Am J Transl Res 2023;15(2):1247-1253
www.ajtr.org /ISSN:1943-8141/AJTR0145929

Original Article

A modified pixel value ratio based on contralateral normal bone: a novel reliable indicator for distraction osteogenesis

Qi Liu[1,2*], Jieyu Liang[1,2*], Ze Liu[1,2], Haibo Mei[3], Guanghui Zhu[3], Hongbin Guo[1,2], Min Wang[4], Yi Zhang[1,2]

[1]Department of Orthopaedics, Xiangya Hospital, Central South University, Changsha, Hunan, China; [2]National Clinical Research Center for Geriatric Disorders, Xiangya Hospital, Central South University, Changsha, Hunan, China; [3]Department of Pediatric Orthopedics, Hunan Children's Hospital, Pediatric Academy of University of South China, Changsha, Hunan, China; [4]Department of Endocrinology, Xiangya Hospital, Central South University, Changsha, Hunan, China. [*]Equal contributors and co-first authors.

Received August 14, 2022; Accepted January 5, 2023; Epub February 15, 2023; Published February 28, 2023

Abstract: Background: The traditional pixel value ratio (tPVR) is subject to disuse osteopenia of the adjacent bone in distraction osteogenesis (DO). Therefore, a modified PVR (mPVR) based on the contralateral normal bone was developed and validated to address this issue. Methods: A total of 79 bone lengthening subjects were recruited in this retrospective study. The difference between the mPVR and tPVR of early callus was identified in the first three months after osteotomy. Moreover, we further investigated the relationship between mPVR and healing index (HI), lengthening index (LI) and external fixator index (EFI). Finally, the potential influencing factors for mPVR of the early callus were analyzed. Results: The mPVR was significantly lower than the tPVR in the first three months after osteotomy, and the difference gradually increased. Interestingly, the mPVR of the early callus in the first two months was negatively correlated with the HI, LI and EFI. Moreover, the age, lengthening site, total bilirubin and mean hemoglobin content were associated with the mPVR of early callus during DO. Conclusion: The mPVR based on contralateral normal bone is a novel reliable indicator for DO, which may be helpful for the clinical management of DO. However, the findings of this study need to be confirmed further by larger prospective research.

Keywords: Bone lengthening, Ilizarov technique, distraction osteogenesis, modified pixel value ratio, plain radiography, external fixator

Introduction

Distraction osteogenesis (DO) was first reported in the 1950s by Dr. Ilizarov [1, 2]. Since then, DO has been widely used for orthopedic surgery, including limb lengthening, bone deformity correction and treatment of bone defects caused by trauma, infection and tumor [3, 4]. Currently, plain radiograph is the most common option to assess the regenerated callus during DO due to the fact that it is inexpensive, convenient and has low radiation levels. Nevertheless, it is relatively subjective and relies on the physician's experience [5, 6]. Thus, an objective quantitative evaluation based on plain radiograph is required.

Pixel value (PV) is utilized to assess the bone mineral density (BMD) in pixels. By comparing the PV of the regenerating callus with that of the adjacent bone, the traditional pixel value ratio (tPVR) is a common indicator for DO [7] (a higher tPVR indicates an increased density of regenerated callus). However, the adjacent bone is subjected to disuse osteopenia [8-14], which may substantially affect the assessment of regenerated callus during DO in the community. In contrast, the contralateral normal bone may potentially be a better reference for the calculation of the PVR and may be more precise and sensitive to assess the regenerated callus [12, 14]. Moreover, the PVR of early callus is rarely discussed. A modified PVR (mPVR) of early callus based on the contralateral normal bone was developed and validated for the first time in this study. The purpose of this investigation was as follows: 1) the difference between the mPVR and tPVR of the early callus in the

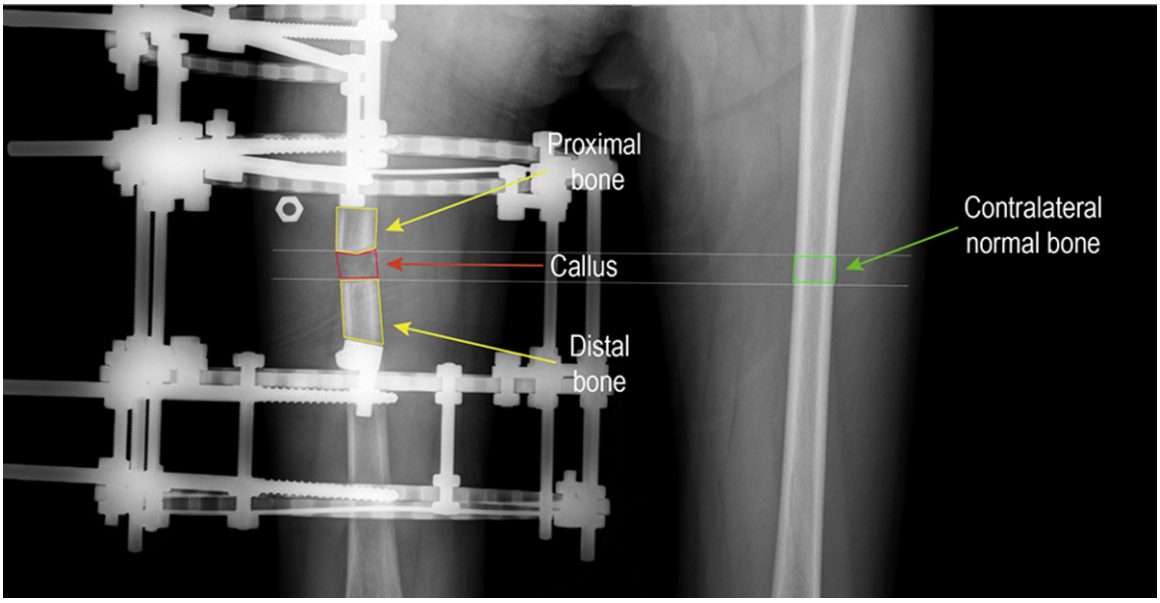

Figure 1. The assessment of mPVR and tPVR of the regenerated callus during distraction osteogenesis. mPVR: modified Pixel Value Ratio; tPVR: traditional Pixel Value Ratio.

first three months after osteotomy; 2) the associations of the mPVR of the early callus with the healing index (HI), lengthening index (LI) and external fixator index (EFI); and 3) the potential influencing factors for the mPVR of the early callus. As a potential novel reliable indicator for DO, the mPVR may be helpful for the clinical management of DO.

Materials and methods

Study population

The ethics committee at Xiangya Hospital, Central South University has reviewed and approved the protocol of this retrospective study (202109038). The clinical data of subjects with limb lengthening were collected and reviewed from Hunan Children's Hospital and Xiangya Hospital, Central South University retrospectively. The inclusive criteria were: 1) Ilizarov technique for the lower limb lengthening; 2) available imaging data for both the adjacent and contralateral bone; 3) primary surgery; and 4) successful external fixator removal (three of the four cortices in which new bone appeared). The exclusion criteria were: 1) bone nonunion or delayed union (at least three of the four cortices have no bridging callus on plain radiographs) with autogenous bone transplantation; 2) skeletal disorders (congenital pseudarthrosis of tibia) that affected the bone healing potential; 3) external fixator without limb lengthening; and 4) the follow-up data was missing. Ultimately, a total of 79 patients were recruited in our study.

Surgical approach

The Ilizarov technique is employed for bone lengthening of the femur and tibia. All surgical procedures were performed by experienced senior surgeons. Stretching started 1 week after osteotomy (rate 0.75 mm to 1 mm/day), and patients received x-rays once a month. The conditions for external fixator removal were listed as follows: 1) three of the four cortices with bridging callus on plain radiographs; 2) the fixation time approximately corresponds to the mean extension index; 3) after nut loosening, normal feeling existed during weight-bearing [15].

The difference between the mPVR and tPVR of the early callus

The picture archiving and communication system was utilized to measure the PV of regenerated, adjacent and contralateral bone in the first three months after osteotomy (**Figure 1**). Importantly, some metal rods are strictly avoided during the measurement. Then, the ratios of the regenerated callus PV to: 1) the contralat-

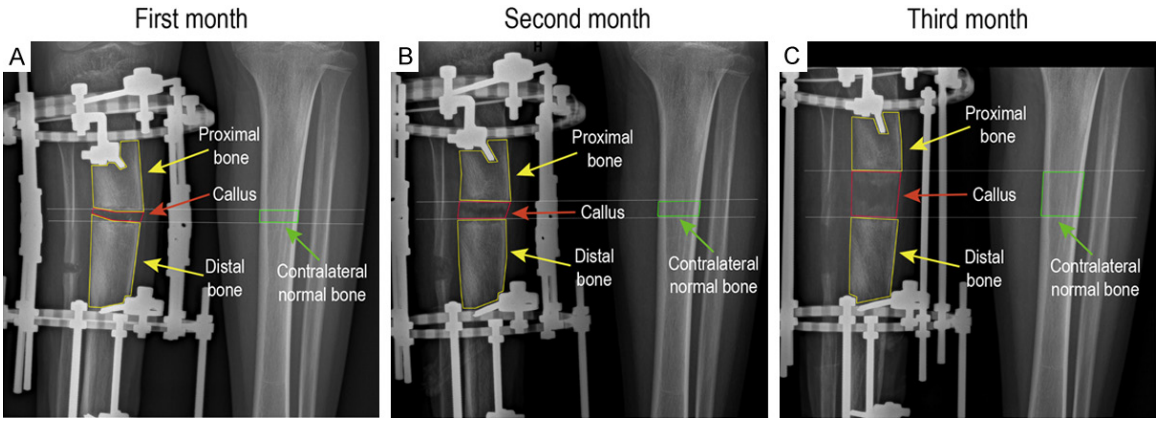

Figure 2. The mPVR and tPVR of early callus during distraction osteogenesis. A. Assessment of tPVR and mPVR based on adjacent bone and contralateral bone in the first month after osteotomy; B. Assessment of tPVR and mPVR based on adjacent bone and contralateral bone in the second month after osteotomy; C. Assessment of tPVR and mPVR based on adjacent bone and contralateral bone in the third month after osteotomy. mPVR: modified Pixel Value Ratio; tPVR: traditional Pixel Value Ratio.

eral bone PV (mPVR); and 2) the adjacent bone PV (tPVR) was analyzed and compared. The higher PVR value indicated that the PV of regenerated bone is close to that of the adjacent/contralateral one. On the contrary, the lower PVR value indicates an inferior maturity of the regenerated bone [16]. The adjacent and contralateral bones were employed for tPVR and mPVR assessment, respectively (**Figure 2**). The formulas for the calculations of the tPVR and mPVR were as follows:

$$tPVR = \frac{\text{Regenerated bone pixel value}}{(\text{Distal nomal bone pixel value} + \text{Proximal normal bone pixel value}) \div 2}$$

$$mPVR = \frac{\text{Regenerated bone pixel value}}{\text{Contralateral normal bone pixel value}}$$

The analysis of indicators for mPVR of early callus during distraction osteogenesis

Basically, HI was the time for full consolidation (three cortices in the regenerated distraction callus) divided by the lengthened length of bone (day/centimeter), whereas LI was the time (months) to lengthen 1 centimeter bone [17, 18]. Moreover, EFI was the time of external fixator usage (days) divided by the lengthened length of bone. The above three indexes were acceptable and reliable indicators for bone healing potential in DO. The mPVR and tPVR were served as primary indicators, whereas the HI, LI and EFI were regarded as secondary indicators. Moreover, age, sex, lengthening site and biochemical indices (white blood cell count, mean hemoglobin content, total bilirubin, ESR, fibrinogen, uric acid, monocyte count, basophilic granulocyte count and total protein) were analyzed as the secondary indicators in our study.

Statistical analysis

The SPSS 26.0 software was utilized for analysis in this study. The difference between mPVR and tPVR values was analyzed by paired t-tests and one-way ANOVA. The associations of the mPVR of early callus with the HI, LI and EFI were assessed by Pearson coefficient analysis. Moreover, the potential influencing factors for the mPVR of early callus were assessed by linear-regression analysis. A $P<0.05$ was considered statistically significant, and the data were expressed as the means ± standard deviations (mean ± SD).

Results

The mPVR was significantly lower than the tPVR of the early callus

The mPVR was significantly lower than the tPVR among subjects who underwent bone lengthening surgery within the first three months after osteotomy (0.75±0.09 vs 0.79±0.09, $P<0.001$; 0.80±0.08 vs 0.85±0.08, $P<0.001$; 0.84±0.09 vs 0.92±0.06, $P<0.001$) (**Table 1**). Intriguingly, the difference between mPVR and tPVR gradually increased during the first three months (0.040±0.005, 0.049±0.001, 0.076±0.025, $P<0.001$) (**Table 2**).

Table 1. The difference between modified and traditional pixel value ratio of early callus

	mPVR value (Mean ± SD)	tPVR value (Mean ± SD)	*P* value
First month	0.75±0.09	0.79±0.09	<0.001
Second month	0.80±0.08	0.85±0.08	<0.001
Third month	0.84±0.09	0.92±0.06	<0.001

mPVR: modified Pixel Value Ratio; tPVR: traditional Pixel Value Ratio; SD: Standard Deviation.

Table 2. The difference between modified and traditional pixel value ratio is gradually increasing

	First month (Mean ± SD)	Second month (Mean ± SD)	Third month (Mean ± SD)	*P* value
Difference	0.040±0.005	0.049±0.001	0.076±0.025	<0.001

SD: Standard Deviation.

The associations of the mPVR of the early callus with the HI, LI and EFI

The mPVR of the early callus in the first two months was negatively correlated with the HI (r=-0.345, P=0.029; r=-0.540, P<0.001), LI (r=-0.321, P=0.043; r=-0.478, P<0.001) and EFI (r=-0.425, P=0.006; r=-0.542, P<0.001) (**Table 3**). This result suggested that a higher mPVR of the early callus may reflect a better bone healing potential and clinical outcome of bone lengthening.

The potential influencing factors for the mPVR of the early callus

Age, lengthening site, total bilirubin and mean hemoglobin content might be the potential influencing factors for the mPVR of the early callus in bone lengthening. However, no significant association between the mPVR and other factors was identified (**Table 4**).

Discussion

As a reference for the assessment of tPVR, the adjacent bone is commonly subject to the issue of disuse osteopenia. For instance, Eyres et al. indicated a decreased BMD of 44.2% (tibia) and 61.0% (femur) in the adjacent bone in children who underwent bone lengthening [8]. Moreover, Maffulli et al. reported that the bone mineral content (BMC) of the adjacent bone dropped below 40% [9]. In addition, Veitch et al. found that disuse osteopenia appeared at both the proximal and distal ends of the fractures [13]. Little et al. found substantial osteoporosis in the bone adjacent to the lengthened segment in a rabbit model [10]. In this case, the tPVR based on the adjacent bone may be seriously disturbed. In our results, the mPVR based on the contralateral normal bone was significantly lower than the tPVR. Furthermore, the difference between the mPVR and tPVR of the early callus gradually increased. Indeed, Eyres et al. found that disuse osteopenia in the second month (BMD reduced by 33%±4%) was more serious than that in the first month (BMD reduced by 23%±3%) after osteotomy, which reached a peak at 12 months [11]. Our study found a significant difference between the mPVR and tPVR. Therefore, disuse osteopenia of the adjacent bone in bone lengthening should be carefully considered in the clinical setting.

Currently, the PVR is usually used to determine the timing of external fixator removal (evaluate the late callus maturity) [7, 12, 14, 16, 19]. However, the evidence for the clinical significance of an early PVR is rather limited. As three important indices for bone healing potential, the LI, HI and EFI can reflect the clinical effect of bone lengthening. Interestingly, the LI, HI and EFI were negatively correlated with the mPVR of the early callus. Our previous study found a moderate negative relationship between the LI and HI and the early tPVR (r≈-0.20) [20]. However, our present study indicated that such a relationship became much stronger for the early mPVR (r≈-0.50). It seems that the mPVR of the early callus may better reflect the clinical effect of bone lengthening. Consequently, it is important to consider mPVR in the community. Unfortunately, our results are limited by the retrospective design. Instead, more and larger prospective studies are still needed to clarify our concerns.

Importantly, several factors were found to be associated with the mPVR of the early callus. With age, the balance of bone metabolism between bone resorption and bone formation changes. Moreover, it is well known that the femur has a richer blood supply than that of

Table 3. The associations of modified pixel value ratio with healing index, lengthening index and external fixator index

Index	mPVR value		
	First month	Second month	Third month
Healing index	r=-0.345, P=0.029	r=-0.540, P<0.001	r=-0.071, P=0.616
Lengthening index	r=-0.321, P=0.043	r=-0.478, P=0.001	r=0.155, P=0.266
External fixator index	r=-0.425, P=0.006	r=-0.542, P<0.001	r=-0.262, P=0.058

mPVR: modified Pixel Value Ratio.

Table 4. The potential influencing factors for the modified pixel value ratio of early callus

Influencing factors	First month	Second month	Third month
Age (adult vs juvenile)	P=0.009	P=0.009	P=0.093
Sex (male vs female)	P=0.111	P=0.677	P=0.945
Lengthening site (tibia vs femur)	P=0.024	P=0.018	P=0.187
Total bilirubin (>17.1 umol/L vs <17.1 umol/L)	P=0.002	P=0.643	P=0.013
Mean hemoglobin content (<27 pg vs >27 pg)	P=0.006	P=0.004	P=0.831
ESR (>26 mm/h vs <26 mm/h)	P=0.179	P=0.070	P=0.424
Uric acid (>416 umol/L vs <416 umol/L)	P=0.822	P=0.324	P=0.155
White blood cell count (>9.5×10^9/L vs <9.5×10^9/L)	P=0.872	P=0.367	P=0.899
Fibrinogen (<2 g/L vs >2 g/L)	P=0.306	P=0.778	P=0.769
Monocyte count (>0.6×10^9/L vs <0.6×10^9/L)	P=0.931	P=0.601	P=0.549
Basophilic granulocyte count (>0.06×10^9/L vs <0.6×10^9/L)	P=0.227	P=0.451	P=0.301
Total protein (<65 g/L vs >65 g/L)	P=0.442	P=0.783	P=0.587

ESR: Erythrocyte Sedimentation Rate.

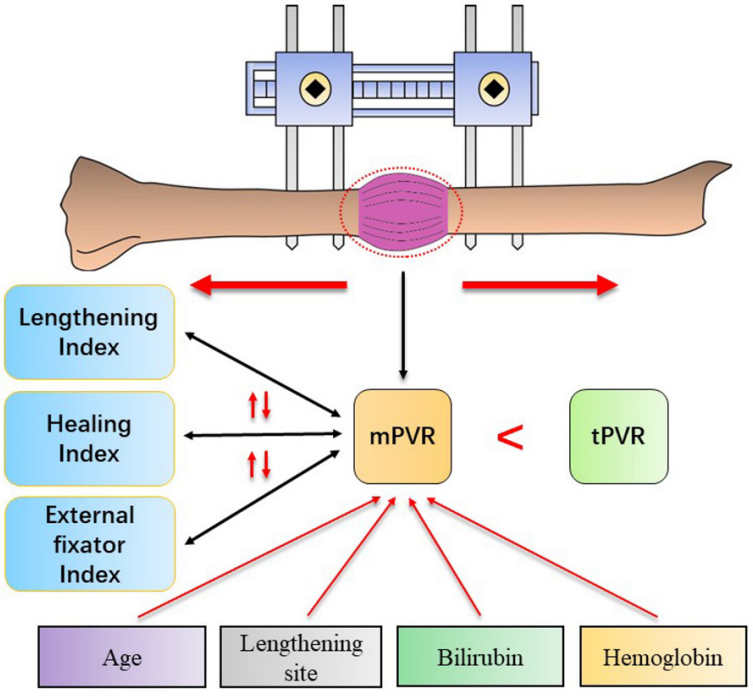

Figure 3. The schematic diagram for the results of this study. mPVR: modified Pixel Value Ratio; tPVR: traditional Pixel Value Ratio.

tibia [21, 22]. Consistently, the mPVR of early callus was associated with age and lengthening site. On the other hand, bilirubin has a negative impact on osteocytes by decreasing viability, differentiation and mineralization and increasing apoptosis [23]. In addition, Bian et al. demonstrated that total bilirubin was inversely associated with osteoporosis among postmenopausal females [24]. Moreover, hemoglobin was reported to be positively associated with bone mineral density [25], and negatively associated with fracture [25, 26], as well as osteoporosis [27, 28]. Indeed, the total bilirubin and mean hemoglobin content were also significantly associated with the mPVR of early cal-

lus in our results. Therefore, these factors may be considered in the assessment of the mPVR.

The strengths of this study can be listed as follow: First of all, it was the first study to report the mPVR based on contralateral normal bone. Second, our results indicated a potential correlation between the mPVR of early callus and the clinical outcome of bone lengthening. On the other hand, our study has several limitations. First, our study was restricted by retrospective study design, which precluded causal relationships. Second, due to the limited evidence available, the issue of bone nonunion could not be considered in this study. Third, the number of recruited patients was relatively small, especially in the analysis for the biochemical index.

In conclusion, mPVR was significantly lower than tPVR of the early callus, which may serve as a novel indicator for the clinical outcome of DO. Moreover, age, lengthening site, total bilirubin and mean hemoglobin content may be considered in the assessment of the mPVR (**Figure 3**). The mPVR is a novel reliable indicator for DO, which may be helpful for the clinical management of DO. However, the findings of this study need to be confirmed by further larger prospective research.

Acknowledgements

This study was supported by the National Natural Science Foundation of China (82-102581, 82270930), National Postdoctoral Science Foundation of China (2021M693562), Provincial Natural Science Foundation of Hunan (2019JJ40517, 2022JJ40843), Provincial Outstanding Postdoctoral Innovative Talents Program of Hunan (2021RC2020), Young Investigator Grant of Xiangya Hospital, Central South University (2020Q14), Fuqing Postdoc Program of Xiangya Hospital, Central South University (176) and Fund of Reform and Practice of Ideological and Political in Xiangya Hospital, Central South University (36, 40).

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Yi Zhang, Department of Orthopaedics, Xiangya Hospital, Central South University, Changsha 410008, Hunan, China. E-mail: zhangyi0205@csu.edu.cn

References

[1] Ilizarov GA. The tension-stress effect on the genesis and growth of tissues: part II. The influence of the rate and frequency of distraction. Clin Orthop Relat Res 1989; 263-285.

[2] Ilizarov GA. The tension-stress effect on the genesis and growth of tissues. Part I. The influence of stability of fixation and soft-tissue preservation. Clin Orthop Relat Res 1989; 249-281.

[3] Shchudlo N, Varsegova T, Stupina T, Shchudlo M, Saifutdinov M and Yemanov A. Benefits of Ilizarov automated bone distraction for nerves and articular cartilage in experimental leg lengthening. World J Orthop 2017; 8: 688-696.

[4] Malkova TA and Borzunov DY. International recognition of the Ilizarov bone reconstruction techniques: current practice and research (dedicated to 100(th) birthday of G. A. Ilizarov). World J Orthop 2021; 12: 515-533.

[5] Anand A, Feldman DS, Patel RJ, Lehman WB, van Bosse HJ, Badra MI and Sala DA. Interobserver and intraobserver reliability of radiographic evidence of bone healing at osteotomy sites. J Pediatr Orthop B 2006; 15: 271-272.

[6] Starr KA, Fillman R and Raney EM. Reliability of radiographic assessment of distraction osteogenesis site. J Pediatr Orthop 2004; 24: 26-29.

[7] Bafor A, Duncan ME and Iobst CA. Evaluating the utility of the pixel value ratio in the determination of time to full weight-bearing in patients undergoing intramedullary limb lengthening. Strategies Trauma Limb Reconstr 2020; 15: 74-78.

[8] Eyres KS, Bell MJ and Kanis JA. New bone formation during leg lengthening. Evaluated by dual energy X-ray absorptiometry. J Bone Joint Surg Br 1993; 75: 96-106.

[9] Maffulli N, Cheng JC, Sher A and Lam TP. Dual-energy X-ray absorptiometry predicts bone formation in lower limb callotasis lengthening. Ann R Coll Surg Engl 1997; 79: 250-256.

[10] Little DG, Cornell MS, Hile MS, Briody J, Cowell CT and Bilston L. Effect of pamidronate on distraction osteogenesis and fixator-related osteoporosis. Injury 2001; 32 Suppl 4: SD14-20.

[11] Eyres KS, Saleh M and Kanis JA. Effect of pulsed electromagnetic fields on bone formation and bone loss during limb lengthening. Bone 1996; 18: 505-509.

[12] Hazra S, Song H, Biswal S, Lee S, Lee S, Jang K and Modi H. Quantitative assessment of mineralization in distraction osteogenesis. Skeletal Radiol 2008; 37: 843-847.

[13] Veitch SW, Findlay SC, Hamer AJ, Blumsohn A, Eastell R and Ingle BM. Changes in bone mass and bone turnover following tibial shaft fracture. Osteoporos Int 2006; 17: 364-372.

[14] Vulcano E, Markowitz JS, Ali S, Nguyen J, Fragomen AT and Rozbruch SR. Assessment of bone healing during antegrade intramedullary rod femur lengthening using radiographic pixel density. J Am Acad Orthop Surg 2018; 26: e388-e394.
[15] Iobst CA, Mohammed W and Colley R. Determining when it is safe to remove the external fixator: results from a survey of the limb lengthening and reconstruction society. Orthopedics 2017; 40: e876-e879.
[16] Zhao L, Fan Q, Venkatesh KP, Park MS and Song HR. Objective guidelines for removing an external fixator after tibial lengthening using pixel value ratio: a pilot study. Clin Orthop Relat Res 2009; 467: 3321-3326.
[17] Wright SE, Goodier WD and Calder P. Regenerate deformity with the precice tibial nail. Strategies Trauma Limb Reconstr 2020; 15: 98-105.
[18] Koczewski P and Shadi M. Factors influencing bone regenerate healing in distraction osteogenesis. Ortop Traumatol Rehabil 2013; 15: 591-599.
[19] Song SH, Agashe M, Kim TY, Sinha S, Park YE, Kim SJ, Hong JH, Song SY and Song HR. Serial bone mineral density ratio measurement for fixator removal in tibia distraction osteogenesis and need of a supportive method using the pixel value ratio. J Pediatr Orthop B 2012; 21: 137-145.
[20] Liu Q, Mei H, Zhu G, Liu Z, Guo H, Wang M, Liang J and Zhang Y. Early pixel value ratios to assess bone healing during distraction osteogenesis. Front Bioeng Biotechnol 2022; 10: 929699.
[21] Kloss FR and Gassner R. Bone and aging: effects on the maxillofacial skeleton. Exp Gerontol 2006; 41: 123-129.
[22] Kizilkanat E, Boyan N, Ozsahin ET, Soames R and Oguz O. Location, number and clinical significance of nutrient foramina in human long bones. Ann Anat 2007; 189: 87-95.
[23] Ruiz-Gaspa S, Guanabens N, Jurado S, Combalia A, Peris P, Monegal A and Pares A. Bilirubin and bile acids in osteocytes and bone tissue. Potential role in the cholestatic-induced osteoporosis. Liver Int 2020; 40: 2767-2775.
[24] Bian LQ, Li RZ, Zhang ZY, Jin YJ, Kang HW, Fang ZZ, Park YS and Choi YH. Effects of total bilirubin on the prevalence of osteoporosis in postmenopausal women without potential liver disease. J Bone Miner Metab 2013; 31: 637-643.
[25] Chuang MH, Chuang TL, Koo M and Wang YF. Low hemoglobin is associated with low bone mineral density and high risk of bone fracture in male adults: a retrospective medical record review study. Am J Mens Health 2019; 13: 1557988319850378.
[26] Kim JS, Choi S, Lee G, Cho Y and Park SM. Association of hemoglobin level with fracture: a nationwide cohort study. J Bone Miner Metab 2021; 39: 833-842.
[27] Xiu S, Mu Z, Sun L, Zhao L and Fu J. Hemoglobin level and osteoporosis in Chinese elders with type 2 diabetes mellitus. Nutr Diabetes 2022; 12: 19.
[28] Kim SY, Yoo DM, Min C and Choi HG. Association between osteoporosis and low hemoglobin levels: a nested case-control study using a national health screening cohort. Int J Environ Res Public Health 2021; 18: 8598.