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FORENSIC EXAMINATION OF SKELETAL REMAINS

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ABSTRACT

The discovery of skeletal remains puts up many questions in regard to the identityof the deceased: Are these really human bones? If so, what would be the gender of theunknown person? What was the age of the person at the time of death? To which geographic location did the person belong? What was the height of the person? What is the time that has elapsed since death? What is the cause of death?

The investigating officers and forensic pathologists, are often times, not properly trained, and do not possess the required knowledge and experience to conduct an examinationof the skeletal remains, thus, a forensic anthropologist best fits the role to conduct an analysis of the recovered skeletal remains and provide answers to the aforementioned questions. The interpretations to these questions can help develop a biological profile of the unknown individual, that will help narrow down the search for the missing person and contribute to a faster and more reliable positive identification of the person.

Even though the body has fully decomposed, the skeletal material and bones provide an abundant and rich source of evidence that is of high medicolegal importance, that will assist in cases of murder, mass disasters, etc.

The present study has been done to review the various techniques that has been used for the examination of parts of a skeletal remain that contribute towards developing a biological profile of the person. Methods for age estimation, stature estimation, gender estimation, and ancestry assessment has been discussed along with their limitations, and suggestions have also been made on how the challenges that are posed towards the accuracy and reliability of such methods may be tackled with. Emerging technologies and methods for the identification of skeletal remains have also been discussed, and the effect of varioustraumas on skeletal remains

has additionally been studied, as these provide major clues to the cause of death. An attempt has also been made towards the end of the study to highlight areasof new research prospects and advancements that can help towards a better and more reliable positive identification of skeletal remains.

Keywords: *Skeletal remains, Forensic anthropologists, Biological Profile, Age estimation, Stature estimation, Gender estimation, Ancestry assessment, Trauma.*

Chapter 1

INTRODUCTION

1.1. <u>The Skeleton:</u>

The skeleton, made up of hard tissues, is the framework that provides support, shape, and structure to the body of a living organism and its function include facilitating the movement and providing protection to vital organs of the body, such as the heart and thebrain. They provide attachment for muscles, and some bones of the skeletal system are also involved in the production of red blood cells. The skeleton in most vertebrates, including humans, is made of bones, cartilage and ligaments. The human skeletal system consists of 206 bones, approximately 900 ligaments, and cartilages.

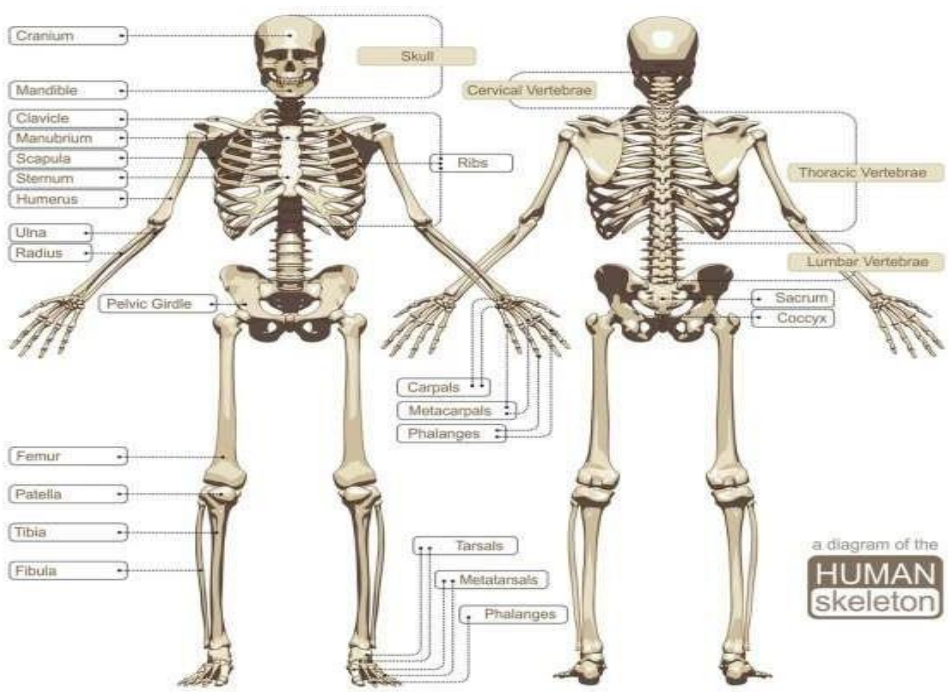


Figure 1.1. The Human Skeletal System. Source: <u>https://www.shutterstock.com/search/human+skeletal+system</u>

1.2. <u>Skeletal System: Formation.</u>

Some parts of the skeleton are formed just after the first few weeks from thetime of conception. The skeletal pattern is formed in the cartilage and the membranes of the connective tissue by the end of the eighth week from conception and thus, ossification, i.e., the process of formation of bones, begins. The process of formation of bones and its development continues after birth up to adulthood.

Bones of the skeleton are grouped into the axial skeleton and the appendicular skeleton. Bones of the axial skeleton protect the vital internal organs of the body. It includes the skull, the thoracic cage and the vertebral column. On the other hand, the bones formingthe appendicular skeleton (e.g., the limbs and the girdles) help in facilitating movement.

1.3. <u>Types of Bones:</u>

There are five types of bones, each grouped according to their respective shape and function. They have been described as follows:

a) **Long bones:** These bones are mostly present in the appendicular skeleton and are their characteristic feature is that they are longer than they are wide. It consists of bones in the lower limbs, viz., femur, tibia, fibula, metatarsals and phalanges and that of the upper limbs, viz., humerus, the ulna and the radius, and metacarpals and phalanges. Long bones have a shaft with two ends. Their main function is to provide structure, mobility and strength.

b) **Short bones:** The characteristic feature of short bones include an equal length, width and thickness with respect to its dimensions, possessing a cube-like appearance. They are found in the hands and the feet. Short bones are usually spongy bones. They arethe carpals located at the wrists and the tarsals, which are located at the ankles. The main function of short bones is to provide support and stability and also some form of limited motion.

c) **Flat bones:** Flat bones are usually thin, flattened and slightly curved. There is usually a layer of spongy bone that is present between two thin layers of compact bones. The function of flat bones includes serving as attachment points for muscles and protecting vital internal organs. These are cranial bones, scapulae, sternum, ribs, etc.

d) **Sesamoid bones:** These bones are named as such, as its shape resembles that of a sesame seed. They are usually round and small. These bones are found to be embedded in the tendons, wherein, there is a great amount of pressure being generated in the joint. They help the tendons to overcome compressive forces and thus, protect them. The number and placement of sesamoid bones varies from person to person, but they are commonly found in the tendons associated with the hands, feet and the knee. The patellae (kneecap) are sesamoid bones and are the only bones of this category thatis common for every individual. They provide an additional shield or cushion to high stress and motion areas of the body.

e) **Irregular bones**: An irregular bone is one that does not have an easily characterizable shape and therefore, thus not fit into any of the above categories of bones. These bones are usually complex in shape. An example of such is the vertebrae that supports our spinal cord and protects it from compressive forces. Most facial bones, particularly the ones containing the sinuses, also falls under the category of irregular bones.

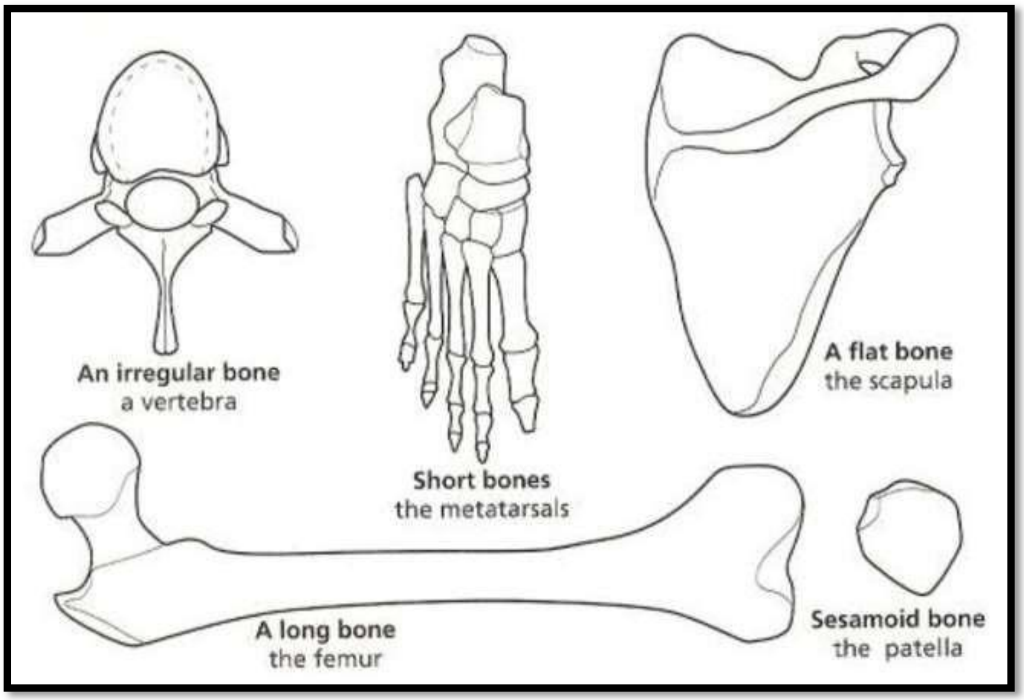


Figure 1.2. Types of Bones in the Skeletal System. Source: Umadevi N. et al (2012).

An infant skeleton comprises of more bones, as compared to that of theskeleton of an adult human. A new born baby usually has about 300 bones at the time of

birth, which gradually fuses to form the 206 bones of the adult skeletal system. A typical example of such a fusion is that of the sacrum. The sacrum is typically five vertebrae withdiscs in between at the time of birth. However, by the fourth decade of the life of the individual, it fully fuses into one bone.

1.4. Skeletal Remains:

The human body is resilient, and can persist for thousands of years. Thedecomposition of the body starts immediately after the death of the person. Normal bodily functions will come to a halt, and the decomposers/microorganisms, such as bacteria, begin spreading throughout the body. The various stages of decomposition cause the body tissues to rupture, and break down, and once all the skin, the soft tissues and other organs have fully been decomposed, all that remains of the corpse, is the skeleton. The teeth and the skeleton may both undergo some subtle changes through the years, depending upon various environmental factors and conditions; however, they have the ability to remain intact, for hundreds to thousands of years, due to their robust nature. The skeleton has the ability to remain pretty intact even when the body has been burned at temperatures over 1000 degree Celsius.

The skeleton of a person undergoes alteration, in terms of shape or chemistry depending upon various factors, such as diet, daily routine or activity, environmental factors, etc, during the course of the person's life. The dentition of a person develops and forms at different stages during childhood, and the bones also remodel at varying rates, therefore, a biological profile, and other important information about a person's life from infancy up until the time of death, can be recovered from these hard tissues.

1.5. Recovery of Skeletal Remains:

A skeletal remain is usually discovered long after the person has died. The most common scenario in cases that involve skeletal remains is that the body of the deceased had been disposed of in such a manner such that it would be hidden, for the purpose of covering up a murder, and/or other similar crime. Another common situation where skeletal remains are recovered, includes the surface of the ground, where the body was simply dumped on the ground, and the body's remains end up being scattered around by wild animals over a certain range of area [73].

Skeletal remains are usually found or recovered from shallow graves, most commonly in regions like marshlands, wooded areas, forests, and other similar areas, where there is little/minimal human population inhabiting the area or passing through it. By the time the skeleton has been discovered, it may have been several weeks, months or even years that the body had been buried. The condition of the skeleton depends upon various factors such as climate, environment, and presence of fauna and insects.

When a skeletal remain is recovered from a crime scene, the main role of the forensic anthropologist will be to develop a biological profile of the unidentified individual whose skeletal remains have been found, in order to help the law enforcement agencies to identify the person. Such a biological profile will consist of the following assessments:

- Ancestry.
- Age of the individual.
- Gender of the individual.
- Estimation of stature, etc.

Upon the discovery of unknown skeletal remains, the following questions are required to be addressed [107]:

a) Are they really bones?
b) Are they human or animal bones?

c) Do they belong to an infant, or foetal child, or any immature individual, or are theybones of an adult?
d) What was the age of the person at the time of death?
e) What was the race of the person?
f) Are the bones that of a male, or female?
g) How tall was the deceased?
h) Do the remains indicate the presence of more than one body?
i) Are any signs of trauma present on the bone?
j) Are there any other unique features present on the bone, that could further assist inestablishing identity?

1.6. Skeletal Evidence as a Means of Identification:

Identification of an unknown corpse involves the process of recognition of the person, based on the examination of various physical characteristics and biological parameters, that are known to be unique to each individual [58]. A body that has arrived the skeletal stage of decomposition, usually only comprises of the bones, the teeth, and the hair. These evidences may contain the genetic material, i.e., DNA, through which an identification can be made. The teeth can provide useful clues as to the identity of the individual, which can be obtained by comparing the evidence against dental records for a match. Presence of any crack or fracture in the bones, such as the arms, or the legs, may be indicative of injuries inflicted during childhood, or at the time of death.

An injury in the skull may indicate that the victim was beaten around his/her head area, and sustained a heavy blow, that is seen in cases of blunt force trauma. In cases where a hole has been located in the skull, it may indicate that the person was probably shot in the head.

The marked differences and variations in the bones, between a male and female, between people from different ethnicity and countries, and between various species as a whole, can all help to make an identification of a person, and help the investigation team to proceed with further findings.

There are many major extensive research works that assess the various methods of performing an analysis based on the aforementioned questions. However, recently, there has been a paradigm shift [105], calling for a focus on the need and necessity for research that will look into the reliability, validity and accuracy of these methods. For this purpose, there is a need to develop quantifiable measurements for quantification of the evidence, and automated techniques that will help strengthen the current forensic methods of identification and evaluation, including the determination of the error rates of the identification process. All these factors combined will throw lights towards the accuracy, validity and reliability of the identification method, and reduce any bias in the results to a fairly large extent [130].

1.7. Estimation of Age from Skeletal Remains:

Aging is a dynamic phenomenon, and it is a continuation of the physiological processes that occur from conception, up until the time of death of the individual. The various factors that serve as indicators of a person's maturity become more identifiable as the person increases in age, for example, gain in weight and size, ossification, formation of permanent tooth, and the appearance and development of secondary sex characteristics. Several of these changes are variable in nature, and therefore, may not be of any reliable use in techniques for age estimation, but the others are fairly constant and have been utilized by medico-legal workers in age estimation [81]. Determining age of the person at the time of death is a key issue faced in correctly identifying an unknown corpse [6]. Estimating age at the time of death from the recovered skeletal remains is a key focus in forensic anthropological cases, and is among the many important assessments to be included while constructing the biological profile of the unknown individual. The selection of appropriate methods for estimating the age at death depend upon what type of skeletal material was recovered from the site/crime scene.

The older the age of the deceased, greater is the challenge that is posed to accurately estimate the age of the individual. Estimating the age of a person who was 20 years or below is fairly more accurate and less challenging as the more the person ages, the growth process slows down and ceases, thus posing some difficulty, and lack of accuracy in the estimation process [107].

During the preliminary examination of a skeletal remain, if it is found that the third molar has completely developed,, the epiphyses of iliac crest, and the sternal end of the clavicle are fused, and the spheno-occipital synchondrosis is fused, then in such cases, it will be appropriate to employ techniques suitable for examining adult bones, as it has been previously professed that techniques that are used for estimating age will be different for an infant or an immature individual, as compared to those techniques that are employed for an adult individual [128]. The aforementioned epiphyses are often the last to fuse, and therefore, if they are discovered to be fused, then the epiphyses of other long bones, such as the proximal tibia or the distal femur are also normally fused.

In cases of a foetal child, the most useful method for age estimation is to examine appearance of the ossification centres, such as the calcaneal ossification centre, the distal femoral epiphyseal ossification centre, etc. The ossification centres in the foetal child becomes visible after 24 weeks of conception.

After birth of the child, age estimation can be done by examining the appearance of epiphyseal fusions and their closure, bone size and the most appropriate being the dental formation, i.e., the eruption of the deciduous and permanent teeth. Analysis of the teeth becomes difficult in adults as the permanent dentition has already been formed in adults.

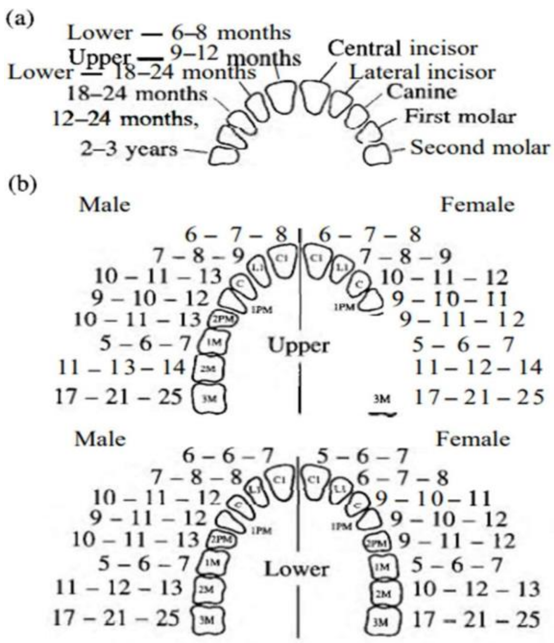


Figure 1.3. (a) Age of eruption of deciduous teeth (average) (b) Age (in years) of eruption of permanent teeth; 3 years shown representing early, average and late eruption, all rounded to the approximate nearest year. Source: Knight BH. Edward Arnold 1991.

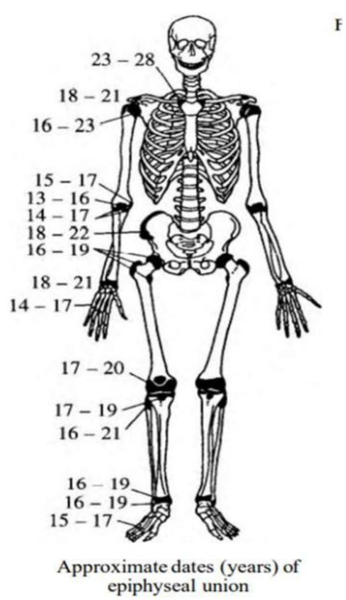


Figure 1. 4. Age of epiphyseal union in the major centres. Source: R SARVESVARAN (1994).

Table 1.1. The table is a guide regarding the epiphyseal union occurring in the major centres, for male subjects (in females, it occurs slightly earlier) in non-tropical climates: the two years given represent partial and complete union (in years years). Source: Knight BH. Edward Arnold 1991.'

Head of femur	16-19
Greater trochanter	16-19
Lesser trochanter	16-19
Head of humerus	16-23
Distal humerus	13-16
Medial epicondyle	15-17
Proximal radius	14-17
Proximal ulna	14-17
Distal radius	18-21
Distal ulna	18-21
Metacarpals	14-17
Acromion	17-19
Distal femur	17-20
Proximal tibia	17-19
Proximal fibula	16-21
Distal tibia	16-19
Distal fibula	16-19
Metatarsals	15-17
Iliac crest	18-22
Primary elements pelvis	14- 16
Sternal clavicle	23-28
Acromial clavicle	18-21

As aforementioned, age estimation becomes more challenging for adults. In order to estimate age of an adult, the examiner may check for the extent of the cranial sutures closure, morphological changes of the pubic symphysis, the histology of the bone, and dental features, bilateral thinning of the parietal bones, sterna rib ends development, osteoarthritis, changes in the acetabulum of pelvis, etc [127].

1.7.1. Age estimation from the teeth:

Developing teeth are one of the most reliable indicators for biological and chronological aging of skeletal remains, because they are less affected than other body tissues by endocrinopathies, and environmental influences, as well as exogenous factors, such as malnutrition or disease.

The formation of the tooth is a complex sequence of events from the first sign of calcification, to that of crown formation, root growth, tooth eruption, and maturation of the apex of root. In addition, tooth formation follows a continuous and progressive process, consisting of a specific sequence of events, that can be used efficiently to assess its maturation [90]. The teeth play a crucial role in the field of age estimation, as it has a prolonged post-mortem longevity, and is resistant to physical, biological, or chemical degradations, and is considered as the most indestructible parts of the body.

The teeth have a unique structure, and it follows a well and clearly defined pattern of sequential development. The age of the teeth can be determined more accurately in young children, because the teeth in this stage is actively undergoing development and mineralization simultaneously. However, most teeth are fully developed by around 14 years of age, however, the third molars mature at a later stage. The root of the third molar is formed around the age of 18-25 years, which is advantageous for estimating the age of those individuals who are over 14 years and above [90].

For children, an age estimate can be made on the basis of the developmental changes occurring in the teeth. On the other hand, for adults, changes in the hard tissue, such as the dentin, cementum, and enamel of the tooth, provide clues for estimating age. Regressive changes such as wear, secondary dentin formation, cementum apposition, and translucency of the root dentin can be used to estimate a person's age. Factors like attrition, and cementum apposition, are strongly influenced by a person's lifestyle, and therefore cannot be considered a reliable parameter. However, many authors consider the translucency of the root dentin, and secondary dentine deposition to be reliable for age estimation [6].

The pulp-dentin complex shows age-related changes, that mainly lead to a reduction in volume of the pulp chamber. This reduction in volume is due to the continuous deposition of secondary dentin in the pulp cavity after the eruption of tooth. As a result, the pulp cavity size decreases gradually with age. Therefore, secondary dentin deposition is an important morphological criterion for assessing age in adult subjects [45].

Some studies have been carried out to formulate the regression equations using secondary dentine as the only parameter. Age estimation can be made using secondary dentine qualitatively on the ground sections of the teeth through the Gustafson's (0-3) scoring system. Quantitatively, it can be done with the help of micrometric measurements, which was suggested by Kedici et al. It is also possible to quantify secondary dentin on radiographs, a method proposed by Kvaal et al [6].

Table 1.2. The Gustafson's (0-3) Scoring System. Source: Jasbir Arora et al. (2016).

Gustafson's (0-3) scoring system

Parameter	SCORE			
	0	**1**	**2**	**3**
Secondary Dentine	No formation of secondary dentine.	Secondary dentine formed up to the upper part of pulp cavity.	Secondary dentine formed up to two-third of pulp cavity.	Diffused calcification of the whole pulp cavity.

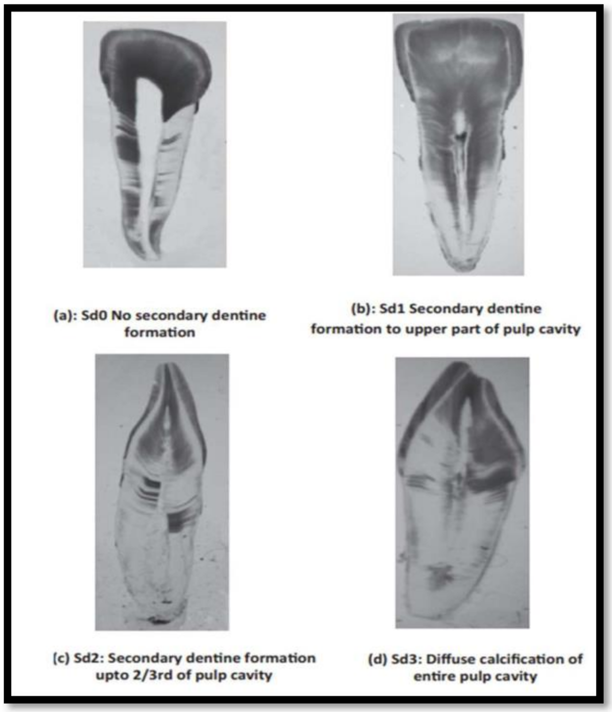


Figure 1. 5. The Various Stages of Secondary Dentine Formation. Source: Arora j et al. (2016)

Dental radiography is a simple, but non-invasive procedure that has recently been increasingly used for age determination, and is considered an essential tool for identification in forensics.

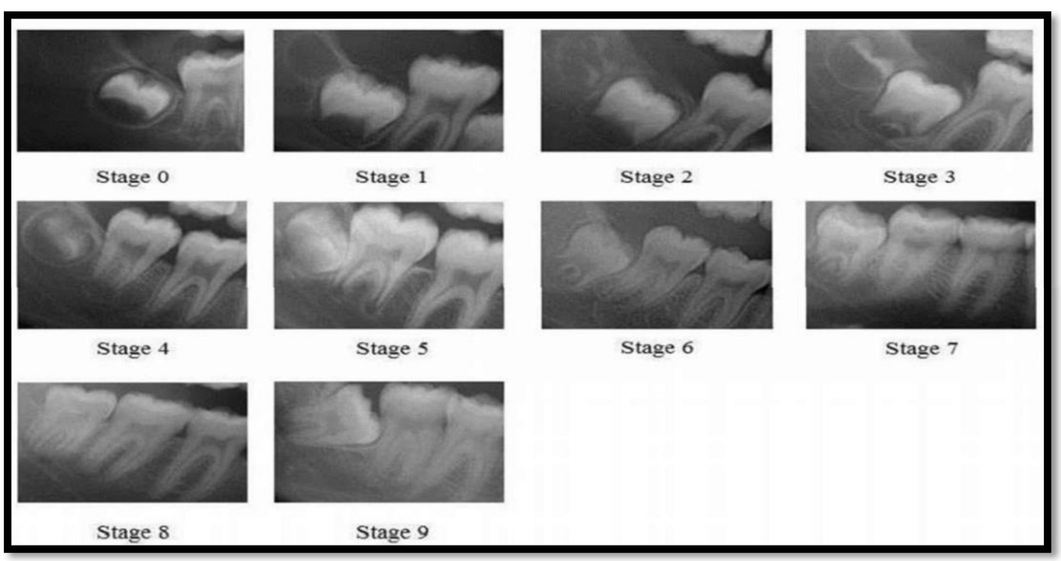


Figure 1.6. A radiograph of the different stages of development of the mandibular third

molar. Source: Phulari Rashmi G.S. et. al.

1.7.2. Age estimation from the skull:

The human skull consists of 22 bones, and its function consists of protecting the brain, and supporting other soft tissues of the head. Most of the bones of the skull are formed through intramembranous ossification, however, the base of the skull is developed by the process of endochondral ossification [103]. During the time of foetal development, the bones of the skull are formed within tough, fibrous membranes and they remain separated from the foetal period through childhood until reaching maturity. The separate bones provide flexibility, and help facilitate the foetal skull to pass through the birth canal. The skull bones begin to fuse as the individual reaches maturity to produce a tough, rigid skull, that protects the soft nervous tissues of the brain.

1.7.2.1. Cranial Sutures:

Between each adjacent bone of the skull, a saw-tooth, or zipper-like articulation intertwines two bones together. It may be present in the midline between the bone edges of two adjacent bones, or may be at the sites of overlapping bones plates (as seen in squamosal sutures). This articulation is known as a suture [103]. Cranial sutures come under the classification of fibrous joints, which means that they lack a synovial cavity, and also, the bones are firmly held together by fibrous connective tissue [58]. The sutures of the cranium perform the following functions:

- They prevent bone separation when external force, such as a trauma, is applied.
- They permit some bone movement during the period of rapid growth of the cephalic viscera [58].

Sutures, along the cranial bone margins, are used as markers for bone growth during craniofacial development. Depending on some undefined molecular mechanisms, some sutures remain open during the time of post-natal development, while some are closed. The formation of sutures appears to be regulated by growth factor signals along the osteogenic fronts of adjacent bones [103]. So far, twenty-four sutures have been reported in forensic osteological literature [4].

Figure 1.7. Cranial sutures. The top left, middle and the bottom right represents ectocranial aspects, endocranial aspects are represented in the bottom left (coronal, sagittal, and lambdoid sutures), A represents bregma sutures, B represents the midcoronal suture. C: Anterior sagittal suture. D: Obelion. E: Lambda suture. F: Mid-lambdoidal suture. G: Pterion. H: Spheno-frontal suture. The top right are facial sutures (I is representative for frontonasal sutures, J denotes nasomaxillary sutures), and bottom right represents maxillary sutures (K and L represent anterior median and transverse palatine sutures). Source: S. Ruengdit et al. (2020).

1.7.2.2. Morphology of Cranial Sutures:

Three different tissues interact to contribute to the suture morphology: the sutural mesenchyme, the dura matter, and the osteogenic fronts. The process begins when the sutural mesenchyme differentiates and deposits extracellular matrix, which are then eventually mineralized. The intervening bones separate the sutural mesenchyme into an outer ectoperiosteal layer, and an inner layer of dura matter. During the prenatal period, signals from the dura matter regulate sutures patency. The postnatal period is dominated by the osteogenic fronts. As aforementioned, the process of suture closure is controlled by some molecular mechanisms, thus, when any kind of disturbance is caused to this mechanism, a malfunction occurs in the suture patency and fusion process, which may lead to serious defects such as cranial disfigurement [135].

1.7.2.3. Cranial Sutures as an Indicator of Age:

From a recovered skeletal remain, the cranium is often the best-preserved portion. The method of age estimation by the study of cranial sutures has been continually used because the fusing of cranial sutures commences with increasing age, however, there is a considerable amount of variation in the rate and patterns of closure. The concept that fusing of cranial sutures occurs as the age progresses, has been in existence since the 16th century. This method has been studied across various populations by introducing several different methods, in order to produce the most accurate results [76].

F. Pommerol in 1869 [58] noted that for each suture, though the period of union varied across individuals, it nonetheless followed a general pattern. He made the following observations:

- People under the age of 35 years had open cranial sutures.
- The closure of the sagittal suture commenced around 40 years of age.
- The closure of the coronal suture commenced around 50 years of age.
- The temporal suture is completely fused by 65 years of age or more.

Table 1.3 gives an estimate of the age in which commencement of some of the sutures begins, and also the age range of termination.

Table 1.3. Cranial suture commencement of closure and termination [58].

Suture	Commencement of closure	Termination
Temporo-parietal suture	56-60 years.	66-70 years.
Lambdoid suture	25-30 years.	66-70 years.
Coronal suture	25-30 years.	56-60 years.
Sagittal suture	Towards the end of 26 years.	61-65 years.

The use of cranial sutures to determine age has always been the subject of considerable debate, and several researchers have not conclusively demonstrated its reliability within the parameter. Many studies have been published in the last few decades discussing the lack of precision and reliability of various methods. Sahni et al. (2005) examined age determination by assessing the closure of cranial sutures in a modern sample of

538 men, and 127 adult women of known ages from North-West India. The sagittal, coronal, and lambdoid sutures were examined at autopsy, and were put into records as “open” or “closed”. They further observed that obliteration commenced earlier in males, was initiated earlier along the endocranial surface, and that the process of commencement and obliteration were so inconsistent that they are not useful for age estimation [104].

Despite possible gender and interpopulation variations, three different modern studies have revived cranial suture closure as an accurate age indicator. Acsadi et al. based their method on endocranial union, however, they pointed out that determining the age from suture closure is possible between broad age limits only. They supported that suture closure is a vital age indicator, mainly when it is used alongside other age estimation methods. Meindl et al. based their method on ectocranial sutures and pointed out that their method is more accurate for people in the older age range. And thirdly, Perizonius suggested that different methods for age estimation should be used for individuals older than 50 years of age, and for those younger than 50 years, and proposed a system that used different endocranial and ectocranial suture sites for the appropriate age group [57].

Catherine A. Key et al. (1994), pointed out in their study, that suture closure aging techniques that are developed on one sample do not necessarily result in accurate aging when applied to another sample. This seems to be particularly true of techniques, such as the Meindl and Lovejoy method, and for the Perizonius system for the elderly individuals, which are based on the closure at ectocranial sutures. Discrepancies in literature where researchers such as Todd, Lyon, and Krogman, have pointed out that no dimorphism exists, while others such as Brooke and Singerg, have maintained that dimorphism exists suggests that there are interpopulation variations in the dimorphism of ectocranial suture closures, thus making endocranial sutures more accurate for age estimation when applied across different populations [57].

The problems faced when using cranial sutures for age estimation, which include variability, dependence on the age distribution of the reference sample, and gender differences, affect most age estimation methods. These reasons, thereby, present to us a challenge for developing more sophisticated methods that will answer questions that arise as to the functions of suture closure, the reasons for their extreme variations across gender and populations, and other environmental factors that affect the rate and order of suture closure [57].

1.7.3. Age estimation from the Pubic Symphysis:

The pubic bones are considered to be the best indicators of age among the macroscopic age estimation methods. The pubic bone, and its articular surfaces, are well preserved, because its anatomical position is a deeply sunk structure, which makes it less susceptible to environmental influences, even if the entire skeleton is destroyed. Assessing age of females by pubic symphysis may not be as accurate as that of males, due to the child bearing feature of females that can cause symphyseal changes.

The first study that correlated the morphology of the pubic symphysis with age was published in 1920 by an American osteologist, Todd. He described ten different phases related to the appearance of the pubic symphysis, which in turn are related to a corresponding age. Todd's methods, however, suffered from some limitations that came into light during the mid-eighties of the last century, and has since gone through several modifications. Today, the most commonly used method for assessing age by the pubic symphysis is the Suchey and Brooks method, which divides the changes on the pubic symphysis into six phases [106].

1.7.4. Recent Advancements in the Field of Age Estimation:

Ubelaker and Khosrowshahi [127] published a study in which they give an in-depth analysis of the historical and modern approaches in forensic anthropology, regarding the estimation of age. They emphasized upon the recent advances that are ultimately being considered for the estimation of age, and discussed each of them briefly. These recent advancements are centred on biochemical analysis, bone histology, technological advancements, and the application of mathematics, taphonomic impact, population variation, dental pulp examination, etc, for the estimation of age. However, there are certain cases in which the conventional methods are more preferred.

It is to be noted that any form of age assessment must consider appreciable differences that exists between populations, and between a male and female, and also on the climate of the region as it has been observed that fusion appears earlier in females, and in tropical climates. This was recognized far back in 1955, when Brooks [17] examined the cranium and pubis for age indicators, while noting the differences in sex, and other varying reliability, and he concluded that there is no one age indicator that is adequate. Also, there is a considerable variation in population in regards to the time of closure of the epiphyses. It is to be kept in mind that the methods that were used in one sample cannot be applied to another sample with the same amount of confidence. Thus, it is very important that an appropriate method and standard be chosen depending on the individual that is being examined, using the many regional tables that has been produced by anthropologists and radiologist as a reference. The nutritional status of the individual, and other socio-economic factors, also has an impact in the ageing process.

1.7.5. Issues with Age Estimation.

Ritz-Timme [95] pointed out in his research that the methods that are employed for age estimation should be able to meet the following criteria:

(i) They should have been presented to the scientific community by the means of peer-reviewed publication.

(ii) Valid statistical publications should have been used to test their accuracy.

(iii) The accuracy of the method should be well defined for routine forensic applications.

The current methods that are commonly used to estimate the age of skeletal remains is typically based on two methods: 1) Those that are based on the dentition, and the developing and growing skeleton, and 2) Those that are based on degenerative changes [19].

Forensic anthropologists usually try to achieve a physiological age estimation, which may be quite different than that of the chronological age of the individual. The degree of this variation will mainly depend upon the quality of preservation, and the availability of the bone elements on which the age estimation is to be performed, and the precision, validity and reliability of the method that is being used. When it comes to age estimation methods, it is of extreme importance that the valid aging method is being applied according to the type of bone elements which survived [19].

Furthermore, there is a lack of consensus when it comes to the methods that are used for age estimation. There arise issues when attempts are made to combine methods (the multifactorial approach), or to use revised methods because the formulations for most of these methods are based on a specific local sample as reference. This, thus, implies that there is a lack of a direct comparison of the reliability of different aging methods that are applied on the same population, and between the results that are obtained by the application of different aging methods on different populations. This creates implications when faced with the decision as to which method should be used in general, and/or for old individuals. This calls for the need to assess the variability between different populations concerning whether the standard that is used for a specific reference group will be the same for an individual who is not from that reference group. It can be concluded that the relationship between chronological age and indicators of skeletal age is neither uniform, nor constant, nor linear, across different populations [19].

For this purpose, knowledge concerning the relevance of aging strategies to samples from numerous populations, and knowledge of population variation in aging processes are elementary to accurate adult age estimation, and still solely few studies have evaluated population variations within the accuracy of aging methods. Similarly, these methods sometimes don't take into consideration the matter of each taphonomy, and post-mortem events, that might alter the preservation of skeletal remains, and the diagnostic bone components concerned [19].

1.8. <u>Estimation of Stature from Skeletal Remains:</u>

The estimation of stature from skeletal remains plays an important role in the examination of unknown human remains, as stature is one of the biological characteristics that can be assessed from a skeletal remain, even several years after death. Lots of intensive studies have been made to estimate stature by using measurements of different parts of the human bones [124].

For the estimation of stature, the technique employed depends upon the type of bone which has been recovered. Studies have shown that it is possible to estimate height with reasonable accuracy from various skeletal remains (such as, the length of long bones, feet, spine, metacarpal bones, metatarsals, and scapula). Over the last decades, anthropologists have used even the size of feet and shoes to estimate height and size of the body. After death, the body is said to increase in size by about 2 cm, due to loss of muscle tone, joint slackening, and tension in the interventricular discs. To calculate the height of an individual, 2½ - 4 cm is added to the total length of the entire skeleton for the thickness of the soft tissues [123].

Standards for stature estimation vary between individuals, as stature is a variable among sex, age and population; therefore, it is important to determine which regression formula is to be applied. Sex differences and population variations have important impacts on the stature of a person, and age also serves an important factor as the more a person ages, the more likely it is for the vertebral discs of the spinal column to compress, thereby, reducing the expected height of the person.

1.8.1. Two Methods for Stature Estimation:

a) **Anatomical Method:** An anatomical method is applied in cases where the complete skeleton has been recovered, and can be achieved using some correction factors. [15, 75]. The axial skeleton is carefully arranged, and gaps are left between the vertebrae for thickness of the intervertebral discs, and gaps are also left between long bones making up space for the articular cartilage. The direct measurement of the whole skeleton can never be accurate to about within 5 cm, as it is not possible to accurately space the bones.

b) **Mathematical Method:** In cases where incomplete skeletons or fragments of skeletons are recovered, stature is estimated mathematically using regression equations and the multiplication factor method [64]. Stature can be estimated from the smallest bones with a great accuracy by regression analysis. Incomplete bone fragments are first used to determine the bone length using a regression equation, after which stature can be estimated by applying the original formula.

Regression analysis is the most preferred method among researchers [2, 113, 46]. The first step is to determine the correlation between stature and the variable of interest, and then fit a line to this correlation to develop a regression formula from the slope and constant of the line. The independent variable is multiplied with the slope, and the coefficient is added to predict the stature. The predicted stature value will differ from the actual stature of the person by some few centimetres, and this difference can be reported in terms of root means square error (RMSE).

Rajesh Verma et al. [130] emphasized that regression analysis and RMSE alone are not sufficient for stature estimation, and that the regression method requires to be approached from a different perspective, as overestimations or underestimations may occur with values that are far greater than the RMSE. They professed that the prediction of confidence intervals (CI) can help to an extent, and the likelihood ratio (LR) approach can help to quantify strength of the level of identification. The likelihood ratio framework was generally introduced by Aitken et al. [3] and Robertson et al. [96], as an approach that sheds light on the similarity of the evidence to the reference and the rarity or common typicality of the evidence in a population which will helps strengthen the reliability of the evidence. For the purpose of stature estimation, the LR approach can help to answer what the probability is to get a bone of a certain length, if it came from a missing person whose height is known, and what the probability is that we will get the same bone from another missing person, or any other person, who is randomly selected from the population of relevance to the missing person [80].

1.8.2. Stature Estimation from Cranial Sutures:

It has been found that the length of sutures can also help in stature and sex estimation. Researchers have tried to use various parameters of the human skull to assess the stature of the dead person. In the past, some researchers, such as Introna et al., as well as Patil and Modi, have attempted to determine the height of a person from cephalon-facial and craniofacial materials. Chiba and Terazawa regressed the length of a cadaver using three skull variables viz., diameter of skull, circumference of skull, and a sum of the skull diameter and circumference, to estimate stature for Japanese sample. Patil and Modi used lateral

cephalometric X-rays to determine gender and estimate height, and derived a regression equation from the length of the skull, which they found to be very reliable in estimating stature [123].

1.8.3. Stature Estimation from Long Bones:

The most common method for estimating the living stature from skeletal remains is to use equation tables that have been developed for the purpose of measuring stature from long bones. [73]. The maximum length of long bones is measured in an osteometric board. The long bone length varies according to the points that are used at each extremity. The measurements are taken from the following points of long bones:

- **Femur:** Maximum length measured as a straight line, from the highest point on the head of the bone, to the deepest point on the lateral medial condyle.
- **Humerus:** Maximum length measured as a straight distance, from the highest point of the head of the humerus, to the deepest point on the trochlea.
- **Tibia:** Maximum length measured as a straight distance, from the anterior edge of the lateral condyle, to the tip of the medial malleolus.
- **Fibula:** Maximum length measured as a straight distance, from the highest point of the head of the fibula, to the deepest point of the fibular malleolus.

- **Radius:** Maximum length is the straight distance, from the highest point of the head of the radius, to the tip of the styloid process.
- **Ulna:** Maximum length is the straight distance, starting from the highest point of the olecranon, to the tip of the styloid process.

Dry bones, which are old, usually tend to be slightly shorter in length than recent ones [107].

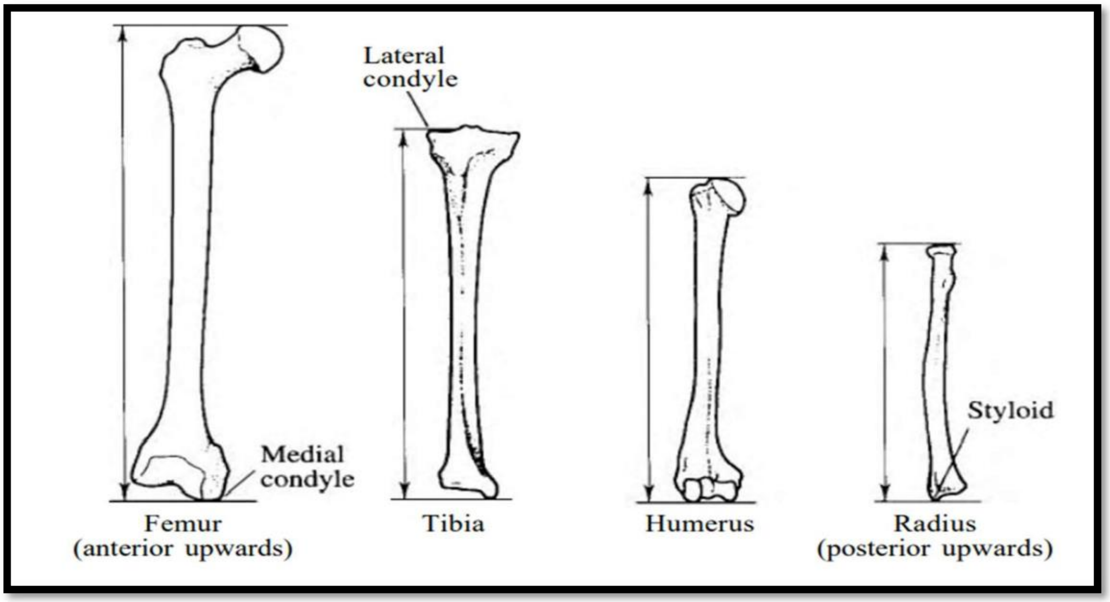


Figure 1.8. Dried bones dimensions for stature estimation. Source: Knight BH (1991).

For anatomical tables that are used to calculate stature, every ethnic group has their own set of tables that were formulated at a particular period of time in the past, which may not be applicable to the modern-day population. Trotter and Glesser professed that the world population is getting taller as time advances, and as such, the formulas require periodical refinement, or may even call for the need of developing fresh new formulas [107].

1.9. Estimation of Sex from Skeletal Remains:

Sex is the biological attribute of species that reproduce sexually based on their reproductive functions, which shows variability across intra and inter populations. Genetic traits vary depending on the functional differences between the sexes that are ultimately represented in body tissues. The function that is taken up by the body tissue will determine the final appearance, and various related responses. Factors such as biomechanical loading and stress, together with the functions that are performed by a body part, determines the morphology of that body part. In addition, secular changes in size and height, as well as, in the size of individual bones have been observed between the generations. In comparison to females, males have a greater stature, more robust skull and facial features, and more muscularity [62].

Sexual dimorphism in bone morphology can be attributed to female reproductive capabilities, variations in body size and proportions, and differences in muscle structure and muscle mass. An accurate estimation of sex is essential as other aspects of a biological profile of an unknown skeletal remain, such as age, stature and ancestry may be dependent on gender characteristics [38].

Sexing skeletal remains is often challenging when infant bones or bones of an immature person are recovered as they are pre-pubertal. However, for adult bones, the skull and pelvis provide the most accurate estimates (about 90% accuracy) for sex estimation, and long bones too, are used in the sexing of skeletal remains. But since it is not often the scenario that complete bones will be recovered from the crime scene, and in most cases incomplete or only fragments of bones are recovered, it is necessary to be able to estimate sex from as many skeletal parts as possible. Researchers now look for alternative methods to determine sex by examining bones, such as the patella, clavicle, mandible, vertebrae, scapula, etc [7, 30, 52, 110, 119, 120, 126].

The pelvis is regarded as the most reliable skeletal element for the determination of sex of human remains, as the female pelvis is designed in such a way so as to provide optimal space for the birth canal, creating a marked difference between the male and female pelvis. Although the skull has traditionally been considered the second-best indicator of sex after the pelvis, recent studies have shown that when the pelvis is missing or fragmented, postcranial factors should take precedence over the skull when assessing gender [29].

Metric analysis of os longum dimensions with multi variate models properly estimates sex at an accuracy of up to 94 percent, while many single methods accurately estimate sex 80-90 percent of the time. For the estimation of sex through metric analysis of postcranial bones, long bones are more preferred because measurement standards are easy to apply, and their preservation is usually better due to their strong and robust tubular structure [38].

Much research over the past years has focused on the femur, and it has shown an accuracy of gender classification of up to 89% for a single measurement, and of more than 93% for multivariate methods. Sexual dimorphism in femur is partly due to the relatively higher absolute axial skeletal weight of the males compared to the females. This weight is mainly carried by the femur in body weight transmission. In addition, variations within the anatomy of the pelvis due to the child reproduction capabilities of a female causes a variable amount of stress to the femur [38].

1.9.1. Sex estimation from the pelvis:

The hip bone has for very long been considered as the most dimorphic bone, especially in the case of adult individuals, and several sex estimation methods have been developed for the hip bone, based on visual assessment, or by recording the linear metric variables of the hip bone. Visual/morphoscopic methods involves assessment of diverse traits, such as the shape of sciatic notch, or preauricular sulcus, or the subpubic angle. After visual assessment, sex is assigned based on a rating that separates males from females. This method, however, has been subject to heavy criticism, as it is highly subjective, requires a highly experienced observer, and tends to be unreliable when the final score and the separating value are close [43].

Some of the notable differences between the male and female pelvis is illustrated in Table 1.4 and Figure 1.9.

Table 1.4. Differences between male and female pelvis [53].

Sl. No.	Pelvis features	Female	Male
1.	Whole pelvis.	Larger, broader, lower.	Taller, narrower, more compact.
2.	Pubic symphysis.	Smaller, less deep.	Larger.
3.	Sacrum.	Shorter, broader, more curved.	Long, narrow, straighter.
4.	Pelvic inlet.	Kidney-shaped.	Heart-shaped.
5.	True pelvis.	Spacious.	Relatively less capacious.
6.	Sub-pubic angle.	Larger, rounder, usually 80-85 degrees.	More angular, around 50-60 degrees.

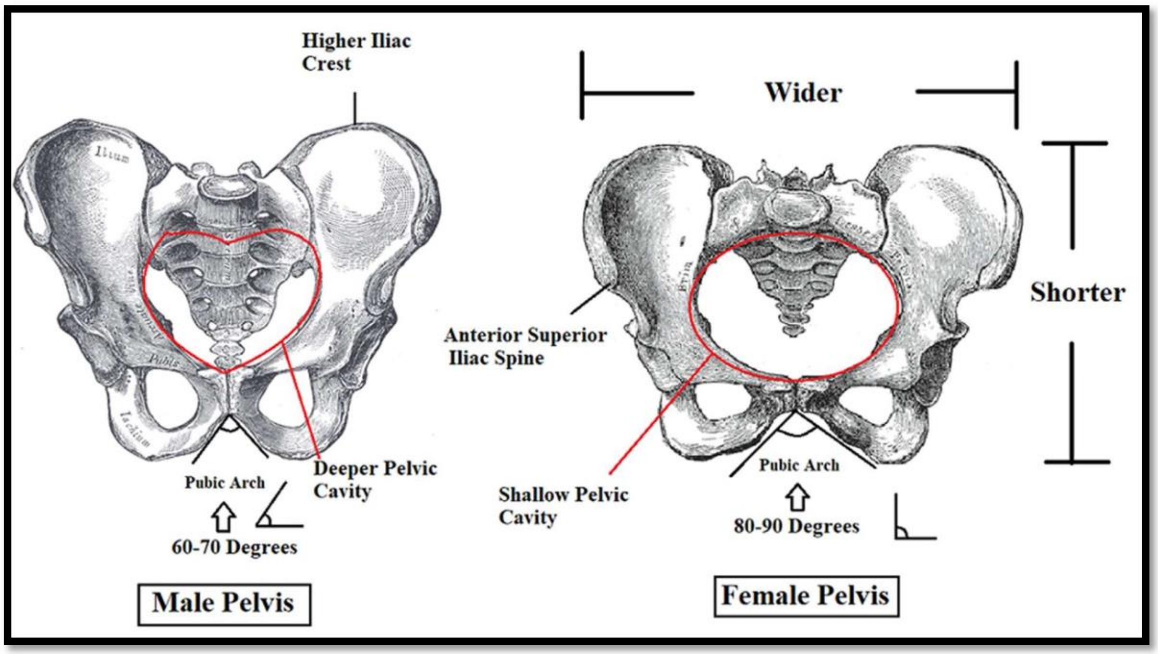


Figure 1.9. Difference between male and female pelvis. Source: https://www.registerednursern.com/male-vs-female-pelvis/

1.9.1.1. Factors influencing pelvis features in sex estimation:

The various factors that influence the process of age estimation from the pelvis have been briefly described below [53]:

a. **Age:** As previously mentioned, it is quite challenging to estimate age from the remains of children, as the skeletal features characteristic of sex has not yet been developed.

b. **Height:** Holland et al. in their study in 1981 observed that there existed a correlation between the height and pelvic size, stating that people who were taller had a larger pelvis. Thus, ethnic groups with characteristically tall people will have different demarking points from those races characterized by people of lower height.

c. **Nutrition:** Ions like phosphorous, calcium, potassium etc., are known to stimulate bone growth. Usually, individuals lacking such ions in their diet have been observed to have anomalies of the sacrum.

d. **Climate:** People from cold or damp regions have been observed to have a stunted growth. While people from hot climate regions have been observed to have a taller height. Since height is related to the pelvis (taller people, larger pelvis), climate also indirectly comes into play as a factor influencing the pelvis size.

e. **Disease:** Degenerative diseases, such as osteoarthritis, and nutritional deficiencies, such as rickets, can cause deterioration to the vertebral column and pelvis, and may even cause distortion.

Other factors that have an influence on the pelvis features include weight of a person, race, heredity factors, etc.

1.9.2. Sex Estimation from the Skull:

The Skull serves as a protective casing for the brain and is usually well preserved. For several years, researchers have relied on the features of the face and the mandible to ascertain the sex of the skull [79]. The various morphological differences of various parts of the skull can differentiate a male and a female, especially in those cases when the skeletal remains are fragmented, posing a challenge for digital examination. Swetha and Thenmozhi (2018) [122] described a number of morphological differences between the male and female skull in their study which is given in Table 1.5 below:

Table 1.5. Differences between a Male and Female Skull [122].

Sl. No.	Skull Parameter	Male	Female
1.	Size and Weight	Thicker, Heavier, Larger.	Lighter and Smaller.
2.	Forehead	Slightly sloping.	Vertical.
3.	Temporal Ridge	More pronounced.	Less pronounced.
4.	Parietal Protuberance	Less.	More.
5.	Supraorbital Arch	More prominent.	Less prominent.
6.	Infraorbital Margin	Blunt.	Sharp.
7.	Mastoid Process	Large.	Small.
8.	Glabella	More prominent.	Less prominent.
9.	Frontal Eminence	Small.	Large.

The male skull is, overall, more massive and rugged, while the female skull is rounder with smoother facial bones and smaller mandible, jaws and maxilla as compared to the male.

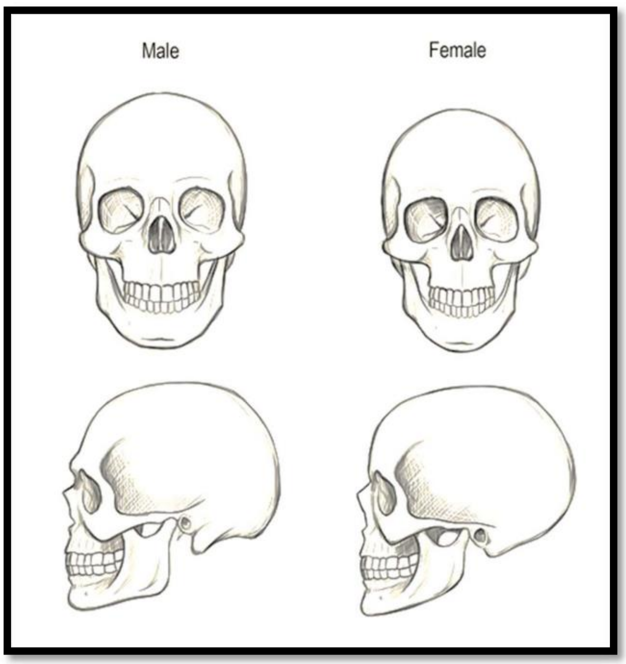


Figure 1.10. Difference between the Male and Female Skulls. Source: https://jeffsearle.blogspot.com/2015/04/the-human-skull.html

1.9.3. Sex Estimation from other skeletal elements:

İşcan [54] in his study suggested that the sternal end of the fourth rib can be a reliable indicator for sex identification, but studies performed by other researchers [25] observed that this showed low rates of success in non-American populations.

Peleg et al. [89] performed a study to estimate the sex of a person by examining the sternum and the ribs, and they suggested that the valid traditional, virtual, reliable, simple, and accessible methods can be used to estimate sex, using the sternum and ribs. They determined success rates for each of the ribs separately which will help in cases where some ribs are missing.

1.10. EVALUATION OF ANCESTRY:

Determining the ancestry of the deceased is required as a precursor to estimate other aspects that make up the biological profile of the unknown individual (such as the age, stature, and sex), as this helps to improve the accuracy of the estimate.

Carolus Linnaeus included humans in his broad binomial classification system of plants and animals [28]. He gave the genus and species *Homo sapiens* to human beings. He furthermore suggested that subdivisions that are based on geographical variations could be recognized. The subdivisions were classified as H.asiaticus for Asians, H. americanus for Americans, H. europaeus for the Europeans and H.afer for the Africans.

The Linnaeus classification was extended by Johann Friedrich Blumenbach, who added the Malayan category and added more detail on head anatomical features [28]. These classification systems reflected the attitudes and perspectives of that time period and thus, all these variations were relatively fixed and static with a religious perspective on human variation that followed a natural order and could be described.

As more information on the subject of human variation accumulated, and with the increase of knowledge in population genetics, the old typologies for racial classification began to fleet. Skin colour varies in different regions of the world, and classifying people into a particular race based upon their behavioural impressions reflected more about the perception and attitude of the classifier, rather than the population's characteristics. However, even though the scientific basis for such a system of classification has been disregarded, the terminologies still continue to persist. Today, the scientific basis for classification of population into a particular race category depends on the gene flow process, and genetic variations within a particular group. Such variations have been shaped by natural selection, genetic drift, sexual selection, size of population, and demography [28].

According to a racial classification developed by Carleton S. Coon in 1962, there are 4 major ethnic groups: Caucasian, Negroid, Mongoloid and Australoid. The identification of race from a recovered skeletal material has become even more challenging in the recent decades, due to ethnic mixing, immigration, etc. [28].

1.10.1. Cultural clues from the skeleton:

Discovering a certain kind of cultural clue from the skeleton is not very often encountered in routine caseworks, however, it can add value to the investigation process, if found. There are various instances of such cultural clues which include modifications to the teeth or the skull, for example, chipping the teeth in specific patterns which was prominent in past American cultures, mainly concentrated in Mesoamerica. These were performed as a tradition that was a part of cultural practices in different parts of the ancient world. [28].

Certain Eskimo dentitions have revealed chipping of occlusal surfaces due to pressure resulting from using the teeth as tools for stressful functions, such as chewing leather, and grinding bones. The teeth of past populations also showed extreme occlusal wear patterns, reflecting ancient cooking methods. All kind of similar situations may indicate that the corpse is ancient and has nothing to do with modern age medicolegal issues [28].

Various cases of modifications to the cranium which were done intentionally, for traditional purposes, are documented in several ancient cultures, such as the Maya, that can help identify specifically, both the period of time, and the group or region from which it originated [96].

The direct dating of the recovered remains makes it possible to distinguish the ancient specimens from the modern ones. The choice method for such a dating is the radiocarbon analysis. Modern percentage of value about 100 indicate the tissue sample formed after AD 1950. Values below 100 indicate that radioactive decay is being detected, and the date before 1950 can be estimated from the extent of this decay [129].

1.10.2. Forensic Significance:

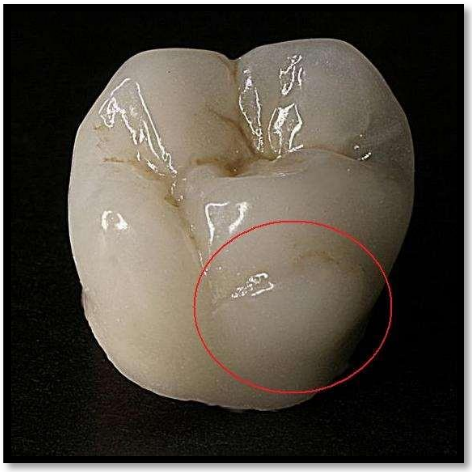
- In some regions, the cultural factors described above can help with group identification. An account of the history of the local population adds value to the assessment in forensic cases. Medical procedures and related technologies can help identify the population. This information is particularly important when assessing unidentified migrants [28].
- Ancestry assessment helps to use the correct reference population for other methods used for estimating age, gender and height, which is why some authors suggest that

this should be the first parameter to be assessed, as the result determines which methods should be used in the other three generic identification parameters [28].

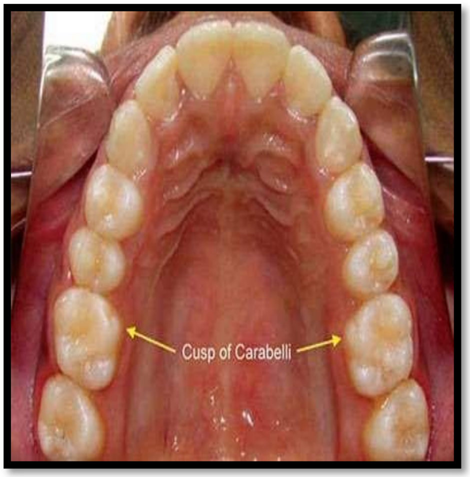
1.10.3. Ancestry Assessment from the Dentition:

The first research that used dental features to estimate the ancestry of an individual was carried out by Lasker and Lee [67]. They reported that shovel-shaped incisors are more common in Mongoloids, while Carabelli’s trait is more common among Whites. However, for Negroids, no common dental traits were recorded. Shovel-shaped incisors and the Carabelli’s trait remain prevalent as the most commonly used dental features in forensic analysis.

It has, however, been lately discovered in recent studies [26] that the frequency of the Carabelli’s trait varies among populations all over the world. Von Carabelli was the first to describe the Carabelli’s trait in 1842. It is one of the most studied morphological characteristics of the teeth. The Carabelli’s trait is characterized by a pit, cusp, or bump, present on the mesio-lingual side of the maxillary deciduous permanent, and pre-molars [108]. In a recent survey that was conducted by Correia and Pina, [26] a report was generated, highlighting the Carabelli’s trait frequency of the first molar. This frequency ranged from 13.5% to 85% of the population from Alaska Natives to White Americans.



a)



b)

Figure 1.11. (a-b). Carabelli's trait.
Sources: a) https://supernumeraryteeth.com/cusp-of-carabelli/

b) https://www.jaypeedigital.com/book/9789350259405/chapter/ch12

Shovel- shaped incisors are characterized with ridges present on the mesial and distal margins of the lingual surfaces. Shoveling is commonly studied as a qualitative variable, however, it has also been examined by some researchers as a quantifiable metric trait, by measuring its depth from the centre of the lingual surface. Shovel- shaped incisors have a frequency that range from 0 percent to 91.9 percent in a wide geographic area range [23].

The frequencies of shovel-shaped incisors in Western Europe, Africa, and Pacific groups are the lowest, while the frequencies in East Asia, North Asia, and Native Americans are the highest. Shovel-shaped incisors are most commonly used to determine whether a skeleton is Native American, or a Hispanic in North America [23].

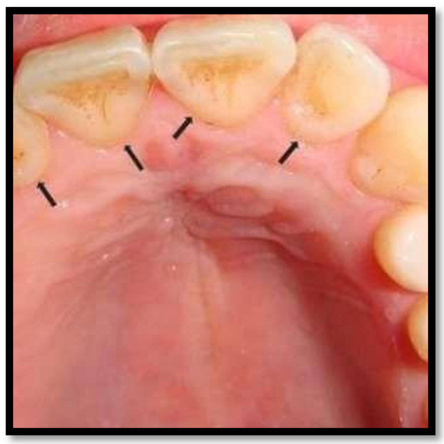


Figure 1.12. Shovel-shaped Incisors. Source: Canger EM et al. 2014 Sep 1.

1.10.4. Ancestry assessment from the skull:

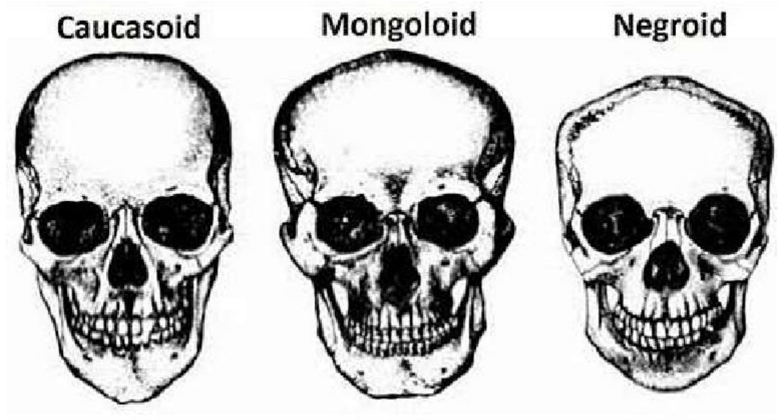


Figure 1.13. The Caucasoid, Mongoloid and Negroid Skulls. Source: https://bonafidebones.wordpress.com/2018/05/08/racial-origin/

- **The Caucasoid Cranium [12]:**
  - The cranium of Caucasoids is long in length, have a narrow breadth and are high in height.
  - It exhibits a round sagittal contour, and they have a sloping forehead, as compared to those of Negroids and Mongoloids.
  - Their occipital profile is also round, and they possess strong nuchal muscle markings (114).
  - The frontal bones of Caucasoid males were found to be thicker than that of Negroid males in a study done by Bass (1979:558).

- **The Negroid Cranium [12]:**
  - The cranium is long in length, has a narrow breadth, and low height.
  - Flat sagittal contour (due to post-bregmatic depression), and rounded occipital profile.
  - Parieto-occipital area thicker than that of Caucasoids.

- **The Mongoloid cranium [12]:**
  - Long in length, but appears round, broad breadth, and an average height.
  - Angular occipital profile.
  - Moderate nuchal muscle markings.
  - Arched sagittal contour due to ‘keeling’ of the skull vault.

Some of the morphological variations observed in the skull and the teeth among the different racial groups [12] are described in Table 1.6.

Table 1.6. Morphological variations in the skull and the teeth among different racial groups Source: i) Modified from Blumenfield 2000, Table 1 ii) Chaurasia A et al. (2016) iii) https://www.futurelearn.com/info/courses/forensic-facial-reconstruction/0/steps/25658

Characteristics	Mongoloids	Negroids	Caucasoids
Cusps of Carabelli	Absent.	Present in the maxillary molars, but not highly prevalent.	Present in high prevalence.
Nasal profile	Concave, less pronounced nasal bridge, heart shaped nasal aperture.	Concave/straight, flat nasal bridge, nasal aperture is wide.	Straight, narrow, nasal aperture is high, nasal bridge is pronounced.
Shape and size of teeth	Shovel-shaped upper incisors. Largest in size.	Usually large with wider spacing between the teeth in comparison to the other two.	Chisel-shaped and smaller in size, exhibiting a smooth lingual surface.
Dental Cusps	No reduction in number.	Usual number.	Reduced number.
Cranium	Long in length but they frequently appear round instead of long. Broad in breadth with an average height.	Long in length, a narrow breadth and low height.	Long in length, narrow breadth and high in height.
Occipital profile	Angular with moderate nuchal muscle markings.	Quite rounded.	Rounded, strong nuchal muscle markings.
Sagittal Contour	Arched.	Flat.	Round, sloping forehead.
Mandible	Robust.	Oblique gonial angle, gracile.	Medium.
Orbital Form	Round.	Round.	Rhomboid.
Chin Projection	Moderate.	Reduced.	Prominent.
Facial Prognathism	Moderate.	Extreme.	Reduced.
Alveolar Prognathism	Moderate.	Extreme.	Reduced.

1.11. Effect of trauma on bones:

Like most materials, the bone is stronger under compressive stress in comparison to tensile stress. The compressive strength of the bone is directly proportional to the density of the bone. The organic components of bones are elastic, and can absorb tensile and compressive forces. The rate and duration of load will determine how bones react to stress. A slow loading stress that is continuous will cause an elastic deformation followed by plastic deformation, and finally leading to failure. On the other hand, a loading stress that is rapid causes bone failure in the absence of elastic and plastic deformation. A slow loading stress is related to a blunt force trauma, while a rapid loading stress is related to trauma caused by firearms [72].

For an accurate fracture interpretation, it is important to understand the onset and extent of the fracture. The starting point (initiation point) of a fracture is where the bone first breaks, and the spread (propagation) of the fracture is the path the fracture takes through the bone. The fracture propagation depends on the direction of the force and the microstructure of the bone and support system. The occurrence and propagation of fractures are highly dependent on the type of impact, the magnitude of the force, and the cortical thickness [72].

1.11.1. Blunt force trauma:

In forensic examination of skeletal remains, understanding the characteristics of bone trauma is a key feature that will assist in the comprehension of nature of deaths that are related to blunt force. Blunt force trauma (BFT), which is also referred to as non-penetrating trauma, is the injury that is caused to the body resulting from a forceful impact, a fall or an attack with a blunt or dull object, for example, a punch to the body is a form of physical attack that results in BFT [31].

The knowledge of the forensic anthropologists in bone biomechanics enables them to fully and accurately assess skeletal trauma, which can provide important information for the medical and legal investigation of death. Through the analysis of the pattern of a blunt force trauma, the anthropologist can determine the minimum number of impacts, the direction of the impact, and information about the impacting surface. It can also help to analyse the tool mark (if BFT was caused by a tool), thus helping to recognise the class characteristics of the tool in question [72].

It is not uncommon for forensic anthropologists and pathologists to examine injuries related to a blunt force trauma, and the interpretation of BFT on bones contribute immensely towards the investigation of trauma related deaths. A common question which is generally asked to a forensic expert in the court is what was the degree of the force that was required to produce the trauma present on the bone, and what was the impact mechanism that was involved. Forensic experts usually categorise the degree of force into three categories viz. mild, moderate or severe, based on factors such as the appearance and severity of the injury that was caused by the trauma with regard to the impact force. However, categorising trauma as mild, moderate or severe, is subjective in nature and thus, lacks empirical support [31].

The analysis of the fracture characteristics, as well as the fracture distribution pattern, often provides information about the events surrounding death. For example, a broken rib pattern that results from falling onto a wide surface, such as a floor, is very different from a broken rib pattern that results from a physical attack with multiple blows in different directions [72]. There are various internal and external factors that has an effect on how a bone is fractured. Some intrinsic factors include the geometry and density of the bone, while extrinsic factors encompass the area of the impacting object, the weight, and the velocity of the load [133].

1.11.1.1. <u>Types of fracture patterns caused by blunt force trauma:</u>

Three types of fracture patterns have been described viz. linear fractures, complex fractures, and comminuted fractures [72]:

- Linear fracture patterns are characterized by two end points. They are usually fairly straight with no displacement in bone, and are the most common fractures observed.

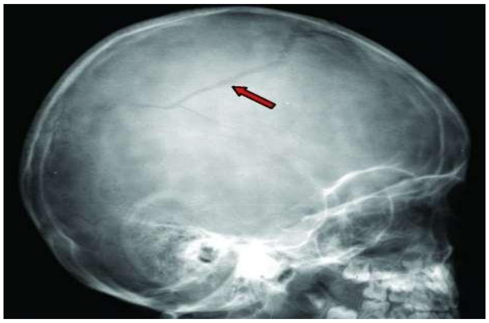


Figure 1.14. A linear fracture on the right parietal skull. Source: Ciurea, Alexandru & Gorga (2011).

- Complex fracture comprises of three or more than three end points. These types of fractures occur in cases where there is a sudden trauma impact, an auto accident, or when a person takes a hard fall from a height. Complex fractures cause shattering or breaking of multiple bones in several places.

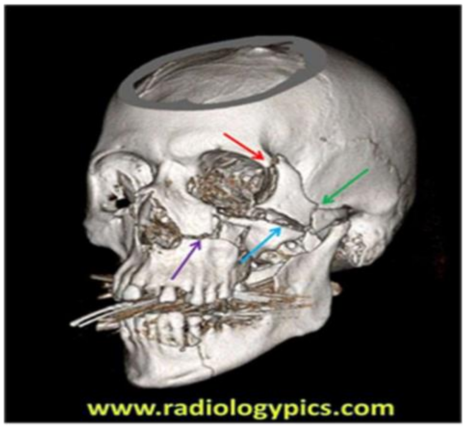


Figure 1.15. A complex fracture. Source: https://radiologypics.com/

- A communited fracture pattern is characterized by fragmentation of the bone. This kind of fracture usually involves a high-impact trauma as a considerable amount of force, and energy is required to fragment a bone. An example of one such high-impact trauma is one that is caused by vehicular incidents.

Figure 1.16. A Comminuted Fracture. Source: Comminuted Fractures reviewed by Richard. W. Kruse, June, 2018.

- Another type of fracture pattern that is commonly encountered is the depressed fracture pattern which is characterized by pieces of a fractured skull pressing inwards. This kind of fracture patterns are caused in cases when the person has been struck with a hammer or rock, etc.

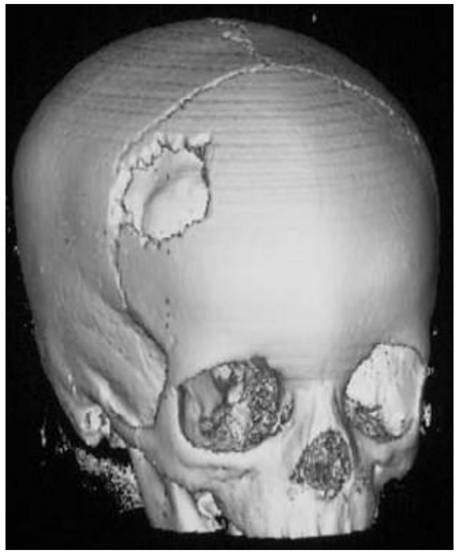


Figure 1.17. Depressed Fracture Patterns. Source: Parizel et al. (2005).

Each of these pattern types provide useful information relating to the impact. Fractures that occur on long bones and ribs also provide information about the direction of force and impact type, and a lot of research has been recently focussing on these bones. Reber and Simmons used a pendulum apparatus to impact 255 femora of sheep, and measured the impact force magnitude with an accelerometer, and recorded the impact site and fracture propagation with a high-speed camera [93]. By evaluating the entire fracture pattern, including complete and incomplete fractures, the authors were able to accurately identify the location of impact in 98% of the samples. The authors described eight types of fracture patterns, each of which consisted of a complete fracture at the point of tension, and a radiating fracture penetrating the bone at different angles. For most of the samples, the complete fracture was at the opposite direction of the point of impact.

Animal models have been utilized in experimental research for hundreds of years to mimic human conditions, with the most used models being porcine head and limb models for trauma studies. This approach has a massive advantage of analysing large sample sizes, thus helping to observe a greater variation is fracture patterns [5].

Love and Simes examined 43 cases of rib fractures caused by blunt force trauma and found that, contrary to current biomechanical knowledge, the anterior area of the ribs (the area where the cortical bones of the ribs are thin) cannot withstand the preload pressure. They termed the fracture type as "buckle fracture" and suggested that the fracture was caused by structural failure rather than material properties. In addition, the authors equated these fractures with anterior to posterior forces exerted on the anterior portion of the chest [71].

1.11.2. <u>Sharp force trauma:</u>

Injuries caused by pointed or sharp-edged objects causes a sharp force trauma. These types of injuries are characterized by a well- defined traumatic tissue separation, which occurs when an object that is sharp-edged or pointed, is stabbed into the skin and underlying tissues. In cases of injuries relating to a sharp-force trauma, the wound has a greater depth than the length of the wound on the skin.

Figure 1.18. A sharp-force trauma on the skull. Source: Steyn et al. (2014). Antemortem trauma.

When a bone or a cartilage is cut with a knife or a saw, the signatures of the cutting edge of the tool gets recorded on the cut surface (Fig. 1.19). A considerable amount of research has focused on the analysis of toolmarks on bone and cartilage [72].

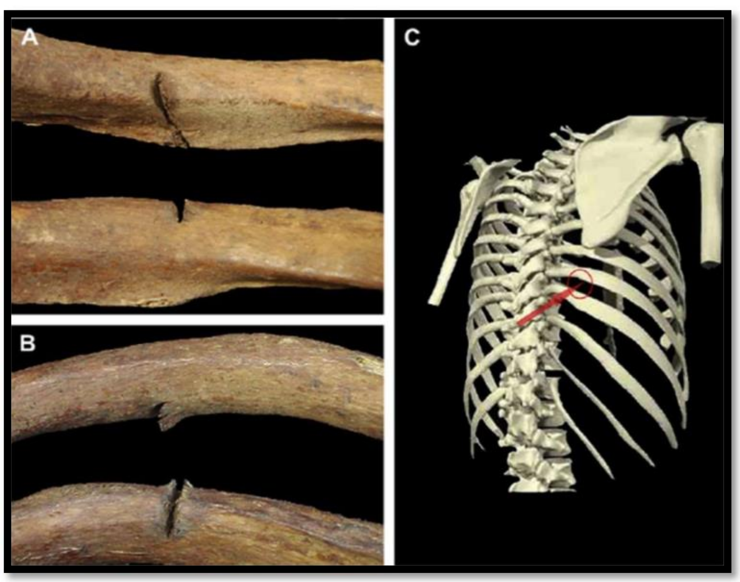


Figure 1.19. Cut marks on right ribs of a female produced by a sharp-double edged weapon. (A) anterior side (B) posterior side (C) location and direction of blow. Source: Jordana (2009).

Symes assessed the bone cuts made with 26 saws and knives with serrated edges, and observed various features that defined the class characteristics of the tools [72]. Some of the class characteristics described by Symes involved design of the saw, the most significant being the tooth set and the respective distance between the teeth.

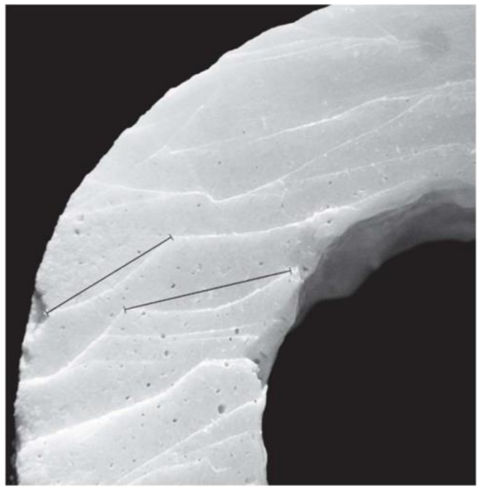


Figure 1.20. Impressions of cuts made by a saw on the bone. Source: Symes SA. (1992).

1.11.3. Firearm trauma:

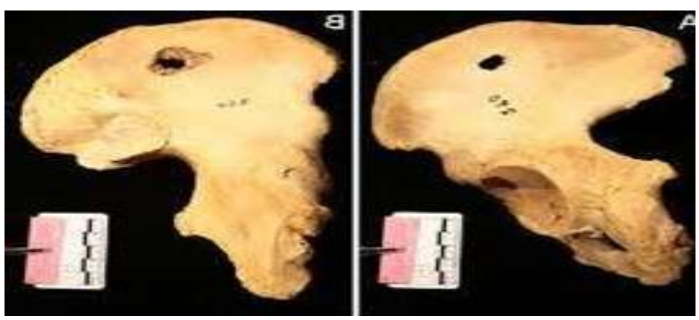


Figure 1.21. Firearm trauma. Source: Humphrey C, Henneberg M. 2017 Jun 27

The specific questions that are asked generally in cases of firearm trauma concern the trajectory of the projectile and the number of individual hits. The literature on the interpretation of ballistic wounds is fraught with problems and has led to a simplistic focus on projectile velocity as the main factor influencing the characteristics of injuries caused by firearms. Penetrative gunshot wounds are usually classified as “low velocity” or “high velocity”. Low velocity, as per ballistic definitions, ranges from less than 400 feet per second to 2000 to 3000 feet per second. According to this definition, most pistols are considered low-velocity, while many rifles are considered high-velocity, but there is a significant overlap between the two. Additionally, velocity alone does not explain the variations in ballistic wounds [35].

Similar to blunt force trauma, both intrinsic (density, geometry, bone, etc) and extrinsic (velocity, size and shape of the impacting object) factors influences a gunshot injury, and this applies to both soft tissues and bones [35]. A considerable focal variability is exhibited by bones, based upon morphology and structure, in its resistance to fractures. Young's Modulus states that the capacity of any material, including the bones, to resist a fracture primarily depends on the rate of applied force. Rapid loads are imposed by firearm projectiles and the bones tend to respond to rapid loading as a brittle material. The harder the tissue, greater is the amount of resistance that is presented to the impacting projectile, and greater is the destruction it will undergo upon failure. Bone is not prone to plastic deformation under high load. Due to the relative lack of plastic deformation, there are usually many bone fragments produced by ballistic impact [72].

The anthropological contribution to the interpretation of ballistic trauma is usually made through anatomical reconstruction of bone fragments and subsequent interpretation of bullet trajectories. Generally speaking, bone loss is less than soft tissue loss, and with careful reconstruction, bones usually provide a detailed record of the characteristics of ballistic wounds [72].

Experimental gunshot wounds on pig ribs were examined by Kieser et al. and several consistent entries and exit characteristics were identified [71]. A cone was created within the rib by the projectile with a smaller defect on the entrance side, and a larger defect on the exit side. In addition, the exit surface was beveled and the radiating fractures extended outward from the wound. It is, however, necessary to exercise some caution while making inferences about the projectile caliber using defect size [60].

1.11.4. Thermal trauma:

The exposure of skeletal remains to extreme heat can cause significant alterations in the bones, thereby posing some challenges to its proper interpretation. Forensic anthropologists have been trained to differentiate osseous from non-osseous material and human from non-human remains, and these are necessary while sorting through a fire debris [72].

The thermal destruction of the bone leads to dehydration, consumption of organic components, changes in color, splitting, shrinkage and deformation; however, important information can be obtained from the burned bone. The deformation of the bone precludes metric analysis to estimate the biological profile, but morphoscopic methods can be used with precision. Often bony pathological features are recognizable in burned bones. In burned bones, despite heat-induced deformation and shrinking, pathological changes in bone shape are still visible. The color of the bone can shed light on the context of the burn and temperature. For example, green, yellow, pink, or red spots on the bone mean that the bone was burned in the presence of metal, such as copper, bronze, or iron, while blackened cortical bone indicates a lower temperature of around 300 ° C, and white cortical bone indicates a higher temperature of approx. 800 ° C [36].

Figure 1.22. Burned bones. Source: Rubio L et al. 2020 Nov.

Anthropologists have focused a remarkable amount of research on the detection of perimortem trauma in burned bones. Fracture patterns resulting from perimortem trauma were studied by Hermann and Bennett. They introduced blunt, firearm (shotgun), and sharp-force trauma on pig femora before subjecting the remains to thermal trauma. Sharp-force trauma were easily recognizable in the burned bones but no radiopaque scatter was observed for the specimens with a shot-gun trauma. They also found it difficult to distinguish between heat-induced fractures and blunt force fractures [49].

1.12. Differentiating animal and human bones:

The problem of differentiating human bone from an animal bone arises in cases when the bones that are recovered have been broken into very small fragments. A trained examiner can easily distinguish whether a bone is human or non-human, if the bones are intact or substantial large bone parts are recovered. However, when the fragments are from the mid-shaft, or small bones of the carpus and tarsus, it may pose a challenge in the identification process as they are not easily distinguishable. Some animal's skeleton remarkably resembles that of a human, such as the bear with a paw skeleton that is almost similar with that of the human hand. In such cases, protein extraction techniques play a significant role by testing the bone against anti-sera samples of humans and various animals. Gel diffusion and electrophoresis methods are also largely employed for the purpose of differentiation [107].

Some notable differences between human and animal bones given by Watson and McClelland (2018) (92) at the University of Arizona are given in Tables 1.7-1.10.

Table 1.7. Difference between animal and human cranium. Source: Watson and McClelland (2018).

Human	Animal
Large bulbous vault, small face	Small vault, large face
Vault relatively smooth	Pronounced muscle markings, sagittal crest Inferior
Inferior Foramen Magnum	Posterior Foramen Magnum
Chin present	Chin absent
Orbits at front, above nasal aperture	Orbits at sides, posterior to nasal aperture
Minimal nasal and midface projection	Significant nasal and midface projection
"U"-shaped mandible (no midline separation)	"V"-shaped mandible (separates at midline)

Table 1.8. Difference between animal and human dentition. Source: Watson and McClelland (2018).

Human	Animal
Omnivorous	Carnivorous; Herbivorous; Omnivorous
Dental formula 2:1:2:3	Basic dental formula 3:1:4:3
Incisors (maxillary) are larger than other mammals	Horse maxillary incisors are larger than human incisors
Canines small	Carnivores have large conical canines; Herbivores have small or missing canines
Premolars and molars have low, rounded cusps divided by distinct grooves	Carnivores have sharp, pointed cheek teeth; Herbivores have broad, flat cheek teeth with parallel furrows and ridges

Table 1.9. Difference between animal and human postcranial elements. Source: Watson and McClelland (2018).

Human	Animal
Upper limbs less robust	Robust upper limbs
Radius and ulna are separate bones	Radius and ulna often fused
Large, flat and broad vertebral bodies with short spinous processes	Small vertebrtal bodies with convex/ concave surfaces and long spinous processes
Sacrum with 5 fused vertebrae, short and broad	Sacrum with 3 or 4 fused vertebrae, long and narrow
Pelvis is broad and short, bowl-shaped	Pelvis is long and narrow, blade-shaped
Femur is longest bone in body, linea aspera is singular feature	Femur is similar length to other limb bones, linea aspera double or plateau
Separate tibia and fibula	Tibia and fibula are often fused
Foot is long and narrow, weight borne on heel and toes	Foot is broad, foot elements are dense, and may have claws or hooves

Table 1.10. Difference between animal and human bone macrostructure. Source: Watson and McClelland (2018).

Human	Animal
More porous cortical bone	Less porous cortical bone
1/4 thickness of diameter of long bone	1/2 thickness of diameter of long bone
Diaphyseal trabecula present	Diaphyseal trabecula absent
Thick diploe in cranial vault bones	More compact cranial vault bones

CHAPTER 2

MATERIALS, METHODOLOGY AND TOOLS

This chapter demonstrates the process of review literature and the inclusive and exclusive criteria that was considered for this study. Apart from that, the laboratory protocol for sample preparation of skeletal remains, and a comprehensive information about the various methods used in estimating age, stature, sex, ancestry and examining skeletal trauma have also been also mentioned.

2.1. Literature Review:

A systematic review of literature has been done by selecting relevant studies regarding the examination of skeletal remains, for the purpose of developing a biological profile of an unknown individual, a situation often encountered in the field of forensic anthropology. For this purpose, a comprehensive search was performed for forensic science journals in the related topics using Google Scholar, Research Gate, Science Direct and PubMed from past years up until 2020. In order to search the literature of relevance with respect to this study, the following keywords were used: i) Skeletal Remains, ii) Examination, iii) Age, iv) Stature iii) Sex/Gender, iv) Ancestry, v) Skeletal trauma, vi) Assessment.

2.2. Research Articles Selection Criteria:

For the selection of research articles for the purpose of this research paper, the inclusive criteria included: i) Current methods used in examination of skeletal remains for the required parameter (age, sex, stature, etc), ii) An English-medium published literature. The exclusive criteria included letters, editorials, news articles and case studies.

2.3. Sampling Preparation of Skeletal Remains in Forensic Anthropology:

The preparation of skeletal remains for sampling and analysis should be done in such a manner so as not to contaminate or cause unnecessary destruction or alteration to any of the skeletal elements [86].

2.3.1. Procedure for Sampling Preparation of Skeletal Evidence:

a) Documentation:

- It is vital to maintain the chain of custody, for the purpose of which, it is required to document the condition of the skeletal remains in its original state, upon its arrival to the forensic laboratory, and throughout the whole sampling preparation process.
- During the process of sampling and analysis, any alterations that are caused to any of the skeletal elements are also required to be documented.
- The appropriate personal protective equipment is recommended while handling the skeletal remains to ensure examiner's safety, and to limit the amount of contamination to the evidence
- Prior to commencing the sampling process, the skeletal remains should be photographed in their original condition.
- The documentation should include details of all the tools used in the sampling procedure.
- Before the sampling process is commenced, all the data collection procedures, for example, visual examination of the skeletal evidence or metric analysis, and the documentation of the skeletal evidence should be done.

b) Sampling:

- Sampling of the skeletal elements or the soft tissue may be required for conducting histology tests for DNA, SEM/EDS, etc. Sampling is done with the prior knowledge and permission of the competent authority, and with a definite aim.
- Sampling of soft tissue should be done prior to skeletal preparation.
- When sampling bone elements for DNA analysis, methods and equipment that help reduce and prevent contamination should be used, including the use of new blades or saws or those that have been treated with bleach or ultraviolet rays.
- A consultation with DNA laboratories should be conducted prior to sampling, and ideally, the entire bone sample should be sent to a DNA laboratory to

check for contamination.

c) <u>Skeletal Preparation:</u>

- When available, radiographs of the remains are taken before the skeletal evidence is prepared in order to identify any foreign material, and secondary ossification centres.
- Prior to processing, consultation with the relevant competent authority regarding the removal of extraneous soft tissue should be made.
- As much adhering soft tissue as possible should be removed, without employing tools. If tools are required to remove soft tissue, preference is given to those made of a material that minimizes alteration, such as wood or plastic, although it should be expected that in certain cases tools, such as scissors or scalpel, may be required.
- There are three main methods for skeletal preparation: mechanical, chemical, and entomological. The various approaches and techniques used in these methods are recognized and accepted. The decision for preparation should consider the type of testing being performed and whether long-term curation is a goal.
- There is always a possibility that additional evidence, such as projectile, or entomological evidence, may be found during the preparation process. Thus, care should be taken to record and process any additional evidence, according to the applicable procedures.
- Any soft tissue maceration method is acceptable as long as it DOES NOT:
    - Alter bone/tooth proportions.
    - Deteriorate bone/tooth surface.
    - Introduce cuts or cracking.
    - Alter bone/tooth structure.
    - Disturb the chain of custody or hamper the integrity of the evidence.
    - Make the sample unfit for any other analysis, such as DNA analysis.
    - Create a situation of mixing of different skeletal remains.

d) Considerations:

- In the event of a reconstruction of the remains, the procedure, and the materials used must be documented. It should be checked that any reconstruction technique that is used, is reversible.
- The following practices are considered unacceptable and should be avoided during sampling preparation of skeletal remains:
    - Using chemicals that damage bone, teeth or DNA, except those for the purpose of curation.
    - Unnecessary alteration to the skeletal element.
    - Performing reconstruction when it is not required.
    - Unnecessarily subjecting the skeletal elements to complete transverse sectioning.
    - Subjecting the skeletal elements to excessive soaking or excessive heat, as it can cause bone, teeth or DNA degradation.

e) Reporting:

- The report accompanying the analysis of samples should document any damage that may have been done to the sample during the process of preparation. The methods used for sampling preparation should be added and described in the case file.

2.4. Methods for age estimation from skeletal remains:

a) Age estimation from macroscopic morphological features:

- In the case of the subadults, age can be determined fairly reliably, since the biological-chronological relationship is clearly reflected in the skeleton of the growing subadult. Therefore, features associated with bone growth, such as the epiphyseal closure, sphenobasilar synchondrosis closure, and the length of the bone (diaphyseal) can be used [37].
- For the adult skeleton, most of the methods are focussed on the non-synovial joints (synchondroses and synostoses), as seen in the innominate and the sacrum [37].
- One of the first methods that was developed was based on cranial sutures. It was described by Broca in 1875, and it has since been extensively tested. However, although there appears to be an age-related drift in complete ossification of the suture

(viewed as obliteration), this trend may be weaker than the other methods described below. There has been challenges to develop a clear and unbiased criterion for the use of sutures in age determination [37].

- The pubic symphysis in particular has a long history of forensic anthropological aging. Todd first described a method in 1920. There have been a number of alterations since then, including the introduction of a common model reference method by Suchey et al., which has become a conventional method for pubic symphysis aging. The basis remains the same, no matter which technique is used: the different phases are described (or provided as models), and they are found to correspond to a certain age range. The researcher compares the pubic symphysis morphology in a particular case against these stages, then estimates the appropriate age range. The stages reflect the changing morphology of the surface, from a youthful appearance that to some extent resembles the epiphyseal surface of a long bone (wavy, undulating surface morphology, with no rim phenomena), to an aged appearance (disappearing waves, replaced by denser and less structured bones). However, a fragmented or destroyed pubic bone can render the method unusable [131].
- The same method of describing age related phases or stages is applied to the auricular surface, though this joint feature a more complicated ligament construction, with a fluid filled space. The method first introduced by Lovejoy et al. and since modified by Buckberry and Chamberlain, uses fairly detailed scoring systems to evaluate various features, which should limit some errors [131].

- A final non-synovial joint is the end of the sternum rib, where the bony rib articulates with the cartilage between the rib and the sternum. Işcan et al. initially gave a rather optimistic precision for this method, which has not been fully confirmed, but the method has the advantage of casts (as with the pubic symphysis). In relation to forensic anthropology, there is one additional benefit as this joint is easily accessible during the autopsy; the rib cage is normally open so that the removal and maceration of the end of the fourth rib is easy, as opposed to the task of extracting and macerating parts of the hip bones to gain access to the pubic symphysis, or the surface of the auricle. The common denominator overall for these age estimates is that joint surfaces and changes have been tabulated, relating them to broad age categories, generally ranging from 10 years of age or older [131].
- Synovial joints may also be used for age evaluation as the arthrosis developments may be an indicator of age. Apart from being clinically age-related, the arthrosis is also related to others factors such as the genetic makeup and nutrition. Stewart published a scoring system for the lipping degree on the vertebral margins, however it is not generally used. However, for an examiner who is experienced, seeing arthritic developments on the joint surfaces will indicate advanced age, and this will likely be part of the final age determination to some extent [131].

b) <u>Microscopy:</u>

- A method was described by Kerley in 1965 where age of death was determined by counting secondary osteons, non-Haversian canals, osteon fragments, and the percentage of the lamellar bone from the midshaft of the tibia, fibula and femur. The method has since been modified and changed by other researchers. Microscopic assessment of cross-sections of the femur or other bones has been considered a well-established method, and many studies have been performed to investigate the effect of diseases, preservation, and also, the importance of sampling location in the bone, e.g., proximal or distal [56].

c) <u>Radiology:</u>

- In this method, the degree of loss of trabecular bone at the proximal epiphyseal end of long bones, such as humerus and femur are quantified. The method was first described on macerated bone, but it can also be used on bone covered with soft tissue.

There may be forensic-anthropological cases where soft tissue removal is not possible or where rapid age determination may be helpful in the early stages of an identification case as in, mass graves and mass disaster, without having to macerate the bones first [131].

- Recently, several morphological methods have been studied as age indicators based on computed tomography (CT) scans and three-dimensional visualizations. The initial studies attempted to directly compare the CT scan or 3D visualizations with the morphological phases (e.g., suture closure, pubic symphysis, etc), however, recently, work has been done to directly describe the various phases/stages based on the information derived from CT. It is expected that in the future, more methods will rely on digital data that is derived from a CT scan in contrast to just visually reconstructed data derived from observing and examining morphological features. This will also lay the grounds for the availability of more reference data [9].

d) <u>Radiocarbon:</u>

- Radiocarbon analysis (carbon-14 dating) is an absolute method for determining age (year of birth). It exploits the variations in the atmospheric carbon-14 levels during the duration of the last 50 years to date crystalline formation of the lens. The carbon-14 concentration in the living tissues will reflect the atmospheric carbon-14 content at the time of growth, due to the reason that cosmogenic carbon-14 in the atmosphere forms carbon dioxide by reacting with oxygen, which will be incorporated in plants, which are then, ingested by animals, and both of which are ultimately ingested by humans. The level of carbon-14, when it is built into tissues and cells that have very minor turnover, such as the dental enamel, shows the atmospheric carbon-14 level [131].
- Until 1955, the carbon-14 amount in the atmosphere was almost constant, however, nuclear bomb testing caused a dramatic rise in its level. The carbon-14 concentration declined rapidly after the Partial Test Ban Treaty of 1963, because atmospheric carbon-14 dissolved into the oceans [131].
- This method enables a very accurate dating as any change in concentration is significant, even that of a yearly basis. Radiocarbon dating, to estimate the time of birth, has also been applied to other tissues and structures with little or no turnover, including neurons in the brain, special proteins in the fibres of the lens of the eye

called crystalline, and also bones [131].

e) **Chemical and Molecular methods:**

- These methods are based on detecting chemical changes in various tissues and molecules.
- All amino acids, except glycine, contain a chiral or asymmetric centre on the α-carbon atom; the two enantiomers obtained are called L-enantiomers and the D-enantiomers. The proteins are completely synthesized by the L-enantiomers. Once the protein is synthesized, the amino acid residues begin to be converted into D-enantiomers in situ, and this racemization finally ceases when 50% of each enantiomer reaches equilibrium. The process, however, is very slow and would take hundreds or even thousands of years for completion depending upon factors such as temperature, the sequence, the amino acid residue, etc. The progress of the racemization process of a given protein can be a measure of the time since protein synthesis, provided that no new protein synthesis takes place, and the protein is metabolically stable [85].
- The linkage between a person's chronological age, and the degree of amino acid racemization from the dental tissue collagen was shown in 1975 [85], and age-dependent amino acid racemization has also been associated with proteins that are found in the eye lens and the vertebral discs. The process of racemization is not directly linked to the aging process of an individual, however, it all depends on when the proteins were first produced in the body [131].
- Several studies have been conducted to quantify the decrease in cross-links between collagen molecules in collagen tissues. These covalent cross-links appear to reflect growth processes and slowly decrease in number, thereby, ultimately forming various compounds that are not all fully known. While the general process has been demonstrated in human bone and cartilage samples from birth to age 80, efforts to make more accurate and forensically useful age estimates have resulted in estimated errors of about ± 15 years [136].
- Another chemical reaction that has sparked interest among researchers is the Maillard or browning reaction, or to be more precise, advanced glycation end products. First described by Maillard in 1912, the reaction is a complex, non-enzymatic series of reactions between free amino groups, and reducing sugars [131].
- The fact that tissue with molecules with a long lifespan (collagen molecules with a lifespan of 200 years) exhibit yellowing / tanning, has been demonstrated for

structures such as the Achilles tendon, intervertebral discs, skin and dental collagen, as well as neurons in the hippocampus. The yellowing of the teeth has been quantified by visual examinations, densitometer, and direct pentosidine measurements. The browning of the skin, as observed on corpses lying in bogs, is also most likely due to Maillard's reactions, probably due to the polysaccharide found in the bog, which combines with amino groups in the decomposing body [131].

- Telomeres are the structures present at chromosomal ends and they ensure correct replication at the disjunction, and correct reading of the DNA strands. Telomere length has been found to decrease with age, presumably due to cell divisions: the more cells divide, the shorter the telomere length, and as the number of cell divisions increases with age, the length of the telomeres could be an indicator of age. Telomere length, however, is unique to each person, and is influenced by genetics and environmental factors. The error reported in the studies is approx. ± 10 years [136].
- It should be noted that biochemical methods depend on biochemical reactions, which means that diagenetic influences, such as temperature, play an important role. Several studies of biochemical methods indicate that results may vary depending on storage. For example, racemization continues post mortem; when the ambient temperature is high, this process (like all biological processes) is accelerated, which can influence the degree of racemization, and as a result, the age estimation [21].

2.5. Methods for stature estimation from skeletal remains:

a) Stature Estimation from a Regression Formula:

- Regression formulae are used to estimate stature from partial or complete skeletal remains recovered from forensic cases. Regression formulae are statistically designed to establish a relationship between a dependable variable and one or more independent variables. The relationship between a scalar variable *y*, and one or more variables denoted *x* is modelled by the linear regression approach. Most often, linear regression refers to a model where the conditional mean of *y*, given the value of *x*, is an affine function of *x*. Less often, linear regression could refer to a model in which the median or some other quantile of the conditional distribution of *y* for a given *x* is expressed as a linear function of *x* [63].
- When using regression analysis for the purpose of estimating stature, stature represents the dependable variable, while the long bone or other body part represents the independent variable. The derived regression equation will reflect the relationship between the body part and stature. The values of constants "*a*" and "*b*" are generated while calculating regression formula, either mathematically or with the aid of computer software programmes; where "*a*" represents the regression coefficient for stature, i.e., the dependent variable, and "*b*" represents the regression coefficient for the bone or body part from which the stature is to be estimated, i.e., the independent variable. Therefore, the regression equation that will be derived is: $y=a+bx$, where y=stature; x= bone or body part measurement [63].
- In 1889, Rollet was the first to investigate the correlation between the length of long bones and stature in a sample of 100 cadavers of mixed sex. Through his study, he provided tables where from the given length of long bones, one could look up the stature. Using Rollet's data, the first regression equations for stature estimation from the length of long bones were produced by Pearson. Regression analysis has since then been successfully used for standardizing the data for stature in populations, and thereby, consequently for estimating stature in forensic and medicolegal cases [63].

b) FORDISC 3.0:

- FORDISC is a windows-based computer software that provides quick and easy analysis of skeletal measurements using the Howell data set and modern forensic data. Based on known samples, FORDISC enables easy classification of unknown individuals, in regards to sex and ancestry. Various measurements of bone length can be used to obtain linear regression equations or stature estimates [55].
- The stature is estimated by entering the long bone lengths in FORDISC 3.0. FORDISC formulas based on forensic databases, and Trotter and Gleser regression equations are most commonly used in forensic anthropology today [55].
- However, FORDISC is still limited in its usability to the populations from which the data was used to build the programme. Hence, the examiner will need a lot more input to ensure that the formulas and data are applicable to other populations around the world [55].
- In some cases, long bones are unlikely to produce results, especially if it has been too badly destroyed or damaged for it to be measured. FORDISC will not be of much use in such cases. This remains especially true for mixed remains which require different body parts to be combined. Factors such as measurement error, ethnicity, age, symmetry, and diurnal variations can influence the stature estimation for an individual [55].

c) Fully's method:

- This is an anatomical method of stature estimation, which enables a direct stature reconstruction by summing up the measurements of the various skeletal elements that contribute to an individual's stature, following it up by adding a correlation factor for skin and soft tissue [14].
- This method was tested by Dwight in 1894 where he placed the skeleton in the anatomical position on a table, and used clay as an interstitial replacement for soft tissue. He then measured the stature of the finished outcome, and estimated 32 mm for the average total soft tissue length [14].
- In 1956, Fully approached this method with a slightly different perspective. He took the sample of 60 French soldiers and independently measured each skeletal element: vertebral height, cranial height, maximum length of tibia

without eminence, bicondylar femolar length, and articulated height of the talus and calcaneous. He then added up the skeletal measurements to obtain the height, and also added the correlation factor for soft tissue to estimate living stature. For the purpose of calculating correlation factor of soft tissue, the average living stature of the samples (extracted from the military records) were subtracted from the average skeletal height. He obtained an average difference of 10.5 cm, which was assessed to be the soft tissue estimate for an average height individual. He recommended 10 cm for short, and 11.5 cm for tall extremes respectively [14].

d) Adjusted Fully Method and Age Correction Factor:

- In 2006, Raxter et al. [92] noted a discrepancy regarding the soft tissue correlation factor of the Fully's sample, i.e., 10.5 cm in contrast to their own, i.e., 12.4 cm, which they obtained when they tested Fully's method on a large documented sample (n=119) from Terry's collection, consisting of American White and Black males and females, between the age of 21-85 years. They also discovered that the original equation of Fully had the tendency to underestimate the living statures in their samples. However, an overestimation of stature occurred when they applied the age correction factor given by Trotter and Gleser, of 0.06 cm per year for the individuals whose age are over 30 years. They, thereby, settled on an age adjustment factor, i.e., 0.0426 cm per year, and new equations were generated based on whether the age was known or not. It is to be kept in mind that Raxter et al. converted the stature of cadaver to the estimated living statures in their samples by the subtraction of 2.5 cm, which was suggested by Trotter and Gleser (1952) [125].

2.6. Methods for sex estimation from skeletal remains:

a) Morphological Methods:

- In this method, a visual assessment of sexually dimorphic traits is done for quick preliminary assessment. Various features such as the shape of the pubic bone, glabellar prominence, mandibular ramus flexure etc, can only be assessed morphologically, as they are challenging to measure, show intra and inter observer errors, difficulties in classification and thus, their analysis is problematic. Better results are obtained from intact bones as the degree of accuracy from morphological assessment decreases when the bones are fragmented or the skeleton is incomplete [62].
- The visual scoring method given by Ubelaker and Buikstra has been used by various researchers for visual scoring on a scale of 1-5, and this method reported a 90 % sexing accuracy for skulls as per Walker. Univariate analysis had a sexing accuracy of 70 %, and multivariate analysis had an 80 % sexing accuracy. In one of the studies that was conducted by Loth and Henneberg, the accuracy of sexing from morphological analysis of the symphyseal region and the anterior part of mandibles of juveniles was 81 %, however, the same method conducted by Scheuer had a decreased accuracy of only 65 %. These shows that visual scoring methods are limited in terms of reproducibility and objectivity [62].

b) Metric Methods:

- These methods usually utilize different statistical methods for the purpose of deriving equations and models, in order to accurately estimate the sex of individuals. The principle of metric methods is based upon the variations that exist in male and female dimensions. The numerical data that are obtained from this method can be easily assessed and interpreted [62].
- The different statistical approaches that are utilized in this method include simple proportions, identification points, logistic regression analysis, sectioning points, discriminant function analysis, etc. Based upon the type of statistical method utilized, the accuracy of sexing will vary accordingly [30].
- Franklin et al. performed a study on five South African tribes outlining interpopulation differences. He took nine measurements on the mandible that

presented sex differences among the group and these differences were compared using ANOVA (Analysis of Variance) [39].

c) Geometric morphometric methods:

- Geometric morphometric analysis is a relatively new method of quantifying the morphology of rigid structures with curves and bulges that cannot be analysed and are largely ignored by conventional methods. This method helps to achieve a detailed morphological trait assessment displaying differences among skeletons. It has the ability to include surface topography using x and y coordinates, which prevents important morphological traits from being overlooked. It eliminates the effect of size as a parameter and considers even the subtle differences, thus increasing its accuracy [62].
- The samples are first photographed and processed into a computer where the landmark coordinates are digitized and analysed by means of generalized Procrustes analysis. The landmark coordinates are represented as points through scaling and alignment. All the specimens are scaled using centroid size (CS). Multivariate analysis is done subsequently by using superimposed coordinates and CS. Principal component analysis with covariance matrix is performed on Generalized Procrustes Analysis Variables. A multivariate analysis of covariance (MANCOVA) is then performed using scores from principal component analysis to check the importance of height and gender on the shape of males and females in each group. These groups are also subjected to discriminant covariance analysis, if no satisfactory results are obtained [62].
- Although this method of geometric morphometric analysis based on landmarks seems to be the most suitable method, it suffers from the disadvantage of insufficiency of available number of landmarks to define a specimen shape. It is also a very time-consuming method [61].

d) Computed Tomography (CT) and Magnetic Resonance Imaging (MRI):

- Computed Tomography (CT) and Magnetic Resonance Imaging (MRI) are non-invasive methods that provide very promising results in the field of sex estimation. Sexing from more deeper and resistant parts can be done using this method [62].

- In their study of craniometric assessment from a Thai population, Roppakhun et al. applied an advanced method on a 3D computed tomography derived data. 3D anatomical landmarks were studied in a digital format on 91 samples of Thai skulls, instead of taking physical measurements. Through the study, it was observed that the male skulls were large in the following parameters: a) Basion-bregma height, b) Maximum cranial length, c) Bizygomatic breadth, d) Nasion-basion length and breadth [98].
- This method, however, is quite expensive to use [62].

e) <u>Digital Radiography:</u>

- The method of digital radiography is an accurate measuring technique for those cases in which the samples are semi-fleshed, degraded, charred or highly decomposed, and maceration cannot be performed prior to its analysis. The radiographs that are taken directly on bones show high sex estimation accuracy results that are similar to those taken from minimum supero-inferior diameter of neck and maximum head diameter of the femur [51].
- Igbigbi and Msamati studied 496 pelvis samples from a population of black Malwawians from individuals within the age range of 18-70 years, and took digital radiographs and X-rays of the femoral head diameter. The vertical and antero-posterior diameters were considered, and identification and demarking points that are used for Nigerians were employed. A high level of accuracy was observed in the results, fewer measurements for the femoral head diameter were sufficient, and it was less sophisticated to perform [51].

f) <u>Diagnose Sexuelle Probabiliste method (DSP):</u>

- Murail et al. developed a virtual sex determination method based on a metric database obtained from 2040 hip bones of known sex from 12 different reference populations. This database is based on the hypothesis that a common pattern of sexual dimorphism exists in all modern human populations. To test this method, four different geographic regions were included, viz. Europe, Africa, Asia, and North America, with one to three subgroups included for each population. Seventeen hipbone measurements were taken of individuals whose sex and age were known, and with the help of discriminant analysis, the pattern of common sexual dimorphism was derived. On the basis of discriminant power and rate or preservation, the variables were selected. This pooled sample of collections from around the world was used to develop DSP. The diagnosis of gender was based on any combination of at least four variables among those proposed. Sex was determined by the comparison of measurements that were derived metrically, and the probability of specimens for males and females was computed [22].

- Chapman et al. examined the accuracy of DSP compared to other virtual sex determination methods and its accuracy for both the hipbone and pelvic girdle to determine its usefulness in forensic casework. An accuracy of 100 percent was achieved between the manual and virtual DSP method [22].

g) <u>Molecular methods:</u>

- Sometimes, morphological or morphometric methods may not help in achieving an accurate sex estimation, especially in cases where skeletal remains are poorly preserved. Molecular methods are the key to solving such problems. Molecular gender typing by means of multiplex PCR [Genderplex] utilizes two amelogenin targets of different sizes, one STY target and four X-linked STRs with short amplicons. It is a very useful technique in sex determination because it uses parallel tests on multiple genomic sites [24].
- These methods are useful for high quality, less degraded DNA samples; however, short amplicons can also be useful in the analysis of severely degraded DNA [62].

h) <u>Models employed in sexing of skeletal remains:</u>

- ***Discriminant Function Analysis (DFA):***
  - This method eliminates subjective criteria for the estimation of sex and is an easy-to-use simple method, even without any prior experience. Discriminant functions especially play a vital role in cases in sexing of poorly preserved skeletal remains. DFA assigns a subject of unknown identity to two or more groups on the basis of linear multivariate analysis. This method is highly population specific, and its accuracy varies from one measurement set to another [62].
  - The first step in this method is to compute classification scores by distinguishing between the groups. The individuals are classified into the

groups for which they show the highest classification score. Cross validations are done in order to ensure accuracy. In cross-validation analysis, cases are classified using the derived functions of all cases except the case to be classified. A series of analysis is carried out in this way where one individual gets excluded at a time [62].

- The results of DFA strongly depend on the sample size, robustness, and sexual dimorphism of the population for which discriminant functions were developed. Sakaue used stepwise DFA and reported that the distal articular surface of the humerus is a good indicator of gender (95 % accuracy), while a study by Mall et al. report that the humeral head is a reliable variable in sex determination with an overall accuracy of 93.15%. This difference in percentages may be attributed to population differences [62].
- DFA has been used to estimate sex from robust skeletal remains such as the femur and talus, and from other bones such as patella, pubis etc. The accuracy of this method ranges from 83.3 percent for patella to a perfect 100 percent for lateral cephalometry radiographs [62].

▪ ***Neural Network Method:***

- This method is also known as the multilayer preceptor method, and it is one of the least preferred methods as it requires extremely high mathematical skills to develop models. However, the derived models give more efficient and accurate results in depicting the relationship among variables in comparison to DFA, and other non-linear multivariate sex estimation techniques [62].
- This method uses a matrix that has values represented as nodes that are similar to the neural network of the human brain, and this shows the relationship among the variables. This network consists of an input layer and an output layer with one or more interconnected layers. All kinds of nonlinear functions between input and output can be modelled without the need for distributional assumptions as in the case of DFA [62].
- Jardin et al. [32] in their comparative study showed that the neural network method with one hidden layer had a higher precision of 93.4% with less asymmetry between men and women in comparison to the DFA methods, and the linear regression method with four parameters obtained from the upper

part of the femur. In a previous study carried out on the patella using the same method, Mahfouz et al. [74] reported a high sexing accuracy of 96 %, while DFA recorded an accuracy of 90.3%.

- ***FORDISC:***
  - FORDISC uses the global database of known sex samples taken from the Forensic databank (FDB) and computes discriminant functions for the purpose of sex estimation, stature estimation and ancestry assessment by utilising various anthropometric measurements. This software also performs similar tests using the Howells data on variations world-wide among past populations. This software works on the basis of the nature of discriminant function analysis, and it implies ancestry of an unknown specimen to get reliable results. The earlier version of FORDISC, i.e., FORDISC 2.0 analyses sex and ancestry as separate units. The newer version, i.e., FORDISC 3.0, selects appropriate groups, uses large combination of measurements, and uses the whole of the FDB, adding up to the accuracy of the software, however reliability is not enhanced [62].
  - The choice of function used in FORDISC depends on the comparability degree of the unknown sample with the reference data bank and the availability of certain measurements that are required [62].
  - One of the limitations of this method include the non-homogenous nature of the group selection process and the FDB [62].

2.7. Methods for assessing ancestry from the skull and teeth:

a) Anthroposcopic/non-metric methods:

- Various traits are considered under the non-metric approach of assessing ancestry. One such that is currently practiced by anthropologists include using cranial morphological traits, which are based on subjective lists of features and traits [50], and experience of the observer. Others include discrete traits which are recorded as absent or present. The list of non-metric features of the skull is very extensive. Among the morphoscopic features, the shape of the suture and the shape of the palate are good examples.
- Hefner [48], proposed an inventory to assess ancestry where the bulk of the traits are set within the middle region of the face, especially in the nasal area. Variation in the lower nose border, nasal aperture along with the anterior nasal spine are among the key features that are to be scored.
- Hefner and Ousley [47] gave the OSSA proposal - Optimized Summed Scoring Attributes. The OSSA is a heuristic method that uses six morphoscopic features (ANS, INA, IOB, NAW, NBC, and PBD) [48]. Since no single characteristic or group of characteristics precisely defines a population, it was necessary to find a way to determine the threshold from which an individual can be considered a member of a particular geographic population. OSSA quantifies the probability that a certain individual belongs to a certain population. The authors provided an evaluation or score sheet on which each trait is scored [28].
- Each of the trait score is dichotomized (e.g., 1,2,3,4 remodelled to 0, 1) to maximise the between-group variations in 2 populations. To accomplish a heuristic optimization of the compressed trait scores, the variables are ordered in a very manner that maximizes the differences between the groups. All the six traits are converted into new binary variables, and the sum of all these traits are calculated that will give individual OSSA scores that range from 0-6. The total of all scores provides the ancestral group [47].
- Analysis of dental features should always be considered, especially when experts do not have a complete skull or when morphological or metric analyses give ambiguous results. Generally, non-metric dental approaches can be helpful when only teeth are available for analysis and support, but it should be noted that dental

studies are limited by the teeth present, and can have large error rates within their equations [28].

- An anthroposcopic method was described by Krogman and Iscan [65] that was used by Todd and Tracy in 1930 for the purpose of determining the racial affinity from Caucasoid and Negroid skulls. Five descriptive traits were focussed upon: I) Glabella. II) Upper orbital margins. III) Fronto-nasal suture. IV) Supraorbital ridges. V) Inter-orbit distance. For each of these five descriptive traits, Todd and Tracy searched for two contrastive variations that would be racially connected. The supraorbital ridges were either mesa-like or undulating, the upper orbital margins were either sharp or blunt, glabella was observed to be either rounded or depressed, the frontonasal suture was either plain or protrusive and the interorbital distance either narrow or wide. Based on these descriptive traits, Todd and Tracy classified skulls into two types: U-type and M type which are distributed throughout both the races [28].
- Recently, using Arizona State University's Dental Anthropology System (ASUDA) models of teeth, Scott and colleagues proposed rASUDAS: a new web-based application for estimating tooth morphology ancestry using the crown and root morphology of the dentition. The reference sample consists of 21 ASUDA-based traits and represents approximately 30,000 individuals from seven geographic regions. The software is available on the Osteomics platform [109].

b) <u>Anthropometric methods:</u>

- Anthropometric methods for determining racial affinity in the skull has been performed using discriminant function statistics. One of the first methods to determine race using the discriminant function statistics was performed by Giles and Elliot in 1962 [41]. In this method, eight skull measurements are taken and multiplied by a certain determined factor. The results are then added or subtracted to give a score that can be rated for racial affiliation.
- In 1984, Gill [42] developed an anthropometric method for determining race, which included six measurements of the mid-facial part of the skeleton and calculation of three indices: maxillofrontal index, zygo-orbital index, and alpha index. Krogman and Iscan [65] were of the opinion that this method is sufficient to distinguish between Caucasoids and Negroids, but it is not suitable to

distinguish Negroids from Mongoloids. However, Gill [42] pointed out that there seems to be no other method that can give such stable and reliable results. He also insisted that Gill's anthropometric method requires the use of an instrument known as the simometer, which was very rarely found during the period of his publication.

c) Forensic Anthropology Databank (FDB):

- The Forensic Anthropology Databank was established in 1986 to solve the problem of poor performance of various methods for determining race and gender, and to solve the problem of old reference collections by providing alternative data sources for the development of forensic methods. It consists of data that were collected from various forensic cases and were submitted by anthropologists. It also consists of samples of individuals. A computer database is not a substitute for skeleton collection, however, the FDB has a monumental analysis potential [87].

d) FORDISC:

- FORDISC is an electronic computer database, created by Stephen Ousley and Richard Jantz, that can be used to determine race or gender. It has several features that make it more useful than any previous discriminant function approaches. It calculates unique discriminant functions on the basis of the measurements obtained from an unknown individual. Posterior and typicality probabilities are then calculated along with the discriminant function score.
- The posterior probability gives a measure of the identity of the group membership, showing that the unknown person is indeed one of the options that was selected.
- Typicality probability is a measure of whether an unknown person belongs to one of the groups selected in the analysis.
- The discriminant function score can induce a position in one of the selected groups; however, a typicality score of 0.05 or lower indicates that the unknown person is not typical for any selected group [87].

e) **Other metric approaches:**

- Another software that is used for the assessment of the ancestry from the skull is CRANID. The skulls are classified on the basis of 29 measurements after comparing them with 74 samples, that include around 3163 skulls around the world [28].
- A new forensic tool that uses a different statistical procedure was recently proposed by the Laboratory of Forensic Anthropology in Portugal by Navega et al. AncesTrees, which currently has a database of almost 3000 people, and it can be viewed as a system to support the decision to classify the human skull based on a machine learning ensemble algorithm, random forest. The system generates several models which are used to arrive at the final decision. The database used in AncesTrees consists of 23 skull measurements. The user enters the measurements that are taken from the skull in the spreadsheet and selects the ancestral group that is to be included. Similar to FORDISC, AncesTrees quantify the likelihood that a particular individual belongs to a particular ancestral group, which serves a crucial benefit in forensic investigations [82].
- Geometric morphometrics (GM) allows a good analysis of the shape of the cranium through a 3D co-ordinate data. It facilitates statistical analysis based on the Cartesian landmark co-ordinates. 3D-ID is a freely available software which assesses ancestry and gender of an individual. To use the software, a digitizer that records the location of particular points is required to collect 3D data. Digital morphometrics can also be used to assess the shape of certain features of the cranium such as the shape of sutures [115].

f) **Molecular techniques:**

- A group of specific molecular markers known as ancestry informative markers (AIMs) have a special potential in the field of ancestry assessment. Over the last few decades, the process of retrieving DNA from a bone sample has considerably improved, making it possible to estimate ancestry by the process of targeted sequencing of small quantities of DNA [28].

2.8. Methods for Interpreting Skeletal Trauma:

a) Recognition and description of preservation:

- The first step involves photography and if necessary and possible, taking an X-Ray or CT-scan in the original condition in which the sample was received, prior to the cleaning process. A detailed description of the preservation of the remains is to be provided regarding its condition and completeness, as these factors will influence the subsequent interpretations and conclusions about the characteristics and type of skeletal trauma, and the events that led to its creation [11].

b) Reconstruction of bones:

- The bones are then cleaned and inventoried for the examination of gross trauma evidence. It is determined whether reconstruction is possible for fragmentary or damaged skeletal remains. The reconstruction process commences the interpretation of the trauma. Easily united bone fragments may be indicative of a firearm trauma (elastic deformation), while warped bone pieces that do not fit back together may be indicative of a blunt force trauma (plastic deformation) [66].

c) Documentation:

- Documentation (written and photographic) should involve details regarding the position of the defect(s) with regard to the anatomical landmarks, and any kind of abnormality in the shape or size. In cases where reconstruction was performed, a description of the fragment size and section should be recorded.
- These descriptions will help form an opinion about the nature and timing of changes in the skeletal sample, in relation to whether the defect is the result of a skeletal injury, or it is a change that occurred post-mortem [11].

d) Interpretation:

- A determination of the type of trauma can be made by using one's knowledge about the biology of bones and biomechanics. An interpretation tries to answer the following questions [66]:
  - Was the injury sustained ante-mortem or perimortem?
  - Where is the point of origin of the trauma?
  - What are the number and sequence of the impacts to the body?

- What was the degree of force?

- What was the class of implement used to inflict trauma?

2.8.1. Forensic Imaging of Perimortem Skeletal Trauma:

For the analysis of skeletal trauma, imaging techniques are utilised to capture bones that are either within whole body or body parts that are covered with soft tissue or as skeletonized remains. Reflective and transmissive imaging modalities can be applied for skeletonized remains, but in those cases where soft tissue is still preserved, transmissive imaging modalities are used [83].

a) Transmissive imaging modalities:

- In this method, the internal structure of human cadaver and bones is captured by passing through the object, and producing a record of the volumetric data [83].
- A major advantage of this technique is that it documents the fracture patterns in their original context, unlike conventional autopsy procedures which may result in the bone fragments being separated, which will later on pose problems in reconstruction. The methodology involved is non-destructive and is minimally invasive, and the obtained images can be kept in records for an extended period of time [83].

b) Radiography:

- It has been illustrated by Brogdon et al. through various case studies on how the conventional X-Ray imaging can help in the post-mortem evaluation of injuries, and for the purpose of personal identification [83].
- A disadvantage encountered in this method is that subtle injuries to the bones may be missed, mainly in cases where complex anatomical structures such as the skull, pelvis, or spine are involved, mostly because of the superimposition of the structures [83].

c) Computed Tomography:

- This is a minimally invasive, reproducible, and quick imaging technique for skeletal analysis (86). For analysing the fractures in individual bone specimens, where it is required to examine the fine details of the bone injury or their histological structure, a micro-CT is used [83].
- A case of dismemberment was presented by Baier et al. where micro-CT was employed for virtually aligning the severed skeletal elements, analysis of tool marks,

and dissection of a charred bone [8].

- CT has a major advantage over conventional radiography techniques in the form of enhanced contrast resolution, and limited superimposition of structures. However, conventional radiography offers a higher spatial resolution. CT allows for orthogonal or three-dimensional view or both [18].
- In a whole-body post-mortem CT (PMCT), a systematic assessment can be done for each skeletal element where the mechanism of injury is unclear. Technological advancements in computer technology have facilitated the routine application of PMCT, enabling storage of large digital files, such as 3D reconstruction models and multiplanar images in the databases, and enabling a smooth ethical exchange between practitioners. PMCT helps in precisely depicting the skeleton and detecting the skeletal trauma [83].
- A challenge encountered in routine PMCT examination is when dealing with severely decomposed cadaver with putrefactive gases, as these gases have the tendency to simulate a fracture especially those of thin bone structures, such as the hyoid bone, thus, producing errors diagnostic in nature [83].
- PMCT finds applications mostly in forensic cases related to blunt force trauma (BFT), which commonly result from falls, motor vehicle accidents, etc [18]. Besides BFT, it finds application in cases of sharp force trauma, thermal trauma, foreign body detection and also helps in matching the objects to the injury. The importance of a PMCT examination for gunshot related traumas have been stressed by several studies, aiming to accurately identify entry and exit wounds, bullet trajectory, and distance of firing [83].

d) <u>Magnetic Resonance Imaging (MRI):</u>

- MRI has limited use in the post-mortem examination of skeletal trauma. Post-mortem MRI has applications in evaluating bone bruises, soft tissue lesions that result from strangulation or blunt force trauma, and it also helps in dating of fractures. MRI has also found forensic applications in the age estimation of the living based on ossification changes [83].
- An overall sensitivity of 68 % for skeletal injury detection with MRI was reported by Ross et al. Sensitivity ranged from 100% for lower extremity fractures to 40% for upper extremity fractures [99].
- The limitations of MRI in comparison PMCT include slower time for acquisition, insufficient amount of bone details, high equipment cost, etc. Additionally, the

presence of foreign metallic objects exhibiting ferromagnetic properties can pose a threat to the personnel and cause image artefacts [1, 44].

- A CT scan prior to MRI was suggested by Ruder et al. to identify such ferromagnetic objects within the body. It is to be noted that bullets are usually not ferromagnetic, and MRI can help in tracking bullet tracks [102].

e) <u>Reflective imaging technique (3-D Surface Scanning):</u>

- Subjecting the surface of the exposed skeletal remains to 3D scanning can directly capture injury related pathological, spatial, and taphonomic data, while surface documentation of the whole corpse or body parts can provide useful information about the relationship between skeletal and soft tissue injury, which may be of relevance to the assessment [33].
- The active reflective imaging techniques include structures light scanning and surface laser scanning. The active surface scanners emit a form of light for detecting the surface of the object. Structured light surface scanning relies on the detection of patterns of light, that have been distorted by the shape of the object being scanned. Laser surface scanners generate image data with a laser beam projected onto the object's surface, and then reflected onto a sensor by the application of the triangulation principle [20].
- The passive reflective imaging techniques include photogrammetry. A series of photographs are taken in passive scanning; thus, an external light source is required [20].
- Photogrammetry is based on using a single camera, or using multiple cameras to acquire multiple images of objects taken from different angles [20].
- Single-camera photogrammetry has a special software that will automatically detect, align and combine image data to create a 3D model, thus not necessarily requiring the need to place reference marks on objects, as in the case with multiple photogrammetric cameras [83].
- Image alignment and fusion is a key step in surface scanning, especially photogrammetry, which may require manual alignment. Process depends on the skill of the operator; thus, it can include subjective aspects. Compared with other surface scanning methods, another disadvantage of photogrammetry is that the model must be scaled after scanning [83].

- Compared to transmissive imaging techniques, reflective imaging offers the advantages of shorter acquisition time, low cost, and high surface model resolution [33, 20, 34]. However, reflective imaging technique has its own set of limitations as only the visible part of the object can be recorded, so, in order to achieve a complete scan, the object may have to be moved a multiple number of times. This is especially required for skeleton elements with irregular shapes, such as the skull and the pelvic bone [33].
- In addition, although data collection equipment can be operated easily relatively, the data processing is far more complex, and requires additional amount of training. Several authors have determined that the high computational power required for data processing is one of the main limitations of daily 3D surface scanning applications, especially when high-quality models are required [34]. Also, redundant data (usually obtained in high-quality laser scanning), or missing data (observed in structured light scanning or photogrammetry), disturbs the proper alignment of images. These are encountered whilst scanning thin bones, thin sharp fracture margins, fine bony spiculae, dental enamel, fatty bones, concavities, or translucent areas [20]. Moreover, foramina or holes are often automatically filled during data post-processing [34].
- Edwards and Rogers [34] investigated the precision of 3D-models of blunt force trauma injuries to the head (hinge, communited, depressed), by taking various types of surface scans, and evaluating different types of printers and the material of the prints for making 3-D skeletal injuries prints. The quality of the digital models had to be compared visually because the automatic comparison systems did not capture enough detail particularly regarding texture, porosity and fine lines (fractures). The authors found that several factors such as resolution, orientation, and fusion, affect the quality of digital models, and consequently the accuracy of the 3D prints. The method of acquisition and the selected settings influenced the effect of these factors. For laser scanning, more accurate models were provided in the standard configuration. Structured light scanning performed better at the user-defined high-quality level. For photogrammetry, the custom setting that allows higher resolution images, produced more accurate models in comparison to the default setting.

2.9. Methods for distinguishing human from animal bones:

a) Morphological methods:

- The response of different taxa to weight bearing and loading leads to variation in bone deposition and remand remodelling, based on their locomotive characteristics. This results in the morphological differences with regard to bone structure and shape that can help in species identification, especially in cases where the whole bone or the easily identifiable regions of the bones are present. Examination of epiphyseal fusion can help to exclude certain group of taxa such as racoons, where fusion termination is similar to that of a human child who would have unfused ends at the long bones. Specimens of larger adult mammals have been observed to have a denser cortical bone, as compared to those of adult humans [84].
- Humans have been found to exhibit more thickness of cortical bones and gracile muscle attachments, attributed to the bipedal feature of humans as compared to other species [84].
- This is a non-destructive technique with useful applications in distinguishing between species from both whole bones and fragmented bones [84].

b) Histological cross-sections:

- The histomorphology of the bone defines bone tissue structure at the microscopic level [84].
- Thin histological cross sections of a complete bone are taken at specific points along the shaft of a long bone [118].
- These cross sections are filled with resin, placed on a glass slide, and observed under the microscope for the ratio of cortical bone thickness, shape of osteons and their consistency, osteon banding, the direction and type of vascularization, etc [27].
- A circular-shaped osteon, for instance, is indicative of non-human bones as humans tend to have elliptical-shaped osteons [84].
- This method has been validated by many studies but it is destructive in nature.

c) **DNA examination:**

- This is yet another destructive technique and requires preservations conditions to protect DNA from degradation [84].
- First of all, the exposed surface of the sample is washed a number of times to remove contamination and is reduced in size by grinding, smashing or using chemicals.
- DNA is then extracted via standardized protocols for extraction and PCR is performed.
- The DNA sample is then sequenced to develop a genetic profile for analysis and interpretation.

CHAPTER 3

RESULTS AND DISCUSSION

A number of various research experiments have been studied for data extraction. The characteristics of all the studies have been summarised and displayed from Tables 3.1-3.6.

3.1. Age Estimation:

Table 3.1. Characteristics of Age Estimation studies in review.

Sl. No.	Study	Intervention	Population	Comparison	Outcome
1.	Catherine A. Key et al. (1994) [57]	Age estimation from cranial sutures using three methods, viz., the Acsidi and Nemeskeri (AN) system, the Meindl and Love joy system, the Perizonius system.	Spitalfields, London.	183 skull samples excavated and examined ectocranially and endocranially to determine the value of cranial sutures as an indicator for age estimation.	-Only broad range of age due to large variability degree observed in suture closure with age. -Endocranial sutures are only useful in estimating age upto 50 years as after this, there is little to no activity in the endocranial suture closure after 50. -The pattern of ectocranial suture closure showed variations among male and female. -They concluded that in order to progress in estimating age from the suture closure, the above factors should be taken into consideration, and future researches should focus on understanding the various functions of suture closure.
2.	Sarah Ghannam (2020) [40].	Evaluate the usefulness of 2D photographs and 3D scans in assessing the fusion stage of	-	Six participants (experienced and inexperienced) analysed the	The intra-observer error pointed out scoring of the medial clavicle using medial

	44physical clavicles,	photographs and 3D scans as a reliable utility for age estimation, and the three-phase method	

		clavicular epiphysis for age estimation.		photographs, and 3D scans to determine whether the fusion stage could be reliably evaluated with digital images.	was shown to be more subjective. This study also showed that experienced examiners produced more reliable scores.
3.	Jasbir Arora et al. (2016) [6]	Compared quantitative (Kedici's) and qualitative (Gustafson's) methods for age estimation using secondary dentine from ground sections of the teeth.	Chandigarh, India.	Samples collected from PGIMER, morphological measurements taken and data entered in Excel spreadsheet and SPSS 17.0 programme for statistical analysis.	-Following measurements taken: Pulp Height (PH), Cervical Total Thickness (CTT), Pulp Width (PW), Labiolingual Pulp Width (LLPW), Height of Pre-dentine (HPD). - ANOVA used to estimate potential difference of absolute mean error of different age groups. -Gustafson's method was found to be more reliable in estimating age from the secondary dentine as compared to the quantitative method.
4.	Molly Miranker (2016) [77].	Suchey-Brooks method, Osborne method, Rissech and Calce aging methods	Knoxville, Tennessee.	Accuracy of four pelvis aging methods tested on 212 pelvis samples of White individuals.	- The Rissech method found to be the most accurate method with least bias and inaccuracy, followed by the Osborne, then Suchey-Brooks, and Calce methods. - Bayesian approach suggested for improved age estimation.

The age estimation methods that employ a regression model allows age to be estimated by observing the diagnostic characteristics of age indicators, the changes in age indicators over time, and the known age from a reference sample series. These methods provide values for mean age, age range, and standard deviation. However, an issue that arises from regression-based methods is the tendency of the test sample to imitate the age distribution of the reference sample. These methods show the tendency to produce age estimates of a sample skeleton that is biased in the direction of known reference sample composition; this situation is often given the term ‘age mimicry’ (37).

A solution to the problems posed by regression-based methods is to use the Bayesian method. The Bayesian method avoids age mimicry and represents the mortality distribution of the population in a better way. Age and age indicators, in this method, are treated as variables that are ordinal non-normal distributed, thus problems of non-normality and non-linearity are avoided. The Bayesian approach gives an advantage to the researcher to pick a reference sample from which prior probabilities can be derived. This allows for age estimation from a more representative population (37).

It is not possible to completely account for the degree and rate of changes that a skeleton undergoes. Thus, there is no single age estimation method that is fully reliable, and the different methods are continually subjected to refinement and re-evaluation.

3.2. **Stature Estimation:**

Table 3.2. Characteristics of Stature Estimation studies in review.

Sl. No.	Study	Intervention	Population	Comparison	Outcome
1.	Suguru Torimitsu et al. (2014) (124).	Multi-detector computed tomography (MDCT).	Japanese.	Measurement conducted on 215 samples, PMCT and statistical analysis via SPSS 21.0 performed.	-The length of an ascending diagonal stroke from the lower left to the upper right of the sternal medullary cavity (RS), and the length of a descending diagonal stroke from the upper left to the lower right of the sternal medullary cavity (FS) were measured. - Both RS and FS positively correlated with stature, irrespective of sex. - RS had higher regression
2.	Dr. Gaurav Tiwari et al. (2020) [123].	Linear Regression Method.	Bhopal, Madhya Pradesh.	500 dead bodies examined utilizing the correlation coefficient and linear	

correlation coefficients than FS. - Standard errors calculated by regression	analysis lower for RS than FS, irrespec	tive of sex. - Sternal medullary cavity	-The followingmeasurements weretaken: i) Length -Data analysed using measurementusing MDCT images suggested as a potentiallyuseful tool fo

				method to determine the relationship between stature and cranial suture length.	SPSS 20.0. -Coronal and sagittal sutures showed positive correlation with supine length, while lambdoid suture did not show significant corelation with supine stature.
3.	Lai Poh Soon et al. (2020) [116].	Computed Tomography (CT).	Kuala Lumpur, Malaysia.	373 sacral CT images collected, and attempt made to correlate morphological measurement of the sacrum with the stature based on ancestry and sex.	-3D sacrum bones segmented from CT images by utilizing the Mimics Research 17.0 software and Microsoft 3D builder used to measure inter-landmark distances with their respective indices computed. -The auricular length found to be the most useful for estimating stature at R>5 among all sacral features investigated in the study. -Only applicable to Malaysian, Chinese, and Indian populations.

Various bone remains and body parts are positively linearly related to height, and have been successfully used to estimate height of the person. This concept has been used to estimate stature in cases such as large-scale disasters, and other forensic research studies. An effort is being made to standardize anthropological data related to different population groups around the world (73).

3.3. Sex estimation:

Table 3.3. Characteristics of Sex Estimation studies in review.

Sl. No.	Study	Intervention	Population	Comparison	Outcome
1.	Barbara Fliss et al. (2019) [38].	Computed tomography (CT) scans, Statistical Shape Modelling (SSM).	Zurich.	-61 femora acquired from PMCT scans. -Statistical Shape Modelling (SSM) used to estimate sex from human femora.	-Femora extracted using segmentation technique. -After SSM is built, linear logistic regression, and non-linear support vector machine technique employed for classification. -76% femora correctly classified by sex using the linear logistic regression and first principal component of SSM. -Rate increased to 87% upon utilizing nonlinear support vector machines and first 20 principal components of SSM. -This method does not perform as well as the traditional linear measurements. -Despite limitations, sex estimation from femur using SSM is promising and can be improved with further research.
2.	Andrew S. Rozendaal et al. (2019) [100].	Discriminant Functions.	Europeans.	Discriminant functions developed from seven cervical vertebrae for an accurate estimation of sex.	-1020 vertebrae were examined using 295 skeletonized adult remains between 20-99 years of age. -Two documented skeletal collections utilized: the Athens collection and the Luis Lopes skeletal collection. -Seven multivariate discriminant functions

					developed using vertebral foramen transverse diameter (CTD), vertebral foramen anterior-posterior diameter (CAP) and maximum body height (CHT). -No population differences observed between the Greek and Portuguese skeletons, which may indicate that this method is not population specific. - Only CTR and CHT exhibit sexual dimorphism that is statistically significant. -The 7 multivariate discriminant functions showed accuracy rates between 80.3% and 84.5%. -Results suggest that accuracy of sex estimation will be enhanced when measurements from all cervical vertebrae are utilized. -This method should be utilized only when more accurate predictors of sex, such as the pelvis and skull are unavailable.
3.	Paula N. Gonzalez et al. (2009) [43].	Geometric Morphometric analysis.	Portugal.	Greater sciatic notch and ischiopubic complex analysed by employing morphometric techniques to accurately estimate sex.	-121 adult hip bones of the left analysed. -Geometric morphometric analysis based on semi landmarks of the ischiopubic and ilium region digitized on 2D images and coordinates of landmarks. -Discriminant analysis

	used to classify individual by sex with the leave-one-out cross validation k-means shape clustering, and shape-size variables. -Average accuracy of 90.9% achieved for the greater sciatic notch with multivariate analysis based on shape variables, while it was 93.4% and 90.1% for the ischiopubic complex from the discriminant and k-means. -Females misclassified more than males, especially with the ischiopubic complex. -Suggested as a reliable method for quantifying the shape difference of the pelvis between the sexes, and can be utilised for sex estimation purposes.

A large number of studies show that measurements made directly on bones have higher reliability and repeatability, thus, direct sex identification methods are considered more reliable than other methods. Geometric Morphometry (GM) and Diagnose sexuelle probabiliste (DSP) are emerging methods and techniques, widely used in forensic anthropology to improve accuracy and reliability. In addition, newer 3D methods have been shown to display specific patterns of sexual dimorphism that are not easily seen using traditional methods. The development of newer and better gender assessment methods and the re-evaluation of existing methods is encouraged to produce more accurate results for sex estimation (52).

3.4. Ancestry assessment:

Table 3.4. Characteristics of Ancestry Estimation studies in review.

Sl. No.	Study	Intervention	Population	Comparison	Outcome
1.	Leandi Liebenberg et al. (2018) [69].	FORDISC 3.1.	Black, White and coloured South-Africans.	180 post crania of black, coloured and white south Africans measured and classified to measure external validity and report realistic performance statistics for sex and ancestry estimation.	-Skeletal elements used in study obtained from Pretoria and Kirsten collections. -Post crania measured and classified by utilizing the custom postcranial database. - A decrease in accuracy observed for sex and ancestry estimations as compared to the original publications. While the original publication using the multivariate approach obtained an accuracy of 85% for ancestry, the accuracy decreased to 79% in this validation study. -Results obtained indicate that postcranial elements are better at identifying sex as compared to ancestry.
2.	Lottering et al. (2020) [70].	3D-ID and FORDISC.	Black, White and coloured South-Africans.	450 crania samples of black, coloured and white South-Africans examined using 3D-ID and FORDISC to compare which method gave more accurate results for ancestry estimation.	-450 crania samples of South African Coloured (SAC), South African White (SAW), and South African Black (SAB) digitized using Micro scribe 3Dx digitiser. -3D-ID and FORDIC utilized to estimate sex and ancestry. -3D-ID classified SAW as European American or Southwestern, SAB as African American or European Southwestern, and SAC as European

	Southwestern. -FORDISC showed higher accuracy as compared to 3D-ID for ancestry estimation of South African populations, however, this could be due to the fact that FORDISC has a custom database for South-African populations, which is not the case for 3D-ID. -3D-ID concluded as not an accurate tool for ancestry estimation with regard to South African populations, however, potential is seen for increased accuracy in future.

Regardless of the method that is used, the precision of the technique always depends on the validation studies. The results cannot be validated until the method developed using a particular sample is applied to another sample. Above all, it is important to ensure that there is a suitable ancestry discrimination tool that includes references to the population from which the individual is descended. In relation to ancestral assessment, two main approaches are considered: the metric and the non-metric. The non-metric approach is less objective in nature, and requires more personal experience. It, however, also gives much more information [15].

For the purpose of determining ancestry, the skull, especially the mid part of the face, is unanimously regarded as the most informative part of skeletal anatomy. However, postcranial elements such as the femur and tibia can also be used for ancestry estimation. It is important to note that skull characteristics and size are always phenotypic characteristics (13), partly affected by genetics and the environment. However, it is important to keep in mind that although there are polymorphisms that are quite distinct to certain geographical regions, there is not one single trait that can be found only in a single population. A pattern of multiple traits only helps determine the most probable group from where the individual originated.

3.5. Skeletal trauma examination:

Table 3.5. Characteristics of Skeletal Trauma Examination in review.

Sl. No.	Study	Intervention	Population	Comparison	Outcome
1.	Amy J. Spies et al. (2020) [117].	Computed tomography (CT), X-rays and Lodox.	Performed using pig models.	10 piglets beaten with a mallet (post-mortem) in every body region to test sensitivity of CT, X-rays, and Lodox.	-CT scans, X-rays and Lodox of each piglet taken after subjecting them to post mortem blunt force trauma. -Piglets macerated to conduct osteological analysis of remains. -No. of present impacts and fracture recorded from each scan and from the dry bone. -CT scans emerged as the most sensitive technique for identifying trauma from all body regions, with a detection accuracy of 73% for fractures, and 79% for impacts. -X-ray and Lodox comparatively less sensitive for detecting fractures (50% and 42%) and impacts (59% and 54%). -Techniques using dry bone and CT scans able to assess direction of trauma, which was unable to be done using X-ray and Lodox. -CT scan has been suggested for examination of skeletal trauma in suspected cases of child abuse, while X-ray and Lodox scans has only been recommended for initial screening procedures.

-Osteological analysis,

				however, still remains as the choice standard for examination, and is recommended whenever and wherever possible.
2. Legrand et al. (2019) [68].	Computed tomography (CT) and autopsy.	Paris, France.	73 PCMT images and autopsy reports collected from May 2014 to January 2017 to check for the agreement between PCMT and autopsy examinations in the assessment of skeletal trauma to the head.	-PMCT images examined for presence of fractures in the skull base, cranial vault, facial bones and axis, and also for intracranial haemorrhage. -Kappa values calculated to measure agreement between PMCT scans and autopsy reports. -The resulting agreement between PMCT and autopsy was almost perfect with k=0.95 for fractures, and k=0.75 for intracranial haemorrhage. -PMCT emerged as the superior technique for the detection of fractures in the facial bones and upper cervical spine, and also for intracranial haemorrhage. However, it tended to miss thin collections of extra-axial blood. -Combination of PCMT and autopsy examination has been recommended for an improved post-mortem evaluation of trauma, as it detects more lesions.

A well-trained forensic anthropologists should be able to understand how bones respond to trauma. For skeletal examination of trauma, the fragmented bones are to be well-constructed, and the pattern of trauma is examined to understand wound characteristics, that can help to interpret the type of force and the number of impacts, and also help identify the type of tool. This helps to reconstruct the events surrounding the manner of death, and helps in the overall investigation process.

3.6. Distinguishing human and animal bones:

Table 3.6. Characteristics of study regarding animal and human bones examination in review.

Sl. No.	Study	Intervention	Population	Comparison	Outcome
1.	Camille et al. (2013) [94].	Computed tomography (CT).	-	2D CT scans of lower limbs (30 femurs, 24 fibulas, 24 tibias) examined and compared within themselves and with the CMI reference values for humans, dogs, sheep, and pigs. to assess the constancy of the corticomedullary index (CMI)	- Measurements were obtained at three different sites from the CT scans, and compared with reference values. -The results were considerably different from those of the reference values. -The percentage of CT scans that were accurately identified as human was only 22.6%. -CMI concluded as not an effective method to evaluate origin of bones.

Encountering animal bones in a crime scene and mistaking it for that of a human is a very common scenario in most criminal cases. Animal bones, are most often, confused to be that of a juvenile. A vigorous study of the various morphological features differentiating an animal from a human bone can help to prevent such misleading events from occurring in an investigation process.

Chapter 4

CONCLUSIONS

In the past several years, forensic anthropology has developed, as both a multidisciplinary, and an interdisciplinary field of study. The growth of modern forensic anthropology can be attributed to its various defining growth characteristics, such as its ability to incorporate new methods and technology into the anthropologist's toolkit. Forensic anthropologists have been increasingly involved in identifying human remains from large-scale disasters, past wars, unreported border crossings, and mass fatality cases. This reflects the development and growth of the field, and the impact and relevance of forensic anthropology on and to the society.

Large-scale challenges to the identification process of the human remains have called attention to the need for advancements in the methodological and technological techniques, emphasizing the use of more advanced statistical analysis methods, advanced software programmes and databases, the need for sophisticated radiography, and 3D scanning programmes, improved estimations for developing the biological profile, estimation of year of birth from isotopes, advancements in DNA technology, and many more.

This review paper has briefly summarized the various personal identification methods from human skeletal remains, from past to current methodologies and techniques, and finally, the future prospective for each identification methods. The different aspects for the construction of the biological profile of an unknown skeletal remain has been aptly discussed. From what has been observed in this study, there is a need to understand the various variations in the human skeleton, within intra and inter-populations, and to understand the interaction between the various variables whilst estimating a particular parameter for the biological profile, so as to get a much broader perspective. It is vital to select and apply statistical methods very carefully, and also to always be aware that the reference samples that is being used can have its own sets of limitations, which needs to be understood and addressed by the examiner.

For age estimation, various researchers have stressed the importance of using population-specific aging standards and to use as many skeletal indicators as possible. Advanced skeletal imaging techniques show a promising scope for future investigations, especially in cases where the skeletal remains are those of a child, as not many skeletal collections include those of sub-adults. Similar to age estimation techniques, methods for stature estimation also require population specific standards, and factors that affect the stature of a person, such as sex and age-related stature decline, are always to be considered. Sexual dimorphism of bones requires to be studied from diverse samples to further refine identification procedures. Traits of the skull and dentition have been found to be apt predictors of ancestry, and various statistical software programmes have been developed to measure morphological traits so as to assess ancestry. Knowledge and experience of the anthropologist to identify various injuries in the skull also helps to easily interpret traumas to the skeletal regions of the body. An anthropologist must be trained efficiently so as to differentiate an animal bone from a human bone, as there has been many cases with false leads due to misinterpretations about the type and origin of bone that was recovered.

In a whole, various imaging technologies are slowly emerging as non-destructive techniques for skeletal identification. Many researchers, irrespective of the parameter (such as age, sex, stature, etc), have stressed over and over again on the need to investigate the various skeletal variations based on human populations, and the necessity to develop population-specific analytical tools. Forensic anthropology is a rapidly growing field, and thereby, forensic anthropologists should receive broad training to understand, utilize, and develop the vast number of forensic skeletal identification approaches. Existing techniques should be refined periodically, to meet the needs of the current ever-growing modern population, and a systematic process for examination is always to be followed.

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