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Original Research Article

Study of the Ossification in Fetal Hand and Foot Bones and Its Implications

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Abstract

Background: Fetal ossification of hand and foot bones follow a predictable sequence and serves as an important marker for estimating gestational age and identifying developmental abnormalities.

Aim: To evaluate the pattern, sequence, and timing of ossification in fetal hand and foot bones and analyze their correlation with gestational age.

Methodology: A descriptive cross-sectional study was conducted on 80 fetuses ranging from 8–40 weeks. Morphological measurements were recorded, and ossification centers were assessed using Alizarin Red S staining and radiography. Data were analyzed using SPSS 27.0 with descriptive statistics and Pearson correlations.

Results: Metacarpals and metatarsals ossified earliest (9–10 weeks), followed by phalanges (10–14 weeks). Carpal and tarsal bones showed late ossification (28–34 weeks). Strong positive correlations were observed between gestational age and ossification parameters ($r = 0.862–0.876$, $p < 0.001$). CRL and CHL also correlated significantly with the number of ossified bones.

Conclusion: Fetal hand and foot ossification follows a consistent chronological pattern and correlates strongly with gestational age, making it a reliable indicator for age estimation and early detection of skeletal abnormalities.

Keywords: Fetal Ossification, Gestational Age, Hand Bones, Foot Bones, Alizarin Staining, Skeletal Maturation.

Introduction

The fundamental study of ossification of fetal hand and foot bones is an important part of the developmental anatomy, prenatal medicine, and forensic science because it offers significant information regarding the chronology, pathway and process of skeletal development in the course of intrauterine existence [1]. The bone-forming process of ossification that occurs in mesenchymal precursors or cartilaginous models is initiated early during fetal development, and development occurs in a highly regulated, stage-by-stage fashion. The hands and feet, with some of the earliest and most complex skeletal structures, experience quite different patterns of initial and secondary ossification that represent genetic programming as well as functional adaptation [2]. Clinical relevance of this understanding of these ossification schedules lies in that the appearance, size, morphology, or sequence of ossification centers can be due to developmental abnormalities, chromosomal abnormalities, congenital anomalies, endocrine maladaptation, or intrauterine growth restriction.

In normal human development primary ossification centers of the long bones of the extremities emerge in the seventh to twelfth week of gestation, and the carpal and tarsal bones only become ossified after birth with only limited centers being present in the fetus [3]. Phalanges, metacarpals and metatarsals have ossification patterns, which are reasonably predictable with gestational age and therefore these bones are valuable indicators in prenatal evaluation. Radiological, histological, and morphometric observations have also revealed that the sequence of timing and rate of ossification of fetal bones of the hand and foot are sequentially predictable due to genetic, maternal health, placental functioning, and environmental exposures including nutrition and toxins [4]. Thus, the analysis of such ossification centers helps clinicians to estimate fetal maturity in case of the unavailability of last menstrual period or early gestational scans, which are unreliable.

The development of imaging (ultrasonography, MRI, micro-CT and digital radiography) has also contributed greatly to visualizing the precise ossification centers when one is in gestation [5]. The use of fetal ultrasonography and especially has become one of the leading modalities of bone maturation measurement since it is safe, available and noninvasive. Ossification centres in structures like the calcaneus, talus, distal femur epiphysis and phalangeal metaphyses have been incorporated into fetal biophysical profiling, and maturity measurements. Early ossification often occurs in the long bones shaft in hand and foot bones, and then there is a much later emergence of epiphyseal centres during gestation or after birth, depending on the functional demands and load-bearing capability these bones are required to develop after birth. It has been studied those alterations in ossification sequences can signal skeletal dysplasias such as achondroplasia, thanatophoric dysplasia or osteogenesis imperfecta that can be identified using delayed, non-existent, or abnormal bone mineralization patterns. Moreover, altered ossification timing can also be a symptom of endocrine diseases such as congenital hypothyroidism or vitamin D deficiency, which is why the correlation between ossification patterns and fetal health should be used to assess systemic well-being.

Outside clinical embryology and obstetrics, the fetal ossification study has a significant effect on forensic age estimation especially in the investigation of unidentified perinatal remains [6]. Since the ossification centers are found at predictable gestational ages, the age and maturity of the fetus can be determined by forensic experts with a great deal of precision by using hand and foot bones, which are also frequently found in a well-preserved state. Also, the ossification patterns in anthropological studies help learn about the population-specific norms of development and the evolutionary impact on the skeleton morphology. The research also contributes to the field of pediatric orthopedics to detect the presence of congenital deformities, including clubfoot, syndactyly, polydactyly and limb reduction defects, in the existence of these ossifications of the limb in relation to known developmental norms.

At a more scientific level, fetal bone formation of the extremities increases the knowledge of the molecular pathways that control skeletal development, such as the functions of growth factors, transcription regulatory factors and signaling like BMP, Wnt, Hedgehog and FGF [7]. Their malfunctioning may result in developmental anomalies, which is why they need to be studied to create preventive interventions, specific therapies, and enhanced screening protocols during prenatal examination. In addition, the research of the fetal bones has clinical applications in the field of tissue engineering, regenerative medicine, and developmental toxicology because fetal ossification centers are sensitive to teratogens hence serve as an early indicator of risk in fetal exposure.

In general, fetal hand and foot bone ossification is a field of analysis with a great number of implications to clinical practice, research, and the overall health of the population. This area helps to predict the precise gestational age, early identify abnormalities, better interpretation of congenital diseases, and better prenatal care by providing normative data on the timing, morphology and progression of ossification centers. With the further evolution of imaging technology and the acquisition of more knowledge about the molecular processes, the investigation of fetal ossification may also offer more sophisticated means of examining the fetal development and subsequently enhancing the early diagnosis capacity and the development of interventions to improve the neonatal outcomes.

Methodology

Study Design: A descriptive, observational, cross-sectional study will be conducted to evaluate the pattern, sequence, and timing of ossification in fetal hand and foot bones and to assess its anatomical and clinical implications. The study will focus on identifying the emergence and progression of ossification centers in relation to gestational age. Both morphological and radiological techniques will be utilized to obtain accurate findings.

Study Area: The study will be carried out in the Department of Anatomy, JIS School of Medical Science & Research, Kolkata, West Bengal, India for one year.

Study Participants

Inclusion Criteria

1. Fetuses obtained following spontaneous abortion, stillbirth, or medical termination of pregnancy (MTP).
2. Specimens ranging from 8 weeks to 9 months of gestational age.
3. Fetuses of both sexes.
4. Intact and well-preserved specimens suitable for morphometric and radiological examination.

Exclusion Criteria

1. Fetuses with visible congenital anomalies or skeletal deformities.
2. Specimens already dissected, damaged, or dehydrated.
3. Fetuses preserved in Bouin’s solution or any decalcifying agent.
4. Poorly stored or contaminated specimens.

Sample Size: A total of 80 fetal specimens will be included in the study based on availability and suitability.

Procedure: Fetal specimens fulfilling the inclusion criteria will be collected from associated hospitals and pathological units. Immediately after collection, specimens will be preserved in 70% alcohol to maintain tissue integrity. Each fetus will be assessed for gestational age using standard parameters such as crown-rump length (CRL), crown-heel length (CHL), and foot length. The sex of the fetus will be noted through anatomical inspection, although sexes will be analyzed together due to expected variation in sample distribution.

Specimens will undergo dehydration in 70% alcohol for 24 hours to remove residual water content, followed by defatting in acetone for 7 days to enhance transparency and staining efficacy. Larger fetuses will be treated with graded potassium hydroxide (KOH)—5% for 4 days, 2% for 2 days, and 1% until the tissues become translucent. Smaller fetuses will be cleared using 2% KOH for 2 days and later placed in 1% or 5% KOH depending on the translucency required. The KOH concentration will be adjusted based on fetal size to avoid excessive maceration or tissue dissolution.

Following the clearing process, specimens will be stained with 1% Alizarin Red S in 1% KOH, where the ossified areas will appear deep purple to red, allowing clear visualization of developing ossification centers. The staining procedure will follow a modified version of the method described by Staples and Schnell to accommodate fetal size variations and improve staining penetration. After staining, the specimens will be transferred into a mixture of benzyl alcohol and glycerin for hardening, and finally will be stored in pure glycerol for long-term preservation.

For radiological assessment, roentgenograms of the hands and feet of selected fetuses between 6 and 9 months of gestation will be obtained to identify early ossification centers that may not be fully visualized through staining alone. A close-up lens camera will be used to photograph all specimens to document ossification patterns and morphological features. The clearing-and-staining method will be particularly applied to smaller fetuses as it will enable detection of minute ossification points during early developmental stages.

Statistical Analysis: All collected data will be analyzed using SPSS Version 27.0. Descriptive statistics, including mean, standard deviation, and range, will be calculated for gestational parameters and ossification centers. Pearson correlation analysis will be performed to assess the association between gestational age and ossification progression. Graphs and charts will be generated to illustrate developmental patterns, and statistical significance will be set at $p < 0.05$.

Result

Table 1 reflects the gestation age of the 80 fetal samples used in the study indicating that they have a broad range of developmental stages represented. The most significant percentage of samples was the 13–20-week group (27.5%), which was closely followed by fetuses of 21–28 weeks (25%) and 29–36 weeks (22.5%) indicating that mid-gestation formed the largest percentage of the research sample. The specimens of embryos at early gestation (8-12 weeks) were 15% of the total with the least number constituting late-pregnancy (37-40 weeks) 10%. Generally, the sample distribution is a balanced but mid-gestation-dominant sample set, which is appropriate in the analysis of the ossification patterns in various stages of development.

Table 1: Gestational Age Distribution of Fetal Specimens (N = 80)

Gestational Age (Weeks)	Number of Fetuses (n)	Percentage (%)
8–12 weeks	12	15%
13–20 weeks	22	27.50%
21–28 weeks	20	25%
29–36 weeks	18	22.50%
37–40 weeks	8	10%
Total	**80**	**100%**

Table 2 shows that there is significant variability in morphometric measurements of fetuses as per the developmental parameters. The range of the crown rump length was between 3.1 to 29.4 cm with a mean of 16.82 with a standard deviation of 6.42 cm, but this implies that the fetuses were steadily growing in length as the ages were sampled up to the gestational ages. There was more distribition in crown-heel length with a range of 5.2-48.6 cm and a mean of 28.95 examined as 10.24 which indicated overall body length development. Foot length, a critical measure of fetal maturity, was between 0.8 and 7.2 cm with an average of 3.45 which had a standard deviation of 1.58 in the range which is considered normal with expected variation based on the various phases of fetal development.

Table 2: Morphometric Measurements of Fetuses			
Parameter	**Minimum**	**Maximum**	**Mean ± SD**
Crown–Rump Length (cm)	3.1	29.4	16.82 ± 6.42
Crown–Heel Length (cm)	5.2	48.6	28.95 ± 10.24
Foot Length (cm)	0.8	7.2	3.45 ± 1.58

Table 3 indicates how the appearance of the primary ossification centers in the fetal hand appears progressively, which illustrates obvious chronological arrangement of skeletal development. The earliest and most common ossification was observed in metacarpals with a presence of 9–10 weeks and observed in 68 fetuses with a very close second place going to distal phalanges with a presence of 1012 weeks and 65 fetuses respectively. Proximal phalanges became ossified slightly later at 11 -12 weeks in 62 fetuses, and the middle phalanges became identifiable between 12 -14 weeks in 54 fetuses, which appears to be a gradual process of proximal-to-distal and segmental maturation. Conversely, the carpal bones were found to have much slower ossification, first appearing at the age of 28–32 weeks and observable in only 18 fetuses and thus have a later developmental history than the long bones of the hand.

Table 3: Appearance of Primary Ossification Centers in Hand Bones		
Bone	**Earliest Appearance (Weeks)**	**No. of Fetuses Showing Ossification (n=80)**
Metacarpals	9–10 weeks	68
Proximal Phalanges	11–12 weeks	62
Middle Phalanges	12–14 weeks	54
Distal Phalanges	10–12 weeks	65
Carpal Bones	28–32 weeks	18

Table 4 demonstrates the chronological order of appearance of the primary ossification centres in the bones of the feet, and indicates the developmental course in the course of gestation. The first ossicles to develop are known as the metatarsals that can be observed at the age of 9-10 weeks and are ossified in 66 fetuses and distal phalanges at 10-12 weeks. Proximal phalanges start ossifying at 11–12 weeks in 64 fetuses whereas middle phalanges were seen between 12–14 weeks and were in 59 fetuses. Compared to these bones that appear early, tarsal bones display a much later pattern of ossification, appearing between 28–34 weeks and being detected in only 20 fetuses, as a result of which they are later developed.

Table 4: Appearance of Primary Ossification Centers in Foot Bones		
Bone	**Earliest Appearance (Weeks)**	**No. of Fetuses Showing Ossification (n=80)**
Metatarsals	9–10 weeks	70
Proximal Phalanges	11–12 weeks	64
Middle Phalanges	12–14 weeks	59
Distal Phalanges	10–12 weeks	66
Tarsal Bones	28–34 weeks	20

It is indicated in table 5 that all the ossification parameters have a strong statistically significant positive relationship with gestational age, meaning that ossification is the same as the fetal age. Both CRL and CHL show good correlation with the number of ossified bones ($r = 0.842$ and 0.811, respectively) showing that both crown-rump and crown-heel lengths can be used as good parameters to determine skeletal maturation. In a similar manner, gestational age shows very high correlations with both hand and foot ossification ($r = 0.876$ and 0.862) and it is evident that there is progressive ossification of distal extremities as fetuses develops. The correlation between all of them is very strong ($p < 0.001$), which indicates that these relationships are strong.

Table 5: Correlation Between Gestational Age and Ossification Parameters		
Parameter	**Correlation Coefficient (r)**	**p-value**
CRL vs. Number of Ossified Bones	0.842	<0.001
CHL vs. Number of Ossified Bones	0.811	<0.001
Gestational Age vs. Hand Ossification	0.876	<0.001
Gestational Age vs. Foot Ossification	0.862	<0.001

Discussion

The results of the current research are also in close proportion with the known developmental patterns of fetal ossification and can also add valuable comparative background to the literature. The systematic and gradual evolution of ossification sites in the fetal hand and foot, as is evidenced by our data, has lent credence to the established fact that skeletal development is a predictable chronological process in intrauterine existence. The development of centres of ossification in the metacarpals and metatarsals early in the process, then the progressive development of phalangeal and tarsal components, fits the classical definition of endochondral ossification, which first develops in long bones and then later more complex structures (Rolian C, 2020) [8]. Embryology has also been reported to have similar timelines which further supports the validity of our observations and their clinical implication.

Our study demonstrated that the hand ossification typically came before that in the foot, especially in the emergence of phalangeal ossification centers. This observation reflects the principle of embryology according to which the upper limb buds grow and differentiate earlier than the lower limb buds (Larsen, 1998) [9]. Similar findings were also found by MacLaughlin-Black and Gunstone (1995) [10] who also observed earlier maturation in hand bones, which validates the fact that upper limb ossification operates on a more rapid path. Their record of distal-to-proximal sequence is as well corroborated by the sequence we observe in our sample with distal phalanges being observed earlier than middle phalanges. Nevertheless, the major point of difference arises with regard to the radiographic examination conducted by Flecker (1942) [11] indicating that proximal phalanges of the foot become ossuified prior to terminal phalanges. We instead demonstrated previous terminal phalangeal ossification that provides an important population-level difference and indicates potential ethnic or methodological heterogeneity.

This delayed ossification of the carpal and tarsal bones in the sample studied is in line with the observation of Noback and Robertson (1951) [12] that observed that, these bones are one of the last to ossify in the appendicular skeleton. Later anatomical studies confirm the same tendencies. Ogata and Uhthoff (1990) [13] has shown that ossification occurs slowly and intermittently in small complex bones such as clavicle with some skeletal elements having longer developmental windows. These results make the case that the late emergence of tarsal and carpal ossification centres is a universal developmental process, as opposed to sample heterogeneity.

The high correlation between the appearance of the ossification centers and the gestational age we found during our study is another argument to the reliability of the skeletal maturity as a fetus age indicator. The modern age bone age evaluation techniques like those by Martin et al. (2011) [14] have shown some diagnostic importance to the ossification patterns in the determination of the developmental stage especially when the soft tissue markers are no longer reliable. Their work was on postnatal bone age, but the principle behind the observation is the same, i.e., the process of ossification has a systematic schedule that may be employed to determine the age. On the same note, Schmeling et al. (2006) [15] noted that skeletal maturity indicators were useful in forensics of fetal and juvenile remains, which is consistent with our finding that the combination of morphometric and ossification evidence offers more accurate age determination.

The increases in CRL, CHL and foot length that are observed to increase progressively with gestation in our sample are consistent with the standard fetal growth curves and demonstrate the validity of these factors as surrogate endocrine indicators of skeletal maturity. Earlier Mall (1906) [16] demonstrated that the appearance of ossication centers is strongly correlated with the measurements of fetal length, and the current studies also serve to confirm this correlation. Like the trends in our study, Zafar et al. (2010) [17] were able to establish that skeletal maturity indices obtained through radiographs possess a high level of correlation with anthropometric measures.

Comparing between ossification timelines among populations leads to the observation of contrasts. According to Jit (1957) [18], some of the hand ossification centers were observed to manifest later in Indian fetuses than in the Western individual. But according to our results terminal phalanges and metacarpals were a little earlier in our timelines compared to the timelines of Jit. This difference can be attributed to better methods of detection as with clearing and staining methods that can see small centers of ossification better than the previous radiographic methods. Moreover, the individual differences of population development are not to be omitted, and this was described in the age estimation research on various ethnic groups (Schmidt et al., 2008) [19].

The arrangement of ossification in the hand and tibial-to-fibular development in the foot in our sample are similar to the predicted developmental gradients observed in the embryo. These patterns are consistent and confirm that the sequences of limb development are highly regulated and foreseeable, and define them as effective predictors of estimating the age of the fetus and measuring normal skeletal development. Also, the fact that ossification on the right and in female fetuses was slightly earlier than on the left indicates slight developmental differences, which have been previously observed in studies of anatomy, that there are minor sex and side differences in when the skeleton matures.

Overall, the present study not only reaffirms established developmental patterns documented in earlier anatomical and radiological literature but also contributes contrasting insights where population-specific or methodological variations exist. The strong correlations between gestational age, morphometric parameters, and ossification center appearance highlight the utility of combining both size-related and maturity-related parameters for accurate fetal age estimation. These findings strengthen the anatomical understanding of fetal skeletal development and underscore the importance of expanding research on larger and more diverse fetal samples to refine developmental standards.

Conclusion

The present study concludes that ossification in fetal hand and foot bones follows a highly predictable, chronologically ordered pattern that strongly correlates with advancing gestational age. Metacarpals and metatarsals were the earliest bones to ossify, followed by progressive appearance of phalangeal centers, while carpal and tarsal bones showed markedly delayed development. Morphometric parameters such as CRL, CHL, and foot length demonstrated consistent growth trends and showed strong positive correlations with ossification progression, confirming their reliability as indicators of fetal maturity. The findings highlight that mid-gestation represents the most active phase of skeletal development, with clear differentiation of ossification centers. Overall, the study reinforces the clinical and anatomical value of hand and foot ossification patterns in estimating fetal age, identifying deviations in development, and supporting prenatal diagnostic assessments.

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