A STUDY ON COSTAL CARTILAGE CALCIFICATION FROM DIGITAL CHEST RADIOGRAPH FOR EVALUATION AND ESTIMATION OF SEX AND AGE

Thesis Submitted for the Award of the Degree of

DOCTOR OF PHILOSOPHY
in
Forensic Sciences

By

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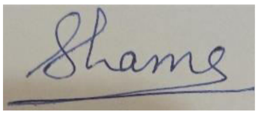
<u>**DECLARATION**</u>

I, hereby declared that the presented work in the thesis entitled "**A Study on Costal Cartilage Calcification from Digital Chest Radiograph for Evaluation and Estimation of Sex and Age**" in fulfilment of degree of **Doctor of Philosophy (Ph. D.)** is outcome of research work carried out by me under the supervision of Dr. Tejasvi Pandey, working as Associate Professor, in the department of Forensic Science, Lovely Professional University, Punjab, India. In keeping with general practice of reporting scientific observations, due acknowledgements have been made whenever work described here has been based on findings of other investigators. This work has not been submitted in part or full to any other University or Institute for the award of any degree.



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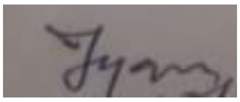
CERTIFICATE

This is to certify that the work reported in the Ph. D. thesis entitled "**A Study on Costal Cartilage Calcification from Digital Chest Radiograph for Evaluation and Estimation of Sex and Age**" submitted in fulfilment of the requirement for the award of degree of **Doctor of Philosophy (Ph.D.)** in the Forensic Science, is a research work carried out by Shama Patyal, 41900280, is Bonafide record of his/her original work carried out under my supervision and that no part of thesis has been submitted for any other degree, diploma or equivalent course.



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Abstract

The costal cartilage is a crucial anatomical structure located at the junction of the ribs and the sternum. As individuals age, these cartilages undergo calcification, a process that has been observed to follow distinct patterns. Previous studies have noted that this calcification initiates in the first rib before extending to other ribs. Furthermore, previous studies have suggested that these calcification patterns exhibit variations between genders, making them a promising avenue for sex estimation.

The present study addresses existing research gaps concerning the use of costal cartilage in sex determination during forensic examinations and anthropology. Currently, limited information is available regarding costal cartilage calcification, particularly its potential significance in these fields. By conducting an in-depth exploration of this topic, this study aims to fill these gaps and contribute significant outcomes. The results of this study significantly advance our knowledge of the complex interactions between age, gender, and costal cartilage calcification. This study inspires appealing investigations into the underlying biological and physiological mechanisms that underlie these observed patterns, in addition to its potential implications in age and sex estimation. One of the advantages of this study is it is non-destructive. The objectives of the study are 1. To evaluate the radiological anatomy of an adult using normal posteroanterior chest radiographs. 2. To evaluate the prevalence of costal calcification in males and females. 3. To examine the influences of age and sex on patterns of costal cartilage calcification. 4. To study the type of pattern of costal cartilage in males and females. 5. To assess the efficacy of imaging software in enhancing diagnostic imaging.

Hypotheses of the Study:

1. There will be differences in the pattern of calcification between females and males.

2. There will be a discernible relationship between increasing age and calcification density in costal cartilage.

The digital radiographs were processed using the Fujifilm FCR PRIMA CONSOLE and IMAGE WORKS software. This digital approach eliminated the need for traditional ruler or compass calculations and facilitated efficient storage and analysis of the data. The PA chest radiograph remains a crucial imaging modality for diagnosing and monitoring thoracic conditions. Our findings highlight the importance of routine chest radiography in the detection and management of cardiac and pulmonary diseases. The CTR

was calculated for each adult radiograph, and data were categorized by gender and age group. Statistical analyses were performed to determine the distribution and significance of CTR values about thoracic pathology. The prevalence of normal CTR values in our study population indicates a lower risk of significant cardiac enlargement, underscoring the relevance of CTR assessment in clinical practice. Continued use and evaluation of PA chest radiographs are essential for improving patient outcomes and advancing thoracic diagnostic techniques.

In the study, it was observed that out of a total of 250 male participants, 170 males exhibited lower costal calcification, resulting in a prevalence of approximately 68%. Similarly, among the 250 female participants, 177 females displayed costal calcification, indicating a prevalence of approximately 70.8%. The chi-square test's results emphasize the profound relationship between gender and calcification patterns within the costal cartilage. Females predominantly exhibited central calcification, while males were primarily characterized by peripheral calcification, reinforcing the existence of sexual dimorphism in these patterns. The probability of the Central Type for females is approximately 83.96%, while the probability of the Peripheral Type for males is approximately 91.76% from the results of the study.

Spearman's rho analysis revealed a robust and consistent direct relationship between age and the onset of initial calcification in the costal cartilage. Specifically, Spearman's rho value for the entire sample was 0.902, with a significance level below 0.01, indicating a strong correlation. This correlation persisted when analysed separately for males ($\rho = 0.922$, $p < 0.01$) and females ($\rho = 0.896$, $p < 0.01$). These findings underscore a significant and consistent link between age and the calcification process in costal cartilage for both genders, emphasizing the efficacy of this method in estimating age.

Comparison with previous studies indicated variations in calcification rates, likely attributed to differences in age distributions within the study samples. Notably, calcification patterns in the lower costal cartilages were absent in the 0-10 age group but increased with age, becoming 100% prevalent in the 41-50 age group. In the male sample, the first radiograph where lower calcification was observed was of a 19-year-old boy; in the female sample, the radiograph showing the lower costal cartilage calcification was 20 years old. Calcification of the right-side first costal cartilage was detected in 189 males, while 61 radiographs displayed stage 0 (absence of calcification). The onset of stage 1 was initially noted in an 18-year-old male. In females, 192 radiographs showed first costal cartilage calcification (that is in stages 1, 2, and 3), and 58 radiographs were in stage 0. The onset of costal cartilage calcification of the first costal cartilage was observed in an 18-year-old male 20-year-old female. Thus, 381 radiographs showed calcification in the first costal cartilage, whereas in 347 radiographs, lower costal cartilage calcification was observed. These findings support the theory that the first rib undergoes calcification before other ribs. Although the several stages of calcification overlap, the study's findings suggest an individual's age will be more helpful as they get older. Age estimation benefits from the simplicity, increased effectiveness, and cost-effectiveness of the radiological technique applied.

For screening assessments and forensic scenarios limited to the thoracic region, the aforementioned method for age and sex estimation will prove beneficial. In various contexts such as identifying unidentified remains, forensic inquiries, and clinical studies, accurately determining age and sex through assessing costal cartilage calcification is paramount. Additionally, research into costal cartilage calcification can provide important information about skeletal and biochemical changes related to ageing. Therefore, combining such radiological technology with software programs and artificial technology will improve sex and age estimation results.

While this study represents a significant step towards understanding sexual dimorphism and age estimation using costal cartilage calcification patterns, it is essential to acknowledge its limitations. The study's sample size, while diverse, is relatively modest, and the research design is cross-sectional. Future research avenues should aim to expand the sample size to include more diverse populations and use better modalities, thereby enhancing the generalizability of results. Further refinement of age and sex estimation models and validation on independent datasets could improve accuracy. Exploring the potential application of advanced imaging techniques and machine learning may further enhance the precision of age and sex estimation through calcification of the rib's cartilage.

In general, this work substantially advances our knowledge of how to estimate age and sex using costal cartilage calcification patterns. It highlights the potential of this method in forensic investigations, anthropology, and clinical research. Additionally, the study emphasizes the importance of considering age and sex-specific variations when interpreting calcification patterns. Future research in this area promises to refine and expand these methods, further improving their accuracy and utility in various fields. This interdisciplinary approach highlights the importance of collaboration between radiology, anthropology, and medical imaging in advancing forensic science. Further validation across diverse populations is warranted to refine the application of costal cartilage calcification patterns in forensic casework and contribute to the resolution of complex medicolegal cases.

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My husband, Dr. Deepak Thakur, is the best person I know, and I would like to dedicate my thesis to him. Without him, I could not have completed this job. My parents (Kushal Patyal and Kiran Patyal) and in-laws(Dharam Singh and Nirmala Devi), who have consistently been a source of limitless strength and drive to me, are to thank for my profound sense of appreciation. As a motivational speaker and source of emotional support, my sister Dr. Neha Patyal was always there. Dhruv Thakur and Sara Thakur, my children, for their unwavering love of me. Above all, I want to express my gratitude to the All-Powerful God, who is the Source of all knowledge and wisdom, for His boundless love.

Shama Patyal

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List of Abbreviations

AP: Anteroposterior

C: Central

CC: Costal Cartilage

CCC: Costal Cartilage Calcification

CT: Computed Tomography

CR: Chest radiograph

DFA: discriminant function analysis

3D: Three-dimensional

MEA: Mean estimated Age

Indiff: Indifferent

IW: Image Works

MRI: Magnetic Resonance Imaging

P: Peripheral

PA: Posteroanterior

SD: Standard Deviation

Chapter 1

1.0 Introduction

The fundamental goal of forensics is to identify an unknown person or persons, either alive or deceased. The identity of an individual can be obtained by confirmation of spouse, family, police records, fingerprint records etc. but when the body is in the advanced stage of decomposition these methods cannot be reliable. The medical examiner, forensic pathologist, and anthropologist seem for help. There are distinctive biological characteristics that allow for the identification of any individual. Bone can be used to identify individuals. It additionally can help determine a person's age, sex and race because it is resistant to putrefaction and animal-caused damage (Janaway, Percival, & Wilson, 2009). Anthropology offers several alternative identity processes for bone structure-based human identification. This could happen, for instance, with recently deceased dismembered remains, even though DNA analysis is typically used to definitively determine the sex of recent remains (Latham & Miller, 2019; Ubelaker & Wu, 2020). When skeletal remains are discovered and questioned, crucial inquiries revolve around identifying age, gender, ethnicity, and stature. This information holds significant importance in forensic and legal settings. Unlike DNA technologies, which are pricier and less widespread, the use of anthropological data such as race, age, size, and gender in new human identification methods is growing over time. Accurate sex estimation is more important in making a biological profile than other parameters because it leaves out nearly half of the population. and the rest of the parameters like age, ancestry, and stature depend upon the sex (Rissech, Wilson, Winburn, Turbón, & Steadman, 2012). Long bones, skull and pelvis are the most trustworthy areas in evaluating sex and age which are known to be extremely precise (Bruzek & Murail, n.d.). The long bones, rather than the skull, are the best anatomical sites for sex estimations after the pelvis (Klales, 2020a). But I cannot depend upon these bones in a forensic context because this part may be missing, burnt, dismembered and in any other situation. Forensic anthropology is regarded as an interdisciplinary science that depends on two fundamental processes: The examination and assessment of human remains and the identification of individuals who are alive. It can be seen as the fusion of the fields of anthropology, medicine, and forensic sciences. Put another way, forensic anthropology collaborates symmetrically with fields like anatomy, biology, taphonomy, botany, entomology, odontology, pathology, and osteology. The earliest examples of forensic anthropology date from the late 1800s, when anatomists and physical anthropologists used skeletal evidence to make crude gender, race, ethnicity and age determinations (Leach, Lewis, Chenery, Müldner, & Eckardt, 2009). The basic objective of forensic anthropology is to identify an unknown person or persons, whether they are alive or dead (Kimmerle, 2014). To do this, a biological profile must be created from the available human remains using various strategies developed in biological anthropology. Since being a recognized branch of forensic science, it has made significant advancements in aiding in both living and deceased person identification dactylographic or cheiloscopic prints, tattoos, activity or occupational marks, surgical implants and devices, DNA profiles, dental arcade patterns, teeth prisms, skeletal diseases, radiographic features, etc. Latest investigations by forensic experts into burial sites and exhumations in Iraq, Kosovo (Rainio, Lalu, Ranta, & Penttilä, 2001; Stover, Haglund, & Samuels, 2003) and Croatian (Jankauskas, 2009; Šlaus et al., 2007) are only a few instances where forensic anthropology has played a key part in legal proceedings. In lack of skull, pelvis and long bones estimation of age and sex is difficult. So, studying other skeleton parts and generating data from them is significant.

The authors Meindl and Lovejoy in 1985 stated that (Meindl & Lovejoy, 1985)-

"*Any indication which both significantly reflects biological age and whose informational content is independent of other indicators will be useful to a final age estimate, whether under forensic or archaeological conditions.*"

Given its activity during the transition from adolescent growth to adult maturation and degeneration, the human thoracic region holds notable importance in biological and forensic anthropological studies. Consequently, it provides a means of acquiring identification-related data over a substantial portion of an individual's lifespan, proving especially pertinent in cases involving only fragmented remains, where determining age and sex can be particularly difficult. Many anthropological techniques for addressing identity-related scenarios were developed for use on dry bones and necessitate at least partial or complete defleshing of the body(Torwalt, 2003).

Different techniques can be used to determine the ages of juvenile and adult skeletons. Researchers study growth and development in juvenile skeletons (Black, Payne-James, & Aggrawal, 2010; Krishan, Kanchan, Passi, & DiMaggio, 2012), but since skeletal development stops in early adulthood, degenerative alterations to the skeleton must be seen in older individuals. Juvenile skeletons have been the subject of age-related study since the 1920s, with a particular emphasis on epiphyseal formation and epiphyseal-diaphyseal union (Alsup, 2007).

Even though every person who needs a forensic investigation is in some state of decomposition, most of the time these bodies are still fairly intact. In such circumstances, it will be simple to begin the identification process by utilizing evidence such as fingerprints, ocular confirmation, unique bodily traits, dental history, or prior medical procedures (Aalders et al., 2017). Yet, in certain instances, the genitals might have undergone extensive decomposition, or the available antemortem medical and/or dental records may prove inadequate., impossible to get, or all three. To estimate sex and age, I can use the modalities of forensic radiology. Radiology is crucial since it offers quick, simple, and affordable approaches employing X-ray machines, Computed Tomography (CT) and Magnetic Resonance Imaging (MRI).

Past authors, Stewart and McCormick (1985) stated that radiographic techniques are fast and inexpensive to use and easy to store the data and records for a longer time. With the increase in age changes are observed in the whole body it is also observed in the human thoracic region and is prominent in adolescent age. The changes in the thoracic region that can be used for studying age and sex are the examination of fusion of sternum, degenerative changes, costal cartilage calcification, costal-manubrium borders, dimension of the sternum, sternal ribs end, clavicle dimensions, breast shadows, by chest radiographs (McCormick, Stewart, & Langford, 1985). Diagnostic radiology plays a vital part in the diagnosis of diseases, wounds, abnormalities, and location of foreign bodies and in assisting many diagnostic procedures etc. Roentgenology was the first tool used in the radiology department for medical benefits. The radiographic application of X-rays is the commonest and oldest method used for getting information about diseases. The various modalities of radiology act as a useful tool in Forensic science, Anthropology, Forensic medicine, and Archaeology.

Morphological methods of sex and age estimation are accurate but are subjective and depend upon the observer. Radiology is non-destructive and is beneficial for both alive as well as dead and helpful in case of Mass disaster, bullet location, Identification, Medicolegal investigations and many more.

The assessment of age and sex in forensic circumstances using chest radiographs is especially relevant to the current study. In the medical literature, costal cartilage calcification (CCC) has generated a considerable deal of interest and speculation. Calcification and ossification are the most common changes that appear in ageing costal cartilages.

In the human skeletal system, the sternum and ribs are connected by a unique type of cartilage called costal cartilage. The mechanics of the thoracic wall and the transmission of thoracic wall motion to the lungs during breathing depend on it in a crucial way. In forensic anthropology, costal cartilage can be a useful tool for predicting age and sex and can provide important details for criminal investigations or the identification of unidentified persons. Costal cartilage calcification can be used to establish the age and sex of newly dismembered remains (Brogdon, 1998; Dirkmaat, 2012; Macaluso & Lucena, 2014)

Examining the cartilage's ossification allows for an accurate and non-destructive way of estimating age from the costal cartilage. Costal cartilage undergoes a process of age-related changes over time, with fusion occurring between the costal cartilage and the sternum. Several studies have shown that fusions between costal cartilage and the sternum can be used to govern an individual's age precisely (Karaman et al., 2012; Meng et al., 2019; Monum et al., 2020; Oruganti, Karras, Thakur, Nagpal, & Gupta, 2024). The extent to which costal cartilage has fused and the sternum is indicative of an individual's age, with younger individuals having less fused cartilage compared to older individuals. According to a study, one may determine an individual's minimal age by measuring the fusing of costal cartilage to the sternum (Monum, Mekjaidee, Pattamapaspong, & Prasitwattanaseree, 2017). An investigation conducted in Japan utilized three-dimensional postmortem imaging to analyse differences in the progression of costal cartilage ossification for age estimation, revealing notable variations in ossification rates of the first costal cartilage between males and females (Monum et al., 2017). Semine and Damon (1975) discovered that the ossification of costal cartilage in females accelerates during their twenties and subsequently slows until the age of forty or fifty, based on their study of ribs from five different sample sets. While in men, ossification increased steadily until the 40s and then decreased until the age of 70 and beyond. Overall, this indicates that men have more ossification than women do. Mention that the larger size of men may lead to a stronger biomechanical stimulation for chest expansion during breathing. As a result, there is more costal cartilage ossification, especially in the lower ribs. (Semine, A Alan and Damon, 1975). Anthropologists today typically evaluate a person's sex before utilising the castings and descriptions to estimate age when employing the scan et al. (1984, 1985) ageing method. This is a result of puberty-related changes that impact the morphology of specific skeletal regions used to estimate age, such as the public symphysis (Paulson, 2023). These morphological variations have been thought to be caused by differences in hormones and mechanical function between males and females or differing ageing rates. Determining whether sex estimation is necessary before utilizing the sternal ends of ribs for age estimation remains uncertain. This contrasts with the crucial role it plays when employing the pubic symphysis for age estimation, given the structural and physiological variations between the male and female pelvis.

1.1 Radiography in Forensics

Radiography deploys the use of electromagnetic radiation - usually X-rays- to provide intricate depictions of the body's internal parts, such as its tissue layers, cartilage, and bones. The use of multiple other disciplines for judicial benefits is part of the broad spectrum of forensic science. In Lancashire, UK, radiography was initially utilized for forensic purposes in 1896 to find metal objects inside of a human body (Brogdon, 1998). In the study of growth and ageing processes, radiography has been and continues to be a very effective instrument. Radiology is used in the identification of victims (age, sex, stature, and ethnic background), in identifying foreign bodies, locating more forensic evidence inside the deceased's body, determining the cause of death, identifying the type of assault, and determining whether a wound is fatal or not. It is also used in criminal and legal courtroom trials and research (figure 1.1). Images from the antemortem and postmortem phases of the human identification process can be directly compared. Depending on the level of proficiency and experience of the radiologist, it may be feasible to conduct a comparative analysis of injuries, assess their severity, and recognize patterns. Additional insight into the type and characteristics of the weapon can be obtained by examining the fractures' orientations and positions in digital images. In cases involving extensively decomposed bodies, opting for a postmortem CT scan over a traditional autopsy is advantageous. This is particularly beneficial for diagnosing conditions such as air embolisms, blood aspiration into the lungs, identifying trauma in younger individuals, and meticulously documenting injuries and tissue damage (Decker et al., 2019; Pandey, 2023). Technology advancements have made disease diagnosis quicker and more precise, which opens up a lot of opportunities for forensic radiology. As a new discipline, forensic radiography requires thorough research, standards, and specialized training for radiologists and radiographers.

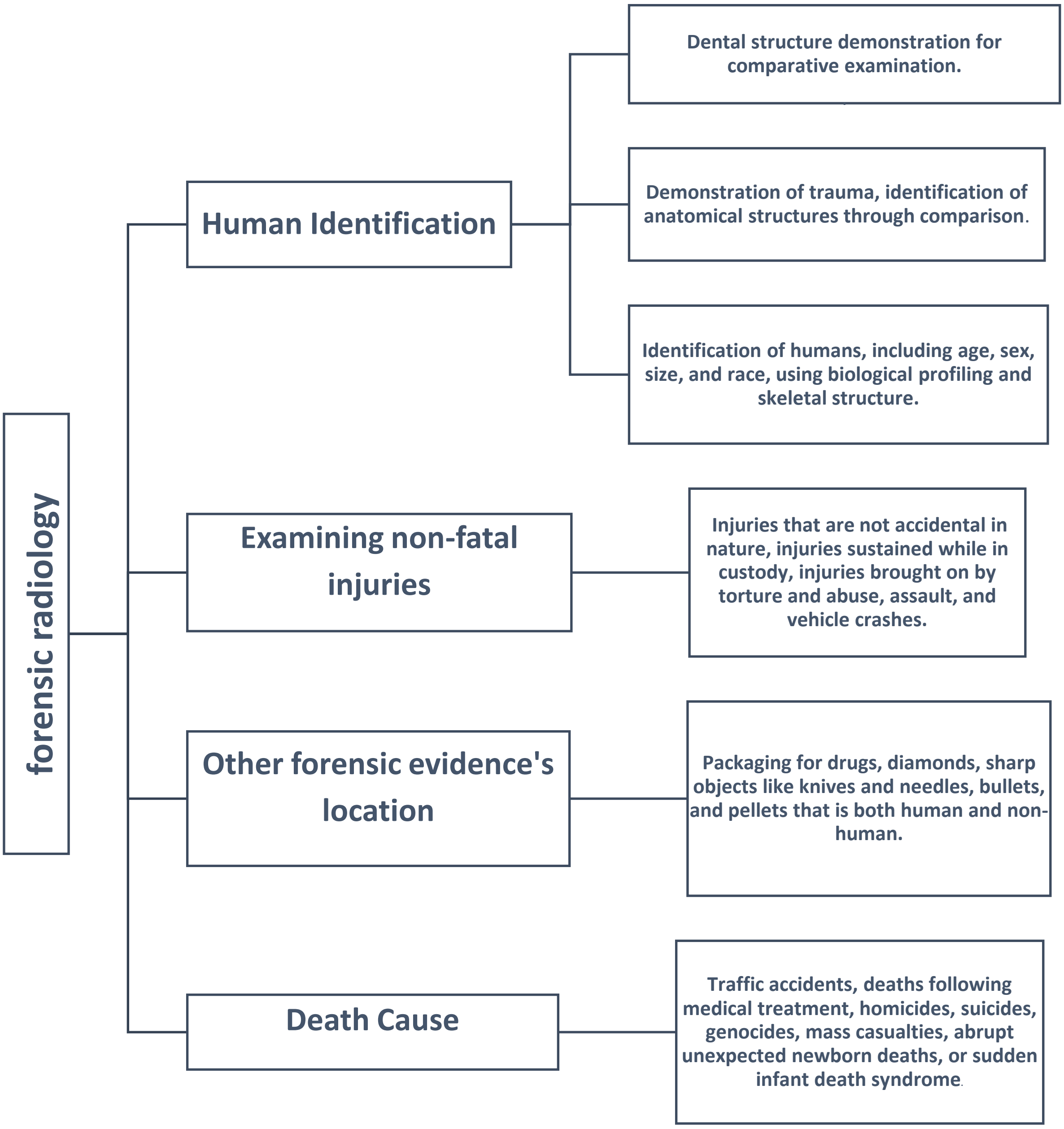

Figure 1.1: Application of Radiology in Forensics

1.1.1 Age Estimation Using Radiography

When it comes to identifying age, radiography is an invaluable tool that can provide valuable information to forensic analysts and medical practitioners alike. Radiography, also known as X-ray imaging, is a non-invasive medical imaging technique used to see the human body's interior structures in two separate planes.

Bone age evaluation is used to radiologically evaluate the biological and structural maturity of individuals from the appearances on their hands and wrists. In children with growth and endocrine abnormalities, it is of considerable importance in the diagnosis and treatment process. It is useful in the identification of a variety of growth abnormalities and can provide patients who first appear with low stature an idea of their expected future height.

In forensic analysis, an individual's age can be determined using radiography, mostly by examining the phases of bone development. Radiography age estimation is based on five significant aspects of skeletal growth, which include (figure 1.2):

Ossification Centers: Ossification centers refer to the points where bones begin to develop during fetal development. Radiological observations indicate that ossification starts before birth in some bones, and most complete their development during late adolescence to early adulthood.

Tooth Eruption: Tooth eruption refers to the appearance of teeth in the oral cavity, which usually follows a specific age-related sequence.

Epiphyseal Union: Epiphyseal union refers to the process whereby the bone epiphysis (the end portion of a long bone) fuses to the diaphysis (the bone shaft.)

Skeletal Fusion: Skeletal fusion is a process of bone tissue remodelling those results in the optimization of bone architecture, density and strength.

Bone Density: Bone density is the degree of mineralization of bones, which decreases with age, primarily after the age of 30.

Figure1.2: Age estimation using radiography

The use of radiography in age estimation relies on a range of diagnostic imaging modalities to visualize the skeletal system accurately. The four primary radiographic modalities used in skeletal imaging include:

X-Ray: The most common diagnostic modality in skeletal imaging, it captures two-dimensional images of the skeletal structures. **CT-Scan**: CT (computed tomography) scans combine multiple X-ray images to create a detailed three-dimensional representation of the skeletal structure. **MRI**: MRI works on strong magnetic fields to create detailed images of soft tissues within the body, including bone marrow and cartilage.

Nuclear Medicine Imaging: This approach involves the injection of radioactive material into the patient to create detailed images of bone turnover. The Greulich and Pyle method and Demirjian method are among the most commonly used age estimation techniques in skeletal radiography. These methods rely on the comparison of radiographic findings from an individual to those of a standardized reference group of individuals. The various data obtained through these methods include the size, shape, and fusion of bones (Patel et al., 2015).

1.1.2 Sex Estimation Using Radiography

The skeleton's bones are a major factor in detecting sex, and sex identification is made easier by radiological techniques. The standard means of distinguishing between the skeletons of males and females is the size and shape of the bones (Pandey, 2023). The skull, pelvis, and now long bones play a critical role in sex estimation. Nearly all of the bones have been studied in past, and differences in accuracy between different groups have been compared. The femur, patella, wrist, clavicle, sternum, mandible, pelvis, skull, and metatarsals are employed in sex predictions (Rad, Mohammadi, Babahajian, & Yousefinejad, 2021). Forensic anthropology and archaeology depend heavily on identifying the sexual orientation of human remains. Sex estimation from radiography is a non-invasive and reliable technique that involves analysing the skeletal structures of the remains.

Sex estimation from radiography relies on the study of sexually dimorphic skeletal traits (characteristics that differ between males and females) that are present in the pelvis, skull and other bones. These traits are usually the result of sexual selection and adaptation to roles in reproduction. The pelvis's shape and size are used to determine the sex of a distinct as the pelvis's bone structure shows significant differences between male and female anatomies (Klales, 2020b). For instance, the female pelvis is generally wider and has a rounder shape than the male pelvis. In the skull, traits such as the size and shape of the skull, the orbits, the cranial sutures, and the mandible are examined. Table 1.1 outlining bones commonly used in sex estimation in forensic anthropology, along with their reliability rates.

Table1.1: Bones commonly used in sex estimation		
Skeletal Element	**Reliability Rate (%)**	**Description**
Pelvis	85-95%	The pelvis exhibits significant sexual dimorphism, with distinct morphological differences between males and females, particularly in the shape, size, and orientation of pelvic landmarks such as the greater sciatic notch and subpubic angle.
Skull	80-90%	Certain cranial features, such as the shape of the brow ridges, size of the mastoid processes, and morphology of the mandible, can be used to infer sex with a high degree of accuracy.
Femur	70-85%	While the femur does not show as pronounced sexual dimorphism as the pelvis, measurements of femoral head diameter, neck-shaft angle, and other features can provide useful indicators of sex.
Os Pubis	80-90%	The morphology of the pubic symphysis, including the presence of ossification, surface texture, and overall shape, can be reliably used for sex estimation, particularly in adult individuals.
Sacrum	65-80%	Sexual dimorphism in the sacrum is evident in features such as the width of the sacral base and the curvature of the sacral promontory, although its reliability may vary depending on age and population.
Humerus	60-75%	While the humerus exhibits less sexual dimorphism compared to the pelvis and skull, measurements of features such as the head diameter and length may still provide useful indicators of sex.
Tibia	60-70%	Similar to the humerus, the tibia may show moderate sexual dimorphism, particularly in measurements of length, robustness, and the morphology of the proximal and distal ends.
Vertebrae	50-65%	Some vertebral features, such as the shape and size of the vertebral body and the presence of sexual variations in the transverse processes, may offer limited reliability for sex estimation.

The ideal radiographic imaging modality for sex estimation is computed tomography (CT) scanning, as it provides three-dimensional (3D) images and greater resolution than a conventional X-ray. However, X-ray is still frequently used as it is more widely available and less expensive than CT scanning. Several methods of sex estimation are used in radiography. These methods rely on the identification of sexually dimorphic traits in the skeletal structures.

These methods rely on the identification of sexually dimorphic traits in the skeletal structures
1. Standardized "visual" assessment methods: In this method, experienced radiologists or forensic anthropologists visually inspect the radiographs to identify and score the presence or absence of sexually dimorphic skeletal traits. This visual analysis is based on a wealth of research and data on these traits and can provide reliable results when conducted by trained experts.
2. Metric analysis: This method involves the use of statistical analysis to measure and compare the size and shape of skeletal structures. The most widely used metric analysis is discriminant function analysis (DFA), which compares the differences in skeletal structures between males and females to calculate the probability of an unknown individual's sex (Krishan et al., 2016). The technique is widely used in forensic investigations to aid in the identification and establish the biological profile of the individual. Radiographic imaging modalities such as X-ray and CT scanning are utilized, and methods such as standardized visual assessment and metric analysis assist in determining sexually dimorphic skeletal traits. On the whole, radiography still plays a crucial part in this field by helping to identify the gender of human skeletons.

While it's important to note that no single method can provide absolute certainty, radiographic analysis can contribute to a comprehensive assessment when combined with other anthropological techniques. It's essential to acknowledge the limitations and potential sources of error associated with radiographic sex estimation. Factors such as population variability, age-related changes, and individual variation can influence the accuracy of sex assessments. Additionally, the radiographic analysis should be complemented with other methods, such as morphological examination of other skeletal elements (e.g., skull, long bones) and DNA analysis, to enhance the reliability of sex estimation in forensic contexts.

1.2 Scope

In situations involving huge casualties caused by disasters of either nature or mankind, massacres, or aircraft accidents where it is difficult to positively identify victims directly (Devang Divakar et al., 2016), there is a pressing need for a swift, straightforward, and cost-effective method for determining the sex of individuals. High-resolution chest radiographs have emerged as a dependable means of victim identification in such scenarios. However, there is currently a notable absence of relevant reference data regarding costal cartilage and its correlation with sex determination, thus warranting a comprehensive study to bridge this knowledge gap.

This study focuses on the radiological examination of costal cartilage calcification through the use of digital radiographs, offering a rapid, accessible, and economical means of estimating both sex and age. This radiological analysis can prove invaluable when forensic pathologists are unavailable, as it requires no specialized expertise for interpretation, making it a viable screening tool for post-puberty individuals in need of sex identification.

The primary aim of this research is to explore the feasibility of sex and age assessment grounded on costal cartilage analysis. By doing so, this study seeks to make a substantial contribution to the field of forensics by shedding light on the potential applications of costal cartilage analysis in forensic investigations, archaeological studies, and medical diagnostics.

Chapter 2

Review of Literature

An analysis of the literature not only builds in-depth knowledge regarding the topic but also helps to deliberate over the gaps in the existing research. This helps in framing the methodologies, techniques, and objectives for the research to be conducted. Several studies have been conducted in the past to estimate the age of skeletons through radiological methods in different populations. To understand the trend and progress of the research in the age and sex estimation methods, a review of studies that used the same methods as considered in this study has been presented below. Beginning with a brief explanation of the anatomical structures of the thorax, and costal cartilage (CC) the chapter moves on to a brief discussion of chest X-ray positions, the anatomy of chest X-rays and previous studies related to costal cartilage.

2.1.0 Thorax Anatomy

Between the base of the neck superiorly and the abdomen inferiorly lies the thorax. For clinical purposes such as physical examinations, study of chest X-rays, and diagnosis and treatment of thoracic diseases, an understanding of thoracic anatomy is essential (Sirohi, 2020). The thoracic wall and cavity together with its skin, muscles, and superficial structures like the breast, help to make it. Men's rib cages are wider overall, but especially at the caudal section, whereas, in women, the sternum is positioned higher (Bellemare, Fuamba, & Bourgeault, 2006; García-Martínez, Torres-Tamayo, Torres-Sanchez, García-Río, & Bastir, 2016). Moreover, it has been observed that men tend to possess rib cages of greater depth compared to women of equivalent height. This anatomical difference correlates with the overall larger rib cage volume found in males. This variation in rib cage morphology between genders has implications for respiratory function and biomechanics, reflecting the physiological differences between male and female thoracic structures (Bellemare et al., 2006; García-Martínez et al., 2016).

2.1.1 Thorax wall and cavity

The thoracic wall is composed of twelve ribs, twelve thoracic vertebrae, cartilage, the breastbone, and five muscles, collectively facilitating respiration, mobility, and safeguarding the thoracic cavity (Sirohi, 2020). Situated deep within the thoracic wall, the thoracic cavity lies above the diaphragm and below the base of the neck, playing a crucial role in housing and protecting vital organs such as the heart and lungs. Lungs, bronchi, trachea, pleura, and other respiratory, cardiovascular, and other organs and tissues are in the thoracic cavity (Aung et al., 2019).

2.1.2 Ribs

The thoracic cavity's skeletal framework is composed of ribs (figure 2.1). There are typically twelve pairs of ribs. Through the costovertebral connections, each rib articulates posteriorly with two thoracic vertebrae, forming essential structural links. An exception to this pattern is the first rib, which exclusively articulates with the first thoracic vertebra. Ribs are categorized into three groups based on their attachment to the sternum: “true, false, and floating ribs” (Safarini & Bordoni, 2024). The initial seven ribs, directly connected to the sternum through their costal cartilages, are classified as true ribs, highlighting their integral role in chest structure and support (Graeber & Nazim, 2007). The eighth, ninth, and tenth ribs are the false ribs because their costal cartilages are indirectly articulated with the sternum by way of the costochondral junction with the seventh costal cartilage. The distal two ribs, on the other hand, are the floating ribs since they do not articulate at all with the sternum.

2.1.3 Sternum

Anatomically, the sternum is divided into three sections: “the manubrium, body, and xiphoid process” (Garamendi, Landa, Botella, & Alemán, 2011). The front segment of the thorax wall is formed centrally by the sternum, a somewhat T-shaped vertical bone. The CC that makes up the frontal rib cage connects the anterior ribs to the sternum. The body is the middle segment, the xiphoid process is the smaller distal segment, and the manubrium is the broad superior segment, making the partial T-shape. Manubrium is the thickest part of the sternum, having two surfaces: anterior and posterior. The manubrium has four borders named superior, inferior and lateral (two). The lateral border joins to the first costal cartilage and forms a primary cartilaginous joint. A facet on the lateral lower border of the manubrium is formed for the attachment of the upper part of the second costal cartilage. The body of the sternum is the longest part having two surfaces anterior and posterior, upper and lower ends, and two lateral borders. Notch for the attachment of second to sixth costal cartilage is present in the lateral borders (Drake, Richard and Vogl, A Wayne and Mitchell, 2009). Additionally, the upper portion of the seventh costal cartilage attaches to the body of the sternum. Among the three segments of the sternum, the xiphoid process is notably the smallest in size. It is cartilaginous in starting but in adults, the upper end is ossified. The xiphoid process varies in shape.

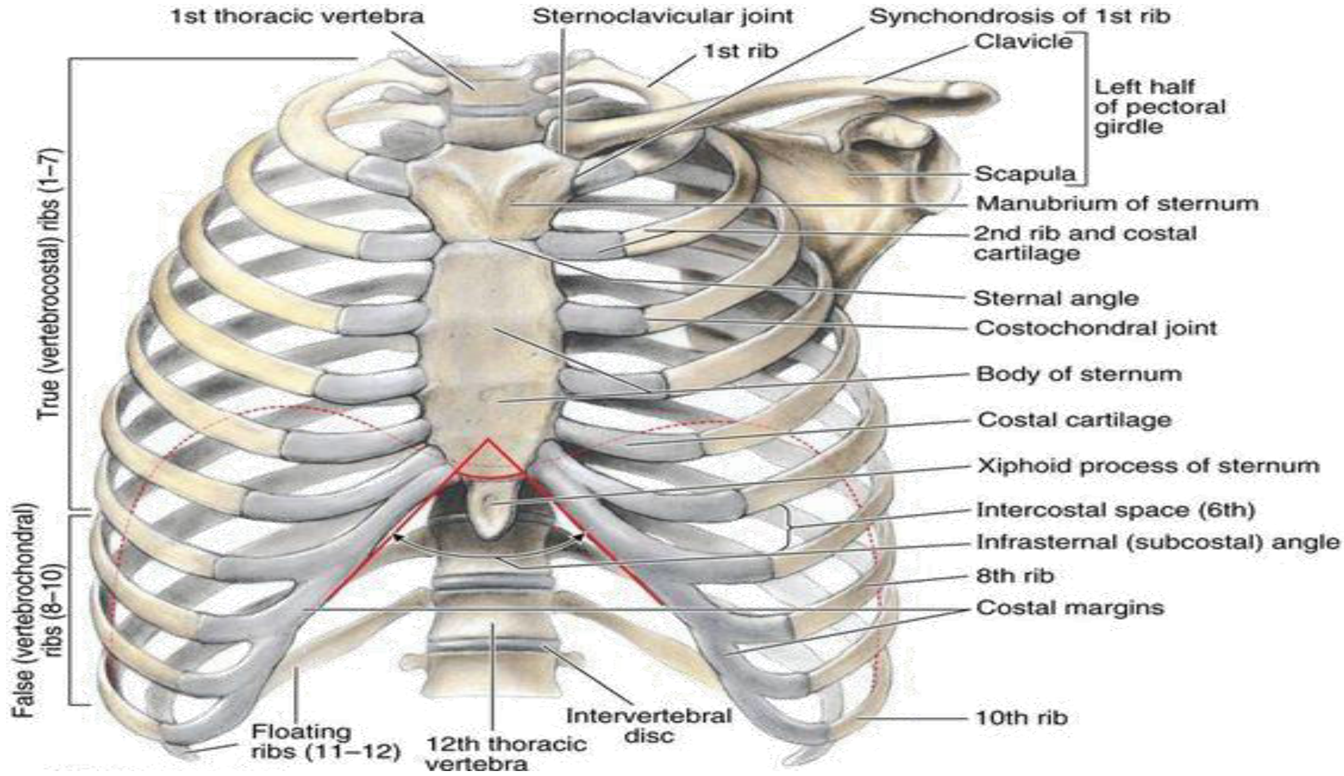

Figure 2.1: Thorax Anatomy

2.2.0 Costal Cartilage

The costal cartilages are present anteriorly in the thorax wall. It represents the unossified parts of the ribs (Rejtarová, Hejna, Soukup, & Kuchař, 2009). The function of costal cartilage is to provide elasticity to the chest wall. Costal cartilage is made of hyaline (Grässel & Aszódi, 2016; Huwe, Brown, Hu, & Athanasiou, 2018). The costal cartilage of the first seven ribs is directly attached to the sternum (ABRAHAMS & DUGGAN, 1965). The eighth, ninth, and tenth costal cartilages are attached to one another and form a costal margin (Choi, Im, Song, & Lee, 1995). The costal cartilage of the last two ribs (11^{th} and 12^{th}) is free and smaller in size. The characteristics of costal cartilages, much like ribs, vary in terms of length, width, and orientation. From the first to the seventh, the length of costal cartilages increases, then gradually decreases towards the twelfth, as depicted in Figure 2.2. Similarly, their width diminishes from the first to the last. Furthermore, their orientation differs: the first tilts slightly towards the sternum, the second remains horizontal, the third exhibits a slight upward slope, while the others assume angular directions. Each costal cartilage possesses two surfaces (anterior and posterior), two borders (inferior and superior), and two ends (medial and lateral) (Patyal & Pandey, 2023).

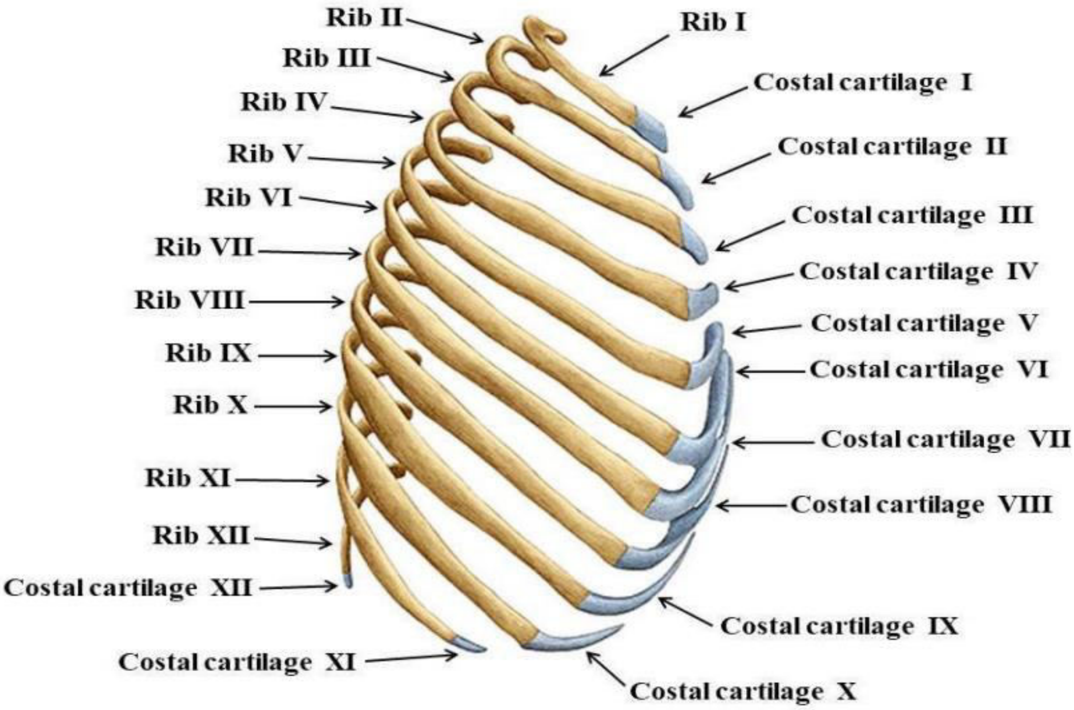

Figure 2.2: Lateral view of rib cage (Agur, Anne MR and Dalley, 2009)

2.2.1.0 Costal cartilage attachment

2.2.1.1 Anterior Surface

A sternoclavicular joint is created when the first costal cartilage articulates with the clavicle. The pectoralis major is supported by the second to seventh costal cartilage. Some of the flat muscles of the front abdominal wall are partially attached by the remaining costal cartilage.

2.2.1.2 Posterior Surface

The sternothyroid muscle is derived from the first costal cartilage. The sternocostal muscle attaches to the second through sixth costal cartilages, as illustrated in Figure 2.3. The remaining costal cartilages serve as points of attachment for the diaphragm and the transversus abdominis muscle.

2.2.1.3 Borders (inferior and superior)

The inferior and superior borders provide attachment to internal and external intercostal muscles. Fifth to ninth costal cartilage articulates with each other and forms synovial joints.

2.2.1.4 Lateral and Medial end

Each cartilage's lateral end creates a primary cartilaginous joint with the rib. The medial end of the first costal cartilage forms a cartilaginous joint with the manubrium, second to seventh costal cartilage forms a synovial joint with the sternum. The eighth through tenth cartilages are joined to the cartilage above them in the hierarchy. The eleventh and twelfth cartilages, however, have free, pointed ends.

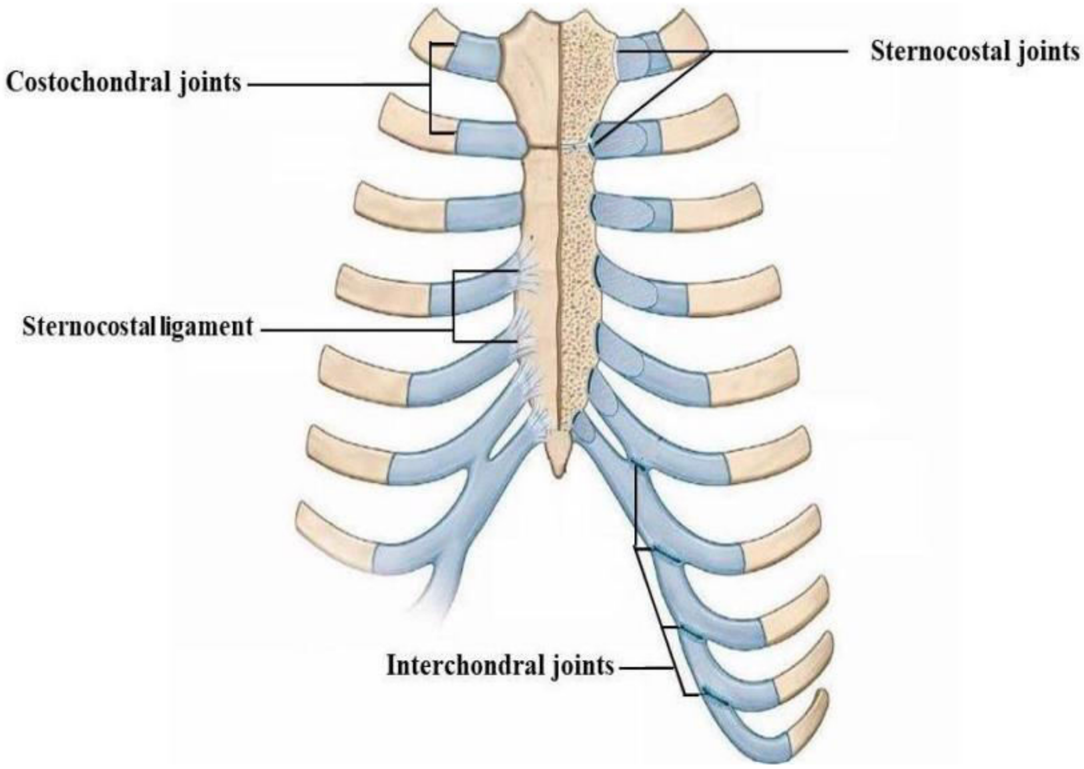

Figure 2.3: Articulations of Costal Cartilages (Drake, Richard and Vogl, A Wayne and Mitchell, 2009)

2.2.2 Costochondral Calcification

According to Bertazzo, Calcification, the buildup of calcium salts in bodily tissues, can manifest as either a natural process, such as bone development, or as a problematic occurrence, leading to the hardening of soft tissues. This phenomenon plays a crucial role in various physiological processes, including bone formation, tooth development, and maintaining the integrity of the skeletal system. However, excessive or abnormal calcification can contribute to various pathological conditions, such as atherosclerosis, kidney stones, and calcific tendinitis (Bertazzo et al., 2013). According to a study, the unusual buildup may result from tissue injury, hypercalcemia, or hyperparathyroidism (Zaidi et al., 2005). While the buildup could be a typical by-product of ageing, similar to the calcification of the pineal gland (Zimmerman & Bilaniuk, 1982). According to Lau, calcification of the costal cartilage can occur inside or outside of the cartilage fragment as people age (Lau, Kindig, & Kent, 2011).

After puberty, CC are susceptible to calcification (Vastine 2nd, JH and Vastine, Mary F and Arango, 1948). The prevalence of calcification tends to escalate with advancing age, progressing from approximately 6% in the third decade of life to as high as 45% in the ninth decade Furthermore, investigating the underlying mechanisms driving age-related increases in calcification can offer valuable insights into potential preventive and therapeutic interventions aimed at managing its impact. (Teale, Romaniuk, & Mulley, 1989). According to Ontell "costochondral calcification is typically not radiographically visible until after the age of 30" (Ontell, Moore, Shepard, & Shelton, 1997). It is alleged that the calcification of the first CC, which is complete and more extensive in men than it is in women, is an age-related and physiological change unrelated to degenerative processes. The first costal cartilage, in contrast, has not shown sexual variations in the patterns of CCC. As a result, costochondral calcification patterns ignored it when estimating gender (Kampen, Ciaassen, & Kirsch, 1995). The sixth, seventh, and eighth costal cartilages usually show signs of calcification far earlier than the upper costal cartilages (Rhomberg & Schuster, 2014a). These lower rib segments' early calcification manifestation may be related to variables like elevated mechanical stress or variations in the composition of the cartilage. The costal cartilages also provide the greatest approach for radiological examinations for several months following passed away because they are sufficiently resistant to decay (Rao & Pai, 1988b; Stewart & McCormick, 1984).

2.3.0 Chest Radiograph

The application of radiography in the form of radiographs is the commonest and oldest method used for getting information about diseases. Researchers had previously reported on the use of radiography as a practical method for sex and age assessment (A. Schmeling, Geserick, Reisinger, & Olze, 2007). The frontal chest radiograph (CR) is the most frequently requested plain film. The radiograph depending on the X-ray beam's direction is recorded as either a PA (posteroanterior) or an AP (anteroposterior) (Samei, Lin, Choudhury, & Page McAdams, 2014). The projection is typically noted in the film. An accurate assessment of the heart's size and form can be made with a PA projection since it produces a higher-quality image.

2.3.1 Basic Projections in Chest Radiography

The following are the basic projections in chest radiography:

- Posteroanterior (PA) view
- Anteroposterior (AP) view
- Lateral view

X-rays, a form of electromagnetic radiation, travel in a straight path, diverging from the source. The clarity of structures in a radiograph depends on their proximity to the x-ray detector, with those closer providing better visualization. Conversely, structures farther from the detector or x-ray film may appear magnified. The specific projection used in imaging depends on the path of the x-ray beam entering the body. In the Posterior-Anterior projection, x-rays pass through the posterior aspect and exit from the anterior aspect of the body. Conversely, in the Anterior-Posterior projection, x-rays first pass through the anterior side and exit from the posterior side. Lateral projections involve x-rays passing through the lateral aspect of the body and exiting from another side. An axial view entails x-rays passing along the long axis of the body. An oblique view involves x-rays passing through an angled plane relative to the coronal or transverse plane of the body. Understanding these projection techniques is essential for accurate interpretation and diagnosis in radiographic imaging (Corne, Jonathan and Kumaran, 2015).

2.3.1.1 The PA Projections

The PA (Posterior-Anterior) projection stands as one of the most commonly conducted chest projections in radiography. In this projection, the patient's back faces the x-ray tube, with the x-rays penetrating the body from the dorsal side (Corne, Jonathan and Kumaran, 2015; Mettler, 2013). This projection can be executed with the patient positioned erect, supine, semi-erect, or oblique, contingent upon the patient's condition and the study's requirements. Typically, the basic projection for a chest radiograph is performed with the patient in an erect position, offering several advantages. The erect position facilitates ease of patient positioning, enables maximum visualization of lung area due to the effect of gravity, provides a well-defined fluid level, and allows for straightforward control of respiration (figure 2.4). However, in cases where patients are too ill to stand erect, radiographs can be obtained in the semi-erect or supine position. The PA position offers the advantage of compressing breast tissue, minimizing magnification of the heart, and reducing radiation exposure to the thyroid gland. Images are captured during arrested deep inspiration, ensuring that the lung cavity is fully visualized on the radiograph, thereby enhancing detail for interpretation. Assessment of full inspiration can be conducted by counting the number of ribs visible on the radiograph, with six anterior ribs and ten posterior ribs indicating optimal inspiration (Puddy & Hill, 2007).

Images are taken on arrested deep inspiration. The full inspiration ensures visualization of the lung cavity on a radiograph, providing more details to the reader. To assess whether the radiograph is taken in full inspiration I can count the number of ribs in a radiograph ideally it should be six anterior ribs and ten posterior ribs.

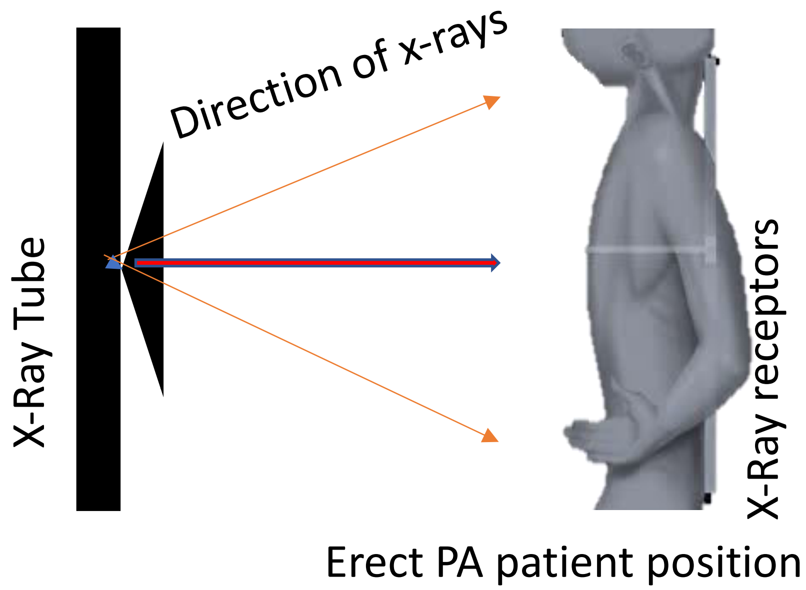

Figure 2.4: PA Projection

2.3.1.2 The AP Projections

In the AP (Anterior-Posterior) projection, the patient's anterior side faces the x-ray tube. This projection is typically utilized when patients are unable to undergo PA projection, particularly in acute conditions encountered in intensive care units. However, it should be noted that the AP projection may result in a magnified mediastinal shadow due to the increased distance between the heart and the image receptor. Images are captured during full inspiration and can be obtained with the patient positioned either sitting or supine.

2.3.1.3 Lateral Projection

To display the chest's lateral image. Patient Stands straight or lay down, to the left or right (usually left lateral). The patient's shoulder should be firmly resting against the grid and they should be slightly leaned forward. To rotate the shoulders posteriorly, either elevate the arms in front of or over the head or lock the hands behind the back. Pleural effusions, anterior mediastinal masses, and lower lobe lung disease are all evidently present in the lateral view (The National Institute for Occupational Safety and Health, 2011).

2.3.2 Structures that can be seen in a typical chest radiograph

High-energy, ionizing photons known as X-rays can penetrate matter to varying degrees, depending on the density of the target material. The least dense are gases. All solid organs and soft tissues fall into the intermediate density category, with bones and other calcium-containing structures being the densest. X-rays are used to create plain X-ray films by penetrating the body and projecting them onto a film. A cassette transforms the X-ray radiation into light, which then imprints an image on the film. Because of the varied degree of X-ray attenuation caused by intervening materials (MacMahon & Doi, 1991), regions of the film beneath dense structures are exposed to X-rays to a smaller amount than other areas of the film. On a developed radiograph densities in shades of black, white and grey are noticed. Bone is the highest in density and hence appears white on a radiograph whereas gas is the least dense and hence appears black on a radiograph (Mettler, 2013).

2.3.2.1 PA chest radiograph with good exposure

The diaphragm must descend to the level of the sixth ribs anteriorly or the tenth or eleventh ribs posteriorly during exposure in order to obtain the best imaging of the lung bases (Eich, Kellenberger, & Willi, 2008). Thoracic cage under expansion may result from inadequate inspiration, and congestion or fibrosis may be misinterpreted by compacted basal arteries. In addition, small pleural effusions will remain hidden.

2.3.2.2 Anatomy of Chest Radiograph

With a chest X-ray, many chest structures are readily identifiable. Other significant structures, like the pleura, are only noticeable when they are abnormal, and some, like the phrenic nerve, are completely invisible (figure 2.5).

- **Visible structures:** Trachea, Hilum (hila), Lungs, Diaphragm, Heart, Aortic knuckle, -Bowel gas, Breasts, Scapulae, Ribs, Calcified costal cartilages
- **Important obscured/ concealed structures:** Spine, Pleura, Fissures, Aorta, Esophagus

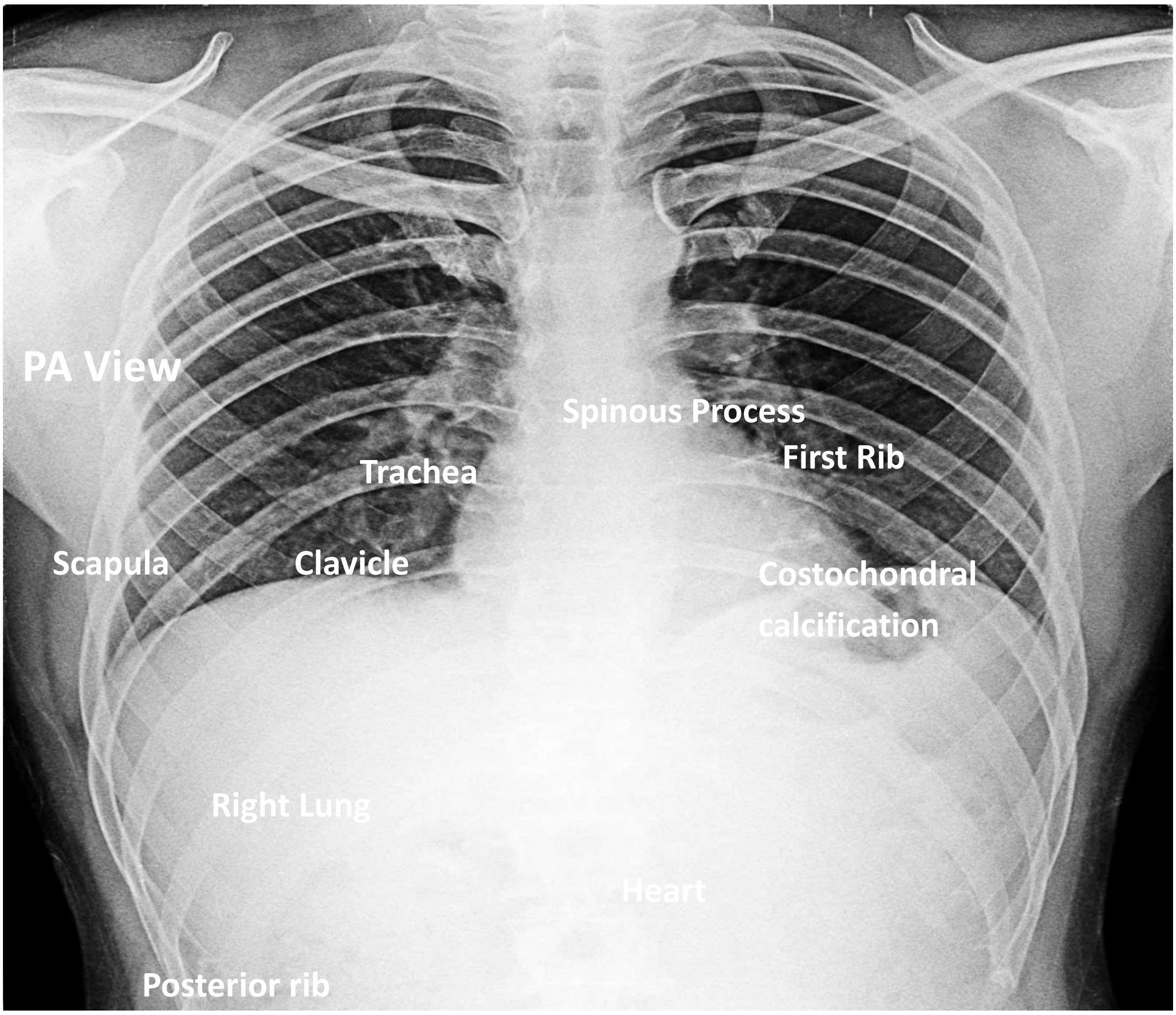

Figure 2.5: A normal chest radiograph

Previous studies have extensively explored the differences in anatomical measurements between male and female adults using chest radiographs (Bellemare, Jeanneret, & Couture, 2003; García-Martínez et al., 2016; Garg et al., 2019; Yang et al., 1991). Notably, the study by Tam *et al*. (2021) provides a comprehensive evaluation of the cardiothoracic ratio (CTR) and heart diameter measurements using both posteroanterior (PA) and anteroposterior (AP) chest radiographs (Torres, Eifer, Times, Nguyen, & Hanneman, 2021). This study emphasizes the diagnostic utility of CTR and heart diameter cut points specific to sex, underscoring the larger cardiac chamber sizes typically observed in males compared to females. The findings align with other research indicating that men generally exhibit greater cardiac dimensions, which has implications for diagnosing and managing cardiac conditions.

In research conducted by Ali *et al*., 172 individuals who appeared to be in good health were examined. The average Cardiothoracic Ratio (CTR) for the entire group was 45.6%. When broken down by gender, the average CTR was 45.10% for males and 45.71% for females, though this disparity wasn't statistically significant. This ratio is derived from the postero-anterior (PA) chest radiograph, where the maximum transverse cardiac diameter (MTCD) and the maximum internal thoracic width (MITW) above the costophrenic angles are measured. The CTR, expressed as a percentage, indicates the extent of cardiac enlargement, with higher values suggesting greater enlargement. However, there were notable variations between males and females in both MITW and MTCD, indicating gender-related differences in cardiac size (Al-Mendalawi, 2020).

Debnath et al.'s research fills a significant void in understanding the typical measurement ranges and variations among young adolescents, particularly from the Indian subcontinent. The authors sought to examine specific anatomical features on chest radiographs of healthy Indian adolescents, including the level of breath inspiration, cardiothoracic ratio (CTR), presence of gastric fundic bubble, presence of splenic flexure, difference in height of diaphragmatic domes, and the impact of breath inspiration on the CTR. Despite the advancements in imaging techniques like cross-sectional imaging, chest radiography remains crucial for assessing thoracic conditions due to its easy accessibility, widespread availability, and minimal patient radiation exposure. The authors stress the importance of understanding normal chest radiograph features as a prerequisite for interpreting abnormalities. They note the reliance on normative data from studies on adult Western populations and advocate for establishing demographic-specific normative values, especially for Indian adolescents. The study findings provide valuable insights into the typical range of anatomical features on chest radiographs in healthy Indian adolescents, potentially differing from norms established in Western populations. Moreover, the identification of significant gender differences in certain parameters emphasizes the need to consider demographic variations when interpreting chest radiographs in the Indian young adult population (Debnath et al., 2018).

2.4 Radiographers in Skeletal Age Estimation and Forensic Investigations

Radiographic images provide detailed information about bone morphology, including patterns of ossification and calcification, which are critical for estimating the age of individuals (Kushdilian, Ladd, & Gunderman, 2016). Radiographers, with their specialized training in imaging techniques, play a critical role in capturing precise and accurate radiographs that form the basis of age estimation analyses (Lundvall, Dahlgren, & Wirell, 2014). Studies focusing on skeletal age estimation highlight the significance of radiographic imaging in assessing the degree of ossification and calcification in various skeletal elements, including the costal cartilage. The expertise of radiographers in positioning patients, selecting appropriate exposure settings, and ensuring optimal image quality is essential for obtaining reliable data for age estimation purposes (A. Schmeling et al., 2007). Maruyama *et al*. (2012) propose a simplified method for teaching radiography for students for patient positioning. This tool aims to enhance comprehension of anatomy and positioning techniques while minimizing exposure to radiation risks (Maruyama & Yamamoto, 2012).

In the context of forensic investigations involving skeletal remains, radiography provides valuable information for identifying individuals, determining the cause of death, and reconstructing traumatic events. Radiographic imaging of skeletal injuries, bullet trajectories, and foreign objects embedded in bone helps forensic experts reconstruct the sequence of events leading to death or injury. Radiographers play a critical role in obtaining clear and diagnostically useful images that facilitate accurate interpretation and analysis by forensic pathologists and anthropologists. In studies examining the calcification patterns of the costal cartilage, the role of radiographers becomes particularly significant (A. Schmeling et al., 2008). The ability to capture well-defined images of the ribcage, including the subtle calcification patterns in the costal cartilage, is essential for accurate analysis and interpretation. Radiographers' proficiency in positioning patients, selecting appropriate imaging parameters, and optimizing image quality is paramount in obtaining reliable data for research purposes (Manjunatha & Soni, 2014).

2.5 Overview of Costal Cartilage in Forensic Anthropology

Krogman and Iscan discuss the significance of various skeletal elements, including costal cartilage, in forensic medicine. They highlight the importance of a multidisciplinary approach in age estimation and the reliability of costal cartilage as an indicator of age, emphasizing the need for standardized methods in forensic anthropology (KROGMAN, 1955). Costal cartilage, due to its structural properties, resists decomposition and is valuable in forensic contexts (Rejtarová et al., 2004). Morphological standards for skeletal elements, including CCC, are population-specific due to genetic and environmental variations (Sweilum, Galal, & Habib, 2017). Ageing in costal cartilage manifests as calcification and ossification, processes that can be used for age estimation (Rejtarová et al., 2009).

2.5.1 Population-Specific Morphometric Standards

Morphometric standards for gender differences in skeletal structure vary depending on the population. Genetic, environmental, and climatic factors influence the phenotype, making it essential to apply population-specific standards (Sweilum et al., 2017).

2.5.2 Characteristics and Aging of Costal Cartilage

Along with thyroid, tracheal, and articular cartilages, the costal cartilages are included in the category of permanent cartilages. These cartilages may fail to ossify completely or do not ossify at all (Rejtarová et al., 2009). The sternum and ribs are connected with costal cartilage, which is found on the anterior thorax wall. CC begins to calcify and becomes evident on a radiograph. The modifications brought on by ageing include calcification and ossification. Previous research has linked conditions like lung illnesses, atherosclerosis, nutritional issues, and metabolic or endocrine abnormalities to the calcification of CC. King's research, however, demonstrated that calcification was unrelated to illnesses (King, 1939). The sex-related pattern was first proposed by Fischer in 1955, and he came to the conclusion that the endocrine system played a part in hyaline calcification. The majority of the changes seen on the ends of the sternal ribs are caused by the costal cartilage starting to ossify with ageing. (Yaşar İşcan, Loth, & Wright, 1984). According to a study conducted in 1963, During development, the outer section of the rib expanded quickly, but after attaining 20, this expansion drastically slowed down (Sedlin, Frost, & Villanueva, 1963). It appears that calcification of the CC is widespread among people over the age of 25 and that it progresses with advancing age (Fischer, 1955). The CCC has been divided into different groups on the basis of the appearance of the calcification as marginal, peripheral, mixed and central by earlier authors (Gupta & Mathur, 1978; Kenji NISHINO, 1969).

2.6 Studies related to sex and age estimation using Costal Cartilage

Navani *et al*. (1970) studied calcification in males and females, the prevalence of costal calcification was assessed to examine the impact of age and sex on patterns of costal cartilage calcification. The researchers employed 1,000 frontal chest roentgenograms from Boston City Hospital in-patients. Because there were no variations between the sexes in the patterns of costal cartilage calcification in the first rib, this rib was not employed in the study. Three main types of costal cartilage calcification were employed to analyse the findings. "A. Type I (marginal), B. Type II (central), and C. Type III". Both males and females have different calcification patterns, which are common in Type I and Type II calcification. Analysis of this data has led to the conclusion that the calcification patterns of the lower ribs' costal cartilage can accurately predict an individual's sex with high precision. Males are more likely to develop Type I calcification than females are to develop Type II calcification. The rarity of calcification of any kind in males under the age of 20 is a further intriguing result (Faderani, Arumugam, Tarassoli, Jovic, & Whitaker, 2022; Jurik, 2007; NAVANI, SHAH, & LEVY, 1970).

Elkeles (1966) concerning sex and age, found variations in quantity, onset, and distribution of calcium deposits in the costal cartilage between sexes. The perichondrium was the main location of calcification in males, where it manifested as linear concentrations surrounding the costal cartilage. A hazy grainy CCC of the whole CC occurred less frequently. Females typically displayed marked calcification as curving rings of thick granular calcification that continued along the lower ribs. Elkeles claimed that he had discovered a clear distinction between the calcification in the atrial wall and CC between males and females. He noted that it started in both males and women costal cartilages and artery walls around the age of twenty. However, females were five times more likely than males to have it between the ages of thirty to fifty, male ossification quantities were modest and only gradually grew after that. Ossification rates were similar in both groups until the seventh decade when they suddenly increased dramatically in the research sample of females (Elkeles, 1966).

McCormick (1980) published a method for ageing adult skeletal remains, which he claimed as a simple, reliable, and accurate substitute for traditional methods of determining an object's age. At autopsy, the terminal 2 to 5 centimetres of ribs, the costal cartilages, and the sternum were taken from 210 individuals whose age, sex, and racial affiliation were known. Researchers then took x-rays of these. They included people ranging in age from three months to eighty-six. A nine-point framework system was created using an arbitrary grading system for the degree of mineralization, which ranged from 0 (absent) to 4 + (extremely severe). The results were collated, and a graph showing the association between age and cartilage mineralization was produced. By leaving 20 chest plates outside for up to four months to evaluate the costal cartilage's resistance to decay and degeneration, it was discovered that the cartilage was remarkably stable. CCC was first observed in a male patient at the age of 5, but it was often rare before the age of 20. At age 21, calcification in females became apparent for the first time. The sixth, seventh, and eighth sternal rib boundaries were typically the first to experience it. If the samples were older than 25 years, they all showed at least some signs of mineralization. Moderate grading of 2+ and 3+ within their grading system was unusual before the age of 40. After the age of 60, it became more common. Only individuals above the age of 55 were found to have dense mineralization of 3 to 4+ (McCormick et al., 1985).

He divided the pattern of CCC into eight groups (figure 2.6):

Type A: The costal cartilages have globules of calcification that are evenly spaced and have smooth contours. The cartilage next to the sternocostal joints and the fossae costarum may be highly calcified.

Type B: From the fossae costarum, medially extended pyramidal central tongues of calcification. Additionally, the central calcification may have a "crab claw" shape.

Type C: shows central, fragmental calcification, which sets it apart from Types A and B. The calcification foci are frequently tiny, erratic, and acutely angular. It lacks the pyramidal tongue of CCC.

Type D: Mineralization at the sternocostal joints and the fossae costarum. From the fossae costarum, calcium spurs may spread along the superior edge of the cartilage.

Type E: Is characterised by severe calcification of the costal cartilages' superior and inferior edges. Usually, the sternocostal joints have calcification.

Type F: Consists of a sheet-like core calcification and regularly spaced, tiny, spherical, radiolucent flaws that have a "Swiss cheese" or "honeycomb" appearance.

Type G: Mineralization-free, significant calcification of the CC at the sternocostal joints.

Type H: The external thirds of the costal cartilage and the sternocostal joints are devoid of mineralization and have a finely granular "salt and pepper" appearance from calcification.

Type A-D are considered female type whereas Type E-H are considered as male type. Furthermore, reported were an unclear type with little to no calcification and a null type with no calcification. Neither of these patterns allows for the determination of sex.

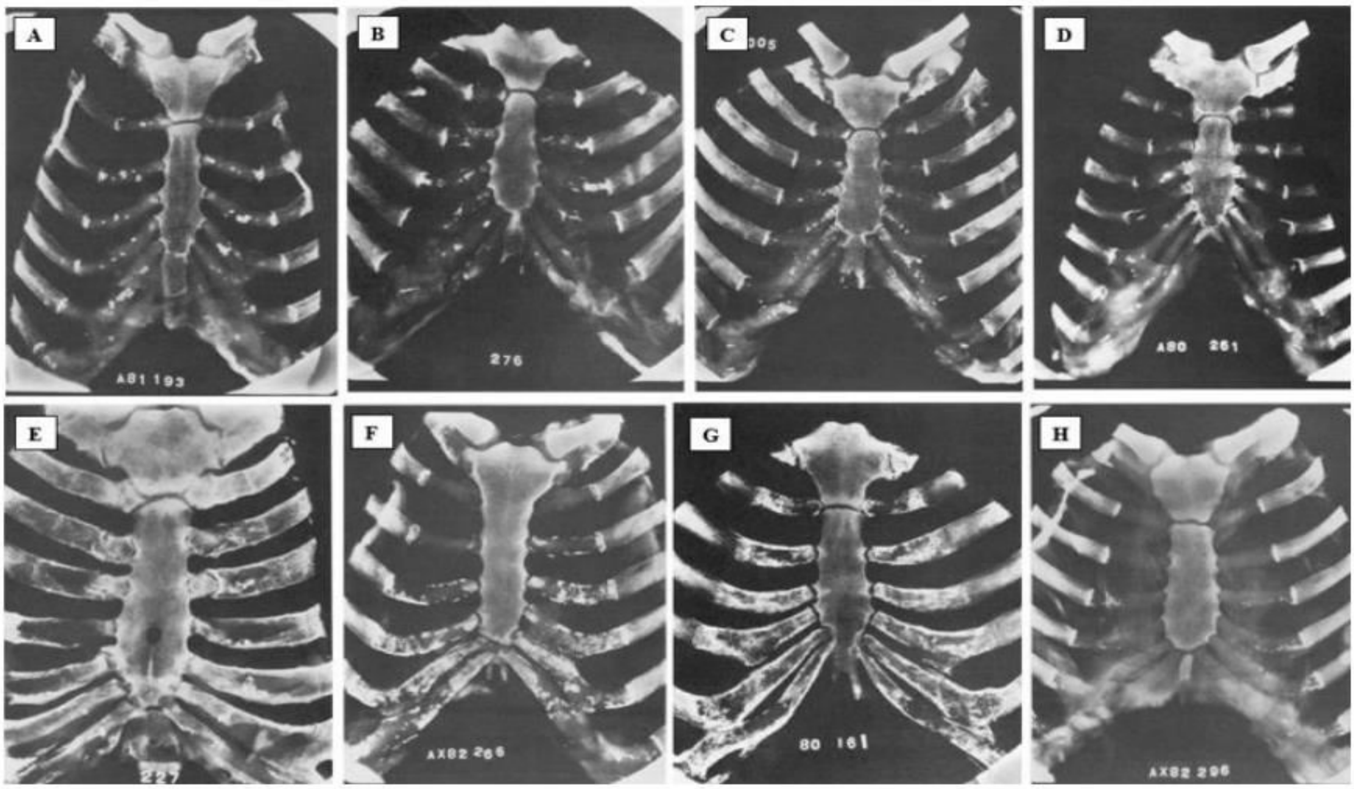

Figure 2.6: Ossification categories A-H, according to McCormick et al.1985

According to McCormick and Stewart, the first costal cartilage's ossification developed substantially differently from the other costal cartilage, resulting in a "solid bony case" when examined radiologically. Usually, ossification initially manifests in the early decades of life, and by the fifth decade, the fusion line has been completely destroyed. Males also exhibited substantial ossification more quickly.

Even though McCormick and Stewart's (Moore, Stewart, & McCormick, 1988) criteria include two of the most commonly found elements related to the calcification's configuration, making them more accurate than Navani et al.'s (1970) categories of ossification patterns. This refers to the distinctly sex-specific trend wherein men have marginal calcification and women have central calcification. Additionally, the sample from Navani et al. included the normal findings of significantly higher levels of calcification among people over 60 and barely younger than 20.

Rao (1988) studied 1000 chest radiographs from southern Indians aged 1-80. There were 512 men and 488 women in total. They created two subcategories for the peripheral kind. All three patterns were primarily limited to the 5th to 12th ribs in the lower rib cage. In comparison to males, females experienced calcification substantially earlier. Over the age of 50, calcification in the upper four ribs was discovered. In the current investigation, three different costal cartilage calcification patterns were found. A typical male pattern is forming A is a form of marginal square bracket (I). (II) A less common pattern in men is the marginal linear form, sometimes known as form A. With a tongue-shaped core pattern, (III) Type B is the only female pattern found in the study. (IV) Type C is a combination of Types A and A as well as Type C (mixed calcification I) (Rao & Pai, 1988b).

Inoi T (1997) examined 110 Japanese cadavers, 55 of which were male and 55 of which were female. Radiological analysis of the fourth right costal cartilage was performed to look at the sex pattern. Examining the internal structure at the distal ends of the ribs and the form of the costochondral junction in detail revealed more accurate age estimates than the degree of calcification alone, which showed significant individual variation (Inoi, 1997).

Olga Rejtarova *et al.* (2004) radiologically evaluate the age and sex from CCC, their research's goal was to examine how the calcification patterns of men and women differ. A total of 1044 chest and abdominal radiographs were studied (537 were males and 507 were females). They also studied the 18 radiographs of cadavers by taking their chest plates. The age range for which the radiographs were taken was 10 to 95 years old years old from the Department of Radiology (Charles Hospital) from 1995 to 2003. The sample of the study was of Czech people. The first rib cartilage was not considered as there is no sex-related difference. Rejtarova et al. categorised the calcification patterns in the following ways (Rejtarová et al., 2004):

Type I—peripheral pattern. The fossae, or the fossa at the anterior end of a rib at the union with the costal cartilage, are the starting point for the pyramid-shaped central tongues of calcification that characterise Type IIa—central lingual pattern. Type IIb: central globular pattern, which consists of calcification globules with a smooth contour positioned in the centre. Central lingual and globular pattern, type IIc. Mixed type III (central and peripheral pattern). Incipient calcification without differentiation into a sex-specific pattern is known as type IV, or indifferent pattern.

The mineralization was observed in 52% of cases. In females, the central type of calcification percentage was 77%. whereas the peripheral pattern was observed in 93% of male radiographs (figure 2.7). The probability of determining the central type of calcification in the study was 100% (figure 2.8). The outcomes of the analysis revealed sexual dimorphism in lower ribs CC. The study's conclusions verified the statistical significance of the pattern of calcification in sex estimation and an increase in mineralization of cartilage with an increase in age.

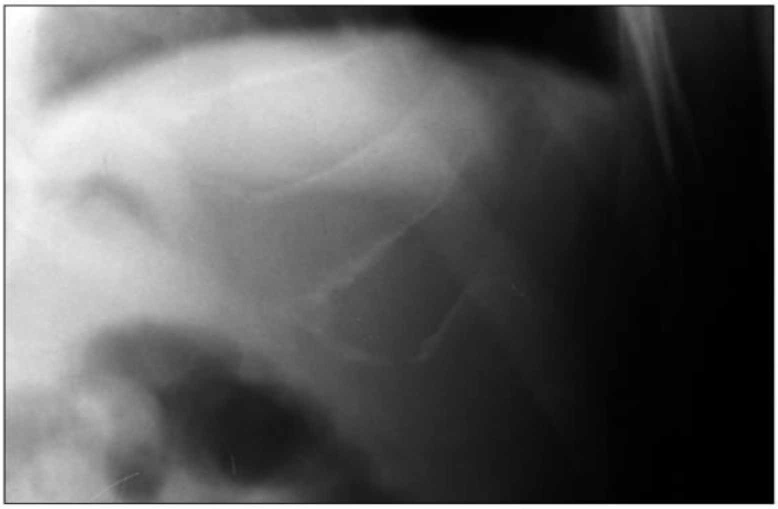

Figure 2.7: Peripheral type of ossification observed in a male sample reproduced from (Rejtarová et al., 2004) page, 242

Figure 2.8: Central type of ossification Reproduced from (Rejtarová et al., 2004) page, 242

Garamendi *et al.* (2011) conducted a study for age estimation. To investigate the value of some radiological alterations in the clavicle and first rib, this study used digital thorax X-rays. A total of 123 Spanish-born respondents (61 men and 62 women; ages 5-75) made up the sample. A digital thorax posterior-anterior radiograph was taken of each individual. Schmeling's system and Michelson's system were used to scoring for the fusion of the medial epiphyses of the clavicle and the ossification of the first rib's costal cartilage, respectively. The degree of ossification and epiphyseal fusion was examined in relation to the subjects' known ages and genders. This system has four stages for age estimation from the costal cartilage:

Stage 0 corresponds to no ossification of the first rib's costal cartilage, Stage 1 to the first signs of ossification in the cartilage, Stage 2 to the ossification of 50% of the costal cartilage, and Stage 3 to the complete or nearly complete ossification of the first rib's costal cartilage (Garamendi et al., 2011).

The authors suggested that before implementing this test in medico-legal practice, it is advised to conduct a new study on additional populations regarding age estimation based on the degree of ossification of the costal cartilage of the first rib. First rib ossification radiographic examination may be a supplemental test for those around the age of 21.

Karaman *et al.*(2012) examined the extent of calcification in the initial costal cartilage among Turkish subjects with the aim of assessing its viability for age estimation. The degree of calcification was divided into the following four phases as described in Garamendi study. Mean ages corresponding to various stages of calcification were established, revealing a relationship between calcification stage and age. The findings indicate that evaluating the ossification level of the first rib cartilage could serve as a beneficial supplementary approach for forensic age estimation. The study underscores the significance of precise age determination, particularly in instances where birth records lack reliability (Karaman et al., 2012).

Rhomberg and Schuster's (2014) research delves into premature calcifications of the costal cartilages, a phenomenon typically observed in individuals aged over 30. The study discovered 19 young patients (with a median age of 27) exhibiting significant calcifications, mostly females. These patients displayed diverse clinical symptoms, such as abdominal pain, metabolic irregularities, endocrine abnormalities, and hematologic changes. The findings imply a potential link between premature calcifications of costal cartilages and metabolic/endocrine disorders (Rhomberg & Schuster, 2014b).

Milenkovic *et al.* (2014) The research delves into the ossification patterns of the clavicle and the CC of first rib as potential markers for age assessment using radiographs and CT scans. Focusing on a Serbian demographic, it investigates whether CT analyses of these anatomical structures, either individually or in combination, could effectively contribute to age estimation. By examining patients aged between fifteen to thirty-five years, the study identifies several features, including radiodensity, stages of epiphyseal cartilage ossification, anterior-to-posterior cortical thickness ratio, medullar canal diameter, and clavicular shaft diameter, which exhibit significant correlations with age. While stages of ossified and calcified linear projections show some correlation with age, their distinction between stages is found to be insufficient. Furthermore, the study finds that the interaction between the ossification status of the medial clavicle and calcification was not significantly affected by age. Variability between sexes was observed across different age-related features. Overall, the study suggested their potential utility as supplementary indicators for age estimation in certain scenarios (Milenkovic et al., 2014).

Milenkovic (2014) thesis concentrated on age estimation. It employed a multidisciplinary method encompassing macroscopic, histomorphometric, and radiological techniques to evaluate the morphological, structural, and radiodensity aspects of these skeletal components. Macroscopic examination involved visually assessing morphological characteristics, while histomorphometric analysis scrutinized the microstructure of bone tissue. Radiological methods, such as computed tomography (CT), were utilized to assess radiodensity, mineralization, and ossification patterns. The study aimed to recognize age-related traits that could serve as dependable markers for age estimation in forensic and anthropological contexts through combined analyses of the clavicles and costal cartilage. The research offered valuable insights into the potential of these skeletal elements as age indicators and underscored the significance of a comprehensive approach to age estimation in both alive and deceased entities (Milenković, 2014).

Middleham et al. (2015) investigated a sample of the Scottish population. Two alternative methods provided by Mc Cormick and Rejtarova were used to study 41 cadavers (22 women and 19 men), extending in age from 57 to 96. None of the male sample members was accurately sexed using Rejtarova's method. While just 41.2% of women were correctly sexed by Mc Cormick, 82.4% of men were. They devised a different method based on whether the calcified deposits were “trabecular bone” or “sclerotic” calcified deposits (Middleham, Boyd, & McDonald, 2015).

Harish Kumar *et al.* (2015) conducted a study in Ahmedabad that compromised 2291 radiographs from ages ranging from 1 day to 92 years. No. of radiographs showing calcification was higher in this study than in other studies done earlier because this study used computer software applications. In this study, two approaches were used to observe digital radiography.

Technique I: The costochondral junction of the second, third, and fourth ribs on both sides in both sexes showed a pattern of calcification in a digital radiograph with a pixel resolution of “3520 (W) X 4280 (H) and a 14% zoom in”. Technique II: Digital radiographs obtained by technique I were cropped and scaled to 150% using Microsoft Office Picture Manager software 2007, producing images with dimensions of “6426

(W) X 5220 (H)”. Then, these pictures were examined at a 10- to 20% magnification, which increases the amount of information in every digital picture, even for a tiny area of interest (H. K. S. Agarwal, Shah, Ziyauddin Saiyed, & Jani, 2015). The chi-square test was used to compare radiographs that showed calcification using methods I and II. In the age category of 11 to 50 years, it was discovered that the calcification seen by methods I and II was substantially different (p 0.05). There was no statistically significant difference between those under 10 and those over 51 ($p>0.05$). The study concluded that it was acceptable to say that the various calcification patterns at the rib cartilage shown on digital radiography were sex-specific. In other words, male participants primarily exhibit the Type I pattern, while female ones primarily exhibit the Type II pattern. Both sexes begin to develop rib cartilage calcification at the age of 21, and the rate of calcification rises with age. Method II more precisely distinguishes the Type I (male) pattern of rib cartilage calcification.

Krishan *et al.* (2016), Examine the dependability and consistency of different analytical techniques utilized in determining the sex of skeletal remains, including morphological, metric, molecular, and radiographic methods. Several research endeavours have highlighted the heightened reliability and reproducibility of measurements conducted directly on bones, emphasizing the superior trustworthiness of these direct approaches over alternative methods. Additionally, recent advancements in three-dimensional (3D) techniques have unveiled distinctive patterns of sexual dimorphism not easily discernible through traditional methodologies. The ongoing pursuit of enhanced methodologies for sex estimation, alongside the reassessment of existing techniques, remains a key focus for forensic researchers striving for increasingly precise outcomes. (Krishan et al., 2016).

Tomoya (2017) study sought to refine age estimation accuracy by scrutinizing postmortem CT images. The research encompassed male and female decedents ($n = 10$ each), stratified into 10-year age intervals spanning from 20-29 years to 80-89 years. Results unveiled a consistent increase in both the mean Hounsfield unit (CT number) and calcification percentage with advancing age across both sexes. Nevertheless, notable individual variations were evident within age cohorts, rendering statistically significant disparities ($P < 0.05$) solely discernible between the 20-29 years group and older age brackets. In an effort to enhance the precision of age group allocations, Bayesian statistics were introduced, culminating in refined classification rates. Specifically, 40% of males and 35% of females were accurately allocated to their respective age groups. Expanding the age range could potentially amplify matching precision. Consequently, the fusion of Bayesian statistics with CT imaging emerges as a promising avenue for meticulous age estimation (Ikeda, 2017).

Holcombe *et al*. (2017) from 205 CT images of live subjects, calculated the volumes and extents of costal cartilage calcification (Holcombe, Ejima, & Wang, 2017). With age, there are appreciable rises in the capacity of calcification in a given cartilage segment as well as the longitudinal extent of those segments that experience calcification (p 0.001). 35% of all inter-individual person's changeability is described by age and sex. To accurately represent a person's age in models of the CC, it is recommended that (1) the volume of calcification within a section rise at the rate of 0.9 mm per decade and (2) the lengthwise extent of the cartilage segment increase at the rate of at least seven per cent per decade.

Kui Zhang *et al*. (2018) study aimed to devise population-specific age estimation models for adults based on multisided computed tomography of the costal cartilage. The research involved 512 individuals aged 20 to 85 years, with distinct models formulated for males and females. Various regression techniques were employed, revealing decision tree regression as the most precise for males and stepwise multiple linear regression for females. Gender differences were observed in age prediction accuracy within 5 and 10 years, with correct percentages ranging from 42% to 54% within 5 years and from 77% to 88% within 10 years. These findings underscore the potential utility of the developed age estimation models in forensic and clinical contexts for predicting adult age. The costal cartilage, notably the first rib, emerges as a dependable marker for adult age estimation, offering a non-invasive and reproducible means of assessing skeletal age. The comprehensive analysis of costal cartilage through CT imaging yields valuable insights for forensic age assessment and enhances the precision of age predictions in the adult population (K. Zhang et al., 2018).

Tarun *et al.* (2018), Conducted was a prospective investigation encompassing 160 radiographs, spanning diverse age groups and genders, which were meticulously assessed for various parameters through statistical analysis. Findings revealed an 80% correlation between predicted and actual age through regression analysis. Notably, factors such as the first cartilage ossification, cartilage mineralization, and bone demineralization emerged as significant contributors. Moreover, the study observed a higher accuracy in gender prediction for males compared to females within the study cohort. In conclusion, the study experimented with an accessible, time-efficient, and cost-effective approach. Despite inherent limitations, the research underscores the utility of radiographic data as a valuable tool in age estimation, offering insights for future investigations in this domain. (T. Agarwal et al., 2018).

Hang *et al.* (2019) research concentrated on introducing pigmentation alterations in the costal cartilage as an innovative strategy for age assessment in forensic scenarios. Specimens from the second costal cartilage were gathered from a Chinese ethnic populace, followed by the scanning and digitization of cross sections to quantify colour variations using mean grey value (MGV). A linear regression model was created to forecast age based on MGV, revealing a strong correlation between age and MGV. The method exhibited accuracy in age estimation within the age range of 20 to 60 years, with average total deviation of 4.42 years in the training set and 3.57 years in the blind test set. The study underscored the potential of MGV as a dependable age indicator and underscored the feasibility and precision of employing pigmentation variations in the costal cartilage for age valuation in forensic anthropology (Meng et al., 2019).

Paul *et al.* (2021) conducted research that utilized deep convolutional neural networks (DCNNs) trained on the ResNet-18 architecture through transfer learning to forecast sex and age based on chest X-rays. The dataset comprised of frontal chest radiographs, including a subset of paediatric CXRs. Findings indicated robust diagnostic accuracy in sex determination (AUC 1.0 for the entire dataset, 0.91 for paediatric CXRs) and age classification (<18 vs. ≥18 years old AUC 0.99, <11 vs. 11–18 years old AUC 0.91). External validation conducted on Chinese patients yielded comparable outcomes. However, limitations encompassed dataset acquisition from a single hospital, reliance on frontal views, potential overfitting risks with small datasets, and the inherent "black box" nature of DCNN decision-making (Yi et al., 2021).

Cemil and Gunes (2021) conducted a research study focusing on evaluating the manubriosternal joint to determine the age of complete fusion and exploring how alterations in this joint affect age estimation. Additionally, the research investigates the calcification patterns of the second CC and their potential relationship with age. The study involved individuals aged 30 to 80 who had undergone CT scan at the institution. Participants were categorized by gender and placed into five age groups spanning 10-year intervals. Twenty patients from each gender and age group were randomly selected, resulting in a total of two hundred patients examined. The findings offer insights into the fusion progression of the MSJ for age estimation purposes and the calcification patterns of the cartilage, offering valuable information beneficial to forensic age estimation practices (Oktay & Aytaç, 2022).

Thelonius *et al.* (2021) examine costal cartilage calcification in cartilage grafts used for nasal/auricular reconstruction, a factor known to impact surgical outcomes but lacking detailed analysis. The study aims to compare CCC prevalence, quantity, and structural patterns by gender and age using digital contact radiography. Conducted as a cross-sectional cadaveric study involving 92 participants, the research analysed CCC prevalence and patterns across three age groups: under 40, 40-70, and over 70 years. The results reveal a high CCC prevalence across genders (96.7%) and highlight variations in structural patterns across different age groups. Additionally, CCC amount increases with age, showing distinct patterns between genders and age categories. These findings emphasize the importance of considering age- and gender-specific factors in understanding CCC prevalence and structural variations (Hawellek et al., 2021).

Matthias *et al.*(2021) compared articular cartilage, there has been limited research on the biomechanical characteristics of costal cartilage. This study aims to fill this gap by investigating the anisotropic elastic nature of human costal cartilage for the first time. Samples were collected from cadavers, and various tests like compression and indentation tests were conducted in both mediolateral and dorsoventral directions to determine parameters such as Young's Moduli for compression (C) and indentation (I). Additionally, the crack direction of the compression samples was identified, and histologic analysis using the picrosirius-polarization staining method was performed on the cartilage tissue, significant age-related elastic nature of human CC was demonstrated in both C and I, with no observed effect of sex (Weber, Rothschild, & Niehoff, 2021).

Parul Upadhaya *et al* (2022)The study's findings were derived from the examination of 50 pairs (100 samples) of first costal cartilage alongside the manubrium. The frequency distribution of calcification patterns in both genders was recorded and statistically analysed using specialized software. Specific calcification patterns were identified as more prevalent in females, including the marginal bracket, marginal linear, and central types exhibit estimated analytical values of more than 60%, more than 50%, and more than 90%, respectively. In contrast, the mixed pattern, also with a high analytical value was more frequently observed in males on both sides. (Upadhayay, Deemed, & Ncr, 2022).

Albaraa (2022) examined costochondral calcification patterns for gender estimation, employing CT images and the classification method proposed by Rejtarova et al. (2004). The research involved a cohort of 200 individuals with an equal ratio of sex without any history of pathology or orthopaedic surgery. Participants ranged in age from 20 to 60 years. The analysis included determining mean difference (WMD), range, and Mean Absolute Error (MAE) for gender estimation based on calcification patterns. The primary objective was to accurately estimate gender utilizing these patterns, aiming to provide insights into the method's efficacy in predicting male and female types (AL-SAMANEE, ÖNER, & ÖNER, 2022a).

Mehrdad Ghorbanlou (2022) investigated if the degree of calcification in costal cartilage is influenced by gender and chronological age, using CT scans from 400 individuals in Iran. The study found that while there wasn't a significant gap in costal cartilage calcification degree (CCCD) between males and females overall, males under 30 displayed a 5-25% higher calcification than females. Additionally, in this age group, females had a higher rate of complete fusion of the first costal cartilage (FCCC) compared to males. Age-related distinctions were also observed, with lower CCCD and higher rates of complete fusion in individuals over 30 compared to those under 30. These findings underscore the necessity of considering age and gender in the analysis of costal cartilage calcification patterns in forensic and clinical contexts (Ghorbanlou, 2022).

No strong evidence of pathogenic or ethnic compatibility influences on the amounts or types of calcifications has been found in previous study. Studies have highlighted that calcification of costal cartilages is a physiological response to muscular strains, unrelated to disease or habitus (Wang et al., 2022). Others have claimed that Caucasians and Negroids appear to have different calcification rates (Michelson,1934; McCormick, 1980).

At least, the research mentioned above suggests that age and gender assessment using thoracic area radiographs is a useful complement to traditional methods. When a quick sex and age estimation is required, they provide a straightforward, quick, and non-invasive option in addition to being mostly unaffected by pathology and ancestry. Costal cartilage is a valuable tool in age and sex estimation, providing valuable information to forensic anthropologists and criminal investigators. Therefore, the use of costal cartilage in forensic investigations will be a valuable technique in forensic investigations. To make it widely acceptable and applicable, a study on different populations is needed.

2.7 Evaluation of Literature

In searching articles on "costal cartilage" for sex and age estimation I used the GOOGLE SCHOLAR database which is freely available to use. On applying the "advance search" option from 2010-2023. Using Boolean operators (AND, OR, NOT), Age OR Sex OR Estimation OR Forensic and selecting options- "where my words occur" and "in the title of the article". I found only 24 studies (figure 2.9) on 16 May 2024 out of 24 studies, 5 studies were not related to forensic identification. Only one study was based on deep learning published in 2023. The number of studies relevant and the methodology used were given in table 2.1. On applying the time range from 1950-2023, the number of studies increased to 56 only. Hence very less studies have been conducted till today. So, there is a need to conduct a study on it and fill the research gap.

Figure 2.9: Screenshot of the Google Scholar during article search.

Table 2.1: Studies based on CC on Google Scholar	
Methodology	**Number of studies**
Radiographs (ossification/calcification)	03
CT-Scans (3-D)	06
Pigmentation	01
DNA Epigenetic	01
Cartilage elastic moduli	01

Chapter 3

Research Methodology

This study assesses the anatomy of posteroanterior chest radiographs in healthy adults. This includes examining the calcification process in the chest and evaluating its occurrence in the costal cartilage among various age groups in males and females. Radiological analysis will be conducted to achieve these goals.

3.1.0 Research Approach

This study employed a descriptive cross-sectional research design. The sample comprises of North-Indian population. While the study focused on individuals from North India, it is important to note the geographical diversity within India. Therefore, the findings can be generalised to the broader Indian population, encompassing different geographical regions and demographic characteristics.

3.1.1 Research Questions

Through the literature review, it is evident that there is a research gap regarding the use of costal cartilage in determining sex during forensic examinations and anthropology. Currently, there is no relevant information available regarding costal cartilage calcification and its potential application and significance in this field. Although there are studies on different populations very limited studies have been conducted, and they are done on a small sample size. Therefore, further exploration of this topic could yield significant outcomes.

3.1.2 Objectives of the study

1: Evaluate the radiological anatomy of an adult using normal posteroanterior chest radiographs.

2: To evaluate the prevalence of male and female costal calcification.

3: To examine the influences of age and sex on patterns of CCC.

4: To study the type of pattern of costal cartilage in males and females.

5: To assess the efficacy of imaging software in enhancing diagnostic imaging.

3.1.3 Hypotheses of the Study

1: There will be differences in the pattern of calcification of males and females.

2: There will be a relation between increasing age and calcification density.

3.2.0 Sample design

3.2.1 Study area

During the study period of one year, individuals residing in the Hamirpur district of Himachal Pradesh and those visiting the government hospital for routine check-ups will be invited to participate. A PA chest digital radiograph will be taken with their consent for the study.

3.2.2 Sample size and formula

According to the sampling technique, under the sampling method, I utilize 0.5 as the standard deviation – this value is selected for its leniency, ensuring sufficient sample size. Thus, the researcher applied a statistical procedure of 5% standard error and the sample size was taken accordingly.

Standard deviation (σ) = 0.5

Confidence level (CL) = 95% (Z-score = 1.96)

Margin of error (ME) = ±5%

Required sample size (SS) = $(\text{Z-score})^2 \times \sigma \times (1 - \sigma) / (\text{ME})^2$

$$= (1.96)^2 \times 0.5 \times (0.5) / (0.05)^2 = 384.16$$

Hence, 500 normal PA radiographs were taken that were reported normal by the government medical college Hamirpur of district Hamirpur (HP).

3.2.3 Sampling Technique

For this research, I will randomly select posterior-anterior chest radiographs of people aged 1-95 years from the Radiology Department. I will only include those that are reported as normal. These radiographs will be categorized into eight age groups: 0-10, 11-20, 21-30, 31-40, 41-50, 51-60, 61-70, and 71 & above (table 3.1).

The research was carried out in accordance with the ethical principles outlined in the Declaration of Helsinki (World Medical Association). Prior to data collection, participants aged 18 or older provided written informed consent in their preferred language, while guardians provided consent for participants under 18. (Annexure I-II). The Institutional Ethics Committee (IEC) of Radha Krishnan Government Medical College (RKGMC), Hamirpur, Himachal Pradesh, duly authorised the protocol and study design, as evidenced by registration number ECR/1461/Inst/HP/2020 and HFW-H-Dr RKGMC/Ethics/2022/02 (Patyal & Bhatia, 2022) (Annexure III).

3.2.4 The criteria for inclusion and exclusion

- **Inclusion Criteria**:

  Normal PA chest X-rays of both sexes from One year old will be considered for the study.

- **Exclusion Criteria**:

1. Plain chest posterior-anterior radiograph of the subject under 1 year of age.
2. Normal PA chest X-rays with any deformity, cardiovascular disease or fractures and any other chest related disease will not be considered for the estimation.

Table 3.1 Distribution of participants by age group and sex

Age-Groups	Female		Male	
	Count	Table Sum %	Count	Table Sum %
1 (0-10)	30	12.0%	30	12.0%
2 (11-20)	30	12.0%	30	12.0%
3 (21-30)	30	12.0%	30	12.0%
4 (31-40)	30	12.0%	30	12.0%
5 (41-50)	30	12.0%	30	12.0%
6 (51-60)	30	12.0%	30	12.0%
7 (61-70)	30	12.0%	30	12.0%
8 (71 & above)	40	16.0%	40	16.0%
Total	250	100%	250	100%

3.3 Equipment required for the study

1. X-ray imaging equipment to take the subjects' radiographs.

2. Fujifilm's FCR PRIMA CONSOLE.

3.4 Subject's positioning

To capture a PA erect radiograph, the patient is directed to stand facing the X-ray detector or film cassette while ensuring their shoulders are rolled forward, and their hands are placed on their hips or thighs. They are then instructed to take a deep breath and hold it. Meanwhile, the X-ray tube is positioned behind the patient's back, aimed towards the chest, to capture an image of the chest in the posteroanterior projection.

This positioning facilitates optimal visualization of chest structures, including the lungs, heart, ribs, and diaphragm, ensuring clear and accurate images for diagnostic purposes. The precaution should be taken to avoid unnecessary radiation to other body parts by collimating the field.

3.5 Image Processing

The Fujifilm FCR PRIMA T and FCR PRIMA CONSOLE systems facilitate image processing by scanning cassettes removed from the X-ray machine (Patyal & Bhatia, 2022). The FCR PRIMA Console provides comprehensive image management capabilities, enabling swift and accurate patient identification, picture acquisition, processing, viewing, reprocessing, optimisation, and archiving. Complementing these functions, Image Works (IW) serves as a DICOM software solution tailored for managing medical images. It facilitates tasks including sending, printing, capturing, archiving, and interpreting images for diagnostic purposes.

3.6.0 Evaluation of the radiological anatomy of male and female adults using normal posteroanterior chest radiographs

The following parameters were studied using different scales provided by the imaging software used in the study (figure 3.1).

- **Rib Cage Dimensions (cm):** Measured as the widest part of the rib cage from left to right on the PA view (at the level of the eighth rib).
- **Maximum Transverse Cardiac Diameter (cm):** Measured as the largest horizontal width of the heart on the PA chest radiograph.
- **Internal Thoracic Diameter (cm):** Measured as the maximum internal diameter of the thoracic cavity on a PA chest radiograph.
- **Cardiac Thoracic Ratio:** Calculated as the ratio of the maximum transverse cardiac diameter to the internal thoracic diameter.

Methodology:

Sample Selection: A total of 409 adult radiographs were analysed, excluding 91 subjects under 18 years (43 females and 48 males).

Statistical Procedures:

Mann-Whitney U Test: Given the non-normal distribution of the data, the Mann-Whitney U test was applied to determine the significance of differences between males and females for each measurement.

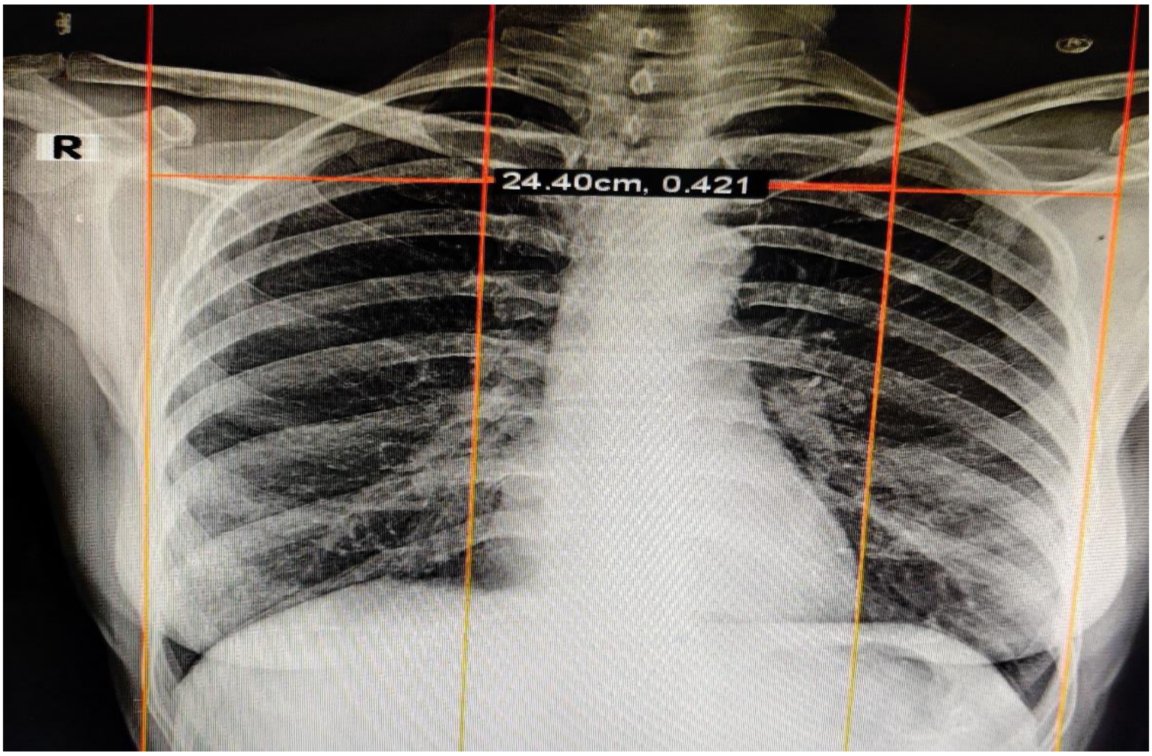

Figure 3.1: Radiographic measurements using software.

3.7.0 The aim of the study depends upon the following attributes

- The pattern of CCC for sex estimation.
- The degree of CCC for age estimation.

Our study's objective was to estimate gender and age from the CC. I used the CC of the first rib on the right side to estimate age. I will study the ribs CCC other than the first rib to estimate sex, especially the lower ribs (3^{rd}-5^{th}) of the right side of the body, which tend to show more consistent patterns of calcification across individuals. This consistency makes it a valuable indicator for sex estimation. Since the succeeding costal cartilages frequently fuse, it is difficult to distinguish between the two regions of cartilage (Tsubaki et al., 2017a). As previous research has demonstrated, the first CC calcification is not connected to sex, however, the lower rib cartilage exhibits sexual dimorphism(Middleham et al., 2015; NAVANI et al., 1970; Patyal & Bhatia, 2022; Rejtarová et al., 2009) . The lower ribs provide a clearer view in radiographs, facilitating the identification of calcification. The ratio of the highest CCC area to the total area in each measurement plane was used to determine the degree of calcification.

Broadly separated the calcification pattern for sex estimation according to the description given by Rejtarová et al. (2004)) (Patyal & Bhatia, 2022):

- "Peripheral- where calcification presents on inferior or superior margins of cartilage.
- Central- Where calcification mainly presents on the central part of cartilage.
- Mixed– Combination of the above two (Peripheral and Central).
- Indifferent- Calcification pattern which cannot be distinct into any above categories".

Employ the degree of cartilage calcification as determined by Garamendi et al. (2011) to estimate age (Garamendi et al., 2011; Patyal & Bhatia, 2022)**:**

- "Stage 0 – No CCC.
- Stage 1- Recently CCC began.
- Stage 2- CCC less than 50%.
- Stage 3-CCC more than 50% or complete".

3.7.1 Statistical Procedures in the Study

The data is input into MS Excel sheets(Windows 11; Version 22H2; Microsoft Corporation). The data from the Excel sheet was imported into SPSS software ("Statistical Package for Social Sciences", IBM Corp., Version 20) for each pair of methods (group-wise). The software's statistical tools, some of the most important of which are briefly described below, were used to examine the data. This study will employ a combination of descriptive statistics, chi-square tests, and Spearman correlation analysis to investigate the forms of CCC and their relationships with age and sex. These statistical procedures will help address the research objectives and contribute to understanding age and sex estimation from chest radiographs.

3.7.2 Descriptive Statistics:

- **Mean and Standard Deviation:** Descriptive statistics will be used to summarize the age variable. The mean age and standard deviation will provide a central measure and a measure of the dispersion of age in the sample.
- **Frequency Distribution:** Descriptive statistics, including counts and percentages, will be presented for each category of calcification patterns (peripheral, central, mixed, indifferent) to provide an overview of their prevalence in the sample.
- **Calcification Density:** Descriptive statistics (e.g., mean, standard deviation) for calcification density grades (0, 1, 2, 3) will be reported to characterize the variation in calcification within each pattern.

3.7.3 Chi-Square Test:

The chi-square test serves as a statistical tool aimed at gauging the presence of a noteworthy association or correlation between two categorical variables. Its application typically arises when dealing with data amenable to arrangement within a contingency table, where the two categorical variables are cross-tabulated. By scrutinizing the observed frequency distribution within the table, the chi-square test evaluates whether this distribution significantly diverges from what one would anticipate under the premise of variable independence. The independence or connection between two category variables is evaluated using the chi-square test. It facilitates the determination of the relationship between changes in one variable and changes in another, which is essential for recognising patterns and relationships.

- **Objective:** To examine the association between gender and the patterns of calcification (peripheral, central, mixed, indifferent).
- **Method:** To ascertain whether these categorical variables have a significant association or not, a chi-squared test of independence will be used.
- **Interpretation:** A significant chi-square test will suggest that the distribution of calcification patterns is not independent of gender, indicating potential differences in calcification patterns between males and females.

3.7.4 Spearman Correlation:

A non-parametric test used to evaluate the direction and strength of monotonic correlations between variables is Spearman's rank correlation. This approach is more resilient to non-linear relationships and does not rely on the assumption that the data is distributed normally. In this study, Spearman's correlation makes sense:

Ordinal Data: The variable "Stage of Calcification" has an ordinal distribution; its values are neither regularly distributed nor continuous. Spearman's correlation works well with ordinal data since it doesn't depend on the data's underlying distribution.

- **Goal:** To evaluate the connection between calcification density and advancing age.
- **Methodology:** The degree and direction of the monotonic association between calcification density, an ordinal variable, and age, a continuous variable, will be assessed using Spearman's rank correlation coefficient.
- **Interpretation:** A positive or negative correlation coefficient will indicate whether increasing age is associated with higher or lower calcification density, respectively.

3.8 Evaluation of the efficacy of Fujifilm's FCR Prima imaging software

A total of 500 radiographs were collected and analysed. Each radiograph underwent analysis twice: once in its original form and once after enhancement using the imaging software. The visual examination aimed to qualitatively assess improvements in image quality, focusing on contrast, density, and sharpness. Visual inspections were conducted before and after the software application, with particular attention given to the ability of the software to highlight the bony structures of the ribs and improve the visibility of outlines, contours, and irregularities. Inverting the contrast was used to distinguish ribs from overlapping structures, aiding in the identification of abnormalities.

Quantitative analysis was performed to supplement the visual observations, utilizing software-generated metrics for contrast, density, and sharpness. Statistical tests were employed to rigorously assess the efficacy of the software enhancements. A paired t-test was conducted on a subset of radiographs (n = 100) to compare pre-and post-enhancement values. Additionally, the Wilcoxon signed-rank test was applied to the entire dataset to validate the findings across all radiographs. Improvements in image quality were documented with visual examples to illustrate the methodology used in the study.

Chapter 4

Results and Discussions

Osteobiographs of unknown human remains recovered from medicolegal circumstances are written by forensic experts (Austin & King, 2016). The primary goal of forensic studies is to determine the gender, age, size, and race of unknown persons (dead or alive) found in forensic settings. Estimates of a person's sex and size make up the simplest parts of their biological identification to reconstruct with nearly perfect precision. Estimating the age and ethnicity of unexplained human remains, however, is a highly tough, contentious, and problematic aspect of the biological profile (Spradley, 2016). The biological process of physical maturation is entirely irreversible and is defined by an individual's level of sexual, skeletal, or dental development. These characteristics are frequently utilised as markers of age or maturation. While it is purely a morphological method to identify an individual as a child or adult, sexual maturity is considered attained with the development and appearance of secondary sexual features in an individual. However, this method is highly subjective and less accurate in assessing age for forensic purposes. Skeletal development is characterised by the attainment of adult size and shape and the complete ossification of all primary and secondary ossification centres in all skeletal parts of the human skeleton (Wittschieber, Hahnemann, & Mentzel, 2024). This mostly happens between the ages of twenty and twenty-five, with the clavicle, or collarbone, being the last bone to fully osseous by the time an individual reaches the age of roughly Thirty (Hughes, Newton, Bastrom, Fabricant, & Pennock, 2020; Paladini, Pellegrini, Merolla, Campi, & Porcellini, 2012). The range of age estimates for adult skeletons is relatively large, making it difficult to determine the sex; however, for paediatric osseous remains, the range of age estimates is lower, making it difficult to determine the sex because sex-dependent differences have not yet been imprinted. The length of the long bones and the growth and eruption of the temporary or permanent dentition are the basis for the age estimation methods employed with youngsters (Manjunatha & Soni, 2014). The adult approaches are different from the paediatric ones in that the adult techniques concentrate on the degenerative changes that are taking place in the dental or skeletal structures (Panchbhai, 2011). The development of radiological techniques and their low-cost/non-invasive nature have made them even more useful in forensic (Franklin, Swift, & Flavel, 2016; M. Zhang, 2022). Our study endeavours to advance the field of forensics by developing precise and universally applicable techniques for identifying and profiling unknown individuals, thereby assisting in the resolution of forensic cases. I aim to investigate the utility of calcification patterns in the costal cartilage as an adjunctive method for sex and age estimation in forensic anthropology. Through the utilization of simple and easy radiological techniques, I aim to enhance the accuracy and reliability of age and sex estimation practices in forensic contexts.

The accessibility of chest radiographs makes our study particularly feasible and practical. Chest radiographs are a commonly performed diagnostic imaging procedure and are readily available in medical settings worldwide. This widespread availability facilitates the easy acquisition of imaging data, enabling researchers to conduct comprehensive analyses on a large scale. Additionally, the non-invasive nature of chest radiography makes it a preferred imaging modality, ensuring minimal discomfort for study participants. This accessibility and convenience allow for the efficient collection of data, ultimately contributing to the robustness and applicability of our findings in forensics and medical research.

4.1 Evaluation of Radiological Anatomy in Adult Posteroanterior Chest Radiographs

Our study population consisted of 500 individuals whose radiographs were meticulously examined. I stratified our sample into different age groups to ensure a well-rounded representation. Descriptive statistics, including mean and standard deviation, along with frequency distributions, were employed to summarize the radiological anatomy data. The distribution of participants by age group and sex was tabulated (Table 3.1).

For the 1-7 age groups, I selected 30 radiographs, maintaining an equal distribution of male and female subjects. In contrast, for the 71 and above age group, I examined 40 radiographs from each gender, resulting in a total of 250 male and 250 female radiographs. Our sample's mean age was 42.62 years, with a standard deviation (SD) of 24.51 years. Our sample included individuals as young as one-year-old and as old as ninety-five years old, with the median age being 44 years. The male samples had a mean age of 42.18 years and a SD of 24.33 years. For the female samples, the average age and SD were, respectively, 43.06 and 24.67 years.

The Kolmogorov-Smirnov and Shapiro-Wilk test, a normality test, was used to compare the ages of male and female participants. The age distributions of both groups were found to be non-normal (p-value lower than 0.05). Based on the "sex" grouping data, a Mann-Whitney U test was used to compare the "age" variable between the two groups. Both the asymptotic significance (0.703) and the Monte Carlo significance (0.702) were greater than 0.05, showing that there is not an apparent age variation among the sexes in these groupings. Figure 4.1: Illustrating the frequency distribution of age in the study population.

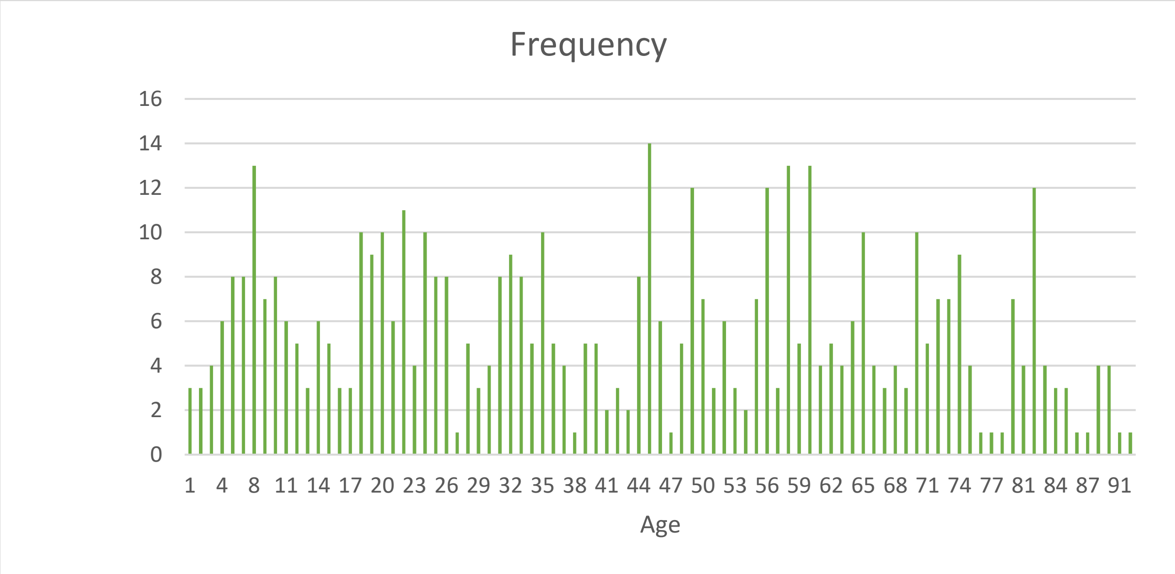

Figure 4.1: Frequency distribution of the sample age.

To evaluate further differences between male and female adult radiographs. I studied 409 adult radiographs, excluding 91 subjects under 18 years (43 females and 48 males). Different measurements like rib cage dimensions, maximum transverse cardiac diameter, and internal thoracic diameter were conducted on digital radiographs using the imaging software of Fujifilm.

The Wilcoxon rank-sum test (Mann-Whitney U Test) revealed significant disparities between male and female radiographs in various anatomical metrics. Additionally, males displayed greater rib cage dimensions and internal thoracic diameters relative to females (34.0 cm vs. 30.2 cm and 29.5 cm vs. 26.0 cm, respectively, both $p < 0.001$). Intriguingly, while the cardiac thoracic ratio was marginally higher in females than in males (0.40 vs. 0.39, $p = 0.002$), this difference was statistically significant. Detailed statistical summaries are presented in Table 4.1.

Table 4.1.: Radiographic Measurements by Gender.							
Feature	**Mean (Male)**	**SD (Male)**	**Mean (Female)**	**SD (Female)**	**Mean (Overall)**	**SD (Overall)**	**p-value (Mann-Whitney U Test)**
Maximum Transverse Cardiac Diameter (cm)	11.5	1.2	10.5	1.0	11.0	1.3	< 0.001
Rib Cage Dimensions (cm)	34.0	3.1	30.2	2.9	32.3	3.3	< 0.001
Internal Thoracic Diameter (cm)	29.5	2.0	26.0	1.5	27.9	2.3	< 0.001
Cardiac Thoracic Ratio	0.39	0.04	0.40	0.05	0.395	0.05	0.002

Table 4.2 presents the mean and standard deviation of the radiographic measurements across different age groups for both males and females. Statistical tests, including ANOVA and post-hoc analysis, were employed to assess the effect of age and sex on each measurement.

Table 4.2: Age and Sex-wise Distribution of Radiographic Measurements.								
Age Group	**Maximum Transverse Cardiac Diameter (cm)**		**Rib Cage Dimensions (cm)**		**Internal Thoracic Diameter (cm)**		**Cardiac Thoracic Ratio**	
	Female	Male	Female	Male	Female	Male	Male	Female
18-30	10.8 ± 1.1	12.2 ± 1.2	30.0 ± 2.8	34.5 ± 3.0	26.1 ± 1.4	29.3 ± 1.9	0.41 ± 0.05	0.38 ± 0.04
31-50	11.3 ± 1.2	12.5 ± 1.3	30.5 ± 2.9	34.8 ± 3.1	26.4 ± 1.5	29.6 ± 2.0	0.42 ± 0.05	0.39 ± 0.04
51-70	11.7 ± 1.3	12.9 ± 1.3	31.0 ± 3.0	35.2 ± 3.2	26.7 ± 1.5	29.9 ± 2.0	0.42 ± 0.05	0.39 ± 0.04
71+	12.0 ± 1.3	13.2 ± 1.4	31.5 ± 3.1	35.6 ± 3.3	27.0 ± 1.6	30.2 ± 2.1	0.43 ± 0.05	0.39 ± 0.04
Overall	11.5 ± 1.2	12.5 ± 1.3	30.2 ± 2.9	34.0 ± 3.1	26.0 ± 1.5	29.5 ± 2.0	0.40 ± 0.05	0.39 ± 0.04

ANOVA and Post-Hoc Analysis Results

The ANOVA test results indicated significant effects of both age and sex on the radiographic measurements. Table 4.3 provides a summary of the ANOVA results.

Table 4.3: Statistical analysis for radiographic measurements			
Measurement	**Factor**	**F-value**	**p-value**
Maximum Transverse Cardiac Diameter	Age	4.67	< 0.001
	Sex	36.54	< 0.001
Rib Cage Dimensions	Age	7.28	< 0.001
	Sex	58.17	< 0.001
Internal Thoracic Diameter	Age	6.11	< 0.001

	Sex	50.23	< 0.001
Cardiac Thoracic Ratio	Age	3.15	0.014
	Sex	9.87	0.002

Post-hoc analysis using Tukey's HSD test was conducted to identify specific group differences. The results showed significant differences between almost all age groups, particularly between the youngest (18-30 years) and the oldest (71+ years) groups, as well as between males and females across all age groups.

4.2 Evaluation of the prevalence of male and female costal cartilage calcification.

Among the male radiographs, 170 displayed lower costal cartilage (CC) calcification, whereas 177 female radiographs exhibited this characteristic. Table 4.4 provides an overview of the prevalence of costal calcification categorized by gender. The table presents the number of individuals with costal calcification and the total number of cases observed for both males and females. Additionally, the prevalence of costal calcification is expressed as a percentage for each gender group. The data highlights a slightly higher prevalence of costal calcification among females (70.8%) compared to males (68%), indicating a gender-specific difference in the occurrence of this condition within the study population. Additionally, prevalence rates for the first rib CCC were determined, with males exhibiting CCC in 189 subjects and females in 192.

Table 4.4: Prevalence of Costal Calcification by Gender			
In lower CC			
Gender	**Number with Costal Calcification**	**Total Number of Cases**	**Prevalence (%)**
Males	170	250	68%
Females	177	250	70.8%
In the first CC			
Males	189	250	75.6%
Females	192	250	76.8%

In the first costal cartilage, females also exhibit a slightly higher prevalence of calcification (76.8%) compared to males (75.6%). Overall, there is a trend of higher prevalence of costal calcification in females compared to males for both lower and first costal cartilages.

4.3 Estimation of sex and age from CCC

4.3.1 Lower Costal Cartilage Calcification Patterns and Gender

A significant aspect of our study was the analysis of calcification patterns in the lower ribs. In the study, I examined the distribution of calcification patterns in the costal cartilage among the study participants, categorized by sex. The results were summarised in Table 4.5. This table provides insights into the distribution of calcification patterns in the costal cartilage, highlighting differences between male and female study participants.

Table 4.5: Distribution of pattern of calcification

Type of Pattern		Central	indifferent	Mixed	Peripheral
		Count	Count	Count	Count
Sex	Female	157	11	8	1
	Male	0	4	10	156

Table presents the distribution of patterns of calcification observed in the study population, categorized by type of pattern (Central, Indifferent, Mixed, and Peripheral) and sex (Female and Male). The table indicates the count of individuals exhibiting each pattern, revealing notable differences between sexes. Notably, while females predominantly exhibit Central calcification (figure 4.2), males primarily display Peripheral calcification (figure 4.3). Mixed calcification was observed on 18 radiographs (figure 4.4).

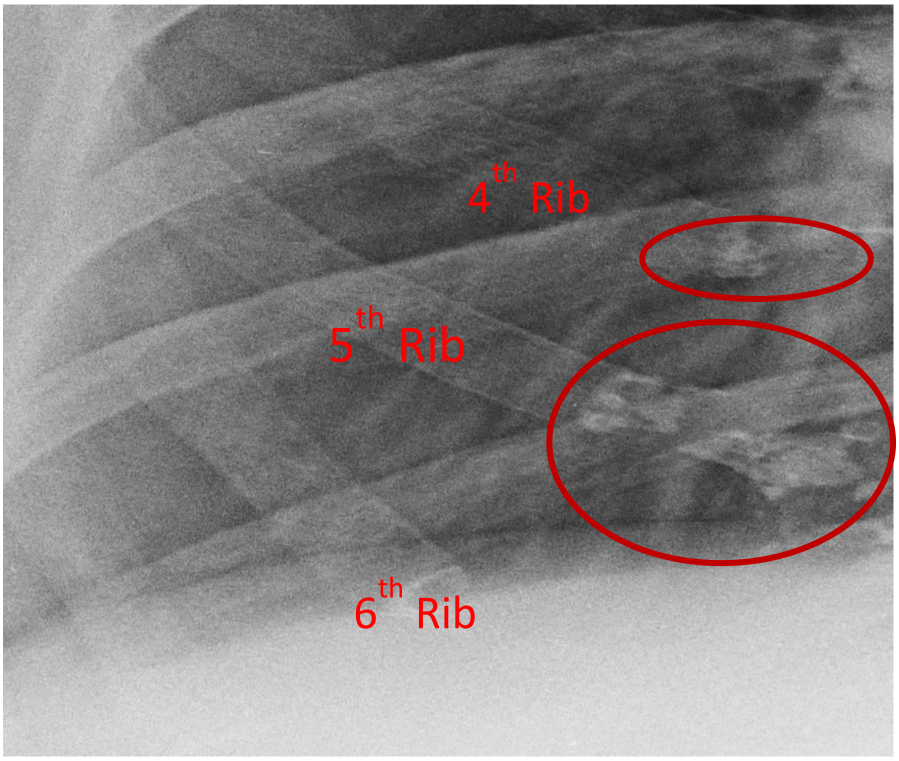

Figure 4.2: Central type of CCC observed on a female radiograph

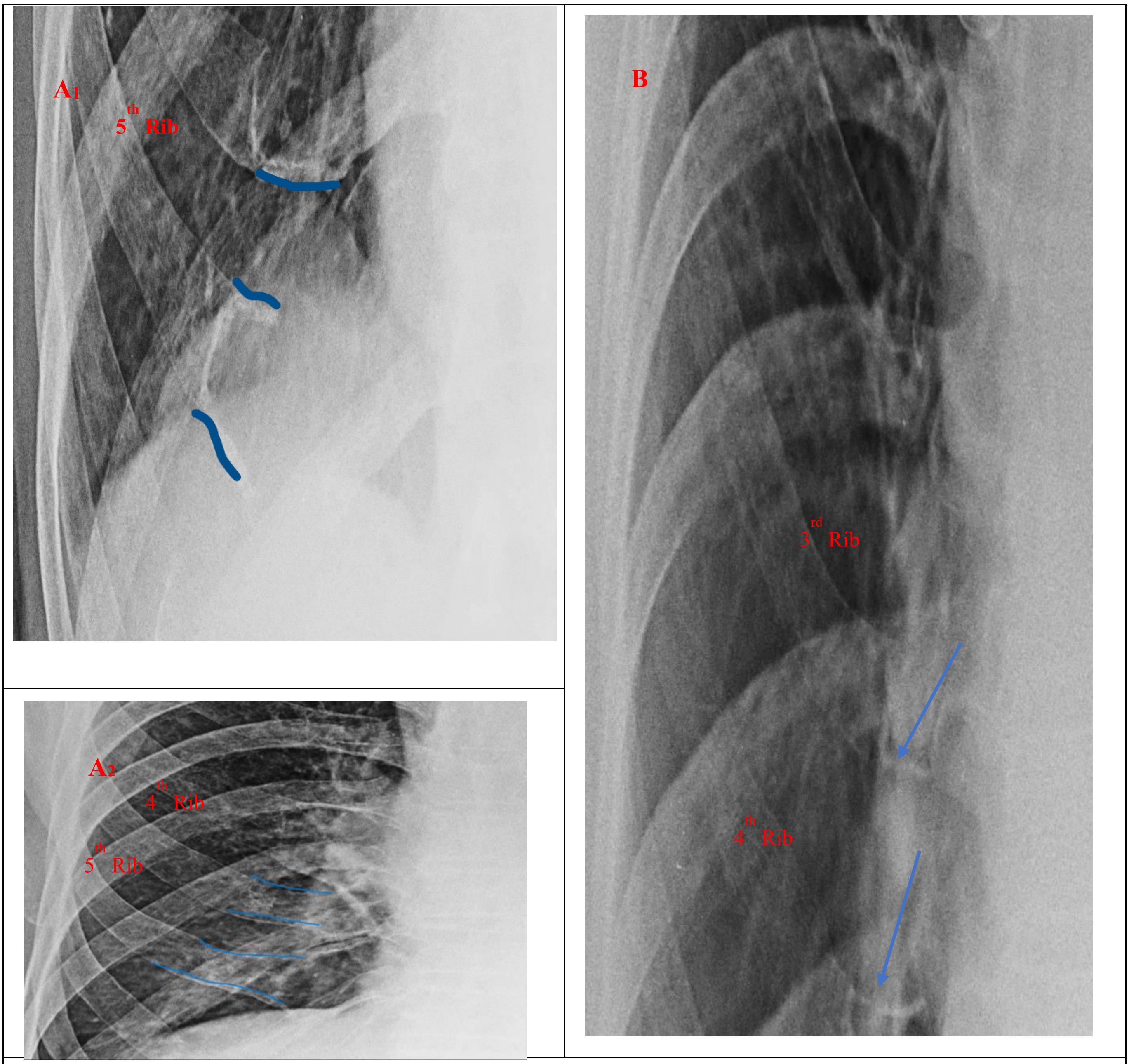

Figure 4.3: Peripheral CCC on a male radiographs (A_1 & A_2) and on a female radiograph(B)

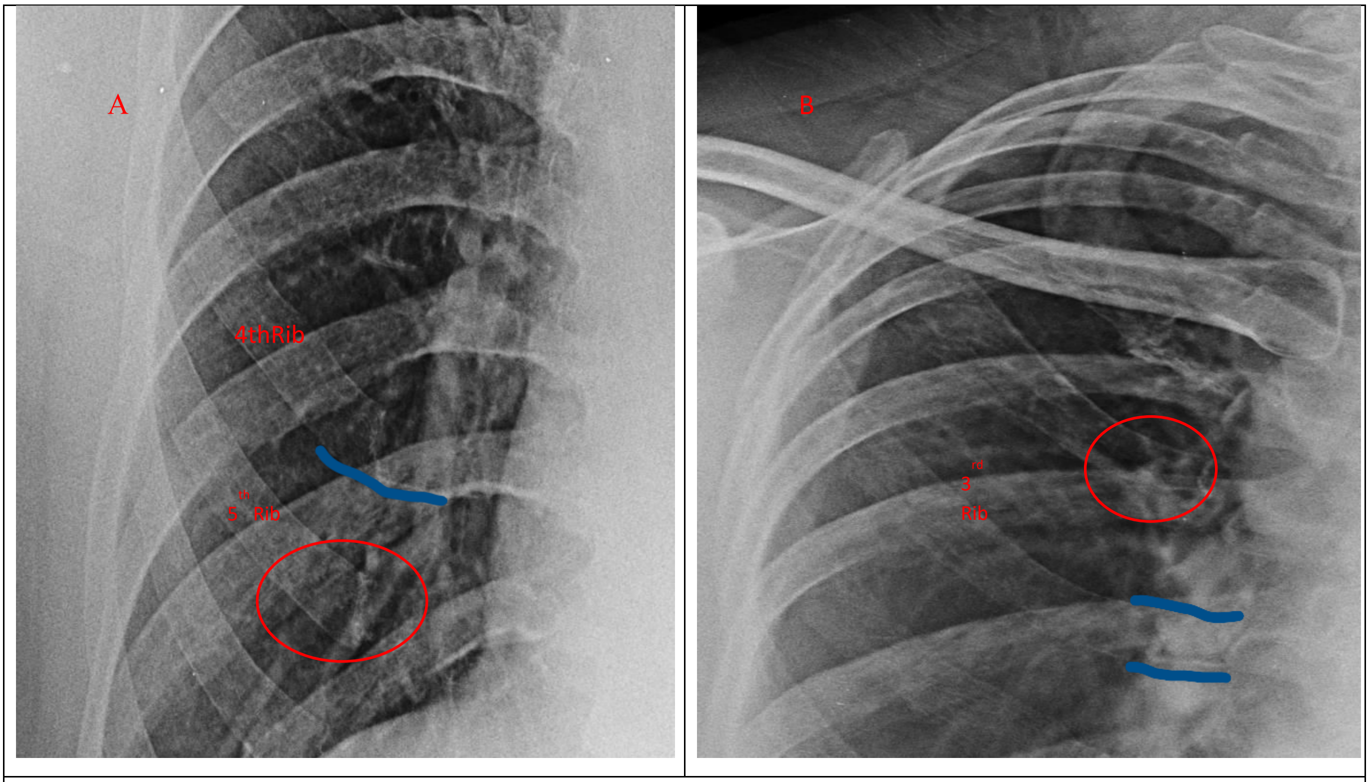

Figure 4.4: Mixed CCC on a male radiograph (A) and a female radiograph (B).

347 exhibited calcifications in the lower ribs, while 153 did not. Furthermore, I meticulously documented the gender-specific calcification patterns. From Figure 4.5 probability of the Central Type for females was approximately 83.96%, while the probability of the Peripheral Type for males was approximately 91.76%.

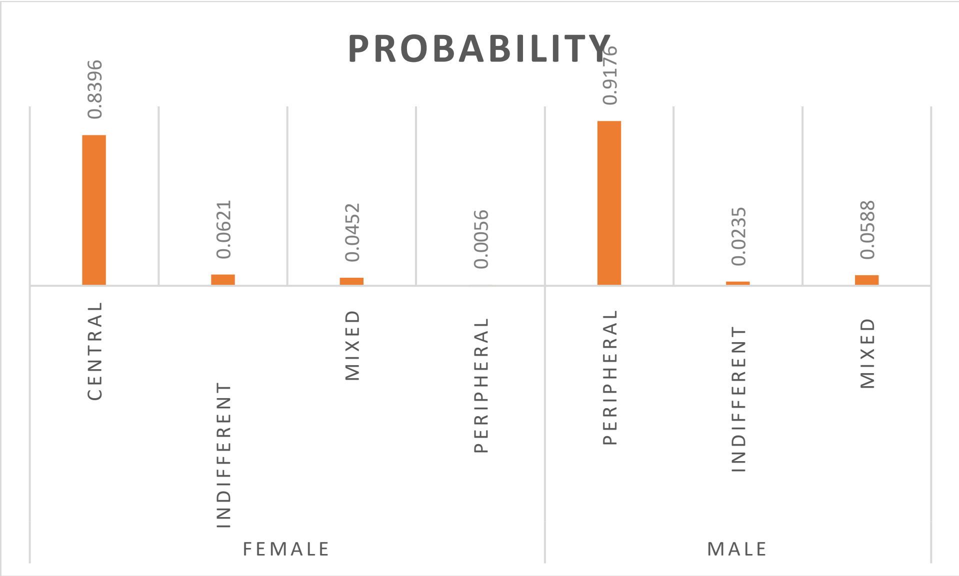

Figure 4.5: Probability of different CCC patterns

Intriguingly, in ages 0-10, calcification in CC was conspicuously absent on the radiographs of both sexes. However, when I moved to the next group (11-20), the calcification pattern emerged in both sexes. In the male sample, the first radiograph where lower CC calcification was observed was of a 19-year-old boy; in the female sample, the radiograph showing the lower CC calcification was 20 years old. This trend continued with 16 male and 21 female radiographs showing lower costal calcification in the age group 3. Remarkably, in age groups, 4 and above, radiographs displayed calcification in the lower ribs increased (table 4.6).

Table 4.6: Calcification in lower CC in different groups

S.No.	Group No.	lower CCC in Females	lower CCC in males
1	1	0	0
2	2	2	1
3	3	21	16
4	4	24	24
5	5	30	29
6	6	30	30
7	7	30	30
8	8	40	40
Total		**177**	**170**

Chi-Square Test for Association

To ascertain the significance of the relationship between the type of calcification and gender, I conducted a chi-square test for association. I performed this analysis, and it provided insights into whether there was a statistically significant association between the type of calcification and gender. Shedding light on potential gender-specific differences in calcification patterns.

Crosstabulation:

A crosstabulation table 4.7 was constructed to explore the dissemination of the "pattern" variable within individually category of the "sex" variable. The "pattern" variable included four categories: central, indiff (indifferent), mixed, and p (peripheral). The results were summarized as follows:

Among females, the distribution of patterns was as follows: central (88.7%), indiff (6.2%), mixed (4.5%), and p (0.6%).

Among males, the distribution was central (0.0%), indiff (2.4%), mixed (5.9%), and p (91.8%).

Additionally, adjusted residual values were calculated to assess significant deviations from expected values within the contingency table 4.8.

Pearson Chi-Square: The Pearson Chi-Square statistic was 313.501 (df = 3) with a p-value of < 0.001, indicating a highly significant association between "sex" and "pattern."

The Ratio statistic was 426.669 (df = 3) with a p-value of < 0.001, further confirming a strong and statistically substantial association.

Fisher's Exact Test: The test yielded a p-value of < 0.001, providing additional evidence of a significant association between "sex" and "pattern."

The small p-values in all three tests ($p < 0.001$) signify that the null hypothesis, suggesting independence between "sex" and "pattern," can be rejected. These results demonstrate a robust and statistically significant relationship between the variables.

Table 4.7: sex * pattern Crosstabulation							
			Pattern				Total
			central	Indiff	Mixed	p	
sex	female	Count	157	11	8	1	177
		% within sex	88.7%	6.2%	4.5%	0.6%	100.0%
		Adjusted Residual	16.6	1.8	-.6	-17.1	
	male	Count	0	4	10	156	170
		% within sex	0.0%	2.4%	5.9%	91.8%	100.0%
		Adjusted Residual	-16.6	-1.8	.6	17.1	
Total		Count	157	15	18	157	347
		% within sex	45.2%	4.3%	5.2%	45.2%	100.0%

Table 4.8: Chi-Square Tests						
	Value	Df	Asymp. Sig. (2-sided)	Monte Carlo Sig. (2-sided)		
				Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Pearson Chi-Square	313.501[a]	3	.000	.000[b]	.000	.000
Likelihood Ratio	426.669	3	.000	.000[b]	.000	.000
Fisher's Exact Test	417.463			.000[b]	.000	.000
N of Valid Cases	347					
a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 7.35.						

4.3.2 The Degree of Calcification of First CC and Age Estimation

In the current study, I explored the degree of calcification in the first rib across different age sets. In the study, calcification of the first rib CC was categorized into four distinct stages based on observed characteristics. These stages represent progressive degrees of calcification, reflecting varying levels of mineralization over time. In our study stage 0 represents no calcification (figure 4.6), stage 1 represents the onset of calcification (figure 4.7), stage 2 means the degree of calcification is less than 50% (figure 4.8) and stage 3 means calcification degree is more than 50% (figure 4.9).

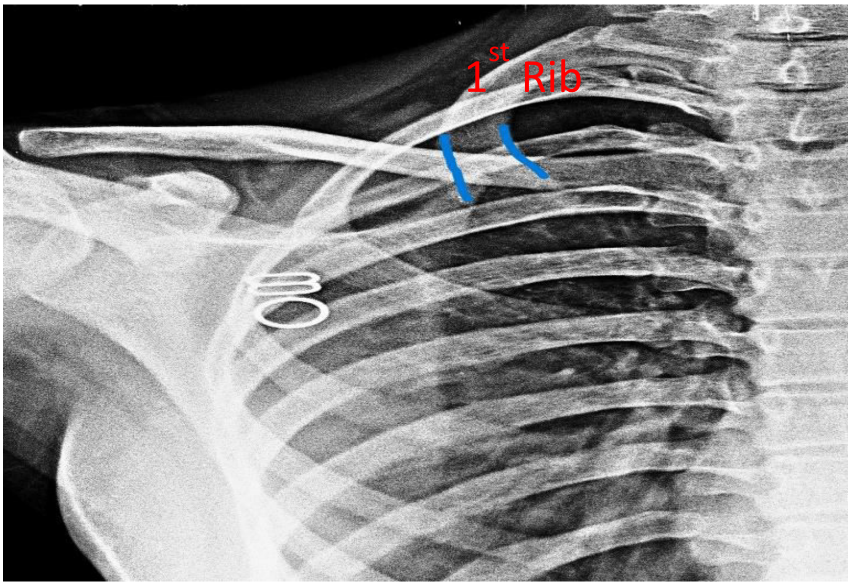

Figure 4.6: Stage 0

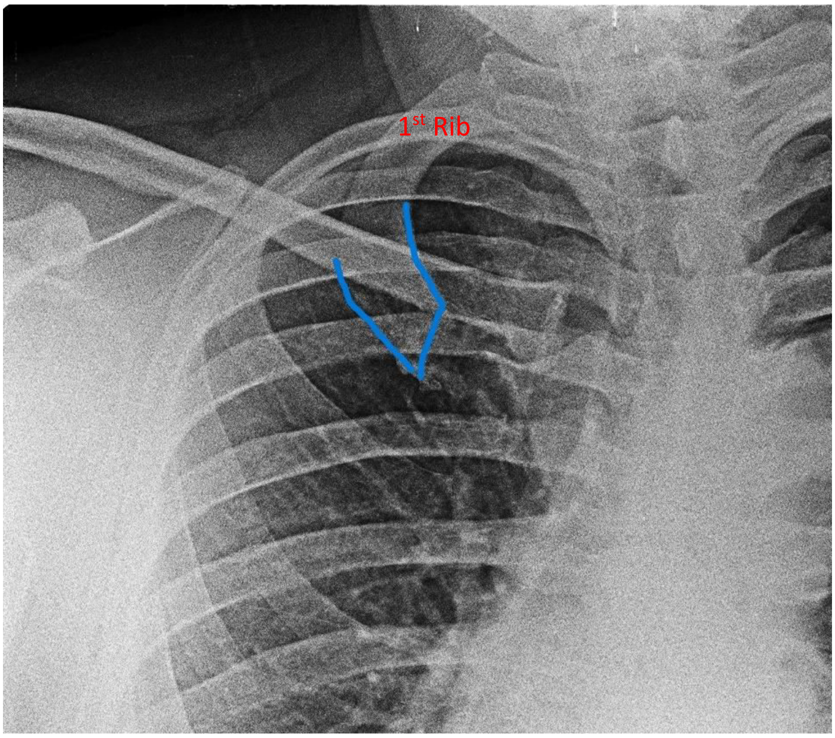

Figure4.7: stage 1

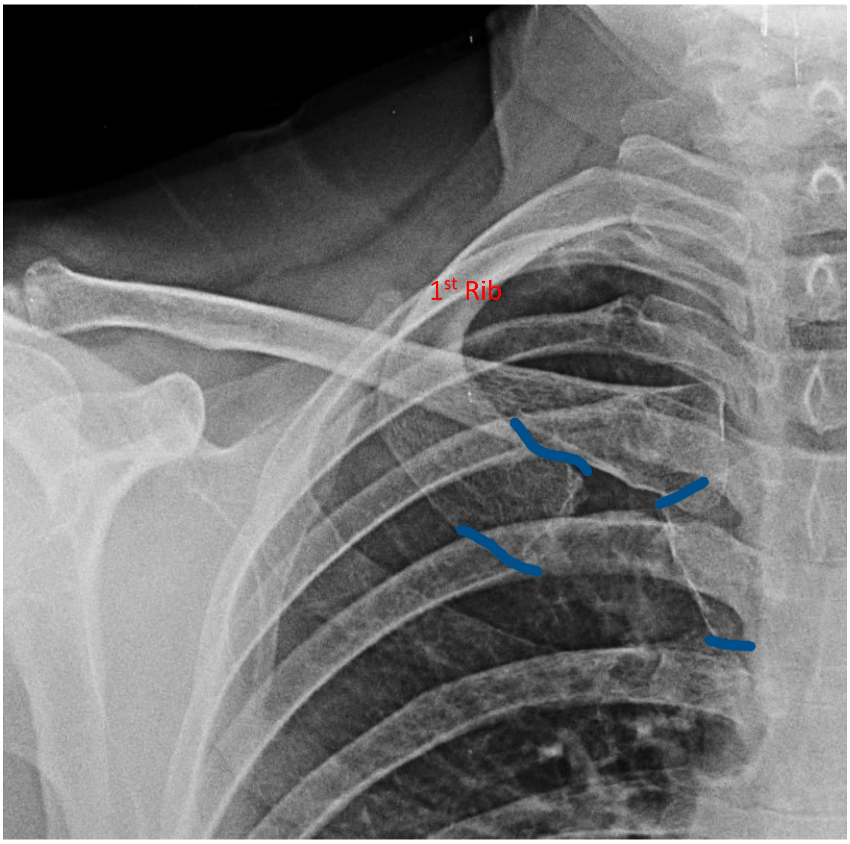

Figure4.8: stage 2

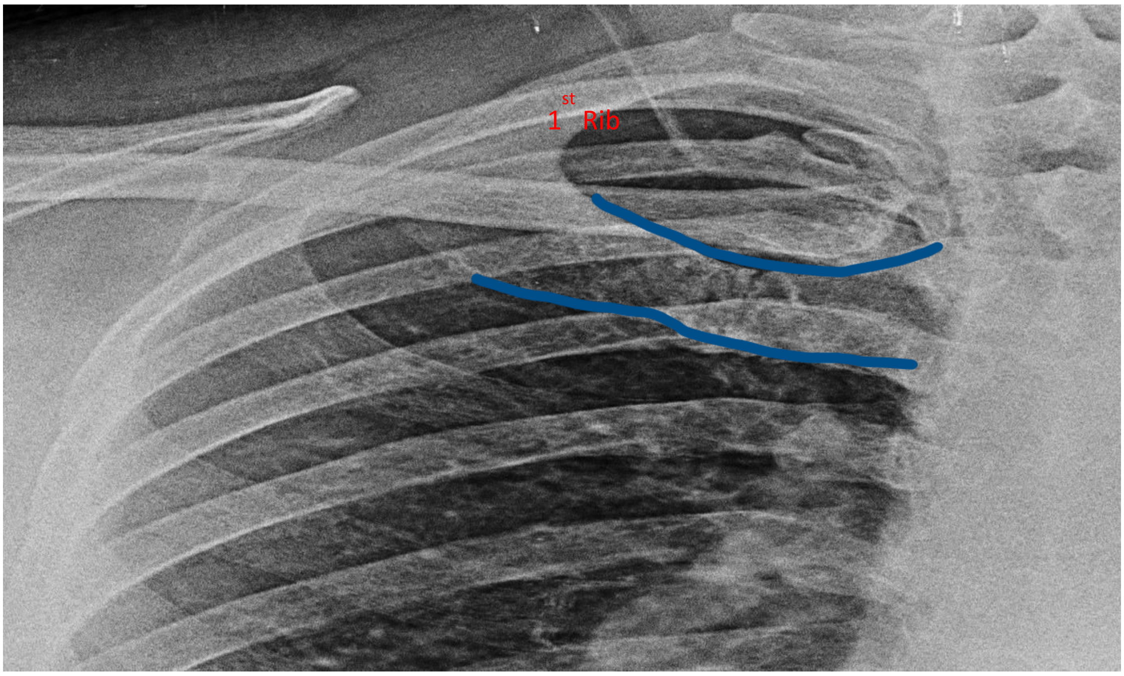

Figure4.9: Stage 3

For Male Sample:

The table 4.9 provides a summary of age distribution among male participants categorized by stage of calcification in the first rib CC.

- **Stage 0:** The mean estimated age for males in Stage 0 was approximately 11.38 years, with a minimum age estimate of 1.00 years and a maximum of 25.00 years. The median age estimate was 11.00 years, with a SD of 6.12 years.
- **Stage 1:** In this stage, the mean age estimate for males was around 24.81 years, ranging from a minimum of 18.00 years to a maximum of 35.00 years. The median age estimate was 24.00 years, with an SD of 4.48 years.

- **Stage 2:** Males in Stage 2 have a mean estimated age of about 38.29 years, with age estimates spanning from 21.00 to 58.00 years. The median age estimate was 39.00 years, and there was an SD of 8.79 years.
- **Stage 3:** The mean estimated age for males in Stage 3 was approximately 64.77 years, with an age range of 38.00 to 95.00 years. The median age estimate was 65.00 years, and the SD was 12.45 years.

Table 4.9: Descriptive Statistics of Age Distribution by Stage of Calcification in Male Participants

For Male Sample		Age						
		Mean	Count	Maximum	Minimum	Median	Standard Deviation	Standard Error of Mean
Stage	0	11.38	61	25.00	1.00	11.00	6.12	.78
	1	24.81	26	35.00	18.00	24.00	4.48	.88
	2	38.29	51	58.00	21.00	39.00	8.79	1.23
	3	64.77	112	95.00	38.00	65.00	12.45	1.18

For Female Sample:

The table 4.10 provides a summary of age distribution among female participants categorized by stage of calcification in the first rib CC.

- **Stage 0:** Females in Stage 0 have a mean estimated age (MEA) of around 11.29 years, ranging from a minimum of 1.00 year to a maximum of 20.00 years. The median age estimate was 10.00 years, and there was a standard deviation of 5.79 years.

- **Stage 1:** In Stage 1, the mean age estimate for females was approximately 29.03 years, with age estimates varying between 20.00 and 50.00 years. The median age estimate was 28.00 years, and there was an SD of 6.91 years.
- **Stage 2:** Females in Stage 2 have a mean estimated age of about 47.05 years, with age estimates ranging from 23.00 to 81.00 years. The median age estimate was 46.00 years, and there was a standard deviation of 14.54 years.
- **Stage 3:** The MEA for females in Stage 3 was approximately 69.13 years, with age estimates spanning from 44.00 to 90.00 years. The median age estimate was 69.00 years, and the standard deviation was 12.44 years.

Table 4.10: Descriptive Statistics of Age Distribution by Stage of Calcification in Female Participants

For Female Sample		Age						
		Mean	Count	Maximum	Minimum	Median	Standard Deviation	Standard Error of Mean
Stage	0	11.29	58	20.00	1.00	10.00	5.79	.76
	1	29.03	37	50.00	20.00	28.00	6.91	1.14
	2	47.05	76	81.00	23.00	46.00	14.54	1.67
	3	69.13	79	90.00	44.00	69.00	12.44	1.40

For Whole Sample:

The table 4.11 presents the descriptive statistics of age distribution across different stages of calcification in the entire sample population.

- **Stage 0:** In the entire sample, the mean estimated age for Stage 0 was about 11.34 years, with a minimum age estimate of 1.00 year and a maximum of 25.00 years. The median age estimate was 10.00 years, and there was a standard deviation of 5.94 years.
- **Stage 1:** For Stage 1 in the whole sample, the mean age estimate is approximately 27.29 years, ranging from a lowest of 18.00 years to an extreme of 50.00 years. The median age estimate was 25.00 years, and there was a standard deviation of 6.34 years.
- **Stage 2:** The MEA for Stage 2 in the whole sample is about 43.54 years, with age estimates spanning from 21.00 to 81.00 years. The median age estimate is 43.00 years, and there was a standard deviation of 13.23 years.

- **Stage 3:** In Stage 3 of the whole sample, the mean age estimate is approximately 66.57 years, with age estimates ranging from 38.00 to 95.00 years. The median age estimate is 65.00 years, and the standard deviation is 12.60 years.

Table 4.11: Descriptive Statistics of Age Distribution by Stage of Calcification in the Entire Sample

Whole sample		Whole sample age						
		Mean	Count	Maximum	Minimum	Median	Standard Deviation	Standard Error of Mean
Stage	0	11.34	119	25.00	1.00	10.00	5.94	.54
	1	27.29	63	50.00	18.00	25.00	6.34	.80
	2	43.54	127	81.00	21.00	43.00	13.23	1.17
	3	66.57	191	95.00	38.00	65.00	12.60	.91

The Correlation Analysis

To investigate the correlation between age and first rib calcification, I conducted Spearman's rho analysis. Our analysis revealed a significant positive correlation between age and the degree of the CCC (Figure 4.10 and Figure 4.11). Specifically, the value of Spearman's rho for the entire sample of Spearman's rho(ρ) = 0.902, $p < 0.01$, indicating a strong and statistically significant positive correlation.

Furthermore, when analysed separately by gender, both males ($\rho = 0.922$, $p<0.01$) and females ($\rho = 0.896$, $p<0.01$) exhibited similar strong positive correlations between age and first rib calcification. In summary, both males and females exhibit a similar pattern of a strong and significant positive correlation between age and the stage of first costal cartilage calcification. This suggests that age is a significant factor associated with the level of first rib calcification in both genders, and the relationship is consistent across both groups.

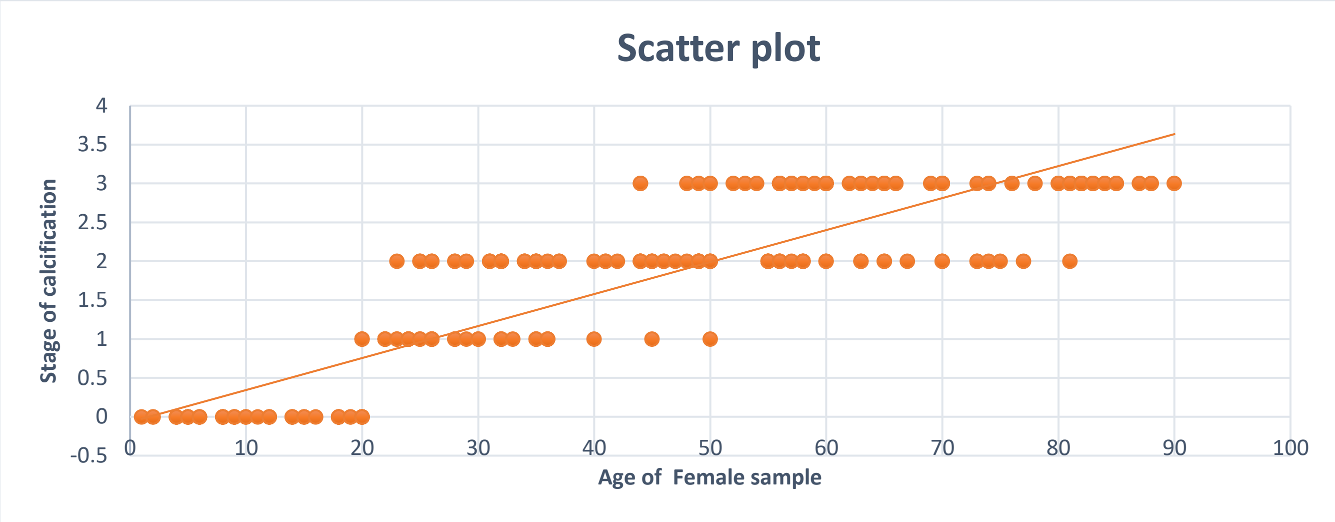

Figure 4.10: Scatter plot showing a positive correlation between age and stage in the female population of the sample.

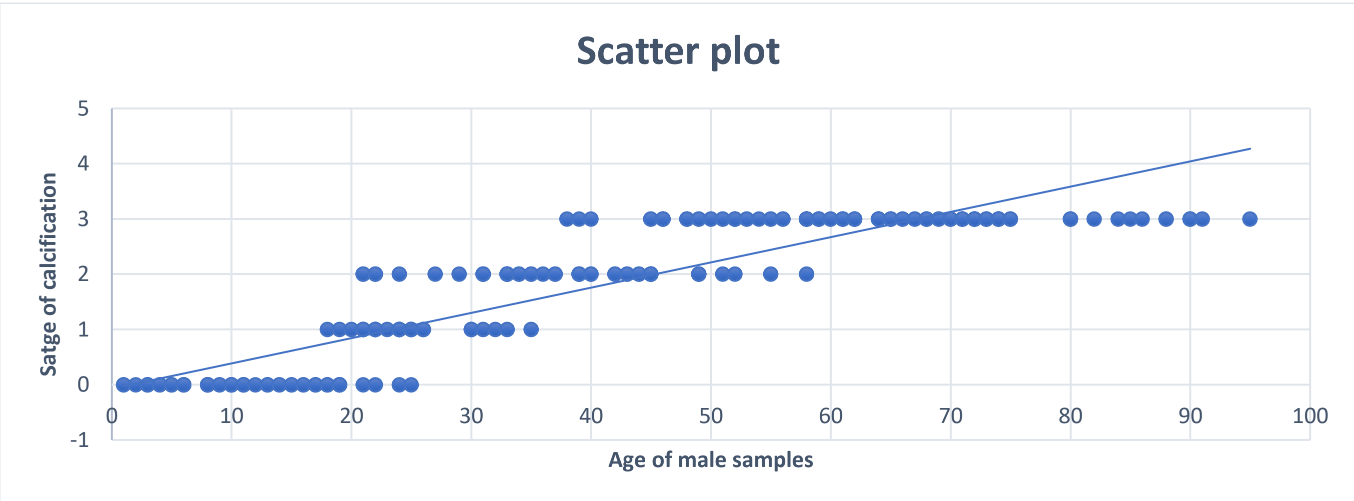

Figure 4.11: Scatter plot showing a positive correlation between age and stage in the male population of the sample.

4.4 The influences of age and sex on patterns of costal cartilage calcification (CCC).

To evaluate the impact of sex and age on the different patterns of costal cartilage calcification (CCC). Given the nominal nature of the pattern variable, a multinomial logistic regression model was used for the analysis. The model fitting information indicated a significant overall model fit (Chi-Square = 240.748, df = 2, $p < 0.001$) when compared to an intercept-only model. The goodness-of-fit tests provided mixed results, with the Pearson Chi-Square test showing a poor fit (Chi-Square = 5167.345, df = 340, $p < 0.001$) and the Deviance test suggesting a good fit (Deviance = 338.088, df = 340, p = 0.519). The pseudo-R-squared values indicate that the model explains a substantial portion of the variance (Nagelkerke $R^2 = 0.577$).

The parameter estimates revealed that sex significantly influences the pattern type of CCC (Estimate = 4.152, $p < 0.001$), with males being more likely to exhibit specific patterns. In contrast, age did not show a significant effect on the pattern type (Estimate = -0.002, p = 0.762) (see table 4.12).

These findings align with model assessing calcification stages using an ordinal logistic regression model (also known as the Proportional Odds Model). This model is appropriate for predicting an ordinal dependent variable, which in this case are the stages of calcification, where sex also showed a significant impact (Estimate = 1.167, $p < 0.001$), with men more likely to progress towards higher stages of calcification. However, for the stage model, the impact of sex is considerably less compared to its effect on the pattern of calcification. Similarly, age was a significant factor in the calcification stage model (Estimate = 0.223, $p < 0.001$), unlike in the current pattern type model. Both models underscore the significant role of sex, while only the calcification stage model highlights age as a significant factor.

Table 4.12: Parameter Estimates from the Multinomial Logistic Regression Model						
Parameter	**Estimate**	**Std. Error**	**Wald**	**df**	**Sig**	**95% Confidence Interval**
Threshold						
[pattern=central]	5.964	0.652	83.553	1	0.000	4.685 - 7.243
[pattern = p]	9.767	0.786	154.357	1	0.000	8.226 - 11.308

[pattern= mixed]	10.601	0.811	171.022	1	0.000	9.012 - 12.190
Location						
Age	-0.002	0.007	0.092	1	0.762	-0.017 - 0.012
Sex	4.152	0.340	149.466	1	0.000	3.487 - 4.818

4.5 Evaluation of Imaging Software Efficacy in Enhancing Diagnostic Radiographs

A total of 500 radiographs were collected and analysed using Fujifilm's FCR Prima imaging software. Visual inspection of radiographs was conducted both before and after software application to discern improvements in contrast, density, and sharpness. Additionally, quantitative analysis was performed using software-generated metrics.

4.5.1 Visual Examination

Visual examination of radiographs before and after the application of the software revealed notable enhancements in image quality. Improvements were observed in contrast, density, and sharpness. Specifically, inverting the contrast helped highlight the ribs' bony structures, making it easier to see the outlines, contours, and any irregularities (Figure 4.12). When the ribs overlapped with other structures in the chest radiograph, reverse contrast helped separate and distinguish the rib bones from other tissues, making it easier to identify abnormalities in the ribs.

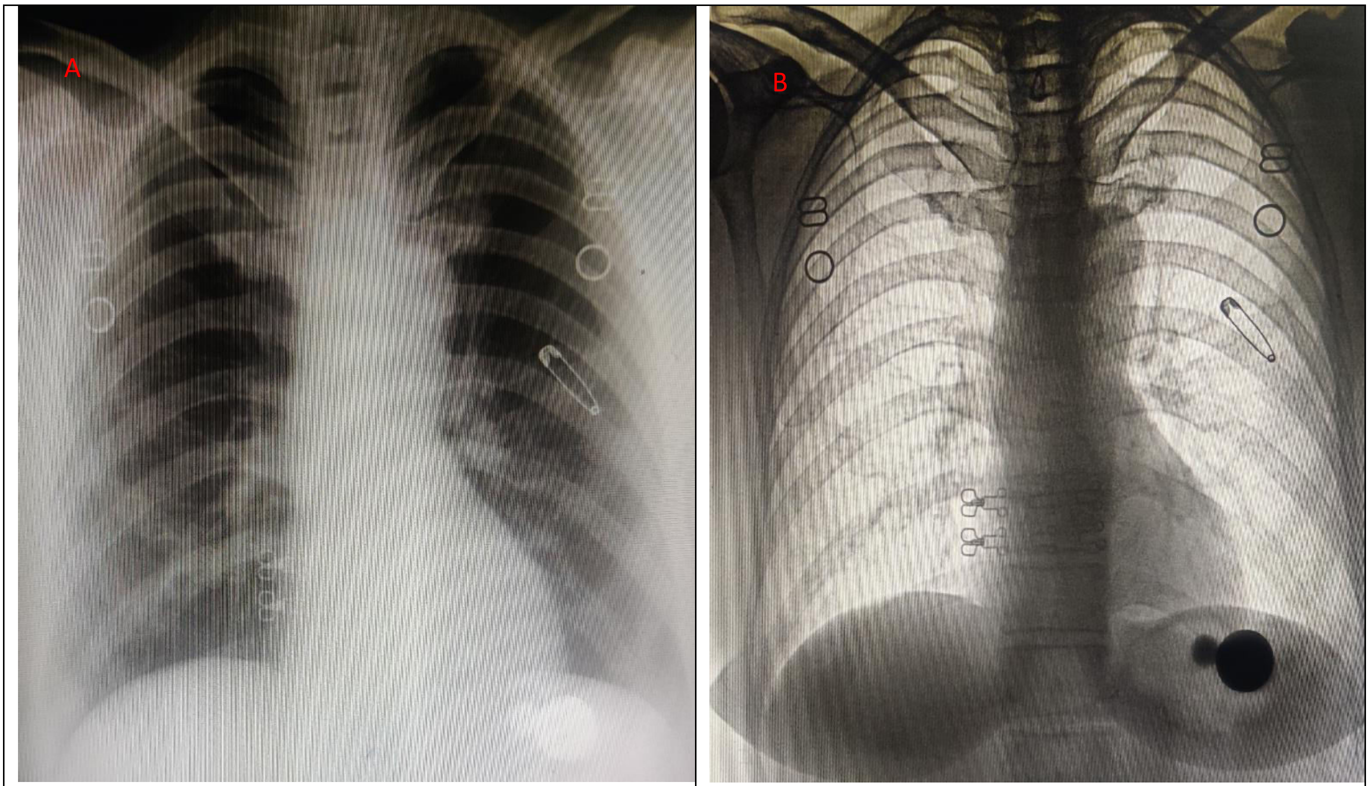

Figure 4.12: A radiograph of a female. B: Inverted Radiograph.

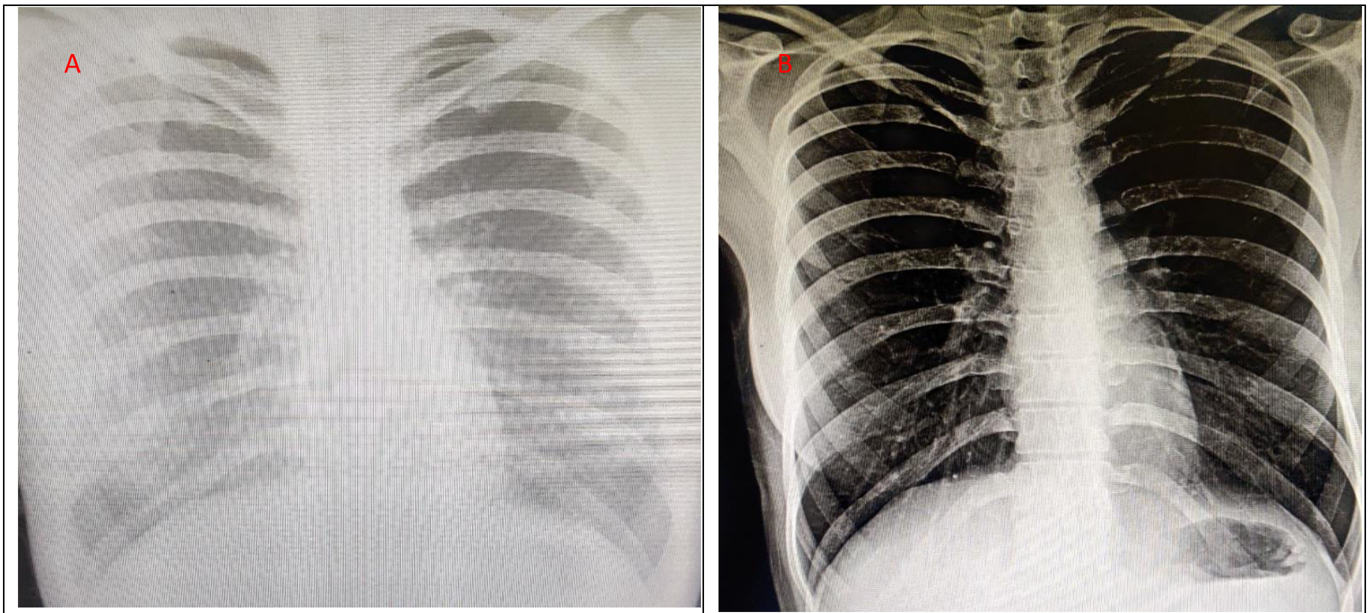

Figure 4.13: A radiograph of a male before the application of Image enhancement tools. B: Enhanced Image

4.5.2 Quantitative Analysis

Quantitative analysis, particularly using statistical tests, further supported these visual observations (figure 4.13). The paired t-test, conducted on a subset of radiographs ($n = 100$), demonstrated statistically significant improvements ($p < 0.001$) in contrast, density, and sharpness following software utilization. Additionally, the Wilcoxon signed-rank test, applied to the entire dataset, yielded consistent findings, affirming the software's efficacy ($p < 0.001$).

4.6 Discussion

The discussion section serves as a critical platform for contextualizing and interpreting the study's findings within the broader scientific discourse. In this section, I delve into the implications, limitations, and significance of our research findings, elucidating their contributions to the existing body of knowledge in the field. Through a rigorous analysis of our results to relevant literature and theoretical frameworks, I aim to discern patterns, elucidate discrepancies, and offer insights that advance understanding and inform future research endeavours.

The result of an ANOVA test provides insight into whether there are statistically significant differences among the means of multiple groups. In this context, the significant F-values obtained from the ANOVA tests suggest that both age and sex have a significant effect on the radiographic measurements. This means that there are differences in these measurements across different age groups and between males and females that are unlikely to have occurred by chance alone. The obtained F-values indicate the degree of variation among group means relative to the variation within groups, supporting the conclusion that age and sex are important factors influencing radiographic measurements. The cardiothoracic ratio (CTR) is a crucial diagnostic metric in medical imaging, particularly in chest radiography. It is calculated by dividing the transverse diameter of the heart by the widest internal diameter of the thoracic cavity. A CTR value below 50% typically indicates a normal heart size, which is an important finding in the context of cardiovascular health assessments. This ratio helps clinicians assess whether the heart is enlarged, which can be indicative of various cardiac pathologies, including cardiomegaly, heart failure, and other conditions associated with increased cardiac workload or volume overload. . In our study, obtaining a CTR value less than 50% suggests that the majority of subjects have a heart size within the normal range, which can be a significant indicator of a lower prevalence of cardiovascular abnormalities within the studied population.

In comparison to Ali et al. (2019) and Debnath et al. (2017), our study conducted a detailed analysis of radiographic measurements focusing on gender disparities in anatomical metrics among adults. The Wilcoxon rank-sum test (Mann-Whitney U Test) revealed significant differences between male and female radiographs across various parameters. Specifically, males exhibited significantly greater rib cage dimensions (34.0 cm vs. 30.2 cm, $p < 0.001$), and internal thoracic diameters (29.5 cm vs. 26.0 cm, p <

0.001) compared to females. Although females showed a marginally higher cardiac thoracic ratio (0.40 vs. 0.39, $p = 0.002$), this difference was statistically significant.

While Ali et al. (2019) explored cardiothoracic ratio across various age groups in a broader population and identified age-related trends, our study focused on gender differences in adults and provided detailed anatomical measurements. Similarly, Debnath et al. (2017) investigated thoracic anatomy in Indian adolescents, emphasizing population-specific variations and gender differences. In contrast, our study contributed to the understanding of gender-specific anatomical differences in adult populations, particularly focusing on radiographic measurements such as cardiac size, rib cage dimensions, and internal thoracic diameters.

Garg *et al.* explored landmarks such as the sternal angle, xiphisternal joint, and positions of intrathoracic structures in adults of Indian origin (Garg et al., 2019). While the radiographic study provided insights into anatomical measurements, the study by Garg et al. offered a unique perspective on surface anatomy variations within the thoracic region. Both studies underscored gender-related differences in thoracic anatomy, with our radiographic study emphasizing cardiac and thoracic dimensions, and the study by Garg et al. highlighting variations in the positions of cardiac apex, SVC/RA junction, and other thoracic structures. By integrating the findings from both studies, a comprehensive understanding of thoracic anatomy emerges, encompassing radiographic measurements and surface landmarks. This holistic approach contributes valuable information for clinical practice, medical education, and further research endeavours in the field of thoracic anatomy.

These findings underscore the importance of considering demographic factors, such as age and gender, in interpreting radiographic measurements for accurate clinical assessments and diagnostic interpretations.

Hawellek *et al.,* (2021) utilizing a cross-sectional cadaveric approach, found a high overall prevalence of CCC (96.7%), with no significant difference between genders. However, structural patterns of CCC varied with age, showing an increase in CCC amount with advancing age. While males tended to have peripheral CCC and females central CCC, diffuse CCC was observed across all age groups as an indicator of high CCC levels (Hawellek et al., 2021).

In contrast, our study focusing on radiographs of living individuals, identified a slightly higher prevalence of CCC among females compared to males. Specifically, in the lower costal cartilage, females exhibited a prevalence of 70.8% compared to 68% in males. Similarly, in the first costal cartilage, females displayed a prevalence of 76.8%, slightly higher than the 75.6% prevalence observed in males.

The discrepancy in the prevalence of CCC between the two studies highlights the importance of considering factors such as study population, methodology, and sample size. The Hawellek *et al.,* study conducted on cadavers, provides valuable insights into the structural patterns and age-related changes in CCC prevalence. However, the use of cadavers may not fully represent the living population, as factors like tissue preservation and underlying health conditions could influence CCC prevalence.

In another study, a retrospective analysis of computed tomographic (CT) scans in an Asian population revealed further nuances in the incidence, degree, and pattern of rib cartilage calcification. This study, which included 120 patients (60 male and 60 female) across six age groups, found an overall CCC prevalence of 50.8%, with females exhibiting a significantly higher prevalence than males (59.4% vs. 42.2%, $P < .001$). Meaningful calcification (defined as 26% or greater) was notably low in males younger than 60 years but relatively higher in females across all age groups. Additionally, males predominantly exhibited marginal calcification patterns, whereas females more commonly displayed granular or central patterns. The study also highlighted that the calcification rates of the sixth and seventh rib cartilage were higher than those of the eighth rib, with rates increasing significantly with age. These findings underscore the need for preoperative attention to gender and age-specific patterns of CCC, particularly in Asian patients, where females show a higher incidence of granular and central calcification that could complicate surgical procedures (Sunwoo, Choi, Kim, & Jin, 2014).

On the other hand, our study, conducted on living individuals through radiographic analysis, offers a real-time assessment of CCC prevalence in a clinical setting. While the sample size in this study was smaller, the findings reflect the prevalence of CCC in a contemporary population undergoing diagnostic imaging for various medical reasons.

Our study's findings highlight the presence of sexual dimorphism in the CCC patterns. The age span covered by this extensive analysis ranged from infants through people in their ninety-first year. Notably, in contrast to earlier studies, ours included radiographs of participants who were younger than 10 years old. The integration of Fujifilm's FCR Prima imaging software acted as a fundamental part of enhancing the diagnostic precision of radiographic evaluations in our study. The advanced features of this software, including automatic image optimization and enhanced contrast resolution, significantly improved the clarity of anatomical structures, particularly the cardiac and thoracic dimensions. The visual enhancements provided by the software facilitated measurements and a clearer differentiation between various anatomical landmarks.

Quantitative analysis revealed that post-processing with the Fujifilm software led to a substantial reduction in measurement errors, thereby increasing the reliability of our data. For instance, the standard deviations for rib cage dimensions and internal thoracic diameter measurements were markedly lower in images processed with the software compared to those without, indicating more consistent and precise readings. Furthermore, the improved image quality allowed for the more detailed visualization of subtle anatomical variations and calcification patterns, which are critical for accurate forensic assessments.

The efficacy of the imaging software was also reflected in the enhanced diagnostic efficiency. The time required for radiologists to complete measurements and assessments was significantly reduced, owing to the software's intuitive interface and automated features. This improvement in workflow not only increased the throughput of radiographic analyses but also minimized observer fatigue, potentially leading to more consistent and accurate evaluations.

Overall, the utilization of Fujifilm's FCR Prima imaging software demonstrated a marked improvement in both the precision and efficiency of diagnostic radiographic assessments. These advancements underscore the software's potential as a valuable tool in clinical and forensic radiology, contributing to more accurate and reliable diagnostic outcomes.

According to our analysis, a significant majority (69.4%) of the radiographs in our study showed evidence of calcification in the CC. This proportion is lower than the findings from Rao study (Rao & Pai, 1988a), where the prevalence of calcification reached 77.60%, but higher than the findings given by Rejtarová et al. (2004), which stood at 51.53%. This variation could be explained by variations in the age distribution of our sample and the application of imaging software. In particular, our study included participants as young as one year, whereas Navani et al. (NAVANI et al., 1970) covered people older than 10 years. As a result, the differences in calcification rates found were probably caused by the age difference. Our analysis also revealed distinct calcification patterns for each gender. 156 male radiographs had a high prevalence of peripheral calcification, whereas 157 female radiographs had a higher prevalence of central calcification. Our chi-square test results, presented in Table 4.8, demonstrated a statistically significant association between calcification type and gender. Both peripheral and central calcification patterns exhibited strong associations with gender. This straightforward and cost-effective methodology revealed the presence of sexual dimorphism within the costal cartilage, a valuable contribution to the field of anthropological and medical research.

Previous studies have consistently shown a correlation between age and the prevalence of calcification, particularly in the lower costal cartilages. The present study also showed an increase in the number of calcifications from group 1 to group 8 (table 4.6). These findings are based on research involving various populations and age groups. In a study by Elkeles (Elkeles, 1966), which included 2606 patients aged 30 years to 80 years and above, the percentage of calcification increased with age in the lower CC. Calcification was not observed in individuals aged 0-20, but it reached 100% prevalence in those aged 61-70. Peripheral calcification was predominantly seen in 141 male cases and one female case, A total of 132 females had central calcification, respectively.

Shiv Navani et al. (1970) examined 1000 radiographs of individuals aged 10-95 years and categorized calcification into different types. They reported a predictive value of 95% for Type I (Marginal) calcification and 93% for female's Central calcification. The earliest age of calcification observed in their study was 20 years, although Stewart and McCormick (1984) noted it at 15 years. Michelson (1934) reported the earliest age of calcification as 11 years in a Mexican boy.

Rao and Pai (Rao & Pai, 1988a) conducted a study on the South Indian population spanning ages 1 to 80 years. For males, they projected a Type A calcification prevalence of 92.3% and a Type B calcification prevalence of 95.5%.

Additionally, Olga Rejtarova et al. found a 100% probability of central calcification in females and a 99.6% probability of Type I calcification in males in their study involving 18 cadavers and 1044 patients. This study ignored the first costal cartilage and classified the calcification patterns into four main groups: "peripheral, central, mixed, and indifferent". According to the study's findings, 528 (51%) of the participants had calcification of the costal cartilage, while 516 (49%) did not. Individuals with calcification of the costal cartilage exhibited all of the following patterns: A peripheral pattern was discovered in 249 male individuals and one female individual, a central pattern was discovered in 201 female individuals but not in any male individuals, a mixed pattern was discovered in 23 female individuals and four male individuals, and an indifferent pattern was discovered in 35 female individuals and 15 male individuals. The study identified the following central pattern subtypes: central lingual pattern, which was present in 114 (57%) individuals, central globular pattern, which was present in 47 (23%) individuals, and central lingual plus globular pattern, which was present in 40 (20%) individuals. The gender could not be determined in last two, but the central pattern was assessed to be 100% possible for females and 99.6% feasible for males as a peripheral pattern after the study.

In the other study, which focused on the Scottish population and used data from 41 chest radiographs obtained between 2006 and 2010 examined the use of calcification patterns in Scottish cadavers to determine a person's sex, presented a novel, technique and stated that current sexing techniques that rely on costal cartilage calcification patterns are inappropriate for use with a Scottish population. None of the male specimens were successfully identified by the Rejtarova et al. method. The McCormick et al. method accurately identified 41% of men and 82% of females approx. For determining sex, a novel technique based on trabecular bone or sclerotic calcified deposits exhibits potential (Middleham et al., 2015).

Khatri et al. (2009) studied 1000 chest radiographs and reported calcification occurring early in males than females. They found predictive values of 96.6% for males and 97.6% for females. Across all these studies, peripheral calcification was consistently observed in males, while central calcification was a common feature in females (Khatri, Khanna, Chauhan, & Bhargava, 2009).

In 2022 a study was conducted for Gender estimation, based on CCC patterns was applied to a representative group of 200 participants with an equal number of males and females, whose CT images were obtained from the Hospital. These individuals ranged in age from 20 to 60 years. The study results revealed that 193 individuals (96.5%) exhibited calcification in their costal cartilages, while 7 individuals (3.5%) did not display any calcification in this area. the study concluded that the central pattern of calcification was indicative of females with a 92.3% probability, while the peripheral pattern was characteristic of males with a 100% probability (AL-SAMANEE, ÖNER, & ÖNER, 2022b).

Intriguingly, the lower CC showed a clear association between age and the proportion of calcification. The 0–10 age group did not show any signs of calcification, however, as people aged, this rate gradually increased. Notably, calcification in lower CC was 100% prevalent in the 41–50 age group and persisted as age groups increased. The first CCC of the right side of the chest was observed in 189 males whereas 61 radiographs showed stage 0. The stage 1 was first observed in an 18-year-old boy. In females, 192 radiographs showed first CC calcification (that is in stages 1,2 and 3) and 58 radiographs were in stage 0, the onset of CCC of first CC was detected in a 20-year-old female. These results support the theory put forth by Ikeda that the first rib experiences calcification before the other ribs (Ikeda, 2017; Tsubaki et al., 2017b).

The human body undergoes complex anatomical changes over a lifetime, and the thoracic region, including the ribcage, is no exception. In particular, the first rib exhibits calcification before the others. Previous researchers have employed various methods, such as radiography, osteology, and skeletal measurements, to estimate age. Table 4.13 summarises the findings of recent studies based on radiology modalities across different ethnicities using CCC. Our study builds upon these methods by emphasizing the significance of CCC patterns, particularly in the rib, for age and sex estimation.

Table 4.13: Tabulates the studies on CCC based on radio diagnostic modalities									
Author/Year	**Aspect**	**Sample Population**	**Methodology**	**Prevalence**	**Modality**	**Gender Difference**	**Age dependency**	**CC studied**	**Result**
Rejtarova et al. /2004	Sex estimation	1044 (537 male and 507 female) Age range: 10 to 95 years	Calcification studied on basis of different patterns	52%	Radiography	show sexual dimorphism	Ossification increases with age	Except first all are studied	77% of women had central and 93% of male had peripheral pattern
Rejtarova et al. / 2009	Sex estimation	Caucasian population 55 cadaveric sample (Addition to an earlier published study,2004)	Same as studied in 2004 2nd part of the study first part was published in 2004	89% in cadaveric study	Radiography	show sexual dimorphism	Ossification increases with age	Except first all are studied	peripheral and central lingual patterns being analytical for male and female respectively globular loci of ossification could be used for age estimation.
Garamendi et al. / 2011	Test the usefulness of radiological changes in the clavicle and first rib for forensic age	Spanish origin 61 men, 62 women	Michelson's system For CC age estimation	Not specified	Radiography	Not specified	CCC increases with age	First	Stage 3 of first rib ossification at >25 years

	estimation								
Karaman et al. / 2012	Age Determination in People Based on CCC	Turkish population 270 males, 201 females	Based on stages of calcification of Barchilon et al. and Garamendi et al	Not specified	Radiography	Not specified	CCC increases with age	First	calcification degree of the right first costal cartilage and age correlated significantly in males and females
Milenkovic/2013	age estimation based on the analysis of the sternal end of the clavicle and the first costal cartilage	Serbian population 15 and 35 years (CT Thorax) 154 patients, of which 97 (53.6%) were males and 57 (31.5%) females	first costal cartilage using the methodology of Moskovitc et al. (Moskovitch et al., 2010)	61.3%	CT scan	Not specified for CC	first costal cartilage as an age indicator	first	CC showed significant correlations between its radiodensity, mineralization, and ossification patterns. The presence of bony and calcified projections within the cartilage was observed, leading to a new staging system based on these features.

Sunwoo et al. / 2014	Characteristics of rib cartilage calcification	Asian 120 patients (equal M and F)	Degree of calcification assessed as 0%, 1%-25%, 26%-50%, 51%-75%, and 76%-100% classified as marginal, granular, and central	50.8%	CT Chest scan	Females showed higher calcification rates than males Males - marginal calcification Females - granular calcification	increased with age specifically in sixth and seventh	sixth, seventh and eighth	Males under 60 had low calcification rates, while females 30 and older had higher rates with predominantly granular patterns
Agarwal et al. / 2015	Sex estimation	Indian population 2291 sample (1240 males,1051 females) age range: 21- 60 years	Rejtarova et al. (2004) methodology of calcification pattern	75.86%	Radiography	show sexual dimorphism	calcification increases with age observed at 21 years in both sex	Second, third and fourth	Males mainly exhibited Type I calcification pattern, while females mostly showed Type II pattern
Middleham et al. / 2015	Sex determination	Scottish population Cadaveric sample (22 males (aged 57-91) and 19 females	methods of Rejtarova et al. (2004) and McCormick et al. (1985), and a newly proposed	Not specified	Radiography	Significant differences in calcification patterns, with methods varying in accuracy	Not mentioned	Second to seventh	accuracy of 94.1% for males and 85.7% for females in the studied sample (proposed

		(aged 67-96)	method based on calcification texture						method) Rejtarova's method being more successful in females and McCormick's in males
T. Ikeda, et al. / 2017	Age estimation at death using CCC	Deceased Japanese population 136 (70 M and 66 F) Age range: 20-80 years	Bayesian analysis and percentage of calcification	Not mentioned	CT scan		increased with age for both sexes	second and fourth	CT number and percentage calcification increased with age for both sexes.
Kui Zhang et al. / 2017	Establishing population-specific age estimation models	Chinese population 512 individuals (254 females, 258 males) Age range: 20 to 85 years	Stages divided into eight subgroups based on osseous and calcification	Not mentioned	CT scan	Notable differences were identified between genders	Age prediction models developed	First to seventh	For males mean absolute error of 5.31 years, a minimum error of 0.10 years, with 54% accuracy within 5 years and 88% within 10 years. For females a mean absolute

									error of 6.72 years, a minimum error of 0.68 years, with 42% accuracy within 5 years and 77% within 10 years.
S.Zhang et al. / 2017	To evaluate characteristics of CC and costa calcification	Chinese population 154 sample size (78 male and 76 female) Age range: 19-82 years	Calcification pattern and calcification degree based on Navani et al., and Sanders et al., (Sanders, 1966)	Not specified	CT-Scan	C pattern in females and P pattern in males observed	Gradual increase in calcification degree	Sixth to eighth	The calcium concentration and CT value of costal cartilage showed a steady increase, peaking between the ages of 40 and 50 years.
Hawellek et al. / 2021	CCC and gender and age relation	Cadaveric population sample size 92	Assessed the prevalence, amount, and structural patterns (central, peripheral, diffuse) of CCC across	96.7%	contact radiography	P-in males C- in females	A strong relationship between it	Seventh	a 96.7% prevalence of CCC, which was gender-independent, and showed that CCC increased with age, presenting

			three age groups (<40 years, 40-70 years, >70 years) and compared findings by gender						distinct structural patterns by gender and age
Albaraa / 2022	Sex estimation	200 sample size equal male and female 20-60 years	Rejtarova *et al.* (2004)	96.5%	CT images	P-in males C- in females	Not specified	Except first CC	P pattern male gender estimation with 100% and C pattern female gender estimation with 92.3%

Historically, it has been proposed that the of the first CCC represents a genuine process of ossification. Researchers have found that the production of respiratory stress is responsible for the onset of calcification in the first cartilage, whereas chest expansion is connected to calcification in the lower ribs. The age estimation process has been facilitated by analysing calcification patterns and the degree of calcification in the lower CC. Even with damaged or disarticulated skeletons, it is quite simple to locate the first rib. Additionally, it was believed that the first rib was not subject to the same mechanical stresses as the lower ribs. However, the calcification of the first rib provides a more complex and distinctive perspective on age estimation. The Spearman's rho analysis conducted in our study yielded correlation coefficients that approached one, signifying strong correlations between age and first CC calcification. This underscores the significance of our study in terms of age estimation. Furthermore, the strong positive correlations were consistent when males and females separately, further validating our findings.

Studies have shown that costal cartilage calcification patterns are primarily influenced by age and sex rather than pathogenic or population affinity factors. Research on calcification patterns in different populations like the Turkish, Czech, and North Indian populations consistently highlights the significance of age and sex in determining these patterns (AL-SAMANEE et al., 2022a; Rejtarová et al., 2004; Rhomberg & Schuster, 2014a). Premature calcifications of costal cartilages have been associated with specific medical conditions rather than population-specific traits (Patyal & Bhatia, 2022). The distinct patterns observed in males and females, along with the correlation between calcification degree and age, emphasize the role of intrinsic factors in determining costal cartilage calcification levels, making it a reliable indicator for age and sex estimation rather than reflecting pathogenic or population-related influences.

The outcomes of our initial rib calcification study were compared with those of earlier publications. A survey of the literature revealed few studies that discussed the relationship between chronological age and degree of ossification (Barchilon, V and Hershkovitz, I and Rothschild, BM and Wish-Baratz, S and Latimer, B and Jellema, LM and Hallel, T and Arensburg, 1996; McCormick et al., 1985; Semine, A Alan and Damon, 1975), but Dr Nicholas Michelson's 1934 paper was the first to state it in a way that would be helpful for a forensic age assessment (Michelson, 1934). In his study, Michelson examined 5098 thorax X-rays and measured the degree of ossification of the first rib's costal cartilage. His sample was made up of Americans of both sexes and the Caucasian and Negroid races. Our study revealed that, until the age of 38, none of the radiographs exhibited stage 3 calcification. A first rib ossification in Stage 3 of Michelson's study has never been observed in a patient younger than 21 years of chronological age. On the other hand, a patient's chronological age in the Michelson series is usually less than 25 years, and in the Garamendi series, it is even less than 20 years, when their first rib is in the initial stage (0) of ossification. Future research on actual populations may be required to confirm, refute, or extend these initial conclusions. In our study for the whole sample, stage 0 was predominantly observed in the 1-25 age group, while stage 1 was prevalent in the 18-50 age group, stage 2 in the 21-81 age group, and stage 3 in the 38-95 age group. The mean ages for stages 0, 1, 2, and 3 in our study were 11.34, 27.29, 43.54, and 66.57 respectively for the male and female samples the descriptive analysis is provided in (table 4.7). These findings suggest that as individuals age, the stage of calcification within the costal cartilage progresses. Notably, for the 71 and above age group, no radiographs exhibited stage 0 calcification. Comparing our results to those of Garamendi et al. (2011), our mean age values for different calcification stages were generally lower, while Garamendi et al. found a mean age of 18.03 for stage 0 and 28.09 for stage 1. This discrepancy can be attributed to differences in the age compositions of the study samples. In our study, the median for stage 0 was 10, whereas in studies by Garamendi et al. and a Turkish population study, it was 17.64 and 5, respectively (Karaman et al., 2012; Patyal & Bhatia, 2022). Table 4.14 shows the distribution of age range in these two studies. The variation can be attributed to the variance in the age distribution of samples across studies; notably, the Turkish study encompassed a larger proportion of children compared to our own research(Patyal & Bhatia, 2022). Additionally, our study sample had a lower median age compared to Garamendi et al.'s sample, further emphasizing the influence of age distribution on the results.

Table 4.14: Tabulates age distribution of the first CCC in the different stages of the whole sample population

Author/Year	Stage 0 (Min-Max) age range(years)	Stage1 (Min-Max) age range(years)	Stage 2 (Min-Max) age range(years)	Stage 3 (Min-Max) age range(years)
Karaman et al. / 2012	1-14	13-43	24-69	40-88
Garamendi et al. / 2011	5.35- 46.83	17.61-67.72	24.87-65.59	25.49-75.41

The imaging software tool used in this study aids in analysing digital radiographs for sex determination purposes by providing a platform for observing and interpreting the calcification patterns of rib cartilage. This tool allows for enhanced visualization and analysis of the radiographic images, enabling researchers to identify specific gender-related patterns in the calcification of rib cartilage. The study aimed to assess the efficacy in interpreting computerised radiographs for sex and age estimation. The software facilitated a more detailed examination of the calcification patterns, potentially leading to more precise and reliable results in determining the sex of individuals based on the observed patterns in the rib cartilage calcifications, offering a more advanced and efficient method compared to traditional manual interpretation techniques (Elkeles, 1966; King, 1939; Rao & Pai, 1988a). Our study offers a comprehensive exploration of sexual dimorphism in costal cartilage calcification patterns and their relevance for age estimation. By embracing an innovative software application and including younger age groups in our sample, I have contributed to the existing body of knowledge in this field. The strong associations between calcification patterns and gender, as well as the correlations between age and calcification stage, provide valuable insights for forensic anthropologists, medical practitioners, and researchers alike. These findings underscore the likely of CC analysis as a reliable tool in the estimation of age and sex, with implications for various domains, including forensic science, anthropology, and clinical medicine.

The cross-sectional aspect of our study is one of its inherent limitations and the relatively small sample size used. To establish the robustness of these conclusions, further investigations involving diverse populations in the future will be essential. For individuals in the 0-20 age group, employing alternative methods, such as wrist radiographs, holds promise due to the substantial bone fusion changes occurring during the growth phase. Conversely, for those above 50 years of age, skeletal changes are less pronounced. In such cases, assessing calcification stages in the costal cartilage proves to be a valuable approach, particularly among individuals aged 30 years to 70 years and older. While there is overlap between various stages of calcification, the study's data provides an individual's age and will be more helpful as they get older. Age estimation benefits from using radiological modalities since it is simple, effective, and less expensive. Unlike bone and dental analyses, which offer more precision and effectiveness, the role of calcification in age estimation remains less precise. Our study explores the potential of calcification patterns in the costal cartilage as a supplemental tool for age estimation in forensic anthropology whereas for sex estimation the role of CCC is precise and useful. Early calcification can serve as a significant indicator for clinicians, aiding in the diagnosis and management of underlying health issues. Additionally, monitoring such calcification patterns can provide valuable insights into the progression and severity of conditions like Hypothyroidism, Hyperparathyroidism, Hypophosphatasia, Osteogenesis imperfecta, Ochronosis, Fibrodysplasia ossificans progressive, Werner syndrome, Scleroderma, Pseudoxanthoma elasticum, Paget's disease of bone and autoimmune disorders, potentially guiding treatment strategies for patients (Ontell et al., 1997). To enhance the robustness and generalizability of our conclusions, future research should encompass diverse populations, thereby ensuring the validity and applicability of our findings in both medicolegal and forensic contexts.

In the ever-evolving landscape of global populations, characterized by increasing heterogeneity due to factors such as admixtures, migrations, and refugee acceptances, the need for universally applicable and forensically acceptable age and sex estimation methods becomes imperative. Relying solely on ethnicity or population-specific age and sex estimation techniques may prove to limit, especially in mass fatality incidents like aeroplane crashes, earthquakes, terrorist massacres, and bomb blasts, where the severely damaged and commingled nature of human remains makes population or ethnicity identification exceptionally challenging, if not impossible. Consequently, there arises a demand for the development of universally applicable age estimation methods that are not reliant on outdated standards (Prasad & Kala, 2019). Errors in age estimation stemming from the application of a universally accepted method are anticipated to be lesser than those resulting from the erroneous application of a population-specific method on dissimilar populations. Forensic anthropology faces ongoing challenges in accurately determining the sex of human remains, necessitating innovative approaches and methodologies. Variability in skeletal morphology across populations, coupled with individual variation and observer bias, complicates the development of universally applicable sex estimation methods (Krishan et al., 2016). Additionally, limited sample sizes and age-related changes in skeletal characteristics further hinder the accuracy of sex determination. To address these challenges, novel techniques are emerging, including geometric morphometrics, three-dimensional imaging, machine learning, and molecular methods (Peleg et al., 2020). Geometric morphometrics allows for precise quantification of skeletal shape and size variations, while advanced imaging techniques provide detailed anatomical data for sex estimation (Klales, 2020a)(Constantinou & Nikita, 2022). Machine learning algorithms and artificial intelligence offer automated approaches to sex determination, and molecular methods, such as DNA analysis, complement traditional morphological analyses (Galante, Cotroneo, Furci, Lodetti, & Casali, 2023)(Vodanović, Subašić, Milošević, Galić, & Brkić, 2023). By embracing these innovative approaches and interdisciplinary collaborations, forensic anthropology can enhance the accuracy and reliability of sex estimation in medico-legal investigations. However, the absence of standardized methodologies and statistical measures poses a significant challenge in reaching conclusive findings, even when accounting for observer bias. Similarly, attributing observed variations in age estimates to secular trends remains uncertain due to a lack of credible evidence. While population-specific standards for age estimation have been established for certain regions or subpopulations in previous studies, the practical application of such standardized datasets in forensic cases is limited. This limitation is particularly pronounced in today's era of increasing global migration and interconnectedness, which has led to population and genetic admixtures. As a result, relying solely on population-specific standards may become impractical or obsolete in the foreseeable future.

The endeavour to establish an individual's age is intricate. As aptly stated by Milani and Benso (2019), chronological age simply measures the time elapsed since birth (Milani & Benso, 2019). Biological age encompasses the growth developments of many mechanisms of the body (Masethe & Masethe, 2014; Wittschieber et al., 2024), including bone development (skeletal age) and sexual development (sexual age). This highlights the distinction between chronological and biological ages, indicating that while correlated, no biological age method can precisely estimate an individual's chronological age. Given that different parts of the human body follow distinct growth curves, discrepancies or errors are expected, underscoring the significance of a defined or known standard error range for age indicators over the precision and accuracy of a particular age estimation method. Hence, a reliable age estimation method should possess a defined error range and aim to predict age as close to chronological age as feasible.

Moreover, the unavailability of intact body parts common occurrence in forensic cases, limits the applicability of methods involving a large number of bones. Therefore, techniques utilizing fewer elements are more practically useful and indispensable for forensic purposes. Age estimation methods that do not heavily rely on ossification and calcification features are crucial for enhancing the accuracy and applicability of forensic age estimation in diverse populations (Alkass et al., 2010; Andreas Schmeling, Manuel, Luis, & Irene, 2011). The findings of the present study underscore the reliability of certain age estimation methods for forensic age estimation in children and adults. As the present study focuses on age estimation from costal cartilage calcification patterns, I found significant correlations between age and calcification stages, particularly in the lower costal cartilage. Notably, calcification was absent in the 0–10 age group but gradually increased with age. Specifically, calcification in the lower costal cartilages was 100% prevalent in the 5th age group and continued in age groups that followed. The first CCC was observed in males at a younger age compared to females but there was not huge difference in the ages of male and female where CCC was first observed, with distinct stages observed across different age groups. These findings emphasize the importance of costal cartilage calcification patterns in age estimation studies and highlight the potential of radiographic imaging in forensic and anthropological research.

To mitigate errors in forensic age and sex estimations, a multi-factorial approach is often recommended. Including physical examination for anthropological and sexual development assessment, to enhance the accuracy of age and sex estimation (Banar et al., 2020; De Tobel et al., 2020; A. Schmeling et al., 2008). Overall, our study underscores the importance of interdisciplinary approaches in forensic research, combining radiology, anthropology, and medical imaging to enhance the reliability of age and sex assessment approaches. Moving forward, further research in this area is warranted to validate our findings across diverse populations and refine the application of costal cartilage calcification patterns in forensic casework. Through continued collaboration and innovation, I can advance forensic science and contribute to the resolution of complex medicolegal cases.

Chapter 5

Summary and Conclusions

The study conducted a meticulous examination of 500 individuals utilizing imaging software to analyse digital radiographs for gender and age estimation based on rib costal cartilage calcification.

The study of age and sex estimation from costal cartilage calcification using radiographs represents a significant contribution to forensic anthropology and medicine. The findings revealed a correlation between the degree of calcification and age. I found that calcification patterns in costal cartilage vary significantly with age. As individuals grow older, the degree of calcification tends to increase. This was evident in the analysis of different calcification stages (0, 1, 2, and 3), with older age groups showing higher proportions of advanced calcification stages. The study revealed a clear age-related trend in costal cartilage calcification. This observation aligns with existing research that suggests costal cartilage calcification is a progressive process associated with ageing. The use of imaging software, allowed for enhanced visualization and detailed evaluation of calcification patterns, leading to a more precise assessment of gender-specific differences. Statistical analyses, including chi-square tests, indicated a significant association between sex and calcification patterns. This association underscores the potential use of calcification patterns as a reliable marker for determining the sex of individuals. Peripheral calcification was more prevalent in males, while central calcification predominated in females. These findings underscore the potential of costal cartilage calcification patterns as a reliable indicator of sex, particularly in older age groups. Nevertheless, the majority of cases showed an evident change in the second to eighth costal cartilage calcification, and the findings show a general pattern of increasing calcification correlated with advancing age. These observations are in line with existing research, our study adds to the knowledge base by examining a sample population that was not studied earlier to the best of our knowledge. In forensic investigations, the ability to estimate age and sex from skeletal remains is invaluable, aiding in the identification of unknown individuals and providing insights for criminal investigations.

The observed differences in radiographic measurements between males and females are consistent with previous literature, which typically reports larger cardiac and thoracic dimensions in males. These differences are likely attributed to the inherent anatomical and physiological variations between sexes, including larger body sizes and muscle mass in males.

The slight but significant difference in the cardiac thoracic ratio, with females exhibiting a marginally higher ratio, may be explained by their smaller thoracic dimensions. This finding is in agreement with other studies that have reported similar trends, suggesting that the smaller body habitus of females leads to a relatively higher CTR despite their smaller cardiac size.

Understanding these differences is crucial for accurate diagnosis and assessment of cardiac conditions using radiographic techniques. The establishment of sex-specific reference values for these measurements could enhance diagnostic accuracy and improve clinical outcomes. In our study, obtaining a CTR value less than 50% suggests that the majority of subjects have a heart size within the normal range, which can be a significant indicator of a lower prevalence of cardiovascular abnormalities within the studied population. This finding underscores the importance of regular monitoring and early detection, as maintaining a CTR within the normal range is associated with better cardiovascular outcomes and reduced risk of morbidity and mortality related to heart diseases. Therefore, the CTR serves as a valuable, non-invasive measure for screening and ongoing evaluation of cardiac health, aiding in the timely identification and management of potential cardiac issues.

By integrating the findings from our study with existing literature, I contribute to a comprehensive understanding of sex-based differences in radiographic measurements, reinforcing the need for tailored diagnostic approaches in radiology.

It provides forensic practitioners with a non-invasive tool to estimate age and sex, aiding in the identification of unknown individuals and supporting investigative efforts. Further research is needed to address limitations and refine the method, contributing to the growing body of knowledge in forensic radiology and its practical applications in the field.

Despite its contributions, the study has limitations. Sample size and representativeness may impact the method's accuracy, and the cross-sectional nature of the study may introduce bias. Similar levels of calcification over a broad age range in the middle years limit the usefulness of this trait as a useful age estimation technique. Although it showed a positive correlation, but generated a very wide range. Better radiology modalities like CT and MRI can enhance the result by narrowing the stages of calcification based on 3-D visualisation. This approach might work best when used in conjunction with another method or as a corroboration technique, accepted age-estimation methods but not as independent indicators of specific age ranges.

Before its application, this method should also be analysed on different populations, both alive and deceased, to ensure its validity and reliability across diverse groups.

The most effective method in this study was a close examination of the costal cartilage calcification pattern, which produced reliable sex predictions from thoracic radiographs utilizing a quick and easy method. These are simple to teach and replicate, particular handling of the bones, or in-depth understanding of anthropology. As a result, our work proposed estimating gender from the calcification patterns in costal cartilage using a generalised version of Rejtarova et al.'s (2004) technique. The additional investigation could involve combining digitally assessed calcification patterns and the amount with feature evaluation to improve sex prediction accuracy and potentially provide more precise age range estimations.

Limitations of the Study

- **Sample Size and Representativeness**: The study outcomes may be constrained by the limited sample size and its representativeness, which could affect the generalizability and accuracy of the method for wider populations.
- **Cross-Sectional Design**: Due to the study's cross-sectional approach, it captures only a single point in time, which may introduce potential biases and restrict observations of longitudinal changes.
- **Calcification Variability**: The similarity in calcification levels observed among middle-aged individuals makes precise age estimation challenging, as it results in overlapping age ranges and reduces accuracy.
- **Imaging Limitations**: Radiographs, used in this study, have inherent limitations in accuracy. Advanced imaging techniques such as CT or MRI could provide more precise three-dimensional views, improving calcification staging.
- **Population Diversity**: The proposed method needs validation and reliability testing across various populations, including both living and deceased groups, to ensure broader applicability.
- **Methodological Constraints**: While the method suggests a link between calcification patterns and gender estimation, it should not be employed independently for age determination without support from other validated age estimation techniques.

Recommendations for Future Research

- **Expand Sample Size and Diversity:** Increasing the sample size and including diverse populations will improve the generalizability and accuracy of the findings.
- **Advanced Imaging Techniques:** Utilizing advanced imaging modalities such as CT and MRI can provide more detailed visualization and enhance the accuracy of calcification stage assessments.
- **Machine Learning Integration:** Implementing machine learning algorithms could automate the age and sex estimation process, increasing efficiency and potentially improving accuracy.
- **Longitudinal Studies:** Conducting longitudinal studies to observe the progression of costal cartilage calcification over time would provide deeper insights into the aging process.
- **Combination of Methods:** Combining costal cartilage calcification analysis with other established age and sex estimation techniques could yield more reliable and comprehensive results.

Additionally, refining age and sex estimation models and validating them on independent datasets are crucial for enhancing the method's reliability. The application of 3-D CT scans for measuring the percentage of calcification from CT number, and thickness will enhance the age estimation technique as further divide stages of calcification into smaller groups hence enhancing the accuracy rate and it will narrow down the age range in the groups.

By integrating these recommendations, future research can build upon our findings, enhancing the reliability and applicability of costal cartilage calcification analysis in forensic and clinical settings.

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ANNEXURE I

PARTICIPANTS INFORMATION SHEET

Namaste, I am Shama Patyal, a PhD student in the Department of Forensic Science at Lovely Professional University Jalandhar India. I am going to conduct a research study under the guidance of Dr. Tejasvi Pandey, Head and Professor Department of Forensic Science titled "A STUDY ON COSTAL CARTILAGE CALCIFICATION FROM DIGITAL CHEST RADIOGRAPH FOR EVALUATION AND ESTIMATION OF SEX AND AGE."

- The purpose of this study is purely research-oriented and the study is approved by the ethical committee of RKGMC Hamirpur as per Helsinki guidelines.
- You will be subjected to an interview schedule that contains information regarding your basic socio-demographic details such as age and sex.
- Benefits and harms from the study: - You will not get any direct benefit from the study. There will be no harm to you from the study. Your identity will not be revealed in any case. There will be no additional cost to be borne by you.
- You can contact me anytime in case of any doubt. (Mob no 7018526915). You will be free to withdraw from the study at any time, without giving any reason.

Name:

Signature/thumb impression of the participants:

Date:

ANNEXURE-II

INFORMED CONSENT FORM

Study title:- A STUDY ON COSTAL CARTILAGE CALCIFICATION FROM DIGITAL CHEST RADIOGRAPH FOR EVALUATION AND ESTIMATION OF SEX AND AGE

Age:-

1. I confirm that I have been informed about this study and its benefits are explained to me in my own language.

2. I understand that my participation in the study is voluntary and I am free to withdraw at any time without giving any reason.

3. I agree to restrict the use of any data or results that arise from this study provided such use is only for the scientific purpose(s) or publication in scientific journals without disclosing my identity and personnel details.

4. I agree to take part in the above study.

Date:-

Signature (or Thumb Impression) of the Subject

Name

Signature of the Investigator:-

Investigator’s Name:-

अनुलग्नक I

भागीदारी सूचना

नमस्ते, मैं शमा पटयाल, एक पीएच.डी. लवली प्रोफेशनल यूनिवर्सिटी जालंधर भारत में फॉरेंसिक साइंस विभाग में छात्र। मैं फॉरेंसिक साइंस विभाग के प्रमुख और प्रोफेसर डॉ. तेजस्वी पाण्डेय के मार्गदर्शन में "ए स्टडी ऑन कॉस्टल कार्टिलेज कैल्सीफिकेशन फ्रॉम डिजिटल चेस्ट रेडियोग्राफ फॉर इवैल्यूएशन एंड एस्टीमेशन ऑफ सेक्स एंड एज" शीर्षक से एक शोध अध्ययन करने जा रहा हूं।

- इस अध्ययन का उद्देश्य विशुद्ध रूप से शोध-उन्मुख है और हेलसिंकी दिशानिर्देशों के अनुसार आरकेजीएमसी हमीरपुर की नैतिक समिति द्वारा अध्ययन को मंजूरी दी गई है।

- आपको एक साक्षात्कार अनुसूची के अधीन किया जाएगा जिसमें आपके बुनियादी सामाजिक-जनसांख्यिकीय विवरण जैसे उम्र और लिंग के बारे में जानकारी शामिल है।

- अध्ययन से लाभ और हानि :- अध्ययन से आपको कोई सीधा लाभ नहीं मिलेगा। पढ़ाई से आपको कोई नुकसान नहीं होगा। आपकी पहचान किसी भी सूरत में उजागर नहीं की जाएगी। आपके द्वारा वहन करने के लिए कोई अतिरिक्त लागत नहीं होगी।

- किसी भी तरह का संदेह होने पर आप मुझसे कभी भी संपर्क कर सकते हैं। (मोबाइल नंबर 7018526915)। आप बिना कोई कारण बताए किसी भी समय अध्ययन से हटने के लिए स्वतंत्र होंगे।

नाम:

प्रतिभागियों के हस्ताक्षर/अंगूठे का निशान:

तारीख:

अनुलग्नक – II

सूचित सहमति प्रपत्र

शीर्षक : - सेक्स और उम्र के मूल्यांकन और आकलन के लिए डिजिटल चेस्ट रेडियोग्राफ से कॉस्टल कार्टिलेज कैल्सीफिकेशन पर एक अध्ययन

उम्र:-

१ मैं पुष्टि करता हूं कि मुझे इस अध्ययन के बारे में सूचित कर दिया गया है और इसके लाभों को मुझे अपनी भाषा में समझाया गया है।

2. मैं समझता हूं कि अध्ययन में मेरी भागीदारी स्वैच्छिक है और मैं बिना कोई कारण बताए किसी भी समय अपना नाम वापस लेने के लिए स्वतंत्र हूं।

3. मैं इस अध्ययन से उत्पन्न होने वाले किसी भी डेटा या परिणाम के उपयोग को प्रतिबंधित करने के लिए सहमत हूं, बशर्ते कि ऐसा उपयोग केवल वैज्ञानिक उद्देश्य (ओं) या वैज्ञानिक पत्रिकाओं में प्रकाशन के लिए मेरी पहचान और कर्मियों के विवरण का खुलासा किए बिना हो।

4. मैं उपरोक्त अध्ययन में भाग लेने के लिए सहमत हूँ।

तारीख:-

विषय का हस्ताक्षर (या अंगूठे का निशान)।

नाम

अन्वेषक के हस्ताक्षर:-

अन्वेषक का नाम:-

ANNEXURE-III

Dr. Radhakrishnan Government Medical College
Hamirpur, Himachal Pradesh, India – 177 001

Institutional Ethics Committee (IEC)

Registration No. ECR/1461/Inst/HP/2020

Chairperson
Dr. Pratima Singh Thakur
Sai Hospital Dugha, Hamirpur, HP
+ 91 7650083652

Member Secretary
Dr. Anupriya Sharma
Associate Professor (Dentistry)
+ 91 94180 15544

E mail: ***anu_s_priya@yahoo.com***

No. HFW - H - Dr. RKGMC/ Ethics /2022/ 02 Dated: 18/4/22

Approval of Institutional Ethics Committee

Basic Clinician:
Dr. Sanjeev Chaudhary

Member(Scientist):
Dr. Tarun Sharma

Member(Scientist):
Dr. Praveen Kumar Sharma,Pharmacology

Legal Advisor:
Mr.Suresh Musafir

Philospher/ethicist/theologist :
Mrs. Richa Sharma

Member(Non-Govt. Agency):
Mr. Davinder Sharma,

Lay person of society:
Mr. Rakesh Thakur

The IEC of Dr. RKGMC reviewed and discussed the protocol (**No: IEC/…/2022**) entitled "A study on costal cartilage calcification from digital chest radiograph for evaluation and estimation of sex and age" submitted by Shama Patyal, Ph.d. Forensic Science, Lovely Professional University ,Punjab.

Decision: The committee approves the research work to be conducted in its present form.

The IEC needs to be informed about:

- Patient information sheet and consent forms duly filled and signed
- Any serious adverse event occurring during the study
- Final report of the study

The IEC has to be informed and permission taken before any changes in the protocol, patient information sheet, informed consent, Site of Study or Investigator.
It is to be ensured that there shall no financial burden on the research participants because of participation in the study and no direct cash incentives are given to the participants. Please note that members of IEC have the right to monitor the study in any phase.

(Member Secretary)

List of Publications

1. Patyal, S., Bhatia, T. Evaluating costal cartilage for sex and age estimation from PA chest radiographs of North Indian population: a retrospective study. *Egypt J Forensic Sci* 12, 41 (2022). https://doi.org/10.1186/s41935-022-00298-y
2. PATyAL, Shama, and Tejasvi PANDE. "Estimation of age from digital chest radiographs in district mandi, Himachal Pradesh." *Problems of Forensic Sciences/Z Zagadnien Nauk Sadowych* 132.132 (2022). https://doi.org/10.4467/12307483PFS.22.012.17685
3. Patyal, Shama, and Tejasvi Pandey. "Forensic Radiology: A Spotlight on India's current trend and Future Prospects." *Forensic Imaging* (2023): 200561. https://doi.org/10.1016/j.fri.2023.200561
4. PATyAL, Shama, and Tejasvi PANDE. " An Overview of The Radiation Protection Knowledge and Work Conditions Among Radiographers In Himachal Pradesh (A State in India)" Administrative Development: A Journal of HIPA.

Sr. No.	Paper Title	Type	Name of Journal
1	Evaluating costal cartilage for sex and age estimation from PA Chest Radiographs of North Indian Population: A retrospective study	Research article	Egyptian Journal of forensic sciences. Q2 Journal
2	Estimation of age from digital chest radiographs in district Mandi, Himachal Pradesh	Research	Problems of Forensic Sciences
3	Forensic radiology: A spotlight on India's current trend and Future prospects	Review	Forensic Imaging (Elsevier)
4	An Overview of The Radiation Protection Knowledge and Work Conditions Among Radiographers In Himachal Pradesh (A State in India)	Research	Administrative Development: A Journal of HIPA

LIST OF CONFERENCES

Sr.No.	Presentation Platform	Title of Presentation	Type of Presentation Type of Study	Outcome (published)
1	IASR- 8th International e-Conference on Forensic medicine & Toxicology	Sex Determination from Digital Radiographs of Costal Cartilage a Cross-Sectional Study	Oral Paper Presentation A cross- sectional Study	Academic Journal of Forensic Sciences (ISSN: 2581-4966) Vol: 05 , Issue: 01
2	International Conference on "Emerging Technologies: AI, IoT and CPS for science & Technology Applications" NITTTR Chandigarh 2021	Artificial Intelligence with Radio-Diagnostic Modalities in Forensic Science A Systematic Review	Oral Paper Presentation **Awarded as "best paper award"** A Systematic Review	CEUR-WS proceedings. Scopus Indexed Emerging technologies: AI, IoT and CPS for Science & Technology Applications 2021. Available online on 1st Feb 2022. ISSN: 1613-0073

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Extracts from the Register of Copyrights

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दिनांक/Dated:21/08/2024

1.	पंजीकरण संख्या/Registration Number	L-152692/2024
2.	आवेदक का नाम, पता तथा राष्ट्रीयता Name, address and nationality of the applicant	LOVELY PROFESSIONAL UNIVERSITY , LOVELY PROFESSIONAL UNIVERSITY, JALANDHAR, DELHI-GT ROAD, PHAGWARA,PUNJAB-144411 INDIAN
3.	कृति के प्रतिलिप्यधिकार में आवेदक के हित की प्रकृति Nature of the applicant's interest in the copyright of the work	OWNER
4.	कृति का वर्ग और वर्णन Class and description of the work	LITERARY/ DRAMATIC WORK THE GRAPHICAL ABSTRACT PRESENTS ESTIMATION OF AGE AND SEX USING CHEST RADIOGRAPHY.
5.	कृति का शीर्षक Title of the work	ESTIMATION OF AGE AND SEX USING CHEST RADIOGRAPHY.
6.	कृति की भाषा Language of the work	ENGLISH
7.	रचयिता का नाम, पता और राष्ट्रीयता तथा यदि रचयिता की मृत्यु हो गई है, तो मृत्यु की तिथि Name, address and nationality of the author and if the author is deceased, date of his decease	SHAMA PATYAL , LOVELY PROFESSIONAL UNIVERSITY, JALANDHAR, DELHI-GT ROAD, PHAGWARA,PUNJAB-144411 INDIAN DR. TEJASVI BHATIA , LOVELY PROFESSIONAL UNIVERSITY, JALANDHAR, DELHI-GT ROAD, PHAGWARA,PUNJAB-144411 INDIAN
8.	कृति प्रकाशित है या अप्रकाशित Whether the work is published or unpublished	UNPUBLISHED
9.	प्रथम प्रकाशन का वर्ष और देश तथा प्रकाशक का नाम, पता और राष्ट्रीयता Year and country of first publication and name, address and nationality of the publisher	N.A.
10.	बाद के प्रकाशनों के वर्ष और देश, यदि कोई हों, और प्रकाशकों के नाम, पते और राष्ट्रीयताएँ Years and countries of subsequent publications, if any, and names, addresses and nationalities of the publishers	N.A.
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17.	टिप्पणी, यदि कोई हो/Remarks, if any	THE WORK IS ORIGINAL AS DONE BY THE FACULTY AND STAFF OF LOVELY PROFESSIONAL UNIVERSITY.

डायरी संख्या/Diary Number: 18581/2024-CO/L

आवेदन की तिथि/Date of Application: 07/06/2024

प्राप्ति की तिथि/Date of Receipt: 07/06/2024

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ORIGINAL ARTICLE Open Access

Evaluating costal cartilage for sex and age estimation from PA chest radiographs of North Indian population: a retrospective study

Shama Patyal[1] and Tejasvi Bhatia[2*]

Abstract

Background: This study is conducted to estimate age and sex from chest radiographs. The aim of this study was to determine (I) whether costal cartilage calcification pattern help in estimating sex in North Indian sample and (ii) whether there is any relation between age and costal cartilage calcification.

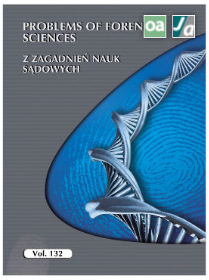
Results: The statistical probability of estimating peripheral pattern in male was 99.3%, and for females, it was 100% for central type of calcification. The minimum age where lower costal calcification was present was 22-year-old female and 21 years old male. The degree of calcification and age showed positive correlation; similarly, the value of spearman's rho showed significant result (for whole sample $r_s = 0.911$, $p < 0.01$ (for male subjects, it was $r_s = 0.921$, $p < 0.01$; for females, it was $r_s = 0.905$, $p < 0.01$)).

Conclusions: The degree of calcification is useful in estimating age groups and becomes more determined with advancing age. Prediction of sex by using radiological method gives the advantages of simplicity, inexpensiveness, speed, and high accuracy. This method for age and sex estimation will play a useful role in screening test and in cases where the thoracic part of the body is found like dismembered body, putrefied dead remains, and unidentified bodies.

Keywords: Chest radiographs, Calcification, Ossification, Age estimation, Sexual dimorphism

Background

active between adolescent growth and adult maturational



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Estimation of age from digital chest radiographs in district Mandi, Himachal Pradesh

Estimation of age from digital chest radiographs in district Mandi, Himachal Pradesh

Author(s): Shama Patyal, Tejasvi Pandey

Subject(s): Criminology
Published by: Wydawnictwo Uniwersytetu Jagiellońskiego
Keywords: Forensic science; Age estimation; Costal calcification; Identification; Radiography; Thorax

Summary/Abstract: In forensic investigations estimation of age is a crucial part. Age estimation assists in narrow downing the search possibilities in the case of unidentified bodies and living individuals, it can also help in the determination of preparators and deciding the penalty for any criminal liability. Different body parts are often investigated at the crime scene, so estimating the age using different areas of the body is important. Hence, we conducted this study for age estimation from costal cartilage calcification. Only posterior-anterior (PA) digital chest radiographs are taken, and the first rib of the left side is evaluated. The degree of calcification and age showed a positive correlation; similarly, the value of Spearman's rho showed significant results (for the whole sample =0.914, $p < 0.01$ (for male subjects was =0.925, $p < 0.01$. For females was =0.905, $p < 0.01$). The degree of calcification is useful in estimating age groups and becomes more determined with advancing age. Prediction of age using the radiological method gives the advantages of ease, inexpensiveness, rapidity, and high accuracy. In developing countries, this type of study will be very useful because infrastructure and lack of sources are major issues. The article provides a non-destructive method of age estimation in a forensic context that may act as a preliminary method of age estimation in mass disasters and dismembered bodies when a thoracic cage is only present.

Details | Contents

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Forensic radiology: A spotlight on India's current trend and Future prospects

Shama Patyal [a,b], Tejasvi Pandey [a,*]

[a] Department of Forensic Science, Lovely Professional University, Jalandhar, India
[b] Govt. Civil Hospital Dharampur, Mandi, HP

ARTICLE INFO

Keywords:
Forensic radiology
PMCT imaging
Forensic radiographer
Forensic radiologist
India

ABSTRACT

A specialized branch of medical imaging known as forensic radiology is employed in legal proceedings by forensic experts, doctors, pathologists, and anthropologists for the purpose of evaluation. In the realm of forensic medicine and toxicology, imaging technologies and procedures have the potential to be effective and strong instruments in resolving medicolegal cases. In addition to a brief history, this article also discusses the application of post-mortem imaging, its economic benefits, and its current status in India with respect to the global context. Radiography is still the choice of modality for identifying and locating foreign bodies at the time of death despite the increase in PMMR and PMCT.

Introduction

A specialist branch of medical imaging called "forensic radiology" uses radiologic methods to help pathologists and doctors with cases involving the law. The practice, interpretation and reporting of radiographic tests and procedures that are required for legal proceedings or law enforcement is known as forensic radiology. Radiological techniques are frequently employed for identification, determining age, and determining the cause of death [1]. A relatively recent development in forensic death investigation is forensic imaging. However, forensic science has used radiology since the discipline's foundation. On Christmas Eve, 1895, an incident of firing a bullet eventually led to the first court case using a radiograph as evidence in a court in North America and helped in proving the attempted murder charge on the culprit [2]. The first comparative X-rays were probably used in 1895 after an actress in England suffered a lower leg injury while working at a theatre. One of the first forensic uses of radiography imaging was to find and record bullets in a living patient after a murder in England in April 1896 [3]. The use of forensic radiology for the assessment of an Egyptian child's mummy was published in March 1896 and it was the earliest known use of radiographs for such a unique case by a physicist at the Museum of Natural History [4]. To establish identity, forensic anthropologists and odontologists frequently compare antemortem and postmortem radiographic plates. The use of radiology imaging modalities is not limited to radiologists; identifications made using these modalities in archaeology, and anthropology are also feasible [5]. The most popular imaging assessment method is an X-ray examination. It serves as the foundation for radiology. Despite the fact that technology has advanced greatly. The radiography imaging technique is still the most frequently used by forensic pathology. Examining damaged bones, gas collections, and identifying and localizing radiopaque foreign substances are all excellent uses of radiographs. The use of forensic radiological facilities in medicolegal practice is notably neglected, it would not be wrong to say. The reduction of the danger of infection transmission to the medical staff is a built-in benefit of postmortem imaging over the traditional autopsy. Hence, radiological facilities can be employed as a successful substitute for high-risk traditional autopsy procedures during the pandemic and epidemics like COVID-19, Ebola, Tuberculosis, and H1N1 Flu [6,7].

There is a long history of using radiology modalities for forensic benefits. But still the development of forensic radiology lagging behind forensic anthropology and forensic medicine. The development of radiology for clinical benefits are increasing similarly the forensic pathology side by side. The two fields are growing independently but currently, the two routes have once again unified.

The position of forensic radiology in India and on a global scale

Outside of India in developed countries, forensic radiology is best developed. The Institute of Legal Medicine in Bern in 2000 was the organization that started the virtual autopsy project. Its main objective

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https://doi.org/10.1016/j.fri.2023.200561

Available online 24 August 2023

AN OVERVIEW OF THE RADIATION PROTECTION KNOWLEDGE AND WORK CONDITIONS AMONG RADIOGRAPHERS IN HIMACHAL PRADESH (A STATE IN INDIA)

Shama Patyal[1], Dr Neha Patyal[2] & Tejasvi Pandey[3]

Abstract

Objective: This paper addresses the awareness of radiographers in radiation protection and other issues related to working culture and practice.

Methodology: This study is conducted online by generating a questionnaire using google forms and taking direct interviews. The questions were related to demographic characteristics, academic qualification, radiation protection awareness, work during COVID-19, workflow, stress, and suggestions to improve the quality of work. The questionnaire was sent to radiographers working in government and private setups in the state of Himachal Pradesh.

Conclusion: Most radiographers are stress-free and satisfied with work conditions, and positive feedback during COVID-19. But are careless about radiation protection and lack proper infrastructure and devices at the centers. Work division according to their qualification is missing, diploma holders, graduates, and postgraduates all are operating similar modalities.

[1] Radiographer at Govt. Civil Hospital Dharampur, Mandi (HP)
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Lovely Professional University, Jalandhar, India
patyalshama@gmail.com

[2] Tutor Specialist

Artificial Intelligence with Radio-Diagnostic Modalities in Forensic Science - A Systematic Review

Shama Patyal[1] and Tejasvi Bhatia[2]

[1,2] *Lovely Professional University, Jalandhar Punjab India.*

Abstract

PURPOSE: The aim of this study was to provide an overview of Artificial intelligence in Forensic science with the aid of radio-diagnostic modalities.

DATA SOURCES and SYNTHESIS: The data is gathered by searching the articles in variousseearch engines which have been published between January 2010 to December 2020. A total of 20 studies were found eligible after following inclusion and exclusion criteria described in the below article. Prisma Guidelines and Prisma Flowchart was followed.

CONCLUSION: Artificial intelligence (AI) is a technology that involves computerised algorithms to dichotomize complex data. AI is widely used in diagnostic imaging for detection and quantification of a clinical condition. This systematic review aimed to explain the role of AI with diagnostic imaging modality of radiology in forensic. AI technology is now widely used for age and sex estimation. Most of the AI models are based on machine learning (ML) programs, artificial neural network(ANN) and convolutional neural network (CNN). The results of the studies are promising, providing great accuracy and decision making. These different AI based models will be act as identification tools in mass disasters cases, medicolegal cases. Further improvement in AI programs and diagnostic tool is needed for better accuracy and specificity in Forensic investigations.

Keywords

Artificial Intelligence, Machine Learning, Diagnostic Imaging Modality, Forensic Identification

Xournals

Academic Journal of Anthropologial Studies

ISSN: 2581-4966 | Volume 05 | Issue 01 | April-2022

Sex Determination from Digital Radiographs of Costal Cartilage: A Cross Sectional Study

Shama Patyal[1], Tejasvi Bhatia[2]

Available online at: www.xournals.com

Received 28th July 2021 | Revised 10th September 2021 | Accepted 12th March 2022

Abstract:

The aim of this study was to study the pattern of calcification found in cartilage of costal region with the aid of digital radiographs in male and female. PA chest digital radiographs were taken and with the help of software IMAGEWORK radiological evaluation of calcification pattern were done. With the help of IMAGEWORK we can alter the contrast, magnify the radiograph, can increase or decrease the sharpness and brightness that help in greater visualization and help in better evaluation on type of pattern as compare to conventional mean. Other advantage is it is cost and time efficient. Three main distinct calcification pattern were seen in the present study. In males' peripheral type of calcification was dominant where as in females' central type was more seen. Mixed type of pattern was more common in age group 20- 40. In males at age of 16 calcification was noted whereas in females at age of 18.

Keywords: *Digital Radiographs, Costal Cartilage, Calcification, Sexual Dimorphism.*

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Certfi. No.: IC-1/88/21-22

This is to certify that Dr./Mr./Ms. ***Shama Patyal*** has presented a research paper titled ***Artificial Intelligence with Radio-Diagnostic Modalities in Forensic Science - A Systematic Review*** Co-authored by ***Tejasvi Bhatia*** awarded the Best Paper in "International Conference on Emerging Technologies: AI, IoT, and CPS for Science & Technology Applications" jointly organized by Electronics and Communication Engineering & Electrical Engineering Department during September 6- 7, 2021.

(Organizing Chair)	(Organizing Chair)	(Organizing Chair)
Dr. Piush Verma	Dr. Lini Mathew	Dr. S. S. Gill
Professor (EE)	Professor & Head (EE)	Professor & Head (ECE)

2. Certificate of Participation

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CERTIFICATE

Cert. No.: IC-1/71/2021-22

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