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Original Research Article

An Observational Assessment of the Ossification in Fetal Hand and Foot Bones and Its Implications

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Abstract

Aim: The aim of the present study was 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.

Methods: The present study was conducted in the Department of Anatomy, Darbhanga Medical College and Hospital, Laheriasarai, Darbhanga, Bihar, India from March 2020 to February 2021 and all the parents of fetuses signed a written waiver of informed consent.

Results: Forty specimens of 20 - 205 mm CRL of both sexes which were subjected to clearing and staining were observed for the presence of ossification centers in the various bones of hands and feet. Our data on the time of appearance of ossification centers in the hand and foot bones was compared with that available in the literature.

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.

Keywords: Alizarin Red S, clearing, defatting, dehydration, KOH, ossification

Introduction

Bone age is an indicator of the skeletal and biological maturity of an individual. This is different from chronological age, which is calculated using the date of birth of an individual. Bone age is often requested by pediatricians and endocrinologists for comparison with chronological age for diagnosing diseases which result in tall or short stature in children. Serial measurements are also used to assess the effectiveness of treatments for these diseases. [1] Formulae have also been designed for computing the final adult height of children from bone age values in normal healthy children. [2]

Calculation of bone age is also employed for estimation of chronological age in conditions were accurate birth records are not available. Absent birth data is a big problem in our part of the world. In South Asia, 65% of all births are not registered by the age of 5 years. [3] Thus need for accurate estimation of age arises in conditions where the age of a child needs to be accurate, such as during immigration [4], in law suits [5] and in competitive sports. [6] In these cases bone age is used to provide the closest estimate of chronological age.

In order to compute bone age various methods have been developed using different skeletal elements and various visualization techniques. The pattern of ossification in the hand and wrist bones is in a fairly predictable manner and age specific until end of adolescence when the elongation of bone is complete. Thus, the standards of bone age have been derived by comparing the level of maturation of hand and wrist bones with normal age levels. Traditionally, the extent of growth and development of hand bones has been visualized by plain wrist radiographs, however newer methods such as ultrasound of hand bones are being tried but have yet

not been validated. Visualization by plain hand & wrist radiographs: There have been great advancements in radiological techniques over the past few decades but to date, plain radiographs of the hand are the investigation of choice for bone age assessment. A standard posterior-anterior (PA) view of the hand and wrist is ideal for visualization of features of hand bones. [7] The hand radiographs are quite safe to obtain as the effective dose of radiation received during each exposure is between 0.0001-0.1 mSV. [8] This dose is less than 20 minutes of natural background radiation or the amount of radiation received by an individual on a 2 minutes transatlantic flight. [1]

Various methods have been developed to compute bone age score from these radiographs by comparing the maturity of hand & wrist bones to idealized standards. The Greulich & Pyle (GP) Atlas: Is a holistic method based on "The Radiographic Atlas of Skeletal Development of the Hand and Wrist", by Dr William Walter Greulich and Dr Sarah Idell Pyle, its last edition published in 1959, is still one of the most commonly used atlas for bone age measurement by radiologists in Pakistan9 and in the West. [10]

The aim of the present study was to determine when ossification centers first arise in both genders' hand and foot bones, both sides and the spread of ossification of embryos and fetuses.

Materials and Methods

The present study was conducted in the Department of Anatomy, Darbhanga Medical College and Hospital, Laheriasarai, Darbhanga, Bihar, India from March 2020 to February 2021 and all the parents of fetuses signed a written waiver of informed consent.

The embryos and fetuses, 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 fetus was visually inspected to determine its sex; however, due to the very limited sample size, the sexes were merged for calculation. Fetuses 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 fetuses. 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 fetuses 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 colored 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. [11]

To check for the existence of ossification centers in the carpal and tarsal bones, roentgenograms of the hands and feet of eight fetuses 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 glycerin 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 favored for smaller fetuses because it may detect small patches of ossification at an early stage of growth. [12]

Results

Table 1: Comparison of time of appearance of primary ossification centers in hand and foot bones

Bones of Hand (CRL in mm at which ossification centers appeared)			
Metacarpals	Proximal phalanges	Middle phalanges	Terminal phalanges
36	39	90	36
Bones of Foot (CRL in mm at which ossification centers appeared)			
Metacarpals	Proximal phalanges	Middle phalanges	Terminal phalanges
70	90	95	45

Forty specimens of 20 - 205 mm CRL of both sexes which were subjected to clearing and staining were observed for the presence of ossification centers in the various bones of hands and feet. Our data on the time of appearance of ossification centers in the hand and foot bones was compared with that available in the literature.

Discussion

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 decom- position 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 fetus, 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 fetal remains because it serves purposes other than personal identification. The biological factors, for instance, may be used to determine fetal viability, which may be significant in later legal concerns. [13]

Early in the embryonic stage, bone formation begins; however, it is not completed until puberty.[14] 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 back- ground of cartilage; the secondary ossification center, on the other hand, refers to the bony area that arises after birth. [14] Forty specimens of 20 - 205 mm CRL of both sexes which were subjected to clearing and staining were observed for the presence of ossification centers in the various bones of hands and feet. Our data on the time of appearance of ossification centers in the hand and foot bones was compared with that available in the literature.

The degree of hand and foot growth between the third and sixth fetal months can be used to determine where a fetus 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. [15] There was no center of ossification was observed at 20 mm CRL except the center for the clavicle and mandible; this observation is confirmed by earlier research. [16] 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. [17] Flecker [18] 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. [19] 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 No back and Robertson. [20]

Conclusion

Hence, it is advised that if attempting to determine the age of fetal 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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