serum β -catenin mRNA

relative expression observed in this study does not preclude the biological importance of local β -catenin signaling within the callus. Clinical observations similarly indicate that long bone fractures accompanied by TBI are associated with faster union and more exuberant callus formation.^[5,20] These findings support the translational relevance of the radiographic association observed in our study and suggest that Wnt/ β -catenin signaling warrants further mechanistic investigation in the context of TBI-associated fracture repair. However, our data do not establish a mediating role for systemic serum β -catenin mRNA. Experimental studies have shown that interventions such as glycogen synthase kinase-3 β (GSK-3 β) inhibition, antisclerostin antibody therapy, and other pharmacological modulators of Wnt/ β -catenin signaling can enhance callus formation and improve bone quality, although their efficacy is critically dependent on timing and dosage.^[11,26] Importantly, these findings are not specific to TBI-associated fracture healing and should not be interpreted as implying therapeutic applicability based on our serum mRNA findings alone. Taken together, our results highlight the complex, phase-dependent regulation of β -catenin during fracture repair and reinforce the need for further mechanistic and translational studies, particularly those incorporating local (fracture-site) pathway analyses.

This study has several limitations that should be acknowledged. First, the sample size was relatively small, which may have reduced the statistical power to detect subtle differences, particularly in the histological analyses. Consequently, modest between-group differences in secondary tissue-level outcomes may have been missed. In addition, group sizes became unequal after exclusions, which may have further reduced statistical power and increased the likelihood of overlooking modest between-group differences. No a priori power calculation was performed; therefore, the study may have been underpowered to detect small histological effects. Second, biomechanical testing (e.g., torsional or bending strength) was not performed, and thus the mechanical competence of the healed femur could not be directly assessed. Third, histological and molecular assessments were conducted at a single time point (week 6), which does not capture the full dynamics of fracture healing across different phases. As a result, subtle or phase-dependent tissue-level differences between the fracture-only and TBI + fracture groups may have gone undetected, and the study may have been underpowered to detect modest histological effects. Fourth, serum β -catenin mRNA relative expression was evaluated only in serum and may not accurately reflect local signaling activity within the fracture callus. Moreover, pre-analytical serum handling variables (e.g., serum separation conditions and storage history) were not independently standardized or recorded, which could influence serum-based RT-qPCR measurements. Because β -catenin is predominantly intracellular, circulating measurements are inherently indirect and may not adequately capture callus-level pathway activation. Finally, as this was an experimental animal study, caution is warranted

when extrapolating the findings directly to clinical practice. Future studies with larger cohorts, multiple time-point analyses, and combined systemic and local molecular evaluations will be needed to better define the role of Wnt/ β -catenin signaling in TBI-associated fracture repair.

CONCLUSION

In this rat model, concomitant TBI was associated with higher radiographic RUST scores at weeks 3 and 6, indicating accelerated radiographic healing as the primary study finding. Histological evaluation at week 6 demonstrated only a non-significant trend toward greater osseous callus formation, and serum β -catenin (Cttnb1) mRNA relative expression did not reveal a TBI-specific systemic signal. Thus, the serum RT-qPCR findings represent an indirect systemic readout and do not establish a mechanistic TBI-specific β -catenin pathway.

Ethics Committee Approval: This study was approved by the Mersin University Rectorate Animal Experiments Local Ethics Committee (Date: 25.03.2019, Decision No: 15).

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DENEYSSEL ÇALIŞMA - ÖZ

Kafa travması ile ilişkili femur kırıklarının iyileşmesi ile serum β -katenin mRNA ekspresyonu arasındaki ilişki: Deneysel bir sıçan çalışması

AMAÇ: Kırık iyileşmesi eşlik eden travmatik beyin hasarından (TBH) etkilenebilir; klinik ve deneysel çalışmalar hızlanmış kaynama ve artmış kallus oluşumunu düşündürmektedir. Wnt/ β -katenin sinyal yolunun bu süreçte rol oynayabileceği düşünülmektedir. Ancak, TBH varlığında serum β -katenin mRNA ekspresyonu ile kırık iyileşmesi arasındaki ilişki belirsizliğini korumaktadır.

GEREÇ VE YÖNTEM: Otuz altı dişi Wistar albino sıçanı dört gruba rastgele atanmıştır: Kontrol, yalnız TBH, yalnız femur kırığı ve kombine TBH ve femur kırığı. Radyografik iyileşme 3. ve 6. haftalarda RUST puanlama sistemi kullanılarak değerlendirilmiştir. Serum β -katenin mRNA görel ekspresyonu gerçek zamanlı polimeraz zincir reaksiyonu ile yapılmış ve histolojik analiz 6. haftada gerçekleştirilmiştir.

BULGULAR: Radyografik değerlendirme tüm kırık gruplarında ilerleyici iyileşme göstermiş, TBH + kırık grubunda RUST puanları her iki zamanda da ($p < 0.05$) yalnız kırık grubuna göre anlamlı olarak daha yüksek bulunmuştur. Serum β -katenin mRNA ekspresyonu kırık gruplarında zaman içinde anlamlı olarak azalmış, kontrol grubunda veya izole TBH grubunda ise anlamlı zamana bağlı değişiklik gözlenmemiştir. Serum β -katenin mRNA ekspresyonundaki düşüş her iki kırık grubunda da gözlenmiş olup, bu bulgu TBH'ye özgü moleküler bir etkiyi doğrulamamaktadır. Histolojik analiz, TBH + kırık grubunda daha olgun osseöz kallus oluşumuna yönelik bir eğilimi düşündürmüştü, ancak farklar istatistiksel olarak anlamlı bulunmamıştır.

SONUÇ: Eşlik eden TBH, radyografik kırık iyileşmesinde artış ile ilişkili bulunmuş ve daha büyük osseöz kallus oluşumuna yönelik istatistiksel olarak anlamlı olmayan bir eğilim göstermiştir. Kırık gruplarında gözlenen serum β -katenin mRNA ekspresyonundaki düşüş, onarım sürecinde Wnt/ β -katenin ile ilişkili aktivitenin faz-bağımlı düzenlenmesini düşündürmekle birlikte, serum β -katenin mRNA görel ekspresyonu indirekt bir sistemik göstergedir ve TBH'ye özgü mekanistik bir yolu kanıtlamaz. Bu bulgular, TBH'nin iskelet onarımı üzerindeki karmaşık sistemik etkisini vurgulamakta ve gözlenen radyografik ilişkinin biyolojik alt yapısını aydınlatmak için, tercihen kırık sahası (lokal) analizlerini içeren daha ileri mekanistik çalışmalarını desteklemektedir.

Anahtar sözcükler: Deneysel sıçan modeli; kallus formasyonu; kırık iyileşmesi; travmatik beyin hasarı; Wnt/ β -katenin yolu.

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