Quantitative analysis of distal femoral epiphysis blood supply in healthy infants with superb microvascular imaging: a pilot study

Abstract

Background/aim: To determine the blood supply of the distal femoral epiphysis (DFE) using superb microvascular imaging in newborns and infants, and to investigate the correlation with ossification center (OC) length, gender and age.

Materials and methods: A total of 140 cases were evaluated in this study. The cases were divided into 2 groups of less than 90 days and over than 90 days. Cartilage blood supply was measured with vascularity index (%) (VI).

Results: The mean OC length and median VI values were measured as 10.20±3.72 mm and 0.80% (0.58-1.50) for boys and 10.03±3.36 mm and 0.70 % (0.30-1.40) for girls, respectively. There was no significant difference in OC length and VI between genders. The mean OC length in Group II was significantly higher than in Group I (12.14±3.14 vs 8.09± 2.64) ($p < 0.001$). The median VI in Group I was higher than the cases in Group II (1.40% vs 0.40%) ($p<0.001$). There were positive correlations between age and OC length ($r=0.716$), negative correlations between age and VI ($r=-0.822$), and between VI and OC length ($r=-0.657$).

Conclusion: Quantitative reference values for DFE blood supply and OC length can guide the diagnosis and follow-up of many skeletal diseases.

Key words: Blood supply, distal femoral epiphysis, infant, newborn, superb microvascular imaging, ultrasound

1. Introduction

Superb microvascular imaging (SMI) is a new Doppler ultrasound (US) technique to detect fine vascular blood flow. SMI effectively separates flow signals from motion artifacts with a new intelligent algorithm. It provides high-quality vascular images not only in lesions with fine blood flow, but also in moving organs in non-sedated children. It has been used for various organs and diseases in the body in the last 5 years [1, 2]. Until recently, conventional color Doppler imaging and power Doppler imaging were widely used to demonstrate tissue vascularity. These methods are very useful for detecting the hypervascular blood flow of lesions such as malignant tumors, metastatic or inflammatory lymph nodes, or vascular malformations. However, these cannot be optimally applied in the assessment of small vessel or low velocity blood flow in young children. Since SMI is a technique developed recently, its application to children is not yet fully documented. However, SMI is a promising tool for the diagnosis and treatment planning of different musculoskeletal diseases [3, 4].

Many pediatric diseases are associated with skeletal retardation. There are many factors involved in bone maturation and differentiation. Hormonal factors, especially thyroid hormones, are very important [5]. Delayed bone maturation expressed by smaller distal femoral epiphysis (DFE) ossification center (OC) has been shown in babies with hypothyroidism. In addition, fractures and inflammatory-septic arthritis may be characterized by articular cartilage loss and may progress with some blood supply or morphological changes [6-8].

Skeletal maturation in infants is generally based on radiographic evaluation of DFE. In the last two decades, interest has grown in the use of musculoskeletal US to diagnose and monitor bone mineralization in young infants because US can depict both

cartilage and bone, making it possible to show even smaller ossification centers It eliminates fear of exposing newborns to radiation. The only ossification centers seen in mature newborns are DFE and proximal tibial epiphysis and these can be evaluated by US.

As far as we know, there is no study in the literature that examines the normal reference blood supply of DFE in newborns and infants with SMI. Determining the normal quantitative values for the vascularity of DFE can be a guide in the determination and treatment of many diseased conditions. Normal epiphysial OC size measurements were made in the intrauterine and early neonatal periods and it was reported that they could be used to determine the estimated gestational week. However, information on this subject is insufficient. There is a need for a study that details normal DFE and OC length reference values in newborns and infants born at term.

The aim of this study is to determine the normal values of DFE blood supply by SMI in postnatal healthy mature newborns and infants and to investigate the relationships between OC length, gender and age. In addition, reference values for DFE OC length will be determined by US.

2. Materials and Methods

2. 1. Study Subjects and Design

This cross-sectional study assessed 140 DFE in a total of 140 mature newborn and infant cases (age interval = 1-180 days [0-6 months]), comprising 70 boys and 70 girls, from June 2020 to September 2020. The cases were divided into 2 groups of less than 90 days and over than 90 days. The study was conducted with ethics approval from the ethics committee, and “informed consent” was obtained from the parents of all cases who accepted participation in the study before SMI investigation.

Among exclusion criteria were being premature, not showing sufficient cooperation, not accepting participation, maternal gestational diabetes, preeclampsia, or a history of difficult delivery, intensive care hospitalization history, hypothyroidism, developmental delay, fever or pain during radiological evaluation. As sufficient image quality and appropriate technique were provided for all cases included in the study, no case was excluded from the study. Since the epiphysis is almost completely calcified, blood supply cannot be evaluated clearly in cases older than 180 days, so these cases were not included in the study. After these were determined, healthy mature cases were included in the study. The radiologic measurements were completed with an experienced pediatric radiologist with more than 3 years of SMI experience.

2. 2. SMI and US Techniques

US and SMI measurements were completed using an Aplio 500 Platinum ultrasound device *(Toshiba Medical Systems, Co, Ltd, Otawara, Japan)* and high-frequency linear probe (frequency range= 5–14 MHz). Radiological examination was performed while the babies were in supine position, calm and motionless. If necessary, help was obtained from the parents to keep the leg stationary. The right leg was preferred when measuring because it is closer to the practitioner. SMI investigation used greater than 50-Hz frame rate. Pulse repetition frequency set was at 220 to 234 Hz for SMI. The color gain was set to 30 to 40 decibels to suppress background artifacts. SMI used color mode. In color mode, a region of interest (ROI) including the whole of the DFE was manually drawn at the epiphysis boundary. Within the ROI, the rate of color pixels in the whole area was automatically calculated by the device in percentages to obtain VI (Vascularity Index) values including arterial and venous total vascularity supply. This method was repeated for DFE with the “VI (percent)” separately measured

in SMI. **(Figure 1).** Transverse measurements of OC in the form of the hyperechoic focus in the center of the epiphysis in 'mm' were performed on ultrasound **(Figure 2).**

2. 3. Statistical Analysis

The fit of quantitative variables to normal distribution was examined by Kolmogorov-Smirnov test. Independent groups were compared with two independent samples t test for variables with normal distribution, and Mann Whitney U test for variables not showing normal distribution. The relationship between quantitative variables was analyzed using Spearman correlation analysis. Descriptive statistics of variables with normal distribution are shown as mean ± standard deviation, and descriptive statistics of non-normally distributed quantitative variables are shown as median (5th-95th percentiles). Descriptive statistics for quantitative variables are specified as frequency (n). Values of $p<0.05$ were considered statistically significant. In the statistical analysis of VI variable according to age groups, the power of the study was found to be 81.6% with a significance level (alpha) of 0.001.

3. Results

Descriptive statistics for age, OC length, VI variables for boys and girls and the comparison results of these variables by gender are given in **Table 1**. There is no statistical difference between the sex groups in terms of age, OC length and VI ($p> 0.05$).

Median age in all cases was 89.50 (7.05-170.00) days. Descriptive statistics for age, OC length and VI variables for cases under 90 days (n=70, Group I) and over 90 days (n = 70, Group II), and the comparison results of these variables according to age groups are given in **Table 2**. As a result of the analyses, it was determined that the age groups were significantly different from each other in terms of all variables. OC length

of cases in Group II were greater than individuals in Group I ($p<0.001$). VI values of Group I were significantly higher ($p <0.001$).

The correlation analysis findings between quantitative variables are given in **Table 3**. Accordingly, there is a positive, intermediate correlation ($r = 0.716$, $p <0.001$) between age and OC length, and a strong negative correlation between age and VI ($r = -0.822$, $p <0.001$). There is a strong negative correlation between age and VI ($r = -0.822$, $p <0.001$). The relationship between VI and OC is negative and moderate ($r = -0.657$, $p <0.001$) **(Figure 3).**

4. Discussion

Human long bones are subject to numerous changes in the uterus due to a number of pathways and cellular signaling mechanisms. The femur is a long bone that develops via endochondral ossification, cartilage is gradually replaced by bone at 8 weeks of gestation [9]. Located at both ends of the bone, the epiphysis (growth plate) is initially cartilage and later develops secondary ossification. The secondary physis provides spherical growth of the epiphyseal ossification center. Any disturbance in epi-metaphyseal development can cause a variety of skeletal abnormalities [10].

While traditional Doppler US methods (color Doppler imaging, power Doppler imaging and advanced dynamic flow) are used during blood flow assessment in young children, especially for the evaluation of respiratory fluctuating organs, the evaluation of minimal blood flow is limited by motion artifacts. Traditional methods often use a wall filter to remove motion artifacts, but this also results in a reduction of low velocity flow components. In contrast, SMI provides excellent blood flow images because it uses a new adaptive algorithm that effectively separates blood flow signals from underlying tissue motion artifacts. This algorithm preserves high resolution and high frame-rate,

low-speed blood flow components without compromising image quality, while only eliminating motion artifacts. SMI is particularly useful in evaluation of non-sedated children. In the pediatric age, SMI studies have been carried out on some organs such as testis, thyroid, and lymph nodes [11- 13]. Small vessels enter the DFE cartilage through the periosteal/subperiosteal regions. Although it is not documented in the literature that cartilage containing vascular structures can be imaged in SMI, we believe that SMI is expected to demonstrate more useful blood flow in evaluation of DFE.

Hip disorders are both highly prevalent and diverse among pediatric patients. Causes include congenital, developmental, infectious, inflammatory, traumatic, and neoplastic processes. Among the best known are developmental dysplasia of the hip, proximal femoral focal deficiency, Legg-Calvé-Perthes disease, toxic synovitis, septic arthritis, juvenile rheumatoid arthritis, slipped capital femoral epiphysis, and tumors [14]. Many of these diseases directly or indirectly affect cartilage, and moreover, cartilage blood supply. Therefore, the reference normal data we determined can be a guide in normal and abnormal diseases. In this study, normal quantitative data for DFE blood supply were obtained. In this respect, it is specific and we think that it provides new contributions to the literature. According to the results of our study, it was found that gender has no effect on VI values. As the age and age group increase, blood supply decreases significantly. Also, as OC length increases, VI decreases. This is an expected result because the epiphyseal cartilage matures and calcifies with the effect of mineral homeostasis, bone turnover, and growth. It is conceivable that cartilage calcification affects the microarchitecture and blood supply decreases.

Skeletal growth and maturation in children are dynamic processes. In this study, DFE OC size was measured by ultrasound in healthy newborns and infants born at term in the postnatal period. There was no statistically significant difference in OC values

between the genders. OC dimension increases with increasing age and age group. Similarly, Windschall D et al. evaluated postnatal skeletal development of the proximal femoral epiphysis, DFE, proximal tibial epiphysis in 178 premature and mature neonates between week 25-47 of biological age using ultrasound [15]. They found that the earliest onset of visible mineralization was at 30 week of maturity in the DFE, 31 week in the proximal tibial epiphysis and 43 week in the proximal femoral epiphysis. In preterm and term neonates, significant correlations were observed for transverse and longitudinal length of the DFE and proximal tibial epiphysis with biological age. Sex and side of measurement did not significantly influence the results.

DFE OC, which can be reliably identified and measured sonographically, can help predict third trimester fetal age. Between 28 and 35 weeks, the percentage of fetuses with DFE gradually increases. Although the mean age of OC appearance is about 32-33 weeks, it can be seen as early as 29 weeks [16]. However, a fetus without an identifiable OC is likely to be less than or equal to 34 weeks of age. Measurements of OC show that its size increases linearly; the age of a fetus whose OC is greater than or equal to 7 mm is most likely greater than or equal to 37 weeks [16]. Similarly, our study, which focuses on postnatal mature cases, shows a positive correlation with age and can be used to estimate age. In addition, it can be an auxiliary imaging method in the determination of diseased conditions affecting cartilage and OC. Birang S et al. measured OC size in 1300 normal single pregnancies at 28 to 40 weeks, for the effect of possible ethnic status in Iranian population [17]. Consistent with the literature, they stated that ultrasonographic imaging of OC and fetal third trimester gestational age is a useful indicator. There are studies that evaluate not only OC studies but also DFE cartilage [18- 20]. Paesano PL et al. reported that ultrasound is a valid alternative to standard radiography for the assessment of skeletal age; not only in fetuses, but also in

infants who are hypothyroid patients [19]. In that study, there were no cases in which a mineralized DFE was visible on X-ray but missed on US.

There are also studies in the literature examining the morphometric analysis of DFE ossification using magnetic resonance imaging (MRI). In a study by Jans LB et al. of 910 children aged 0.7-16.9 years, they stated that ossification variability in femoral condyles is common and should not be confused with abnormal processes [21]. Variability in femoral condyle ossification should be differentiated from osteochondral lesions and bone lesions such as infarction, especially in older children or patients receiving steroid therapy [22]. However, MRI requires sedation in newborns and young children and the examination takes a long time. Computed tomography (CT), which evaluates the bone structure in detail, involves high ionizing radiation and should not be used in children in this age group as much as possible. US and SMI can be used safely in these cases. In fact, it can be routinely added to examination of babies who require musculoskeletal ultrasound for various reasons. It can contribute to the radiological diagnosis of many diseases such as growth retardation, metabolic diseases, hypothyroidism, and skeletal dysplasia by addition to community screening protocols.

There are some limitations to our study. These include the limited number of cases. In selecting healthy children, blood tests that may identify systemic diseases, vitamin or mineral deficiency, and thyroid hormone disorder were not completed, which is another limitation. They were included according to normal history and physical and sonographic examinations. In addition, as measurements were made by a radiologist, intra- or interoperator reliability and repeatability were not evaluated.

In this study, normal quantitative reference data were obtained by measuring DFE blood flow with SMI and DFE OC length with ultrasound in healthy newborns and

infants born at term. OC length and VI values are independent of gender. As the age and age group increase, the OC length increases, and the blood supply decreases significantly. There is a negative moderate correlation between blood supply and OC length. These data can guide the diagnosis and follow-up of hormonal, congenital or acquired disease processes of the DFE.

Ethical statements: The study was approved by the Medical Ethics Committee of the Hospital, and all subjects provided written informed consent.

Conflict of Interest: The author(s) declare(s) that there is no conflict of interest.

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Figures

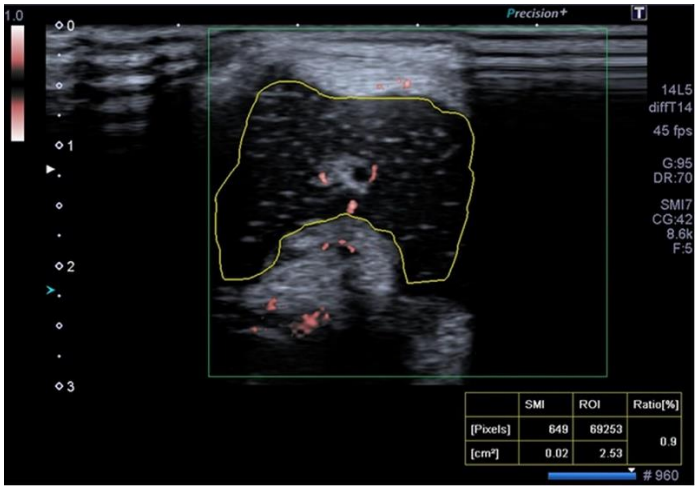

Figure 1. Measurement example of distal femoral epiphyseal blood supply using SMI in a 7-day-old baby girl. VI is obtained by manually drawing the borders of the cartilage tissue (ROI) and the ratio of the perfused tissue (colored parts) to the total area was 0.9% for this patient.

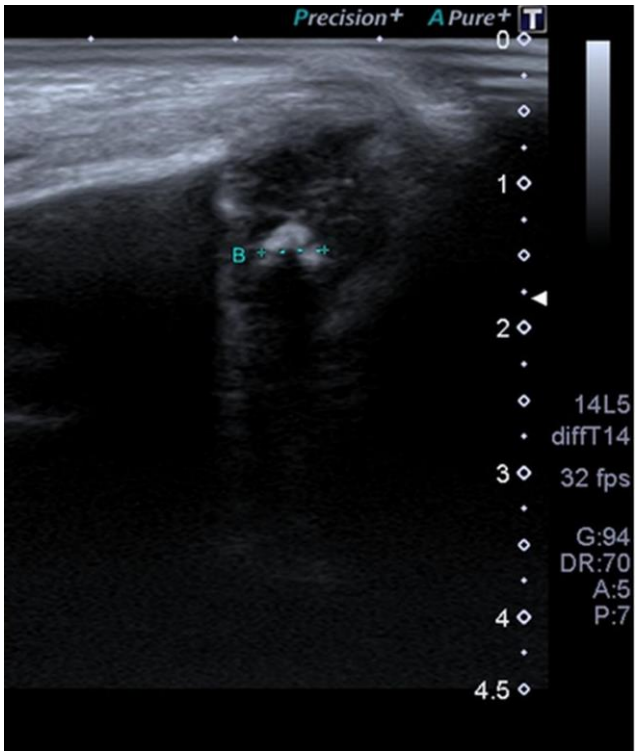

Figure 2. In the same case, measurement of the ossification center on gray scale ultrasound is shown. The measurement was obtained as transverse dimension.

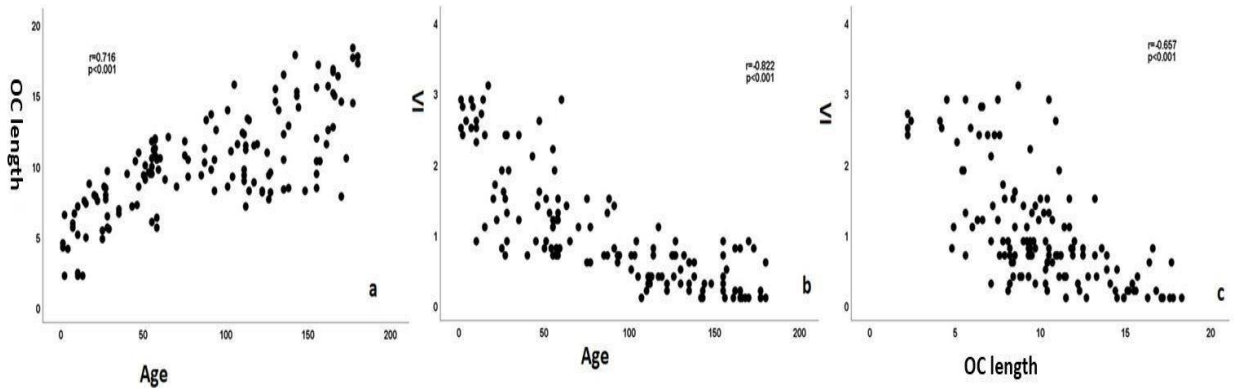

Figure 3. Relationship graphs between age with OC length (a), age with VI (b) and OC length with VI (c).

Tables

Table 1. Descriptive statistics for quantitative variables by gender and comparison results

Variables	**Gender**		**p**
	Boys (n = 70)	Girls (n = 70)	
Age (days)	84 (7.55-171.35)	90.50 (5.65-173.15)	0.636
Ossification center (mm)	10.20±3.72	10.03±3.36	0.781
Vascularity index (%)	0.80 (0.10-2.85)	0.70 (0.10-2.75)	0.235

Table 2. Descriptive statistics and comparison results of quantitative variables by age group

Variables	**Age Groups**		**p**
	Group I (n=70)	Group II (n=70)	
Age (days)	45.50 (2-85.90)	133.50 (93-177)	<0.001
Ossification center (mm)	8.09±2.64	12.14±3.14	<0.001
Vascularity index (%)	1.40 (0.70-2.90)	0.40 (0.10-0.90)	<0.001

Table 3. Correlation analysis results between quantitative variables

	Ossification center length (mm)	**Vascularity index (%)**
Age	r= 0.716	r= -0.822

Vascularity index	r= -0.657	

1