Assessment of diaphyseal long bone fracture healing in cats using radiography, ultrasonography and doppler impedance index

ABSTRACT

Color Doppler ultrasonography evaluates blood flow and neovascularization in vessels, assessing Pulsatility Index (PI) and Resistive Index (RI) values. The primary objective of this study is to monitor the healing periods of fractures using color Doppler ultrasonography index values, thereby preventing complications through objective data. The emphasis is particularly on establishing the foundation for studies aimed at early detection of delays in vascularization associated with fracture healing. The research involved 20 cats of various breeds and genders, aged 1 to 4 years, diagnosed with femur and tibia diaphyseal fractures. Mediolateral and anteroposterior radiographs were taken on postoperative days 0, 10, 20, and 30. The fracture site was examined using color Doppler ultrasonography. As a result of the study, using Doppler ultrasonography and radiography, neovascular areas from fracture formation to callus formation were converted into objective data through vascular indices. Particularly noteworthy was a significant increase in RI and PI values observed on the postoperative 10th day. It was deduced that the detection of fracture healing could occur earlier with the assistance of these index values when compared to radiographs taken on the 10th and 30th days. In conclusion, PI and RI values may be important parameters in evaluating the healing process in complex fractures, especially in determining complications and monitoring the progress of healing. Moreover, Doppler indices might be employed to complement traditional diagnostic approaches in the early detection of pathological bone conditions

Keywords: Color doppler, femur, pulsatility index, resistive index

INTRODUCTION

Radiography, commonly favored for diagnosing and treating fractures, proves highly advantageous in imaging bone anatomy, evaluating postoperative periosteal reactions, and assessing changes in bone mineral density (Kealy et al., 2011; Thrall, 2013). In the early stages of pathologies such as periosteal reactions or avascular necrosis, where bone opacity is minimal, changes in opacity may only become apparent through radiography approximately 7 to 10 days later (Hammond, 2016). Postoperative radiographs taken during the fracture healing process are established as a standard procedure. However, the periosteal reactions obtained through radiography are quantitative assessments. The evaluation of mineralization and callus formation is subjective and may vary depending on the assessor. To enhance objectivity in evaluations, diverse imaging techniques should be employed. Ultrasonography is one of the diagnostic methods utilized for soft tissue monitoring of extremities and the assessment of fracture healing. Bone tissue appears hyperechoic and is visualized as a straight line in ultrasound imaging. In fractures, the imaging of surrounding soft tissues allows for the evaluation of muscle tears, hematomas, and the healing processes of soft tissue (Wawrzyket al., 2015).

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Research Article

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The process of bone formation varies depending on the vascular supply to the region. Color Doppler ultrasonography can be employed to monitor the neoangiogenesis process surrounding the fracture site and the formation of callus (Caruso et al., 2000). This diagnostic method is also valuable for assessing secondary fracture healing in simple fractures, cases of non-union, and situations of delayed union (Kealy et al., 2011; Kramer, 2011). Doppler spectrum contributes to the evaluation of vascular flow characteristics (Kim, 2020; Varshney et al., 2022). Parameters used to assess the resistance of blood flow in the vascular lumen and the perfusion of organs are available. Quantitative information obtained from these parameters aids in diagnosis (Chung et al., 2020; River et al., 1997). The pulsed wave waveform concurrently displays blood flow velocity and changes, facilitating the calculation of indices (Gorgas, 2011; Kim et al., 2020; Varshney et al., 2022). Doppler waveforms, specifically Pulsatility Index (PI) and Resistive Index (RI) values obtained from them, are utilized in assessing blood flow resistance in the vascular lumen and perfusion of blood circulation in organs (Rawashdeh et al., 2001; River et al., 1997). While RI examines the negative relationship between vascular resistance and vascular perfusion, PI is a parameter related to the flexibility of arteries (Bude et al.,1999; Ginther, 2007; Gosling et al., 1974).

This study aims to evaluate the healing process of diaphyseal fractures of the long bones of the hind limb of cats by radiography, ultrasonography and Doppler ultrasonography. It is aimed to increase the usability of the index results obtained from neovascular areas especially with Doppler ultrasonography in the evaluation of healing periods in clinical practice. With the objective data to be obtained, standardisation of neovascularisation occurring in the fracture line will be established, thus early detection of vascularisation-induced healing delays will be provided in the future.

MATERIALS AND METHODS

Selection of cases

The study material comprised a total of 20 cats, aged between 1 and 4 years, admitted to the Ankara University Faculty of Veterinary Medicine Animal Hospital and Aksaray University Faculty of Veterinary Medicine Animal Hospital due to single-piece fractures along the mid-diaphyseal line of the femur and tibia bones. Ethical approval for the study was obtained from the Ankara University Local Ethics Committee for Animal Experiments (Approval no: 2020-5-36). Moreover, the owners of the animals were adequately informed about the study, and their consent was obtained prior to their participation in the research.

Operation method

General anesthesia was provided with Medetomidine HCl (80 micrograms/kg, intramuscularly, Domitor®, Finland) and Ketamine HCl (5 mg/kg, intramuscularly, Keta-Control®, Türkiye). Isoflurane (with 100% oxygen, Isoflurane®, USA) was used for anesthesia maintenance. Butorphanol tartrate (0.1mg/kg subcutaneously, Butomidor®, Austria) was administered for analgesia. Balanced electrolyte solutions (Ringer's lactate, 50 ml/kg/h, intravenously) and cefazolin (25 mg/kg, intravenously, Eqizolin®, Türkiye) were administered intraoperatively. In the postoperative period, antibiotic treatment was provided with amoxicillin/clavulanic acid (25 mg/kg, oral, Augmentin®, Türkiye) for one week.

In all cases, reduction of the fractures was achieved using the retrograde intramedullary pinning method. After complete anatomical reduction, fracture fixation was performed by a routine surgical approach using a Steimann pin or a Schanz pin in a retrograde technique according to the diameter of the medullary cavity (to fill approximately 70 % of the diameter of the medullary canal) (Roe, 2005**)**. In addition to the fracture fixation method, cerclage wire was

applied to prevent rotation of the fracture ends. In the postoperative days, the affected extremity was supported with a bandage for 2 weeks to minimise movement, and the bandage was renewed on control days. Postoperatively, area restriction was applied during the recovery period.

Radiographic examination

Radiographic and ultrasonographic assessments were performed on postoperative days 0, 10, 20, and 30. Direct radiographs were obtained in mediolateral and anteroposterior positions for radiographic evaluations. The assessment included the examination of fracture healing, fracture reduction, intramedullary pin position, and callus formation. These evaluations were based on the radiodensity of the bone cortex and the condition of the cortical bone.

Ultrasonographic examination

Fracture line assessment in all cases was conducted using B-mode ultrasonography and color Doppler ultrasonography with 5 and 7.5 MHz sector and linear probes. In pulse Doppler ultrasonographic examination, Doppler color gain setting (G) was set between 046-050, wall filter mean (WF) 100 Hz, sampling rate (PRF) mean 2.2-2.6 KHz, sampling interval 1-1.5 mm and Doppler angle ≤ 60°. Evaluation involved a lateral approach for the femur and a medial approach for the tibia. The ultrasound probe was positioned parallel to the bone axis of the relevant extremity, with ultrasound beams oriented perpendicular (Figure 1). During ultrasound evaluation, any anesthetic agent was not administered to the patients.

Color Doppler ultrasonography was used to evaluate the vascular space surrounding the fracture line (the vascular network formed by the periosteal arteries in the area for fracture healing). The spectral waveforms obtained from these regions in pulsed wave mode provided pulsatility and resistance indices by automatically measuring their values through the instrument software. Measurements were taken from the surrounding tissues and neovascular areas of the periosteal vessels during fracture healing, with samples obtained from the fracture line for each case.

Figure 1. Placement of the linear probe along the axis of the tibia.

Statistical analysis

The SPSS 21 (Version 28.0, IBM Corp., NewYork, USA) package program was employed for the statistical analysis of the acquired data. The normal distribution of the data was assessed using the Shapiro-Wilk test, and the assumption of sphericity was examined through Mauchly's Sphericity Test. The Resistive Index and Pulsatility Index data were subjected to analysis using the Repeated Measures Analysis of Variance Test with respect to time groups. A significance level of $p < 0.05$ was considered to determine statistical significance.

RESULTS

The study included a total of 20 cats, consisting of 6 females and 14 males. The average age of the cases was determined to be 1.35 ± 0.17 years, with an average weight of 3.24 ± 0.24 kg (ranging from 2 kg to 6.5 kg) (Table 1). Trauma

(n=10) emerged as the predominant cause of fractures based on anamnesis. Fractures were evenly distributed between femurs and tibias, each accounting for 50% of the cases. Specifically, among femoral fractures, 2 were spiral and 4 were oblique, while tibial fractures included 4 oblique and 6 transverse fractures. Cerclage wire was applied in all cases except case 2.

Table 1. Signalment and postoperative index values for the cases.

Case	Fracture Bone	Signalment				Day 0		Day 10		Day 20		Day 30	
		Breed	Age (year)	Weight (kg)	Gender	RI	PI	RI	PI	RI	PI	RI	PI
1	T	Tabby	1	3.0	M	0.59	0.89	0.54	0.82	0.75	1.66	0.76	1.73
2	T	Bombay	4	6.5	M	0.49	0.86	0.68	1.23	0.35	0.42	0.42	0.57
3	F	Tabby	1	4.0	M	0.58	0.89	0.62	1.18	0.55	0.92	0.56	1.01
4	F	Tabby	2	3.6	M	0.51	0.77	0.67	1.23	0.53	1.00	0.72	1.83
5	T	Tabby	1	2.8	M	0.63	0.97	0.45	0.63	0.59	1.20	0.39	0.52
6	F	Tabby	1	4.0	M	0.47	0.68	0.52	0.75	0.73	1.25	0.49	0.79
7	F	Tabby	1	2.7	M	0.48	0.99	0.60	1.02	0.45	0.98	0.68	1.22
8	F	Tabby	1	2.9	M	0.42	0.64	0.48	0.64	0.51	0.71	0.55	0.85
9	T	Bombay	1	4.1	M	0.59	0.98	0.60	1.03	0.62	1.04	0.62	1.09
10	T	Tabby	1	3.0	M	0.43	0.56	0.52	1.01	0.68	1.22	0.63	1.13
11	F	Tabby	1	2.0	M	0.58	0.96	0.59	0.95	0.57	0.92	0.59	1.01
12	T	Tabby	2	3.2	Fe	0.40	0.51	0.45	0.61	0.48	1.20	0.52	0.89
13	T	Tabby	2	3.2	Fe	0.43	0.66	0.62	1.13	0.48	1.20	0.54	0.86
14	T	Tabby	2	5.0	M	0.51	0.82	0.65	1.16	0.38	0.91	0.70	1.48
15	T	Tabby	1	2.2	Fe	0.55	0.87	0.67	1.13	0.53	0.83	0.50	0.68
16	F	Angora	1	2.0	Fe	0.49	0.70	0.65	1.10	0.62	1.19	0.65	1.18
17	F	Tabby	1	2.5	Fe	0.63	1.08	0.66	1.12	0.66	1.28	0.57	0.94
18	F	Tabby	1	3.0	M	0.52	0.75	0.64	1.05	0.63	1.18	0.59	1.02
19	T	Van	1	2.8	Fe	0.37	0.64	0.55	1.05	0.60	1.13	0.62	1.07
20	F	Bombay	1	2.3	M	0.51	0.73	0.69	1.16	0.56	1.02	0.66	1.15

F: Femur, T: Tibia, M: Male, Fe: Female, RI: Resistive index, PI: Pulsatility index.

Radiographic examination findings

In all cases, it was observed that appropriate anatomical reduction was achieved at the fracture site on postoperative day 0, and the intramedullary pin was correctly positioned with the appropriate diameter. By postoperative day 10, it was noted that the fracture ends lost their sharpness and became rounded. On the 20th postoperative day, a reduction in the distance between fragments was observed, and periosteal callus formation around the fracture site was evident. Furthermore, periosteal reactions in regions away from the fracture line were noted in three cases (Case no: 1, 4, and 8): periosteal reaction was observed 10 mm proximal to the fracture line in case 1, 6 mm distal to the fracture line in case 4, and towards the distal region of the bone in case 8 (Figure 2). Cases 3, 5, and 18 exhibited pin migration, and in case 6, an exuberant callus formed.

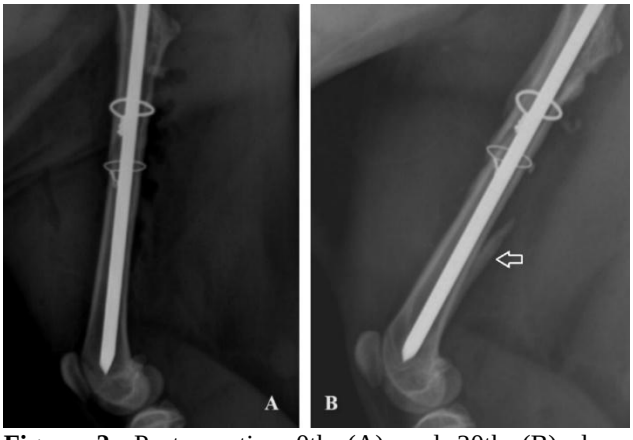

Figure 2. Postoperative 0th (A) and 20th (B) days radiographic images of Case 8. White arrow: callus.

By postoperative day 30, it was observed that the fracture line became indistinct, and the amount of callus tissue increased, becoming more prominent compared to the previous checkup. Specifically in case 2, it was noted that the fracture ends rounded and closed, yet the fracture line remained distinct, and cortical integrity was not maintained. Consequently, treatment for this case continued for some time due to the lack of union. On postoperative day 50, union finally formed, leading to a delayed union diagnosis for Case 2 (Figure 3). In case 2, cerclage wire application was not performed and it was thought that delayed union may have occurred due to the inhibition of rotation movement, which is the disadvantage of intramedullary pin, and movement of the fracture ends. None of the other cases had any complications during the recovery period.

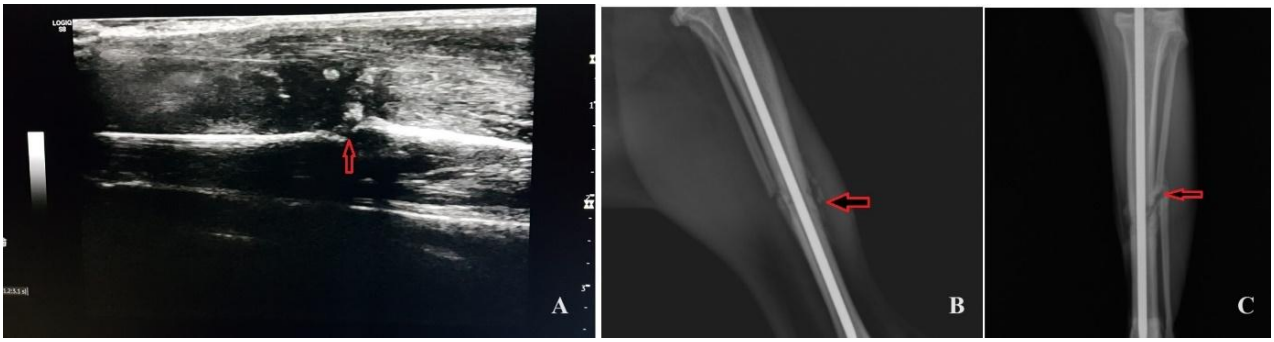

Figure 3. A. Postoperative 20th day B-mode ultrasonography image of Case 2. Red arrow: Fracture line. B. Postoperative 20th day mediolateral radiographic image. Red arrow: callus C. Postoperative 30th day anteroposterior radiographic image. Red arrow: Fracture line.

Ultrasonographic examination findings

On the postoperative 0th day, B-mode ultrasonography of all cases revealed a hyperechoic, longitudinal bone structure. The bone line, visualized as a hyperechoic straight line, was followed, and the interruption of the line was identified as the anechoic region, representing the fracture site. When the area around the fracture line was examined with color Doppler ultrasonography, the presence of small-diameter vessels was identified as a neovascular area, with the signal coming towards the probe appearing in red and the signal moving away from the probe appearing in blue. While a high-resistance spectral waveform was observed in most cases, a low-resistance spectral waveform was detected in Case 2. A decrease in downstroke was observed in late systole.

On the 10th day postoperatively, there was an observed increase in small-diameter vessels displaying both blue and red color flow, signifying an increase in neovascular areas around the callus and its surroundings (Figure 4). On postoperative days 20 and 30, a decrease in neovascular areas was observed, and challenges in pulsed wave blood flow acquisition persisted. In Case 6, the presence of exuberant callus irregularly appeared as a hyperechoic bone line on days 20 and 30. A high-resistance and turbulent flow were noted in the spectral waveform.

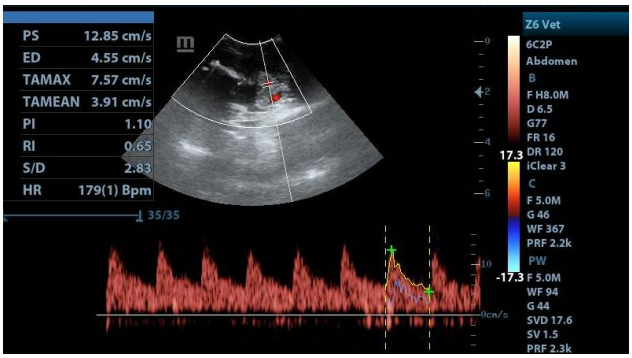

Figure 4. Postoperative 10th day doppler ultrasonography image of case 16

In Case 2, on postoperative day 20, it was observed that the fracture ends became more distinct, and the fracture line was anechoic.

During the 30th-day follow-up, it was noted that cortical bone integrity was not maintained. Consequently, on the 50th day, a reduction in the fracture line interval and a shift to a high-resistance spectral waveform were notable.

Index findings

According to the index analysis results, on postoperative day 0, the mean RI value was determined to be 0.509 ± 0.017, and the mean PI value was 0.798 ± 0.035. The data indicated similar results among the cases on postoperative day 0. On postoperative day 10, a statistically significant increase was observed in the arithmetic mean of RI values by 0.1 units compared to day 0, along with a 0.255-unit increase in PI values. On postoperative day 20, it was observed that, compared to day 10, there was a decrease of 0.05 units in the arithmetic mean of RI values for all cases, and an increase of 0.04 units in PI values. Examining the postoperative 30th-day RI analysis results, a 0.05 unit increase was noted compared to the postoperative 20th day. The variance analysis of RI-time intra-group factors has been indicated in the table for repeated measurements (Table 2). Upon general examination of the cases, it was observed that the RI values showed a statistically significant increase on the 10th and 30th days. According to the data, it was observed that the increase in PI value between the 0th and 10th days is more significant compared to the rate of increase on other days, while the difference in PI between the postoperative 20th and 30th days was found to be less pronounced (Table 3).

Table 2. Change in Resistive Index values over time.

Resistive index	n	Art.Mean ± Std. Deviation	Median (Min-Max)	P value
RI (t0)	20	0.509 ± 0.017[a]	0.510 (0.370 – 0.630)	0.010
RI (t10)	20	0.593 ± 0.017[b]	0.610 (0.450 – 0.690)	
RI (t20)	20	0.564 ± 0.023[ab]	0.565 (0.350 – 0.750)	
RI (t30)	20	0.588 ± 0.021[ab]	0.590 (0.390 – 0.760)	

[a,b]: Different letters in the same column indicate statistically significant differences (p<0.05). Art.Mean: Arithmetic mean value, Std. Deviation: Standard deviation, Min: Minimum, Max: Maximum, RI: Resistive index.

Table 3. Change in Pulsatility Index values over time.

Pulsatility index	n	Art.Mean ± Std. Deviation	Median (Min-Max)	P value
PI (t0)	20	0.798 ± 0.035[a]	0.795 (0.51 – 1.08)	0.002
PI (t10)	20	1.000 ± 0.045[b]	1.050 (0.61 – 1.23)	
PI (t20)	20	1.063 ± 0.057[b]	1.085 (0.42 – 1.66)	
PI (t30)	20	1.051 ± 0.075[b]	1.015 (0.52 – 1.83)	

[a,b]: Different letters in the same column indicate statistically significant differences (p<0.05). Art.Mean: Arithmetic mean value, Std. Deviation: Standard deviation, Min: Minimum, Max: Maximum, PI: Pulsatility index.

Upon individual examination of cases, it was noted that on the 20th and 30th days of Case 2, on the 10th and 30th days of Case 5, on the 30th day of Case 6, and on the 10th day of Case 8, both RI and PI values were lower compared to other cases (Table 1). The statistical significance level was determined to be $p < 0.01$ for the RI value and $p < 0.002$ for the PI value, and a significant change in the data obtained on control days was observed.

DISCUSSION

Fractures can result from various factors such as trauma, metabolic diseases, age, and others. The diagnosis of a fracture, the methods employed in treatment, and postoperative follow-up processes are typically determined through radiography. Following the trauma period, the evaluation of images termed "callus" on radiographs, which appear adjacent to the bone opacity around the fracture, plays a crucial role in determining the process of fracture healing. However, this assessment is subjective and relies on the knowledge and expertise of the physician interpreting the radiographic images. Ultrasonography, as an imaging method, offers

more detailed results in the early stages of fracture healing compared to radiography. Ultrasonography not only enables the visualization of the fracture line and bone tissue but also provides information about the soft tissues affected by trauma (Maffulli, 1995).

Color Doppler ultrasonography is a significant diagnostic method that identifies the presence and direction of blood flow in tissues, along with the normal and abnormal vascularization structure in organs. While widely utilized in various fields, it is commonly employed in the physiological and pathological evaluations of bone tissue (Kealy et al., 2011; Kramer, 2011). In a study assessing fracture healing, Doppler signals were identified until the 50th day, but a decrease in the signal was observed after the 30th day. Concurrently, reductions in the vascular area also occurred during this process (Risselada, 2006). Similar findings were obtained in the conducted study, where an increase in neovascularization was observed in the fracture area on the 10th day after surgery, while a decrease was noted on the 20th and 30th days. The increased angiogenesis during the inflammatory phase is thought to contribute to the proliferation of osteoprogenitor cells, leading to more neovascular area signals on the 10th day compared to other days in color Doppler ultrasonography.

Su et al. (2013), reported that bone union was inadequate in cases with decreased index values. In the research, periosteal reaction was identified in the radiographic images of case 2 on the 10th and 20th days, indicating that the fracture line was beginning to blur. Based on the radiographic examination results, the healing process of the fracture was considered to be progressing normally. However, during the assessment on the 30th day, it was observed that the fracture ends started to close, and the fracture line became more pronounced. In the radiographic findings, the ultrasound images of the case with complete anatomical reduction were notable for the anechoic appearance of the fracture line. On the postoperative 20th day, the anechoic appearance of the fracture line became even more apparent. Considering the ultrasound evaluation of the fracture line, it is suggested that delayed union could be diagnosed earlier compared to radiographic images. In the index assessments of the same case, it is noteworthy that the PI and RI values were lower on the postoperative 20th and 30th days compared to other cases. Taking into account the existing neovascular areas and index values, it is believed, similar to the findings of a previous study (Caruso, 2000; Su et al., 2013), that these assessments are crucial for the early diagnosis of callus complications.

In a human study reported an inverse relationship between the RI value and the amount of callus, although no significant difference was observed within the group (Su et al., 2013). Conversely, another study concluded a direct proportionality between bone integrity and the RI value (Wawrzyk et al., 2015). Discrepancies in the study results have been interpreted as potentially arising from variables such as the assessment of different extremities, the inclusion of middle-aged adults and children in the study, and variations in vessel diameters. Upon reviewing the conducted studies, it is evident that cases achieving bone integrity, particularly by the 10th day, exhibit a notable increase in the RI value (Wawrzyk et al., 2015). The direct proportionality between the development of bone integrity and the increase in the RI value aligns with the findings of the current study. This observed proportional increase in the RI value with callus formation can be attributed to heightened vascular resistance, especially in microvascular areas where vascular walls are shaped. This is particularly evident on the 10th day when osteoblastic activity and neoangiogenesis are

intense. The subsequent decrease in the RI value on the postoperative 20th day can be interpreted as a reduction in resistance due to a decrease in cell density compared to the 10th day. In the healing process on the postoperative 30th day, there was a decrease in extraosseous blood supply compared to other days. Nevertheless, the diminished neovascular area lead to an increase in flow in other small vessels. Ongoing cellular activity contributed to an increase in resistance in the vascular wall. Consequently, there was an observed increase in the RI value on the 30th day compared to the value on the 20th day.

The pulsatilite index is associated with the flexibility of arteries and provides information about the cardiovascular system. The flexibility of large arteries plays a role in tolerating vibrations caused by left ventricular contractions. The expansion of the aorta during each heartbeat and the storage of a portion of its volume allow smaller vessels to be less exposed to pulsatile stress (Michel and Zernikow, 1998; Wielicka et al., 2020). In our study, an increase in the pulsatility index was recorded throughout the fracture healing process. Especially on the 10th postoperative day, microvascular structures were exposed to pulsatile stress due to the increase in blood flow. The increase observed in the PI index on this day, when osteoblastic activity and inflammation are active, can be interpreted as the presence of high blood flow and perfusion in the distal tissues. The increase in neoangiogenesis in proportion to the fracture healing process leads to a significant increase in the vascular network formed by the main artery and a decrease in the flow per vessel. This was interpreted as a decrease in pulsatile stress in the vessels and a decrease in the pulsatility index on the 30th postoperative day.

Intramedullary pin application minimally traumatises the periosteal circulation compared to plate application because it is placed in the medullary cavity (Decamp et al., 2016). In this study, the microvascular structures that provide extraosseous blood supply and callus formation are investigated by imaging methods during the healing process. The fracture fixation method to be applied was decided in line with this purpose, therefore intramedullary pin application was preferred. In addition the cerclage wire affected the periosteum from a single site and was ignored because it was thought that the extraosseous blood supply would not be affected as much as the plate.

Fracture fixators utilized in reduction are typically made from inert materials. However, in certain instances, it becomes necessary to remove these fixators. Based on the data obtained in this study, normal values for index values during fracture healing have been established. According to the data, it was determined that the average unit value of RI on the 30th day for cases is 0.588 ± 0.0021, and the average unit value of PI is 1.051 ± 0.075. If both indices exceed the normal values on the 30th postoperative day, early consideration may be given to the removal of fixators. This is particularly crucial in cases where the growth plate is affected or to alleviate the stress factor created by the bandage. Early detection of a decrease in index values in cases where fracture healing is delayed, possibly due to hormonal imbalances or inadequate nutrition, can prompt consideration for nutritional supplementation with fortified foods.

CONCLUSION

The study produced statistically significant results regarding vascular index values throughout the period from the emergence of neovascular areas to callus development in the healing stages of long bones. The conclusion drawn was that PI and RI values could serve as crucial parameters for evaluating the healing process in complex fractures, especially in promptly identifying complications and monitoring recovery progress. Furthermore, it has been suggested that alternative Doppler indices could be utilized to supplement

traditional diagnostic approaches for the early detection of pathological bone conditions.

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Author contributions: NA and IE contributed to the project idea and design. NA contributed to the execution of the study. NA and YS contributed to the data collection. IE and YS critically reviewed the manuscript and supervised the study. All authors read and approved the final version of the manuscript.

Availability of data and materials: Data supporting the findings of this study are available from the corresponding author upon reasonable request.

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