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Original Research Article

Pattern of Major Sites of Ossification in Foetal Hand and Foot Bones: Implications for Estimating Foetal Maturity

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ABSTRACT

Background: The estimation of the foetal age at death is challenging in many ways. Therefore, determining foetal maturity is frequently a more plausible solution to the dilemma of foetal viability. In an endeavor to create a maturity spectrum, this research aims at the pattern of major sites of ossification in the foetal hand and foot bones.

Materials and Methods: The onset of main ossification centers in hand and foot bones was examined in 48 foetuses between 8 weeks and 9 months gestational age. Larger foetuses of 6 to 9 months of gestation (31.0 to 45.0 CMS CH lengths) were analyzed by roentgenography and by clearing procedure. Smaller foetuses of 20 to 205 mm CR length were cleared in potassium hydroxide, stained with Alizarin Red S, and examined for the appearance of ossification centers in the bones of the hand and foot.

Results: In this investigation, the centers were first formed in the hand bones before the foot bones, on the right side before the left, and in females before males. Ossification spreads from the radial to the ulnar side of the hand and from the tibial to the fibular side of the foot.

Conclusion: Relying on both size related and maturity related information will only boost the chance of getting close to genuine age when attempting to determine the age of foetal remains. Consequently, it is advised to use as much information as possible rather than taking a simple strategy, such as depending just on body size.

KEYWORDS: Alizarin Red S, clearing, defatting, dehydration, KOH, ossification.

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INTRODUCTION

A base line biological profile, which includes the person's sex, age of death, stature, and ethnic affiliation, must be created in forensic investigations as the first step toward identifying the deceased. Practically, soft tissue preservation is good only when the death occurred recently, which suggests that a fully accurate biological profile may be obtained. The rate of decomposition normally rises with the period after death, and as the remains approach skeletonization, the biological profile's accuracy declines. Whether the remains are those of an adult, child, or foetus, the accuracy and precision of this profile are essential to the outcome of any inquiry. The biological profile has a distinct role in the case of foetal remains because it serves purposes other than personal identification. The biological factors, for instance, may be used to determine foetal viability, which may be significant in later legal concerns [1].

Early in the embryonic stage, bone formation begins; however, it is not completed until puberty [2]. The majority of bones form through a process called endochondral ossification. With this sort of ossification, minerals, and collagen are deposited on a cartilaginous model to form bone. The time it takes for bones to develop varies. The primary ossification center, which often forms before birth, is where bones first begin to form on a background of cartilage; the secondary ossification center, on the other hand, refers to the bony area that arises after birth [2].

Eight short bones called carpal bones are located at the wrist in two rows, each containing four bones. Whereas seven bones in the tarsal are arranged in three rows. In hand, the scaphoid, lunate, triquetral, and pisiform bones make up the proximal row, from lateral to medial side, while the trapezium, trapezoid, capitate, and hamate bones make up the distal row [3]. These bones ossify in cartilage during the second month of intrauterine life at the wrist [4]. The pisiform cartilage is the last to develop, while the capitate cartilage is the first. The carpals are still cartilaginous before birth because the ossification of these carpal cartilages is not initiated until birth. Skeletal age assessment is frequently used to determine a person's real age because it represents their level of maturity. In addition to tooth eruption, the development of the wrist bones is particularly significant for determining age in early children [5]. Carpal bones can be used to estimate one's skeletal age and assess their growth. This is of utmost significance for pediatricians when it comes to detecting metabolic or endocrine diseases. In cases when a birth certificate has not been issued, it is required to estimate the bone age medicolegally [6,7]. The carpal bones mature in a precise fashion, according to classical anatomy textbooks. During the first year of life, the capitate and hamate bones are the first to ossify. The lunate then makes its appearance in the fourth year of life, followed by the triquetral in the third. In the fifth year, the scaphoid forms, and in the sixth year, the trapezium and trapezoid emerge. The pisiform at the 12th year of life is the last carpal bone to appear [8]. Whereas some other books describe that pisiform bone ossifies in the ninth year of life [3].

The ossification of the carpal bones and other hand bones often starts earlier in females than in males. Populations vary in terms of the timing of the emergence of ossification centers in various hand and wrist bones. For various demographic groups, including American Caucasians [9], Indians [10-12], Pakistanis [13], Nigerians [14], Saudi Arabians [15], Sudanese [16], and Western Australians [17], the precise timing of the development of bones is documented in the literature.

In the foetal foot, talar cartilage is first identified at 7 weeks [18]. Talus ossifies from a single ossification center that appears at 6 months of intrauterine life; calcaneus is the only tarsal bone that always has two ossification centers. Along with the main ossification center, a scale like posterior apophysis covers most of the posterior surface and part of the plantar aspect. The primary center appears prenatally in the third month, whereas the posterior apophysis appears in the sixth year in females and the eighth year in males [3].

The navicular ossification center appears during the third year, whereas the cuboid frequently begins to ossify before birth, and the primary ossification center appears just before birth. The medial cuneiform may have two separate ossification centers, which appear during the second year of life. The ossification center for intermediate cuniform appears during the third year of life, and the lateral cuneiform ossifies during the first year of life. The first metatarsal has two centers of ossification, one in the shaft and the other in the base (unlike the other metatarsals, in which the secondary ossification center is distal). They appear during the 10th week of prenatal life and the third year of life, respectively, and fuse between the 17th and 20th years. The first metatarsal head may have a third center [3].

For the first, second, third, and fourth metatarsals, there are two centers of ossification, one in the shaft and one distally in the metatarsal head. Ossification of the shaft starts during the ninth prenatal week. Whereas for the fifth metatarsal bone, there are three centers of ossification: one at the base in the region of the tuberosity (an apophysis), one in the shaft, and one distally in the metatarsal head. Ossification of the shaft starts during the 10th prenatal week [3]. Phalanges are ossified from a primary center for the shaft and a basal epiphysis. Primary centers for the distal phalanges appear between the ninth and 12th prenatal weeks and somewhat later in the fifth digit. Primary centers for the proximal phalanges appear between the 11th and 15th weeks and later for the middle phalanges, but there is a wide variation. Basal centers appear between the second and eighth years (usually second or third in the great toe), and union with the shaft occurs by the 18th year. There is considerable variation in ossification and fusion dates [3].

In addition, some authorities described that ossification begins in the talus between 25 and 31 weeks gestation, in the calcaneus between 22 and 25 weeks gestation, in the cuboid bone between 37 weeks gestation and 3 months after birth, and in the navicular bone between 2 and 5 years [19].

Estimating the gestational age of the foetus has significant medical and legal implications(20). It is crucial to the scientific examination of crimes against foetuses such as foeticide and illegal abortion. It must be established that the infant was born alive and had a distinct life from the mother, that it was capable of a separate existence (i.e., more than 28 weeks gestation), and that a deliberate act of commission or omission resulted in its death [21].

The purpose of the present study is to determine when ossification centers first arise in both genders' hand and foot bones, both sides and the spread of ossification of embryos and foetuses. This was done by observing when these centers first appeared during the prenatal period.

MATERIALS AND METHODS

Ethical approval: The SV Medical College, Tirupati, India, granted the institutional ethics committee permission for this study (reference number: SVMC/Institutional Ethical Committee/161/SRC/2013, Dated 05/12/2013). All the parents of foetuses signed a written waiver of informed consent.

The embryos and foetuses, either aborted or after Medical Termination of Pregnancy ranging from 8 weeks to 9 months gestational age and of both sexes, were collected in 70% alcohol. They were procured from various hospitals in South India. Each foetus was visually inspected to determine its sex; however, due to the very limited sample size, the sexes were merged for calculation. Foetuses that were dissected, injured, dehydrated, abnormal, or kept in Bouin's solution that decalcifies were excluded from this study. The ultimate sample size, therefore, was 48 foetuses. The specimens' sex, crown rump length (CRL), and crown heel length (CHL) were recorded. Dehydration in 70% alcohol over 24 hours and defatting in acetone for 7 days were applied to 40 specimens of CRL ranging in size from 20 mm to 205 mm. The larger foetuses were cleaned in 5% KOH for 4 days, 2% for 2 days, and 1% until translucent. Less developed embryos were held in 2% KOH for 2 days and then in 1% or 5% until they

became translucent. To prevent excessive maceration and specimen dissolution, the concentration of the KOH solution depends on the size of the specimen. Careful inspection is thus required. The ossified regions of the cleaned specimens coloured purple after being stained with 1% alizarin red S in 1% KOH solution. We changed the approach outlined by Staples and Schnell for processing the specimens [22].

To check for the existence of ossification centers in the carpal and tarsal bones, roentgenograms of the hands and feet of eight foetuses between 6 and 9 months of gestation were collected. These samples were subsequently cleaned and stained as previously mentioned. After staining, all the specimens were placed in a solution of benzyl alcohol and glycerine to harden before being kept in pure glycerol. The close up lens was used to take photos of the specimens. The clearing and staining technique was favoured for smaller foetuses because it may detect small patches of ossification at an early stage of growth [23].

OBSERVATIONS

Forty specimens of 20 - 205 mm CRL of both sexes [Table 1], which were subjected to clearing and staining, were observed for the presence of ossification centers in the various bones of hands and feet [Table 2]. In a 20 mm CRL (seventh week) male foetus, two centers for clavicle and one center for mandible were observed on each side. The limb bones showed no ossification center [Figure 1]. Even at 25 mm CRL (eighth week), the hand and foot bones contained no ossification center in either sex.

The spread of ossification in hand bones was observed from the radial to the ulnar side, and in the foot bones, it was observed from the tibial to the fibular side. The centers for carpal and tarsal bones were not present until 6 months except in one female foetus of 107 mm CRL (16th week) where the capitate, hamate, and triquetrum on both sides, trapezium, and trapezoid on the left side [Figure 2], ossification center for metacarpal appeared ninth week (36 mm CRL and 45 mm CHL) foetus, proximal phalanges in the 10th week (39 mm CRL and 50 mm CHL), middle phalanges in the 14th week (90 mm CRL and 125 mm CHL), and terminal phalanges in the ninth week (36 mm CRL and 45 mm CHL). Hence, the ossification center for terminal phalanges appears earlier than proximal phalanges, followed by middle phalanges.

The calcaneus and talus on both sides and navicular and cuboid on the right side showed centers of ossification in 107 mm CRL foetus (16th week) [Figure 3]. Metatarsals ossification center appeared in 12th week (70 mm CRL and 90 105 mm CHL), proximal phalanges in 14th week (90 mm CRL and 125 mm CHL), middle phalanges in 14th week (95 mm CRL and 135 mm CHL), and terminal phalanges in 10th week (45 mm CRL and 70 mm CHL). In female foetuses, the centers were observed earlier than in male foetuses. Hand bones were ossified earlier than foot bones. Ossification started earlier in the right side bones than in the left side bones. Roentgenographs of the hands and feet of eight foetuses of 6 - 9 months show no centers for carpal bones. Centers for calcaneus, talus, and cuboid were observed in one male and two female foetuses at 9 months gestational age.

Table 1: Gestational age, sex, size, and number of specimens observed.

Specimens Observed					
Gestational age in weeks	Size of foetus in mm		Sex and number of specimens		Total number
	CRL	CHL	Males	Females	
7	20	-	1	-	1
8	25	30-40	2	4	6
9	36	45	-	1	1
10	39	50	-	1	1
10	45	70	1	-	2
12	70	90-105	1	1	2
13	77	100	2	-	1
13	81	125	1	-	1
14	90	125	-	1	1
14	95	135	2	1	3
15	99	138	-	1	1
15	105	150	-	1	1
16	107	161	-	2	2
16	110	155-180	1	1	2
17	125	190	1	-	1
18	140	208	1	-	1
18	145	212	2	2	4
20	172	202-216	4	4	8
22	205	310	1	-	1
24-36	206-310	310-450	3	5	8
Total			23	25	48

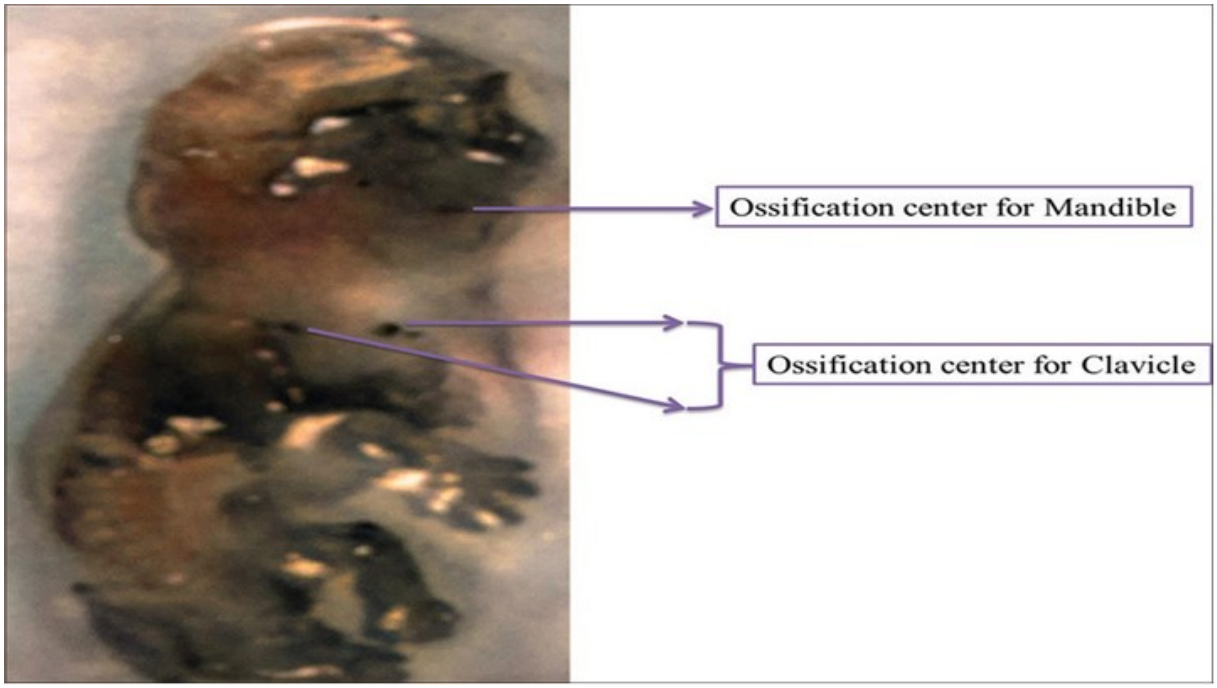

Fig. 1: Representative photograph of Alizarin Red S stained 20 mm CRL male foetus.

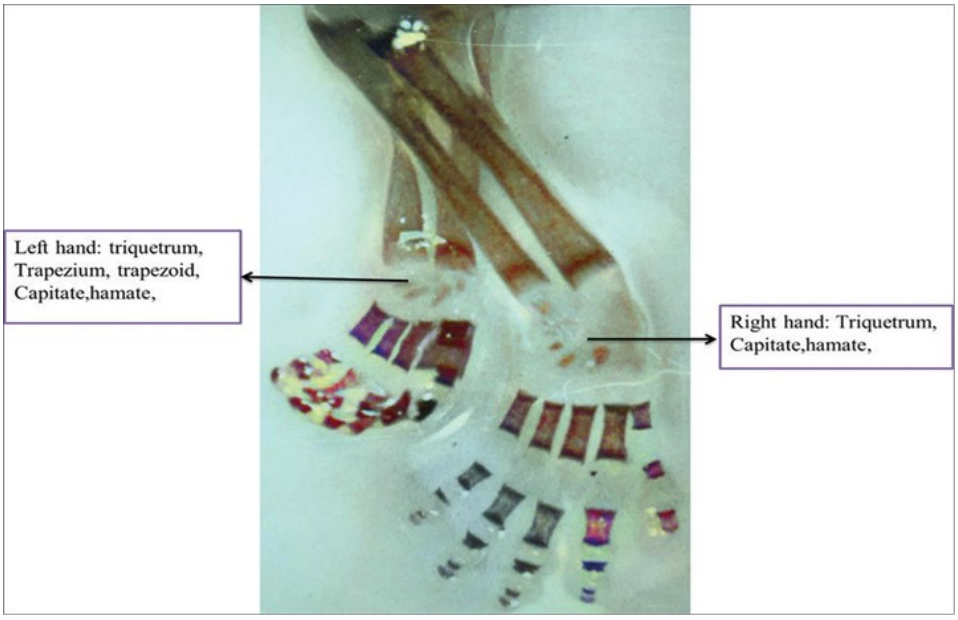

Fig. 2: Representative photograph of Ossification centers of hand Roentgenogram of 107 mm CRL female foetus.

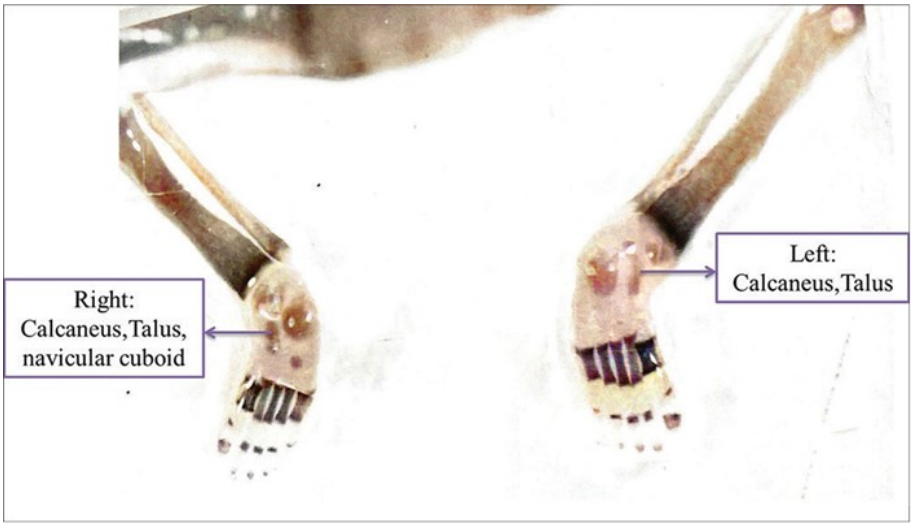

Fig. 3: Ossification centers of foot -Roentgenogram of 107 mm CRL female foetus.

DISCUSSION

Since the beginning of the 20th century, there has been a growing interest in studying the ossification of carpal bones [15] and tarsal bones [24]. Numerous research from various populations has provided information on the period of ossification of carpal bones. This study aimed to determine whether ossification patterns in the fetus's hand and foot might be used to determine its maturity. The degree of hand and foot growth between the third and sixth foetal months can be used to determine where a foetus is on a maturity continuum. It is commonly known that the hand ossifies before the foot as the upper limb buds begin to grow early in the embryo [25]. The primary centers of ossification appear before birth, and secondary centers of ossification appear after birth. In female foetuses, the appearance and the fusion of ossification centers are earlier than in males. This is the general rule of ossification described in anatomy textbooks [3]. In our study on prenatal ossification, the hand and foot bones show differences in the time of appearance and progression of ossification.

There was no center of ossification was observed at 20 mm CRL except the center for the clavicle and mandible [Figure 1]; this observation is confirmed by earlier research [26]. Centers for the clavicle and mandible appear at 15 mm CRL, and primary centers for long bones also appeared before 20 mm CRL, which is in the same line with existing literature [27].

At birth, carpal bones are cartilaginous, although ossification may have started in the capitate and hamate. The capitate and pisiform carpal bones ossify from the same center, with the remaining carpal bones having a different sequence. The order varies according to sex, nutrition, and possibly race [3]. For instance, ossification of carpal bones in the population of North India occurs 1 2 years later than in Europeans, and the appearance of the ossification center is earlier in females than males [10].

Occasionally, an ossification center occurs between the scaphoid, trapezoid, and capitate bones; during the second prenatal month, it is a cartilaginous nodule that usually fuses with the scaphoid. Lunate and triquetral elements may occasionally fuse [3]; such variation is not found in this study.

Our data on the time of appearance of ossification centers in the hand and foot bones was compared with that available in the literature [Table 2]. The centers for carpal and tarsal bones were not present until 6 months. This is in agreement with the study conducted in Saudi Arabia. The centers for terminal phalanges of the hand appeared earlier than the proximal and middle phalanges, similar to existing literature [3,28]. According to Hill [29] who studied 500 foetuses by roentgenography, proximal phalanges of the hand and foot ossify earlier than the terminal phalanges. Flecker [30] studied the time of appearance of both prenatal and postnatal ossification by the roentgenographic method and concluded that the proximal phalanges of toes ossify earlier than the terminal phalanges. These findings are contrary to our findings. Centers for terminal phalanges, metacarpals, and proximal phalanges of the hand and terminal and middle phalanges of the foot appeared earlier in our study. They differed with a study on the Punjab population reported by Jit [31].

The hand bones show ossification earlier than the foot bones except in the carpal and tarsal bones. Tarsal bones ossify earlier than carpal bones, which agrees with that of Noback and Robertson [32]. Furthermore, our study shows that the centers for the right side bones appear earlier than the left side bones and the spread of ossification in the hand is from lateral to the medial side, and in the foot bones it is from the medial side to lateral side. Jit did not notice any sexual difference in the appearance of ossification centers [31]; in this study, a female specimen of 107 mm CRL [Figures 2 and 3] the centres for carpal and tarsal bones showed left sided predominance in hand bones and right sided predominance in foot bones, probably a genetic factor was responsible. This is in agreement with the latest study, which stated that the ossification of carpal bones and other hand bones occurs earlier in females than in males [15].

Table 2: Comparison of time of appearance of primary ossification centers in hand and foot bones reported in the literature with the present study.

literature with the present study				
Bones of Hand (CRL in mm at which ossification centers appeared)				
Metacarpals	**Proximal phalanges**	**Middle phalanges**	**Terminal phalanges**	**References**
45	60	68	37	[31]
33	34	57	31	[27]
37	50	60	29	[27]
40	50	80	30-40	[3]
36	39	90	36	**Present study**
Bones of Foot (CRL in mm at which ossification centers appeared)				
Metacarpals	**Proximal phalanges**	**Middle phalanges**	**Terminal phalanges**	**References**
47	68	125	56	[31]
34	69	-	34	[27]
38	69	116	38	[32]
40-50	80-120	120	40-90	[3]
70	90	95	45	**Present study**

This study may be used as a guide to figure out the skeletal age of the hands and foot of the South Indian population. The findings can aid in diagnosing and treating pediatric orthopedic problems as well as guiding and facilitating the evaluation of growth abnormalities brought on by illnesses that interfere with the growing process. The results of this study might have limitations because they cannot be generalized to the entire country, because of less sample size. Therefore, a larger study involving multiple hospitals in various cities across the nation should be carried out to accurately represent the population's skeletal age.

CONCLUSION

Hence, it is advised that if attempting to determine the age of foetal remains, referring to both size related and maturity related information can only enhance the likelihood of approaching real age. Consequently, it is advised to use as much information as possible rather than taking a simple method like depending just on body size. In this investigation, the centers first developed in the hand bones before the foot bones, on the right side before the left, and in females before males. On the other hand, the ossification extends from the radial to the ulnar side, whereas in the foot, it extends from the tibial to the fibular side. Additional investigation necessitates conducting the study on a larger sample size, including other markers of skeletal maturity, to elevate the range and value of this method.

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Author Contributions

SD: Concept and Analysis & interpretation of the findings **RK:** Collection of data and literature review **YD:** Collection of Data and literature review **MS:** Collection of Data and literature review **AK:** Manuscript critical review and correspondence with the journal

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