



BIOMEDICAL SCIENCES

Forensic age estimation from ossification centres: a comparative investigation of imaging and physical methods

DAMINI SIWAN, KEWAL KRISHAN, VISHAL SHARMA & TANUJ KANCHAN

Abstract: Age estimation is a crucial component of human identification in forensic science. It has a vital role in forensic anthropology, including examinations of skeletal remains, disaster victim identification, and locating missing individuals. Present communication focuses on the age estimation through the examination of ossification centers of bones and its significance in identifying the age of 18 years old, a recognized age of majority in many countries. The process of ossification is integral to biological development and serves as critical standard for age estimation in forensic identification. This study reviews relevant literature from well-known databases such as PubMed, Scopus, Web of Science, and ScienceDirect. Additionally, the present review elaborates various classification methods used by authors to classify the stages of ossification centers of bones. The objective of this communication is to assess the effectiveness of both imaging and physical methods for age estimation and to provide a critical comparison to determine the superior approach. The findings suggest that imaging methods are more reliable for the estimation of age from ossification centers. Staging methods introduced by Schmeling et al, Kellinghaus et al, Dedouit et al, Vieth et al, and Kvist et al. are found to be the best methods for age estimation.

Key words: bone age estimation, ossification, Human Identification, Forensic anthropology, disaster victim identification.

INTRODUCTION AND BACKGROUND

Age estimation is a pivotal element in many forensic investigations. Age estimation of skeletal remains and living persons plays an important role in personal identification in medico-legal cases and disaster victim identification (DVI) for forensic and humanitarian purposes. In addition to this, forensic age estimation is frequently called upon by the courts of law and government authorities to estimate an individual's actual age in many criminal and civil cases. This practice serves to ensure that individuals are not unfairly disadvantaged due to misconceptions about their age (Schmeling et al. 2016). According to the recommendation of International

Interdisciplinary Study Group in Forensic Age Diagnostics (*Arbeitsgemeinschaft für Forensische Alters diagnostik*; AGFAD 2023), age estimation is primarily conducted through physical examination. Subsequently this process involves an examination of X-Ray of left hand and lastly dental examination. Furthermore, AGFAD, offers recommendations for age estimation of living persons, facilitating applications in criminal, civil, asylum, and old-age pension proceedings, as well as in assessing the sex and age of skeletal remains.

While age estimation holds significance in all stages of life, its importance is particularly accentuated at the age of majority i.e., 18 years of age. The age of majority signifies the legal

transition to adulthood. For instance, as per The Majority Act 1875 in India, the legal age to attain maturity is 18 years for both the sexes (Gulsahi et al. 2016, Cipriani 2016, Majority Act 1875). This is also true for many other countries where the age of majority is considered as 18 years. In addition to this, 18-years of age is also a threshold in medico-legal or forensic cases e.g. unaccompanied adults requesting asylum in developed countries, estimating biological age of living person claiming to be younger than 18 years old (Houpert et al. 2016), child marriages (Cameriere et al. 2020), child labor, sexual assault, prostitution and sometimes for determining actual age of athletes (Davidson 1999, Audu et al. 2009, Timme et al. 2017).

Age estimation is not as straightforward as sex estimation which can be done directly with the help of morphological characteristics (Alkass et al. 2010). There are several methods that may be employed for age assessment such as medical history and physical examination (contains the records anthropometric data like height, body weight, and body mass), visual sexual maturity, dental examination, X-Ray examination of left hand, clavicle, epiphysis of bones, examination of bone mineral density etc. (Schmelting et al. 2006, 2016, AGFAD 2023).

Age estimation from bones, also known as 'bone age', is one of the important methods as the bones show biological changes corresponding to the increasing age; the principle may be used to estimate the biological age of a person. Biological age can be calculated by using bio-physiological measures to determine the age of an individual more accurately (Diebel & Rockwood 2021). This is different than chronological age, which is calculated by using date of birth of an individual. Difference in biological and chronological age may give an idea about the abnormality in development and growth of the child. Biological age is much more important and informational

than the chronological age. Bone age estimation (BAE) can be done by studying many aspects of the bone such as appearance and fusion of the centers of ossification, increase in length of bone and change in bone mineral density (useful predictor of age-at-death). In living individuals, BAE can be done by studying ossification of bone (epiphyses) (Satoh 2015), however, after death, BAE can be done by studying bone mineral density and simply by the examination the bone length and development (Bethard et al. 2019).

Ossification is a process of bone formation which starts at third month of fetal life and completed at the age of around 25 years in most of the bones of the human body. There are two types of ossification – intra-membranous ossification and endochondrial ossification. Former takes place in flat bones and later in long bones. In adolescence, endochondrial ossification of long bones continues until only a small strip of cartilage—known as the epiphyseal plate—remains at both the ends of long bone. Epiphyseal plate persists until the bone reaches its full adult length and it finally gets replaced with the bone (Encyclopedia Britannica 2020). There are two main types of ossification centers in endochondrial ossification namely primary ossification center and the secondary ossification center. Primary ossification center forms in the diaphyseal region and secondary ossification center develop in the epiphyseal region after the birth, as shown in figure 1 (Breeland et al. 2023). These epiphyseal ossifications can be studied with the help of different imaging methods like, X-Rays, CT- Scan (Computerized tomography), and MRI (Magnetic Resonance Imaging) of bones. However, the physical methods for bone age estimation from skeletal remains can be done simply by observing epiphyseal union of long bones (Cardoso 2008a).

This communication aims to shed light on the application of imaging techniques for age

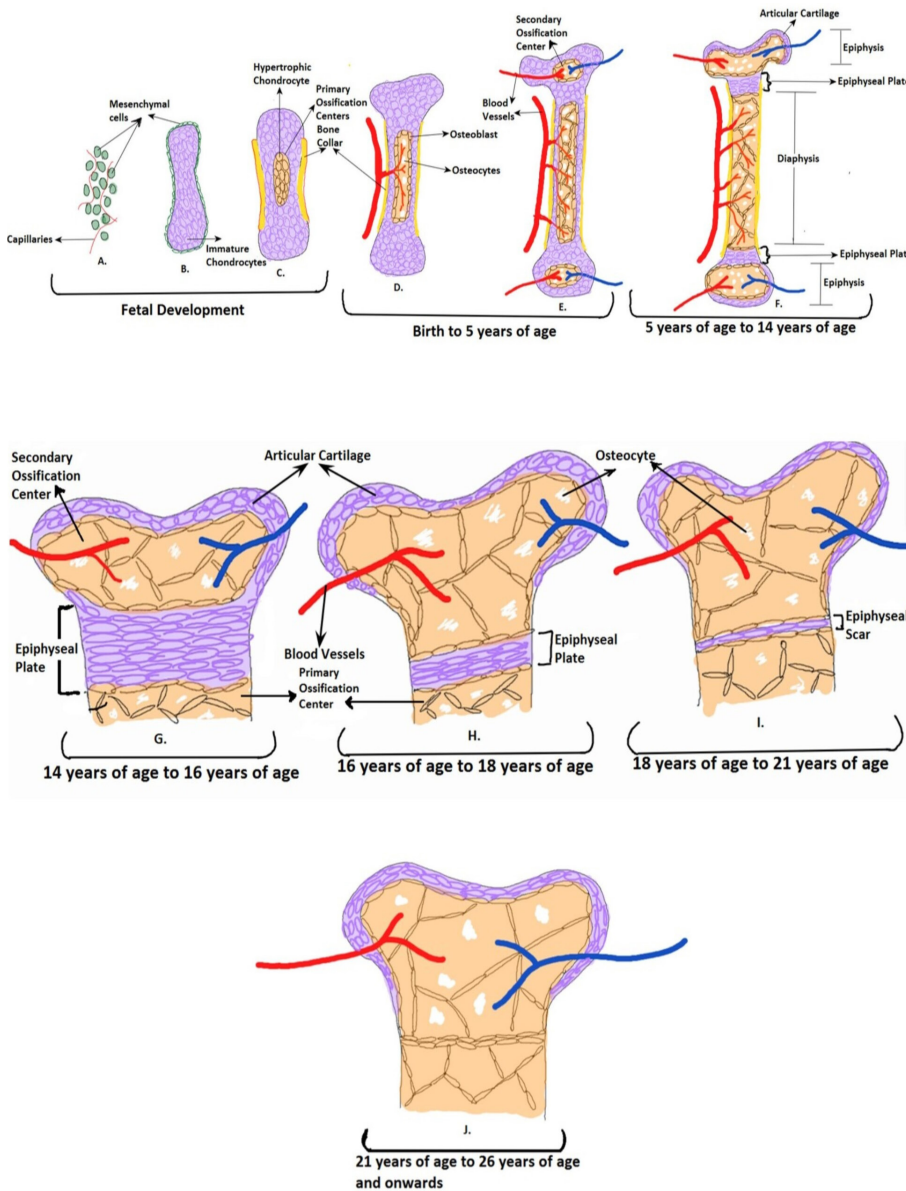


Figure 1. Endochondrial Ossification in Long Bones: a. Mesenchymal cells, b. Immature Chondrocyte, c. Appearance of Primary Ossification Center (POC), d. Development of POC, e. Appearance of Secondary Ossification Center (SOC), f. Development of POC and SOC, g. Stage I of endochondrial ossification (Epiphyseal plate not ossified yet), h. Stage II or III (Epiphyseal Plate starts to ossified), i. Stage IV (Appearance of Epiphyseal Scar), j. Stage V (Fully Ossified Bone with no epiphyseal scar).

estimation, focusing on secondary ossification in three prominent epiphyseal regions: the medial clavicle, distal radius, and distal femur. Additionally, the paper provides a concise overview of prior research related to age estimation through ossification centers, with a particular emphasis on determining ages near 18 years. It further engages in a critical comparative analysis between physical and imaging methods for age estimation. Refer to figure 2 for a visual representation of all the classification system

given by different researchers for imaging as well as physical methods.

MATERIALS AND METHODS

For the present study, we have used the databases such as *PubMed*, *Scopus*, *Web of Science*, and *ScienceDirect* to look into the usage of various physical and imaging techniques for estimating age. Keywords used for the search were, “ossification and bone age estimation”, “forensic age estimation through ossification”, “Bone age

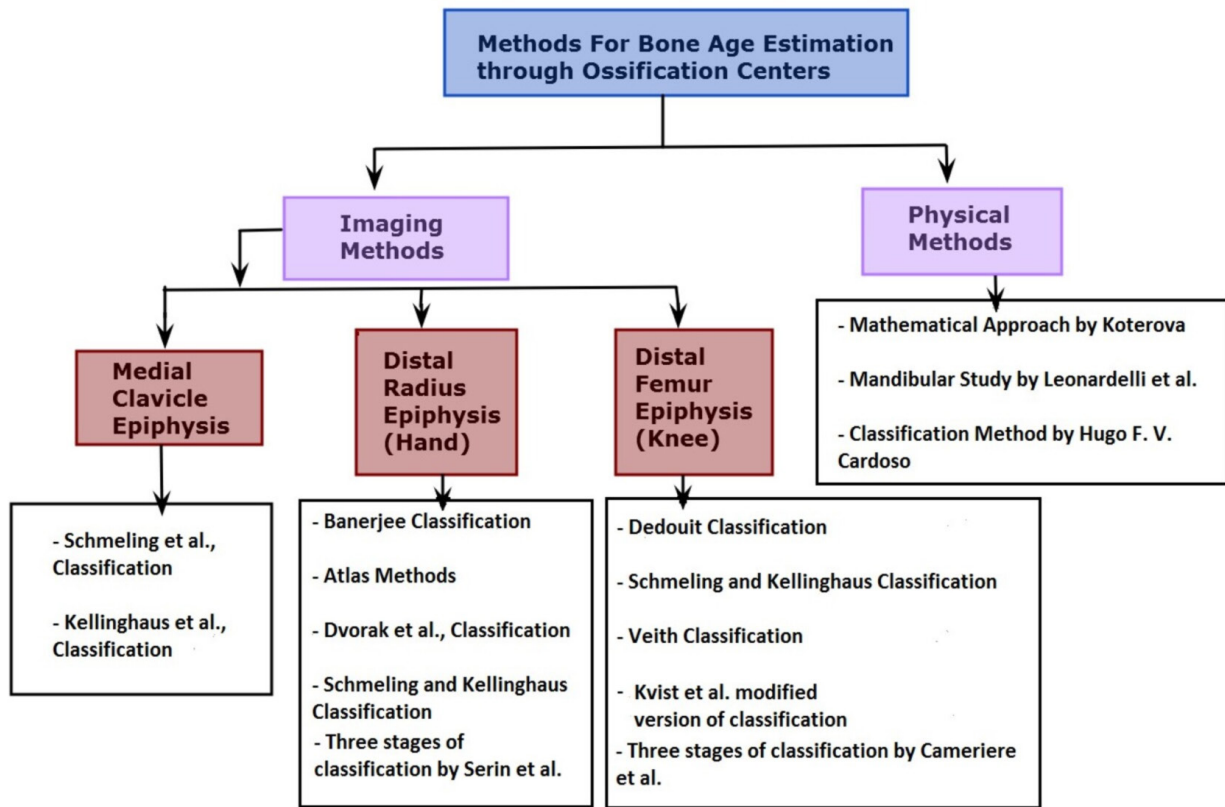


Figure 2. Classification Methods of ossification stages used for bone age estimation introduced by many researchers in their studies.

estimation and forensic science”. By using these keywords, a total of 14976 articles were shown by the databases. After the application of all-inclusive and exclusive criteria, 65 articles were selected for the present study. The articles were thoroughly studied for their methodology and results.

a) Inclusive criteria for searching the research articles;

- Ossification centers like medial clavicle, distal radius, and distal femur,
- Age range of sample falls between 12 to 30 years old,
- Imaging methods like CT scan, MRI and X-rays,

b) Exclusive criteria for searching research papers were with;

- Ossification other than medial clavicle, distal radius, and distal femur, (except for physical methods),
- Age range less than 12 years old (papers with post-natal and child age estimation).

IMAGING METHODS

Imaging methods such as X-rays, CT scan (Computed Tomography scan) and MRI (Magnetic Resonance Imaging) have been used by many researchers for the purpose of age estimation from bones (Schmidt et al. 2007 ,2016, Bassed et al. 2011, Brown et al. 2013, Schmeling et al. 2004, Kellinghaus et al. 2010). “The Tanner and Whitehouse” (TW2, TW3) and “the Greulich and Pyle (GP) procedures”, both of which are based on radiographs (specifically X-ray), are currently

the most widely used methods globally (De Sanctis et al. 2014, Fan et al. 2016). Later on, CT scans were used instead of X-rays due to less amount of exposure of radiation to the subjects. MRI recently came into use due to no exposure to radiations and seems less harmful than X-rays and CT scan (Fan et al. 2016, Dedouit et al. 2012). Three epiphyseal regions of the bones i.e., medial clavicle, distal radius and distal femur were examined by different researchers for giving more precise age estimation in males and females separately.

Medial clavicle epiphysis

Medial clavicle epiphysis plays an important role in forensic age estimation (Scendoni et al. 2022). Over the last hundred years, different investigators have studied the development and fusion of the medial epiphysis of the clavicle. These studies include both imaging assessment (CT, X-Ray, and MRI) as well as direct skeletal observation (physical examination) method (Bassed et al. 2011, Brown et al. 2013, Schmidt et al. 2016). Clavicle consists of laminated bone in embryological stage. The first ossification center in clavicle arises during 7th and 12th week of embryogenesis. However, the complete fusion takes place during adolescence (Schulze et al. 2006). Bassed et al. (2011) mentioned in his study that medial clavicle and molar tooth are the only sites which are realistically available for the estimation of age specifically 18 years old.

Nevertheless, only a small number of X-rays and MRI studies on medial clavicle epiphysis for age estimation have been performed in comparison of CT examination. There are two classification methods which classify stages

of ossification in medial clavicle region i.e. Schmeling et al. (2004) classification and Kellinghaus et al. (2010) classification.

i) Schmeling et al. classification: - In this classification, Schmeling et al. have given “5 stages” which clearly represents the variations occur in the epiphyseal cartilage of the medial clavicle during the ossification process (Table I) (Schmeling et al. 2004). Supplementary Material - Figure S1 depicts stage 3 where the participants in each study have reached or are approaching the age of 18.

ii) Kellinghaus et al. classification: - This classification is just the extension of classification method given by Schmeling et al. in which stage 2 and stage 3 among 5 stages are further classified into sub-stages 2a, 2b, 2c and 3a, 3b, 3c respectively (Kellinghaus et al. 2010). These sub-stages are giving more close prediction of the age of the person from medial clavicle epiphysis (Table I). Figure S2 depicts the sub-stages 3b and 3c where the subjects in each study have reached or are approaching the age of 18.

These classification methods were used by different researchers on the bone images, taken by using imaging methods (Schmidt et al. 2007, 2016, Brown et al. 2013, Schmeling et al. 2004, Kellinghaus et al. 2010, Schulze et al. 2006, Schulze et al. 2005, Wittschieber et al. 2014, Ramadan et al. 2017). Tables II and III summarize all of the studies conducted by various researchers using the classification methods of Schmeling et al. (2004) and Kellinghaus et al. (2010) for age estimation in males and females.

Table I. Ossification stages given by Schmeling et al. (2004) and Kellinghaus et al. (2010).

STAGES	OSSIFICATION
Stage 1:	the ossification centre has not yet ossified
Stage 2:	ossification centre has ossified; the epiphyseal cartilage has not ossified <ul style="list-style-type: none"> • Stage 2a: The lengthwise epiphyseal measurement is one third or less compared to the widthwise measurement of the metaphyseal ending • Stage 2b: the lengthwise epiphyseal measurement is over one third until two thirds compared to the widthwise measurement of the metaphyseal ending. • Stage 2c: the lengthwise epiphyseal measurement is over two-thirds compared to the metaphyseal ending
Stage3:	the epiphyseal cartilage is partially ossified <ul style="list-style-type: none"> • Stage 3a: The epiphyseal- metaphyseal fusion completes one third or less of the former gap between epiphysis and metaphysic. • Stage 3b: The epiphyseal-metaphyseal fusion completes over one third until two thirds of the former gap between epiphysis and metaphysic • Stage 3c: The epiphyseal-metaphyseal fusion completes over two thirds of the former gap between epiphysis and metaphysic.
Stage 4:	the epiphyseal cartilage is fully ossified
Stage 5:	the epiphyseal cartilage has fused completely and the epiphyseal scar is no longer visible

Distal radius epiphysis (hand)

Human hand and wrist consist of many bones and a number of epiphyses which mature in a well-defined progression over time (De Sanctis et al. 2014). Among all these epiphyses, epiphyseal union of radius bone in distal region is one of the most useful regions for age estimation. This region has not only been used for estimating age for medico-legal purposes but also for defining the age of certain categories the players in various sports competitions (Schmidt et al. 2015, Dvorak et al. 2007a, b). X-rays (previously) and MRI (recently) are two main imaging methods used by most of the researchers for age estimation through wrist radiographs.

Case studies in which Distal Radius Epiphysis used for age estimation:

i) Banerjee Classification: In a study conducted by Banerjee (Banerjee & Agarwal 1998), X-Ray films of 180 individuals (90

girls and 90 boys) were taken and divided them into two groups. Group 1 shows those having incomplete union and Group 2 showing those having complete union. Results show that in females, the complete union occurs in all the subjects in age group 18-19 years and for males in the age group 19-20 years. According to this study, if lower end of radius and ulna is fused in both the sexes, then they may be above 18 years of age (Banerjee & Agarwal 1998).

ii) Atlas Methods: In some pioneering studies, radiographic atlases of bone age for hand and wrist have been given by “**Greulich and Pyle**” (Greulich & Pyle 1959), **Tanner** (Tanner 1962), **Fels** (Chumela et al. 1989). The researchers and the scientists are using these atlases for comparing the radiographic images of their studies with the atlases and they estimate the age of the person and may give their specific opinion in a medico-legal

Table II. Appearance of different ossification stage of medial clavicle in the different age of male and female.

Author with year	Sample size/ Age group(age in years)	Imaging Method used	Stage 2(age in years)	Stage 3(age in years)	Stage 4(age in years)	Stage 5(age in years)
Schmeling et al. (2004)	873/16-30	X-Ray	NA	16Δ(appears) Maximum at 27 O and 24 □	20 O 21 □	26Δ
Brown et al. (2013)	1035/16-32	X-Ray	16Δ	17 to 30 O 18 to 31 □	20 O 21 □	30 O 31 □
Schulz et al. (2005)	629/15-30	CT Scans	15Δ	16 O 17 □	21Δ	21 O 22 □
Schulze et al. (2006)	100/16-25	CT Scans	Upto 21 Δ	Younger than 21	Older than 21Δ	NA
Kellinghaus et al. (2010)	592/10-35	CT Scans	13 O 14 □	16 O 17 □	21Δ	26Δ
Wittschieber et al. (2014)	572/10-40	CT Scans	NA	15-26 O 16-36 □	21.1 O 21.6 □	26.7 O 26.6 □
Ramadan et al. (2017)	601/10-35	CT Scans	Between 13 and 23Δ	Between 16 and 27	20Δ	25Δ
Schmidt et al. (2007)	54/6-40	MRI	15.0Δ	16.9Δ	23.8Δ	NA
Schmidt et al. (2016)	395/10-30	MRI	NA	NA	21.0 O 21.5 □	26.6 O 25.8 □

○ Female, □ Male, Δ both male and female, and NA- No data given.

case. However, due to some limitations, these atlas methods were replaced by other methods of age estimation (Dvorak et al. 2007a).

iii) Dvorak et al. Classification: Dvorak et al. (2007a) introduced another classification criterion for ossification of distal radius. In this classification, researchers have mentioned different grades for ossifications. Grades 1 to 6 are as follows; **Grade I:** Completely unfused, **Grade II:** Early fusion, **Grade III:** fusion of <50% of the radial cross-sectional area, **Grade IV:** fusion of >50% of the radial cross-sectional area, **Grade V:** Residual physis, <5 mm on any one section and **Grade VI:** Completely fused. Legal age

of players for age-based tournaments was checked through this grading system (Dvorak et al. 2007a, b). According to the study of Dvorak et al. the complete fusion cannot take place before the age of 18. For instance, the combination of radiographic images given in figure 3 shows all the grades of ossification given in the grading system of Dvorak et al. (2007a).

iv) Schmeling and Kellinghaus Classification: Ossification Staging system, which was previously given by Schmeling et al. (2004), and Kellinghaus et al. (2010), (Table I) for clavicle, later on used by some other researchers as well for estimating age through distal radius epiphysis (Schmidt et

Table III. Appearance of ossification stages and sub-stages in different age of male and female.

Author	Sample/age groups in years	Method	Stage 2a(age in years)	Stage 2b(age in years)	Stage 2c(age in years)	Stage 3a(age in years)	Stage 3b(age in years)	Stage 3c(age in years)
Kellinghaus et al. (2010)	185/13-26	CT Scan	13.1 ○ 14.4 □	15.4 ○ 16.4 □	15.6 ○ 17.1 □	16.8 ○ 17.5 □	17.8 ○ 18.3 □	19.5 ○ 19.7 □
Wittschieber et al. (2014)	572/10-40	CT Scan	NA	NA	NA	15.5 ○ 16.4 □	16.4 ○ 17.6 □	19.4 ○ 19.0 □
Schmidt et al. (2016)	395/10-30	MRI	14.0 ○ 15.0 □	15.5 ○ 16.0	15.8 ○ 17.3 □	16.4 ○ 16.3 □	16.4 ○ 16.5 □	19.3 ○ 19.0 □
Ramadan et al. (2017)	859/10-35	CT Scan	NA	NA	NA	NA	17 △	18 ○ 19 □

○ Female, □ Male, △ both male and female, and NA- No data given.

al. 2015, Baumann et al. 2009, Timme et al. 2017). Some of the studies are:

- **Baumann et al. (2009)** taken hand radiographs from 554 male and 388 female subjects aged 10-30 years. In his studies, the minimum age for attaining stage 4 (given by using Schmelting et al. (2004), and Kellinghaus et al. (2010), method of classification) is 12.9 and 14.5 years in females and males respectively. Stage 5 appears at the age of 16.2 and 18.7 years in females and males respectively.
- **Schmidt et al. (2015)** used same classification method for estimating age of players participating in U-17 FIFA tournament. For his studies MRI has taken of 152 German males of age 18 to 22 years old. Result of his studies show that stage 2c and 3a were established exclusively in 18-year-old age group, whilst ossification stage 3b found always above the age of 18- and 19-year-old (Schmidt et al. 2015). The limitation of the study indicated that

there was no data for female players all the MRI were taken of males only.

- **Timme et al. (2017)** used the same method for the purpose of age estimation by using MRI scans of wrist in males and females. He obtained 668 MRI scans (333 females and 335 males) of the subjects ranging in age from 12 to 24 years. In addition to this, they used the sub-division of stage 4 i.e., 4a and 4b, for more accurate estimation of 18-year-old of age. According to the study, minimum age which is greater than 18 was observed in Stage 4b of males and stage 4 in males and females. Youngest male was of age 18.6 years on which ossification Stage 4b was observed. In addition to this, stage V was observed at the age of 23.1 and 22.3 years in males and females respectively.
- v) **Serin et al. (2016)** did a similar work and gave their own ossification staging system for wrist radiographs, these stages are: - **Stage 1:** non-fusion, **Stage 2:** partial fusion and **Stage 3:** Complete fusion. Observation

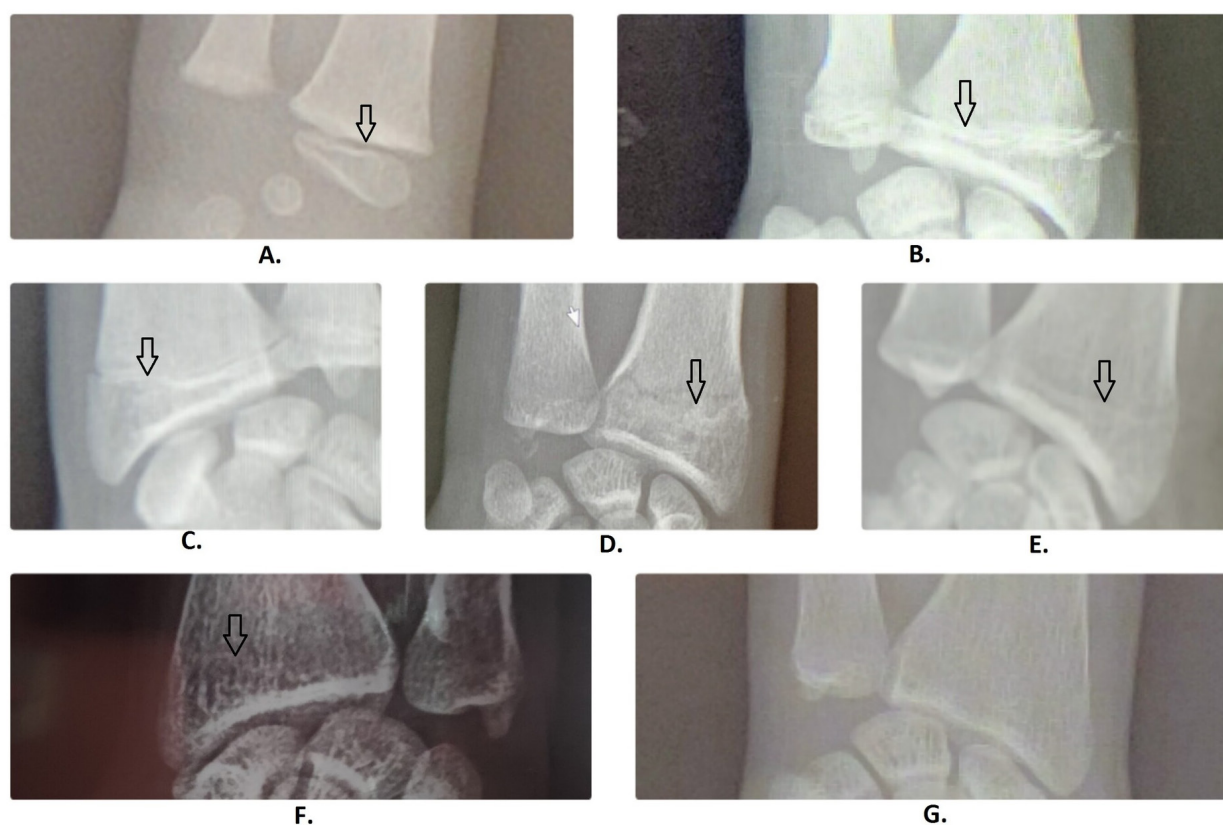


Figure 3. X-ray (radiographic) images of distal radius epiphysis following grading system given by Dvorak et al.; a. Grade I, completely unfused; b. Grade II, Early fusion; c. Grade III, fusion of less than 50%; d. Grade IV, Fusion more than 50%; e. and f. Grade V, Residual physis less than 5mm (epiphyseal scar); g. Grade VI, completely fused.

of the study says that, the highest age for stage 1 was 12 years old in females and 13 years old in males, highest age for stage 2 was 17 years old for females and 19.5 years old in males, while lowest age for stage 3 was 15 years in females and 16 years in males.

In comparing the age estimation methodologies of Serin et al. (2016) and those utilizing the classification methods of Schmeling and Kellinghaus, the three-stage approach by Serin appears less precise when contrasted with the five-stage method. The five-stage classification provides a more nuanced and refined age estimation, as well as greater subject categorization detail. In contrast, Serin's three-stage method provides a more generalised

categorization, which may result in a less precise age estimation. This highlights the importance of methodological choices in forensic age estimation studies, as the selected classification system significantly influences the accuracy of age assessments.

Distal femur epiphysis (knee)

Human knee joint is composed of four bones including distal region of femur (DF), proximal region of tibia (PT) and fibula (PF) and the patella (P). Except patella, all other regions of epiphyses show their ossification over the course of development. However, patella begins to ossify into bone at approximately 3 years of age (Cunningham et al. 2016, Maggio 2017).

A number of radiological and MRI studies on knee region for the estimation of age have been

conducted by many researchers. According to these studies, fusion in the center of epiphyseal plate of knees falls between the age of 14.5 years in females and 19 years in males (Davies & Parsons 1927, Paterson 1929). These previous studies have less precision of age estimation as they didn't have any classification methods by which one can rely on them. Later on, some classification methods were introduced by researchers, which classify the stages of ossification specifically for the knee region. These grading or classification methods are:

- Dedouit classification (Dedouit et al. 2012, Altinsoy et al. 2020, Uygun et al. 2021),
- Schmelting and Kellinghaus classification (Kramer et al. 2014a, Ottow et al. 2017, Ekizoglu et al. 2021),
- Vieth classification (Vieth et al. 2018, Gurses & Altinsoy 2021, Alatas et al. 2021),
- Dedouit, Kellinghaus and Schmelting modified version of classification: - This classification was given by Kvist et al. (2020) which was the combination of Dedouit, Kellinghaus and Schmelting classification,
- Three stages of classification by Cameriere et al. (2012).

a) Dedouit classification Method

Dedouit et al. (2012) introduced a grading method for epiphysis in knee region with 5 stages. These stages are as follows (Figure 4); **Stage I** – Stripe like continuous horizontal cartilage present between the metaphysis and the epiphysis (thickness greater than 1.5 mm), with multilaminar appearance, **Stage II**- continuous horizontal line present between metaphysis and the epiphysis (thickness greater than 1.5 mm), without multilaminar appearance, **Stage III**- continuous horizontal line present between metaphysis and the epiphysis (thickness less than 1.5 mm), **Stage IV**- discontinuous horizontal line present between metaphysis and the

epiphysis, with discontinuous increased signal intensity, **Stage V**- No increased signal intensity between the metaphysis and the epiphysis. Dedouit et al. (2012) and Altinsoy et al. (2020) used this classification for studying knee region (specifically distal femur region) and concluded the age in years of the individuals where each stage appears (Table IV).

b) Schmelting and Kellinghaus classification method: -

Kramer et al. (2014b), Ottow et al. (2017) and Ekizoglu et al. (2021) evaluated the age of the subjects by using classification method formerly given by Schmelting and Kellinghaus for clavicle. Table V lists all of the studies as well as the participants' ages in years at each stage of classification.

c) Vieth Classification method: -

Vieth et al. (2018) in his research have given improved version of grading system, as he claimed that other grading system didn't meet the requirements of AGFAD's recommendation. In his MRI Classification, 6 stages are given as follows (Figure 5); **Stage 1**- unfused, **Stage 2**- Continuous band of intermediate signal intensity is visible, **Stage 3**- discontinuous band of intermediate signal intensity is visible, **Stage 4**- discontinuous thin and serrated line of intermediate signal intensity between epiphysis and the diaphysis is visible, **Stage 5**- continuous thin line of intermediate signal intensity between the epiphysis and the diaphysis is visible (epiphyseal scar). **Stage 6**- continuous thin line of intermediate signal intensity between the epiphysis and the diaphysis is visible (complete fusion). Table VI shows the studies in which the Vieth classification method for femoral ossification was used by researchers, as well as the age in years of the subjects at each stage.

Kvist et al. modified version: -

Kvist et al. (2020) introduced grading system which is a modified version made by the

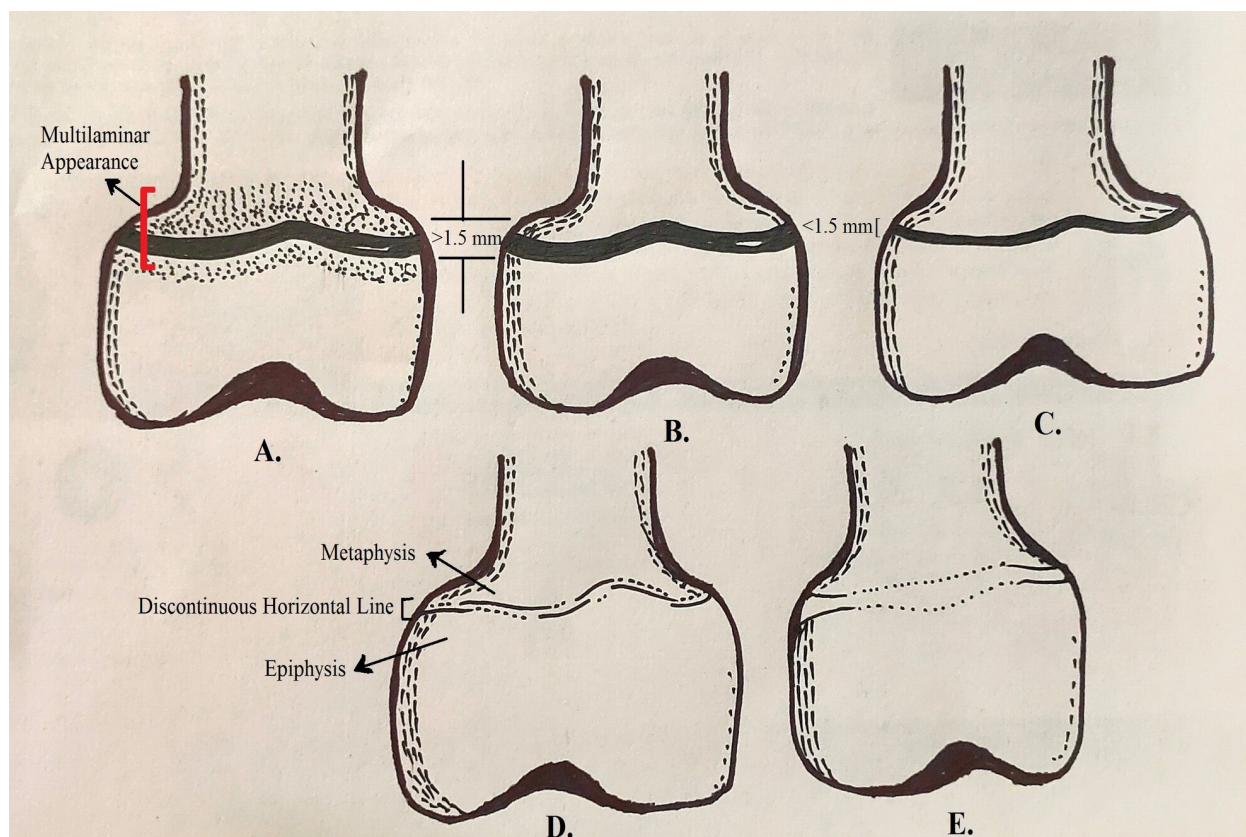


Figure 4. Dedouit et al. classification method : a. **Stage I** – Stripe like continuous horizontal cartilage present between the metaphysis and the epiphysis (thickness greater than 1.5 mm), with multilaminar appearance (ML), b. , **Stage II**- continuous horizontal line present between metaphysis and the epiphysis (thickness greater than 1.5 mm), without multilaminar appearance (ML), c. **Stage III**- continuous horizontal line present between metaphysis and the epiphysis (thickness less than 1.5 mm), d. **Stage IV**- discontinuous horizontal line present between metaphysis and the epiphysis, with discontinuous increased signal intensity, e. **Stage V**- No increased signal intensity between the metaphysis and the epiphysis

combination of two grading systems i.e. Dedouit et al. classification and and schmelting kellinghaus classification. In this, Kvist et al. used all five stages classified by Dedouit et al. and further divides the stage 4 into sub-stages viz., Stage 4a, 4b and 4c. Kvist et al. collected data on 395 males and females as healthy subjects aged between 14.0 and 21.5 years. They found that the maximum age on which stage 4b appearing was 18 years old for males and 17 years old for females, maximum age for stage 4c was 19 years old for males and 17 years old for females. Three stages of classification by Cameriere et al. 2012

Cameriere et al. (2012) performed radiographic study on 215 subjects for showing epiphyseal fusion at knee joint for the estimation of age. They introduced three stages of classification such as; **Stage 1**, epiphysis is not fused; **Stage 2**, epiphysis is fully fused and epiphyseal scar is visible; **Stage 3**, epiphysis is fully ossified and epiphyseal scar is not visible. According to the study stage 2 and 3 of above classification always fall above the age of 18 (Cameriere et al. 2012). For instance, figure 6 shows the radiographic images of distal femur where stage 1, 2 and 3 are shown by images A, B and C respectively.

Table IV. Dedouit classification method for age estimation from knee.

Author	Sample Size/ Age Range	Stage I (age in years)	Stage II	Stage III	Stage IV	Stage V
Dedouit et al. (2012)	290/ 10.1-30.9	10.1-13.6 ○ 10.3- 16.1 □	11.0-15.7 ○ 12.1-18.9 □	13.6- 25.1 ○ 14.8-25.7 □	16.6-29.6 ○ 17.8- 30.0 □	22.1-30.9 ○ 22.6-30.3 □
Altinsoy et al. (2018)	472/ 10-30	10.26-14.03 ○ 10.23-16.70 □	11.48-16.09 ○ 12.73-18.51 □	13.43-22.39 ○ 14.94-26.70 □	16.31-30.48 ○ 17.17-30.10 □	21.23-29.68 ○ 21.83-30.98 □

○ Female, □ Male, △ both male and female, and NA- No data given.

Table V. Schmeling and Kellinghaus classification method for age estimation from knee.

Author	Sample size/Age range	Stage IIc (age in years)	Stage IIIa (age in years)	Stage IIIb (age in years)	Stage IIIc (age in years)	Stage IV (age in years)	Stage V (age in years)
Kramer et al. (2014)	290/10-30	10.1 △	11.4 ○ 12.2 □	15.0 ○ NA □	15.6 ○ 15.0 □	16.2 ○ 18.3 □	22.1 ○ 22.6 □
Ottow et al. (2017)	658/12-24	12.11 ○ 12.05 □	13.39 ○ 13.68	14.73 ○ 17.77 □	14.53 ○ 16.13 □	16.13 ○ 17.46 □	24 △
Ekizogluet al. (2021)	649/10-30	10.1 ○ 10.0 □	12.8 ○ 12.7 □	14.6 ○ 15.1 □	14.6 ○ 15.8 □	15.4 ○ 17.0 □	NA

○ Female, □ Male, △ both male and female, and NA- No data given.

PHYSICAL METHODS

Age estimation with the help of physical methods can be done in living as well as in dead. In living individuals, physical methods for age estimation include measurement of height and body weight and comparing it with the standard databases such as World Health Organization, and Centers for Disease Control and Prevention. In addition to this, physiological changes and externally visible sexual maturity characteristics can be seen for estimation of age (Schmeling et al. 2016). Before applying any physical method for age estimation, an initial medical examination is conducted to ensure the absence of any physical growth-related disorder (Schmeling et al. 2016).

In addition to this, age of living individuals or age of individual immediately after the death cannot be estimated directly from bones without using imaging methods because bones

are not explicitly visible, however in the case of decomposed body where bones can be visible clearly to the naked eye, expert's examination on ossification center can be useful (Cardoso et al. 2014).

There is a shortage of available literature on physical examination for age estimation specifically on medial clavicle, distal femur and distal radius epiphysis. Therefore, present study evaluated all the previous studies where bones (any) from skeletal remains were used by many researchers for the age estimation by using physical methods. For instance, some of the studied are as follows:

i) Mathematical Approach by Koterova et al.: Koterova et al. (2018) carried out an estimation of age-at-death from hip bones. They have used different mathematical approaches (multi-linear regression and collapsed regression model) to reach more accurate and reliable age estimation results.

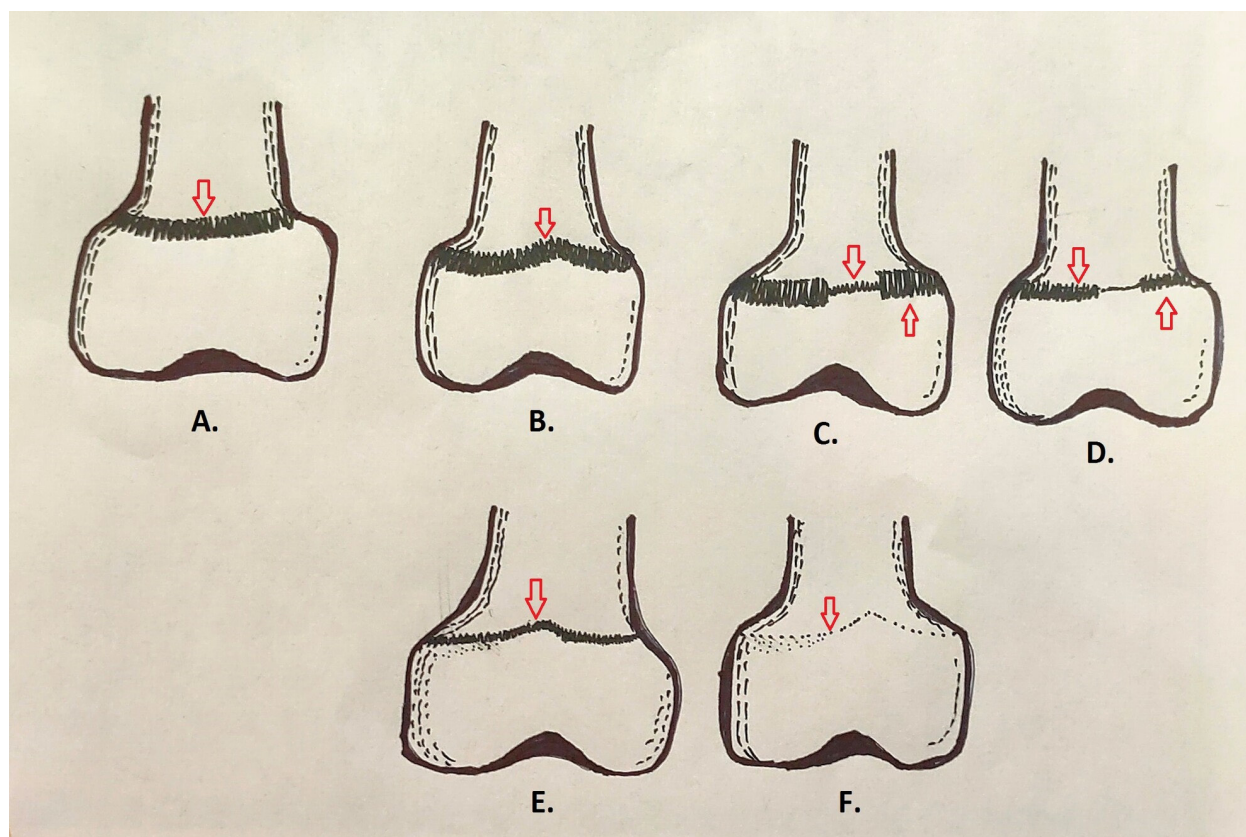


Figure 5. Vieth Classification method: a. Stage 1- unfused, b. Stage 2- Continuous band of intermediate signal intensity is visible, C. Stage 3- discontinuous band of intermediate signal intensity is visible, d. Stage 4- discontinuous thin and serrated line of intermediate signal intensity between epiphysis and the diaphysis is visible, e. Stage 5- continuous thin line of intermediate signal intensity between the epiphysis and the diaphysis is visible. f. Stage 6- continuous thin line of intermediate signal intensity between the epiphysis and the diaphysis is visible (complete fusion).

Accuracy rate of age estimation by using these models ranged from 32% and 72.3%.

ii) **Mandibular Study by Leonardelli et al.:** Leonardelli et al. (2021) used mandible from skeletal remains which undergoes remodeling and morphological alterations throughout the life of an individual, which can be used for age estimation. In addition to this, authors have mentioned the bigonial width and gonial angle measurements changes with age (Leonardelli et al. 2021). However, there are very less studies which have done on direct examination of ossification centers in skeletal remains.

iii) **Classification method by Hugo F.V. Cardoso:** Pleasantly, three of such studies were conducted by Hugo F.V. Cardoso (Cardoso et al. 2014, Koterova et al. 2018, Cardoso 2008b) on epiphyseal union, where he evaluated epiphyseal union at the lower limb, upper limb, scapular girdle and sacrum. He had given three stages of epiphysis viz; stage 1 (no union), stage 2 (partial union) and stage 3 (complete union). By examining lower limb (Innominate, femur, tibia and fibula), upper limb and scapular girdle; stage 1 always present when age is <18, stage 2 present when age is ≤ 18 and stage 3 always present when age is >18 (Cardoso et

Table VI. Veith Classification method for age estimation from knee.

Author	Sample size / age range	Stage 2 (age in years)	Stage 3 (age in years)	Stage 4 (age in years)	Stage 5 (age in years)	Stage 6 (age in years)
Veith et al. (2018)	694/ 12-24	12.11 ○ 12.95 □	12.16 ○ 12.13 □	14.33 ○ 15.49 □	14.82 ○ 15.71 □	20.65 ○ 21.24 □
Gurses et al. (2020)	598/12-30	12.08-14.75 ○ 12.08-15.33 □	12.92-16.08 ○ 12.92-19.50 □	14.33-19.67 ○ 15.08-20.67 □	14.75-29.42 ○ 15.83-30.50 □	20.58-30.92 ○ 20.58-30.92 □
Alatas et al. (2020)	709/12.01-27.55	12.01-14.53 ○ 12.02-16.28 □	12.01-17.22 ○ 12.34-18.92 □	13.77-19.08 ○ 14.84-21.96 □	14.77-25.61 ○ 15.81-26.71 □	14.77-25.61 ○ 20.76-27.37 □

○ Female, □ Male, △ both male and female, and NA- No data given

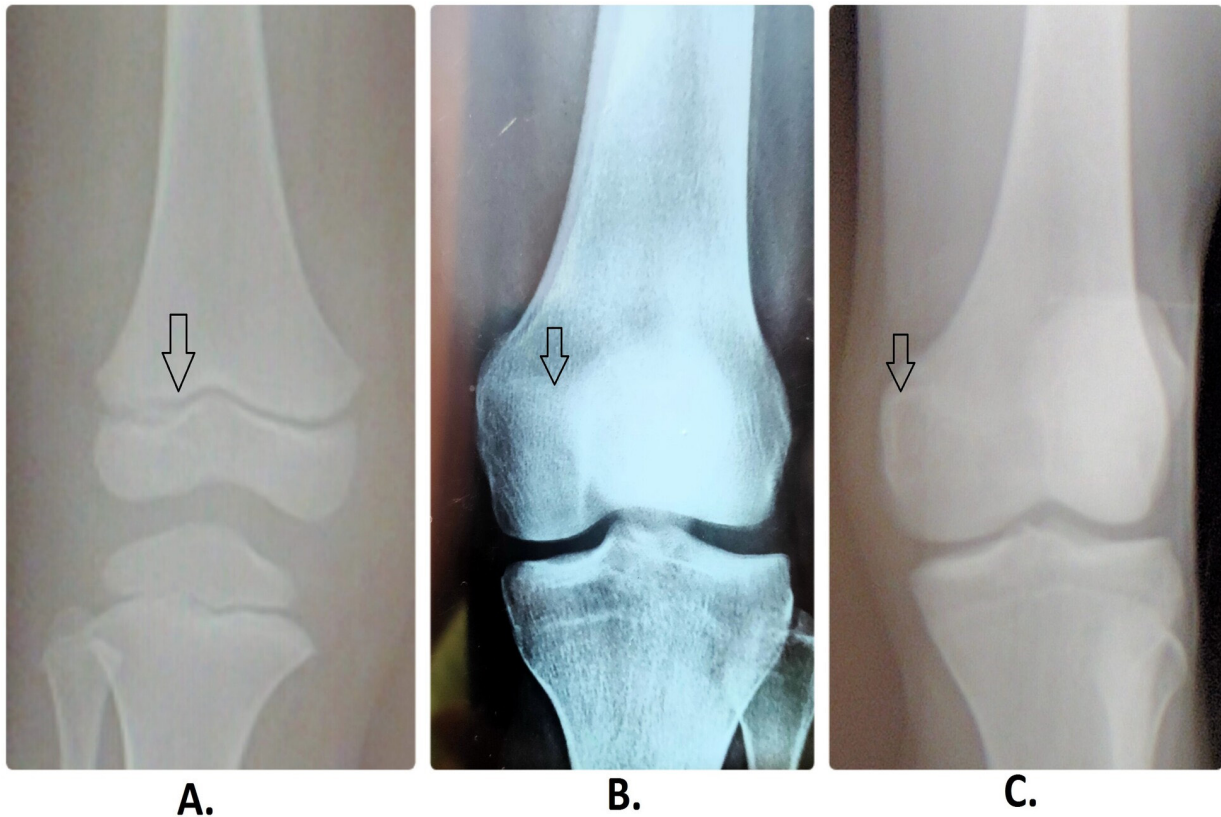


Figure 6. X-ray images of Distal Femur Epiphysis following three stages of classification by Cameriere et al.; a. Stage 1, epiphysis is not fused; b. Stage 2, epiphysis is fully fused and epiphyseal scar is visible; c. Stage 3, epiphysis is fully ossified and epiphyseal scar is not visible.

al. 2014, Koterova et al. 2018, Cardoso 2008b) Cardoso et al. (2014) concluded the age in years after examining three secondary ossification centres of the sacrum, namely the Annular, Sacro-iliac, and Lateral margin, in Table VII.

DISCUSSION

Examination of ossification centers are used for precise estimation of age since late 1900s. It is a process of bone formation which has a fixed time for its chronological progress throughout the life of human. Accordingly, it can be used for age

Table VII. Stages of ossification examined physically in ossification centers of sacrum by Cardoso et al. (2014).

Epiphysis	Sex	Stage 1 (Age in year)	Stage 2 (Age in year)	Stage 3 (Age in year)
Annular	Female	≤16	15-21	≥18
	Male	≤18	17-21	≥16
Sacro-iliac	Female	≤19	16-21	≥18
	Male	≤18	17-21	≥16
Lateral margin	Female	≤19	16-19	≥18
	Male	≤21	18-20	≥18

estimation of an individual before or after the death. Examination of these ossification centers can be done directly in the case of skeletal remains (Cardoso 2008a, b), and in the case of living individuals or post-mortem examinations there is a requirement of radiographic technique to make these ossification centers visible (Schulze et al. 2006, Schulz et al. 2005, Monum et al. 2020). Moreover, Physical examination for age estimation includes the records of anthropometric data, such as height, weight, and body type, as well as externally visible sexual maturity characteristics (for boys, genital development, pubic hair, underarm hair, beard growth, and laryngeal prominence; for girls, breast development, pubic hair, and hip shape). However, the AGFAD recommended that physical examination procedure begins by taking the medical history, because pre-existing illnesses can affect the natural sequence of growth. Some disorders do not affect only adult height and sexual maturation, but also the skeletal maturation. Some of endocrine disorders are; precocious puberty, Adrenogenital syndrome and hyperthyroidism. Therefore, physical examination cannot be normal, in some cases like gigantism, acromegaly, dwarfism, virilization in girls, dissociated virilism in boys, goiter etc. (Schmeling et al. 2016). Some studies show physical examination in which ossification centers are directly observed in skeletal remains (Cardoso et al. 2008a, b). However, it does not provide the precise age of majority; rather,

it provides an approximate idea of whether the bone belongs to a person under or above the age of 18 years old. Additionally, physical methods were used by fewer researchers, in the case of examination of ossification centers; the possible reason behind this can be less availability of samples of skeletal remains or other methods like tooth examination, ribs examination, skeletal growth and cranial suture might be taking the front seat (Franklin 2010).

On the other hand, imaging methods provides the highlighted image of epiphyseal region which shows ossification centers more clearly and the different staging methods given by researchers can examine these ossification centers more efficiently. For more orderly representation of all the studies mentioned in the present review, Table VIII illustrates these studies along with the exact stage where subject is near to or equal to 18 years of age. Moreover, these staging methods can be used by the forensic investigators for estimating age of highly decomposed body by taking radiographic images or MRI of the body. This review focuses more on three ossification centers viz; medial clavicle, distal radius and distal femur, however, the staging methods given by Schmeling et al. (2004) and Kellinghaus et al. (2010) can be useful for different ossifications centers as well, such as, humeral head (Ekizoglu et al. 2019) and proximal tibia (Kramer et al. 2014a).

This review can act as a guideline, firstly to compare physical methods with imaging

Table VIII. all the studies in present review along with the exact stage of ossification where age is near to or equal to 18 years old.

S.No.	Ossification region and classification methods	Stage of ossification	Age of the person (Specifically near to or equal to 18 years old of age)
1.	MEDIAL CLAVICLE EPIPHYSIS		
	Schmeling et al. classification	Stage 3 Stage 4	16 Δ ≥18 Δ
	Kellinghaus et al, classification	Stage 3b Stage 3c	17 O, 18 □ 18 O, 19 □
2.	DISTAL RADIUS EPIPHYSIS		
	Banerjee classification	Group 2	18 – 19 O, 19 – 20 □
	Dvorak et al. classification	Grade VI	≥18 Δ
	Schmeling and Kellinghaus classification Bauman et al. Schmidt et al. Timme et al.	Stage 5 Stage 2c and 3a Stage 3b Stage 4b Stage 4	16.2 O, 18.7 □ 18 □ >18 O 18 □ 18 Δ
	Serin et al.	Stage 2	17 O, 19.5 □
3.	DISTAL FEMUR EPIPHYSIS		
	Dedouit classification method	Stage IV Stage V	16.6 O, 17.8 □ 22.1 , 22.6 □
	Schmeling and Kellinghaus classification	Stage 4 Stage 5	16.2 O, 18.3 □ 22.1 O, 22.6 □
	Veith classification method	Stage 5 Stage 6	14.82 O, 15.71 □ 20.65 O, 21.24 □
	Kvist et al. modified version of classification	Stage 4b Stage 4c	17 O, 18 □ 17 O, 19 □
	Three stages of classification by Cameriere et al.	Stage 2 and 3	>18 Δ

O Female, □ Male, Δ both male and female.

methods used for age estimation, and secondly for checking different staging methods which are in use till date, as the selected classification system significantly influences the accuracy of age assessments. Furthermore, this review covers almost all of the stages of ossification that occur at or near the age of majority. In forensic investigations and other medico-legal cases where the age of majority is important, ossification staging methods can provide very precise age (close to 18 years old), which can add value to the justice.

CONCLUSIONS

The present study is an attempt to evaluate the comparison between imaging and physical methods used for age estimation through ossification centers. The findings of the present communication suggest that imaging methods are more reliable if we are looking for more precise age. Staging methods introduced by Schmeling et al. (2004), Kellinghaus et al. (2010), Dedouit et al. (2012), Vieth et al. (2018), and Kvist et al. (2020) can be directly used for this purpose. When a person reaches the age of majority (18 years), they enter stage 3 and sub-stages 3b and 3c of the staging systems described by Schmeling et al. and Kellinghaus et al. and most of the studies agree with this. Even though the advanced and further work is emphasized in order to refine these methods and increase the accuracy in age estimation. Additionally, Ossification centers other than medial clavicle, distal radius and distal femur can also be examined by using same staging methods. After this point, it is appropriate to assert that ossification centers have a lot of potential to develop into the primary area for age estimation by using the methods mentioned in this review. Therefore, ossification centers can be utilized to create a biological profile of

the skeletal remains or for providing age-based justice for the living person.

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SUPPLEMENTARY MATERIAL

Figure S1-S2.

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DAMINI SIWAN¹

<https://orcid.org/0009-0007-9643-7010>

KEWAL KRISHAN²

<https://orcid.org/0000-0001-5321-0958>

VISHAL SHARMA¹

<https://orcid.org/0000-0002-5130-1626>

TANUJ KANCHAN³

<https://orcid.org/0000-0003-0346-1075>

¹Panjab University, Institute of Forensic Science and Criminology, Sector-14, Chandigarh-160014, India

²Panjab University, Department of Anthropology, Sector-14, Chandigarh-160014, India

³All India Institute of Medical Sciences, Department of Forensic Medicine and Toxicology, 160014, Jodhpur, India

Correspondence to: **Kewal Krishan**

E-mail: gargkk@yahoo.com; kewalkrishan@pu.ac.in

Author contributions

Damini Siwan: Conceptualization, data collection, data entry, writing original draft, review & editing, final approval; Kewal Krishan: Writing, review & editing, data analyses and interpretation, final approval and supervising the work; Vishal Sharma: Writing, review & editing, data analyses and interpretation, final approval and supervising the work; Tanuj Kanchan: Writing, review & editing, data analyses and interpretation, final approval and supervising the work.

