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Identification of Human Skeletal Remains through Analysis of Skeletal Pathologies

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Declaration

Acknowledgements

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List of Abbreviations

AD -	Anno Domini
BC -	Before Christ
CASP -	Critical Appraisal Skills Programme
CT -	Computed Tomography
DISH -	Diffuse Idiopathic Skeletal Hyperostosis
DNA -	Deoxyribo Nucleic Acid
HFI -	Hyperostosis Frontalis Interna
HPO -	Hypertrophic Pulmonary Osteoarthropathy
PRISMA -	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
UNIFESP -	Federal University of Sao Paulo

Abstract

Background: The analysis of skeletal lesions in human remains is not only important for the investigation of pathological manifestations in the bones, but may also assist in the identification of dead and missing persons using the discipline of forensic anthropology. Several human diseases leave extensive clues in the skeleton in the form of lesions, which can be used to reconstruct the lives and pathologies of people.

Aim: This study aims to explore the pathological diseases that affect the human skeleton that can be detected in human skeletal remains, and characterize their types, occurrence, range, and manifestations. It also aims to describe specific characteristic features of these pathological diseases that can help in the identification of the person to whom the skeletal remains belong.

Methods: The PubMed database was used to search for original research articles using the keywords (Bone Pathology OR Paleopathological Evidence) AND (Human Skeletal Remains OR Human Identification) AND (Forensic OR Paleopathology OR Crime Scene Investigation). The inclusion criteria were articles between the years 2000 and 2020, studies that described pathological manifestations in the skeleton, and original articles and case reports. The exclusion criteria were articles before the year 2000, articles that were a discussion and/or analysis of other original articles, and articles that did not directly use skeletal pathology to add value to human identification or disease characterization.

Results: Applying the inclusion and exclusion criteria, 23 studies were selected for the final review. A data extraction table was prepared summarising the key features, aims, and results of all the studies. Only 3 articles used skeletal pathologies to create a biological profile that could help confirm the identity of the deceased,

whereas the remaining studies characterised skeletal lesions pertaining to specific disease conditions.

Discussion: The literature regarding use of skeletal pathologies for identification of the deceased is extensive and elaborate. Several studies and case reports present detailed descriptions of skeletal lesions that extend our understanding of skeletal pathologies and their differential diagnoses. These can then be used to create a unique biological profile which, when combined with other physical and situational factors, can aid in positive identification of the deceased individual.

Chapter 1

BACKGROUND

1.1 Introduction

The study of disease in human and animal remains is known as paleopathology and it is a subdomain in the wider study of archaeology. It involves collecting evidence either from skeletal remains or from artificially preserved bodies in order to acquire information about the lives, deaths, and diseases of humans and animals (Roberts 2016). The discipline of paleopathology was born in 1774 when Johann Friederich Esper studied the remains of a cave bear and presented a diagnosis of osteosarcoma. Slowly, doctors and pathologists started recognizing the importance of studying pathological lesions in human skeletal remains to understand the mechanisms and manifestation of diseases better. Despite the fact that the science of paleopathology had been around since the 17th century, the term 'paleopathology' was coined by R. W. Schufeldt in 1892. By 1918, paleopathology had become a well-established field acquiring the interest of several young and budding pathologists (Grauer 2017).

Human skeletal remains are one of the most significant evidence available to us for understanding and examining how people in the past lived and died. Paleopathology, in particular, helps researchers understand the status of health and well-being of people and the relationship between people's living conditions and their health. Currently, there are several challenges in understanding human disease mechanisms and information obtained from human skeletal remains can help us address at least some of these challenges. Trauma and disease has been present since generations and it affects, not only quality of life, but also the physiological mechanisms that lead to death. As a result, the study of paleopathology is significant in enlightening us on the various pathological diseases that affect humans (Roberts 2016). As the

number of these diseases is enormous, this thesis aims to focus on those pathological diseases that affect the skeletal system in humans and describe the range and manifestations of these diseases. It also aims to describe how the study of skeletal pathologies in human remains can aid in the construction of a biological profile, using which the identity of the person may be determined.

As paleopathology is the study of diseases in human and animal remains, it is paramount to have a clear and in-depth understanding of the skeletal system, its components, and its important structural features. This thesis focuses on pathological diseases of the human skeletal system and their role in human identification, and hence, the structural and functional features of the skeletal system in humans are described in detail below.

1.2 Human Skeleton

The human body comprises of nearly 37 trillion cells, 4 different tissue types, and 11 organ systems of which the skeletal system is one of the most important structural systems of the body (Hossain 2018). The study of the skeletal system forms the crux of the study of human anatomy and provides knowledge about the structure and form of living organisms. The skeletal system comprises of bones, joints, and the associated cartilages, and it is the hard core framework around which the entire body is present. As the skeletal system is a hard structure, joints enable movement at different points of the skeleton so that human beings can walk, eat, work, and perform several other seemingly simple and yet structurally complicated tasks. The number of bones in the human body is 270 at birth which decreases to 206 by adulthood as a result of the ossification and/or fusion of several bones (Hossain 2018).

1.3 Bone Composition

Bones are composite tissues of the body that comprise of 25% organic material, 50% inorganic components and mineral salts, and 25% water (Robson and Syndercombe Court 2018). The organic matrix or osteoid tissue of the bone comprises majorly of type-I collagen fibres and, to a lesser extent, other components such as glycoproteins, proteoglycans, and osteocalcin. This matrix contributes to the hardness of the skeletal framework around which the inorganic components are deposited to give structural support in a process known as ossification (Tortora and Derrickson 2009). Among the inorganic constituents of bones, calcium and phosphate are the most important salts that are present in the form of crystals of hydroxylapatite. Apart from these, other inorganic materials present in bones include fluoride, magnesium, carbonate, acid phosphate, and citrate (Safadi et al. 2009).

The balance of organic and inorganic constituents of the bones is extremely important to give them tensile strength and flexibility so that they can withstand mechanical forces and are not broken easily. These properties are mainly conferred by collagen fibres and also by the minerals within and around these fibres. In the event that the quantity of collagen is lesser than the required amount or there is over-mineralization in the bones, the bones become extremely fragile and brittle, and the risk of them breaking is much higher as compared to normal bones. An example of a genetic disorder that leads to extreme brittleness in bones is osteogenesis imperfecta where the tendency of the bones to break is quite high (Ralston and McInnes 2014).

1.4 Bone Structure

There are two types of bone tissues that make up the entire bone structure, and these are cortical bone and cancellous bone. Cortical bone tissue is a dense compact tissue that covers and protects the inner cancellous tissue. It is made up of three important constituents – the periosteum which is a fibrous outer covering, the endosteum which is the inner tissue, and the intracortical area between these two layers (Bartl and Bartl 2017). The outer periosteum layer is mostly continuous, except for regions over the joints that are composed of hyaline cartilage. It has several vascular connections, and attachments of tendons and ligaments on the outer surface and osteoblasts and osteoclasts on the inner surface. Osteoblasts are cells involved in bone formation and osteoclasts are cells involved in bone resorption, both of which are involved in bone remodeling processes. The most important function of the periosteum is bone protection, nourishment, and repair (Robson and Syndercombe Court 2018). The intracortical areas are made up of cylindrical structural units known as osteons comprising of lamellar bone tissue. The main function of this region is to provide structural support and strength to the cortical bone tissue. The cell type that is found in this region is osteoclasts that are matured bone cells present in small gaps within the lamellar tissue. In contrast to the thick periosteum layer, the inner endosteum layer is thinner and made up of connective tissue (Bartl and Bartl 2017).

The second important region found in the bone is the cancellous bone tissue which is a spongy tissue found immediately inner to the cortical region. It is made up of structural units known as trabeculae that are arranged in an irregular pattern so that there are huge gaps between adjacent units. This characteristic honeycomb arrangement of the trabeculae is meant to confer lightness to the

bones so that mobility becomes easier for the human body. The orientation of trabeculae is such that bones can resist mechanical stresses and decrease the risk of acquiring fractures. Hematopoietic tissues are also present in the spaces between trabeculae which comprises of red and yellow bone marrow, and red blood cells, white blood cells, and platelets (Tortora and Derrickson 2009).

1.5 Structural Classification

Based on structure, bones can be broadly classified into five categories – long, short, flat, sesamoid, and irregularly shaped bones. The differences in their structure arise due to differences in their formation mechanisms during embryonic development (Safadi et al. 2009).

- i. *Long Bones* – These bones consist of a cylindrical hollow tube or the diaphysis in the centre bounded on either side by extremities or epiphysis and a transitional zone between the hollow tube and each extremity, also known as metaphysis. Examples of long bones include tibia, femur, radius, and ulna (Safadi et al. 2009).
- ii. *Short Bones* – These bones are cube-shaped comprising of spongy bone tissue in the centre surrounded by thin compact bone tissue. Examples of short bones include tarsal and carpal bones of the foot and the hand respectively (Safadi et al. 2009).
- iii. *Flat Bones* – As the name suggests, these bones are flat comprising of a flat layer of spongy tissue surrounded on the upper and lower sides by compact bone tissue. Examples of these bones include those present in the skull, scapula, and sternum (Safadi et al. 2009).

- iv. *Sesamoid Bones* – These bones are similar to short bones and they are mainly found in the tendons. Examples of sesamoid bones include pisiform and patella (Safadi et al. 2009).
- v. *Irregularly Shaped Bones* – These bones lack a specific symmetry or structure apart from the presence of inner spongy tissue and outer compact tissue. Examples of these bones include vertebra, ethmoid, and scapula (Safadi et al. 2009).

1.6 Bone Development

Bone development begins in the embryo where connective tissues are formed first which is called the skeletal template. These tissues are then converted into bone tissue either by intramembranous ossification processes or endochondral ossification processes. Intramembranous ossification refers to the formation of bone tissues directly from the primitive connective tissues through the differentiation of stem cells into osteoblasts and ossification of fibrous tissues. Eventually, the newly formed osteoblasts are organized into the bone matrix subsequently leading to the formation of new bone tissues (Walker 2020). Endochondral ossification is the process by which the cartilage tissues are gradually replaced with osteoblasts in bone formation processes. As a part of this process, a primary centre of ossification and a secondary centre of ossification are formed which serve as sites of cartilage tissue formation. The primary centre of ossification serves as the site for the initial development of cartilage tissue and it lies in the centre of the bone. As the endochondral bone development process spreads on either side of the primary ossification centre, secondary centres of ossification are formed on either ends of the bones, which eventually become the epiphysis. The primary centre of ossification eventually becomes the diaphysis with a layer of cartilage being present

between the epiphysis and the diaphysis. This cartilage layer forms the epiphyseal growth plate which serves as the site for continual bone tissue formation and repair (Safadi et al. 2009).

1.7 Bone Mineralization

Mineralization of bones occurs through the deposition of calcium hydroxylapatite crystals in soft tissues in response to injuries, traumas, myelomas, and metastases. Three major processes are involved in bone mineralization, which include the normal physiological process, the pathologic dystrophic process in response to injury, and the metastatic process. Deposition of hydroxylapatite crystals occurs in the spaces between adjacent collagen molecules without disrupting the bone matrix. On the contrary, pathologic mineralization is characterized by intracellular deposits of calcium ions in the mitochondria due to tissue injury or trauma. Sometimes, the excess calcium ions from the cytoplasm are pinched off in the form of vesicles leading to extracellular calcium deposits (Safadi et al. 2009).

1.8 Bone Remodeling

Fully developed adult bones constantly undergo remodeling processes mediated by osteoblasts and osteoclasts, wherein old and worn bone tissues are replaced by new bone tissues. This is important because mineral salts such as calcium and phosphate in the bones need to get mobilized regularly for the maintenance of serum homeostasis and for preparation of the skeletal system to withstand various mechanical forces and stresses over the years. Osteoclasts are specialized cells that cluster in areas of active bone growth and remodeling and secrete lysosomal enzymes that break

down old bone tissues and the minerals present in the region. Once the minerals are mobilized and removed, the broken down bone tissues are processed and phagocytosed by the osteoclasts in a process known as bone resorption. Following this, the osteoblasts are recruited at these sites to lay down new bone tissues including the organic matrix and inorganic minerals found embedded in the matrix. The new bone tissues are formed around the osteoblasts trapping them within the organic bone matrix. Once trapped, these cells differentiate into osteocytes or mature bone cells that are incapable of forming new bone tissues (Walker 2020).

1.9 Pathologic Diseases that affect the Human Skeleton

The range of pathologic diseases that affect the human skeletal system may either be due to external injuries or trauma, or due to intrinsic factors and mechanisms that lead to diseases of the bones and joints (Davidson et al. 2010). The major diseases of importance in skeletal pathology that aid in the examination and interpretation of human skeletal remains are described below:

1.9.1 Trauma

Trauma occurs when any external force is applied to the human skeleton that may cause irritation, inflammation, and damage to the bone tissues (Davidson et al. 2010). Based on the source and the type of the force applied, trauma may be categorized into the following types:

i. Blunt Force Trauma

This type of trauma may affect either the internal spongy bone tissue or the outer cortical tissue, and may occur through compression, shear, or torsional forces. The manifestation of trauma in the human body may be either fractures or dislocations. A fracture is an interruption in a bone's structural integrity and may vary in depth and type, resulting in cracks, fissures, or breaks in the cortex. Examination of human remains generally show evidence of healing and callus formation at fracture sites if the fracture has taken place before death. The time of the fracture can also be deduced by analyzing the collagen composition of the bone which contains the fracture site (Davidson et al. 2010).

ii. Sharp Force Trauma

This type of trauma occurs through the application of force using a sharp-edged object on a very small area by means of compressive forces. This type of trauma can be identified in human remains most easily, and it is also the type that can be easily confused with postmortem damage. The types of trauma that occurs by using sharp objects include punctures, clefts, and incisions (Davidson et al. 2010).

iii. Ballistic Trauma

This type of trauma encompasses bullet wounds and other weapons that hit the human body in a projectile motion. The type and extent of the wound depends on the weapon used as well as

elasticity, hardness, and composition of the affected bone tissue (Davidson et al. 2010).

iv. Explosive and Burning Trauma

This occurs when heat energy is directly applied to the human body in the form of convection, induction, or radiation by means of explosions and fire. This type of trauma is the most difficult to examine in human skeletal remains as it destroys all DNA and fingerprint evidence from the body (Davidson et al. 2010).

1.9.2 Infectious Diseases

Any disease in the body is marked by a robust inflammatory response which involves capillary dilation and infiltration of large quantities of cytokines and growth factors at the injured site. Eventually, the coagulation cascade is activated and blood clotting is promoted that leads to several significant changes at the tissue level. Consequentially, if death occurs due to an infectious disease, it becomes very difficult to discern the cause of death because the changes rarely penetrate into the skeleton. The information that we have currently regarding infectious diseases is of those that do leave behind traces of evidence in the skeleton (Ochsner et al. 2016) and some of the more important of these are discussed below:

i. Osteomyelitis

This is a disease of the skeletal system where pyogenic bacteria such as *Staphylococcus* or *Streptococcus* cause infection in the bones. It usually occurs secondary to wound infections and

surgeries and may affect any part of the body; however it is most commonly seen in the skull and limbs. Osteomyelitis affects only the outer bone lining and the cortex and does not penetrate into the internal spongy tissue. It manifests as a zone of tissue deposition around the primary wound at the cortical level in a localized manner (Ochsner et al. 2016).

ii. Periostitis

Although uncommon, it is most often chronic, and directly affects the continual bone deposition processes at the periosteum. Over time, the bone tissue that is deposited in the affected regions appears irregular, variable in thickness, and having a rough surface. Its manifestation resembles a plaque formation that is extremely thick and rough like an ulcer. The challenge in analyzing periostitis in human skeletal remains is that often there is no information available regarding the underlying infectious or non-infectious reasons for periostitis making it difficult to understand its pathological features (Ochsner et al. 2016).

iii. Brucellosis

Brucellosis is caused by three pathogenic species of the bacteria, *Brucella*, which results in a severe and chronic lung disease in humans. In a small percentage of cases, the skeletal system is involved and the commonly affected sites include the spinal column and the pelvic region. The lesions that are formed as a result of this disease are found embedded in the vertebral column and projecting from one vertebra into another. The identification of brucellosis in human skeletal remains points to an endemic and the presence of

domestic vectors such as sheep, goats, horses, dogs, and pigs (Ochsner et al. 2016).

iv. Leprosy

Leprosy is caused by *Mycobacterium leprae* and until a few decades back, it used to be prevalent in every region of the world except the north and south poles. Primarily, it causes infection in the skin, mucous membranes, nerves, and soft tissues. When it reaches the skeleton, it results in periostitis, osteomyelitis, neurotrophic lesions, and arthritis. Given the widespread prevalence of leprosy, analysis of human skeletal remains that showed signs of this disease have been extremely insightful in identification of the body parts that were affected. For the most part, these included limbs, face, nasal bones, palate, and skull with signs of chronic inflammation (Ochsner et al. 2016).

1.9.3 Other Bone Conditions

i. Paget's Disease

Paget's disease, scientifically known as osteitis deformans, is a chronic condition that affects localized regions of the human skeleton. It was first described by Sir James Paget in 1876 who extensively studied the characteristic features and manifestations of the disease such as increase in skull size, severe bone pain, fractures in bones, deformities, and development of malignant lesions with the progression of the disease. The lesions of this disease first begin in the proximal end of the epiphysis and slowly spread across the entire bone. Radiographically, this lesion is observed as a V-shaped lytic wedge which is representative of

osteoclastic resorption. It occurs due to disturbances in the bone remodeling processes and may involve all external and internal layers of entire bones. An important characteristic feature of this disease is that it affects both osteoblasts and osteoclasts simultaneously, resulting in excessive bone remodeling and a characteristic mosaic pattern observed under the microscope (Cundy 2017).

ii. Myositis Ossificans Progressiva

This is a hereditary disease that affects the connective tissues leading to progressive ossification of ligaments, tendons, fascias, and striated muscles. It develops congenitally mostly affecting the toes and manifests as progressive heterotopic osteogenesis restricting movement at specific points in the body. The lesions of this disease are exacerbated at sites of trauma or bone injury; however, it does not affect the tongue, diaphragm, smooth muscles, and cardiac muscles of the body. As this is a genetic disorder, there is no permanent cure available for this disease and the symptoms of this condition can only be managed. As a result, studying the lesions of myositis ossificans in human skeletal remains has thrown light on several features of the progression and manifestations of this disease (Rathee et al. 2016).

iii. Postparalytic Deformities

These are structural deformities that follow a neuromuscular paralytic attack in childhood or adolescence. These deformities mostly affect the spinal column leading to imbalance and scoliosis. As muscles are one of the significant factors that provide support to the skeletal system, a paralytic attack that affects the muscles

causes the bones in that region to lose its structure, size, and shape. Affected extremities can become slender, hip bones alter their angles, and the neck can become elongated (Ochsner et al. 2016).

Although the above mentioned skeletal conditions are by no means representative of all conditions that cause diseases of bones and joints, they do give a broad overview of the types of diseases that may affect different parts of the bones in different ways.

1.10 Methods Used to Analyze Human Skeletal Remains

Diagnosing skeletal conditions in living patients is challenging due to lack of identifiable evidence and suitable analysis methods for skeletal lesions. In contrast, the depth and extent to which well-preserved skeletons can be examined is immense in contrast to living persons (Lundy 1998). Before proceeding to examine a skeletal lesion in human remains, it is important to collect background information about the skeleton that will help in including and eliminating several possibilities of diseases that may have affected the bones of the skeleton. This information, based on the circumstances, may include age, biological sex, ethnicity, geographic location, and time period of survival and death. Age of the person at the time of death may be estimated by analyzing the extent of skeletal remodeling and associated changes in the skeleton, either by naked eye or under the microscope. The estimation of the sex of the person is very easily discerned by examining the pelvic bones – in women, the pelvic canal is significantly larger as compared to men as it is specifically designed for reproduction and child bearing. Apart from the pelvic region, differences in other bones between men and women can also serve as important indicators of the biological sex of a person; for instance, the bones of the skull in women are smaller

and more slender as compared to those of men (Srivastava et al. 2014).

Apart from age and gender, the geographical location of the skeleton is known and using the cues present, sometimes it may also be possible to identify the ethnic background and other demographic characteristics. Once all identifiable features have been accurately noted, gross examination of the skeletal remains is performed by direct inspection. This is done in excruciating detail by noting down every abnormality in every bone, its location, distribution, and morphological description. Attempts are made to identify if the morphological abnormality has originated in the osteoblasts or the osteoclasts, and the underlying mechanisms and pathology that may have given rise to the skeletal condition. Structural abnormalities are also noted that include changes in contour, shape, and size of bones reflecting loss or gain of bone tissue (Randolph-Quinney et al. 2011).

The most basic and widely preferred method used to examine skeletal lesions is through X-ray films or other imaging modalities such as CT. As this is a non-destructive method, it is the first technique of choice for examining human skeletal remains before moving on to more invasive techniques. If a specific disease condition needs to be evaluated, then X-rays of all bones of the skeleton may be necessary. However, if secondary bone conditions need to be identified, then X-rays of specific parts of the skeleton are obtained so that they can be compared with X-rays of healthy skeletons. In order to maintain a standard method of radiographic analysis of human skeletal remains, the bones are arranged in the anteroposterior or the mediolateral direction with the help of small pieces of foam. The information that can be obtained through X-ray film analysis includes the extent of bone tissue that is diseased

internally and characteristic features of lesions present in bones due to underlying disease (Wiersema 2016).

Although biochemical tests form a routine part of diagnostic tests for living people, samples used for these tests such as blood and urine are not available in human remains. Despite this, chemical analysis of bone tissue can still provide several insights into changes introduced in bone proteins as a result of underlying diseases. An example of such a disease is osteogenesis imperfecta that disrupts synthesis of proteins in the bone and results in an increase in quantity of neutral amino acids. Another disease that results in the aggregation of abnormal residues of amino acids is fibrogenesis imperfecta ossium. Chemical investigation, when possible, is done for analyzing changes in amino acid residues, bone mineralization, levels of fluorine (in fluorosis), and levels of strontium, and data obtained from these investigations can provide significant information about chemical changes in the skeletal remains (Wiersema 2016).

Finally, microscopic analysis is done to add to knowledge obtained through other means. As this is a method that destroys the sample in the process, it is only performed if it is considered valuable and if the available sample is sufficient for analysis. Additionally, microradiographs of normal healthy bones should also be available to allow an in-depth comparative analysis. Microscopy of human skeletal remains reveals changes at the cellular level pointing towards abnormal activity of osteoblasts and osteoclasts. Other findings include changes in the quantity of resorption spaces, formation of osteons, and occurrence of osteoid seams (Wiersema 2016).

1.11 Aims and Objectives

This study aims to explore the pathological diseases that affect the human skeleton that can be detected in human skeletal remains, and characterize their types, occurrence, range, and manifestations. It also aims to describe specific characteristic features of these pathological diseases that can help in the identification of the person to whom the skeletal remains belong. In order to accomplish this, an in-depth and extensive systematic review will be performed and the results will be extracted and classified appropriately to answer the questions posed above.

The objectives of this systematic review are three-fold:

1. To provide a general overview of the different pathological diseases that are identified in human skeletal remains and classify them based on type, range, and occurrence
2. To provide an extensive description of the manifestations of these pathological diseases in human skeletal remains
3. To analyze the literature and provide a comprehensive description of how pathological changes in human skeletal remains may be helpful in the identification of the person to whom the remains belong

The results of this systematic review are significant in terms of providing a detailed description of the pathological conditions that are manifested in human skeletal remains and ways by which these pathological changes can help in the identification of the deceased individuals. This comprehensive analysis will throw light on the depth and breadth of literature available on skeletal pathology in human remains. It will expand our knowledge of the pathological diseases

that affected the skeleton systems of people in the past and provide insights into the prevalence and epidemiology of these diseases in specific geographical locations.

Chapter 2

LITERATURE REVIEW

2.1 Introduction

Physical anthropology or the study of human skeletal remains depends on the identification of several parameters at death such as age, sex, stature, ancestry, pathological processes evident upon the hard tissues of the body, and other individuating features such as medical prosthetics and unique dental restorations that constitute a person's biological identity. However, these characteristics might be shared between thousands of individuals worldwide and are not suitable for a positive identification of the deceased individual. Therefore, specific features, pathological or non-pathological, of the human skeletal system can aid in the construction of a biological profile of an unknown person using which the identity of the deceased person may be established. Not only can the pathological features of bones aid in identification, but they can provide valuable insights into the physiological features that led to the death of the person (Cunha 2006). The objective of this literature review is to examine the available literature regarding the use of skeletal pathology features in the understanding of disease and death, and how they can be used to assist in the identification of the deceased persons.

2.2 Classification of Skeletal Pathologies

In the evaluation of skeletal pathologies, several levels of analysis are performed. The first is to identify the difference between pathological changes that have taken place ante-mortem or post-mortem. Fossilization processes may introduce changes in human remains that may often be confused with skeletal pathologies that have occurred as a result of disease. Examples of pathologies that occur as a result of forensic taphonomy include bites from carnivore scavenging, the action of microfauna such as nematodes and small arthropods, interaction with roots of plants and trees, and effect of extreme environmental conditions such as temperature and high-

speed winds. Hence, these pseudopathologies need to be identified and eliminated from the study (Ubelaker 1988).

Once the pathological alterations that have taken place before death are identified, they need to be characterized as morphological or pathological changes. Morphological changes in the skeleton are of four major types and these include accessory ossicles, accessory foramina, nonfusion disorders, and other changes that comprise of all features not present in the other three categories. These features need to be carefully identified as they may sometimes be confused with pathological changes in the skeleton (Cunha 2006).

Once pseudopathologies and morphological changes are eliminated, the pathological changes in the bones need to be identified, examined, and characterized. Identification of skeletal pathologies is always a challenge because skeletal remains can be less informative when compared to a living person, or even the body of a person who has recently died, or been well preserved. Also, most diseases or trauma do not leave lasting marks on the skeletal system and this is often the last system to be affected in response to a disease. Hence, when dealing with pathologies in skeletal remains, it is often said that the absence of evidence is not evidence of absence (Skinner et al. 2003). If no skeletal pathologies are present, the inference cannot be that the person did not suffer from any disease; rather, it should be noted objectively that no pathologies in the skeletal remains were observed (Skinner et al. 2003).

2.3 Pathological Features of Bone Diseases

According to the available literature, the characteristic pathological features of bone diseases that can be identified in human skeletal remains have been described below.

2.3.1 Joint Diseases

2.3.1.1 Osteoarthritis

Osteoarthritis is a degenerative disease that affects a huge number of people worldwide. Although its presence alone is not very useful in identifying a person, the location of the lesions, pattern of distribution, severity of the lesions, and presence of primary or secondary osteoarthritic lesions can provide significant indicators regarding the identity of the person. Osteoarthritic lesions on the spinal column are never identical between two people and their characteristic features such as colour, form, and extent of the margins can provide insights for the identification of an unknown individual. However, the researcher needs to have access to X-rays of the lesions taken prior to death to perform a comparative analysis of the post mortem lesions to provide a positive identification (Angyal and Derczy 1998). Additionally, the study of osteoarthritic lesions in human skeletal remains can provide valuable insights regarding overuse of a certain part of the body, for example, an osteoarthritic lesion on the hip might be indicative of problems with body balance and gait (Valenzuela 1997).

2.3.1.2 Rheumatic Diseases

These diseases cause inflammatory reactions in the spinal column and sites of attachment of the peripheral joints to the bones and the most common of these are seronegative spondyloarthropathies. Of these arthropathies, ankylosing spondylitis is the most prevalent disease and its uniform pattern of calcification and ossification throughout the spinal column is quite easily identifiable. Another characteristic feature of ankylosing spondylitis is sacroiliitis and abnormalities in the sacroiliac region point towards an early stage of the disease. In one case study of a male person, the total vertebral column, thorax, pelvis, and both clavicles were fused

and skeletal patterns of ankylosing spondylitis were used for identification of the person (Alava et al. 2000). Apart from identification, ankylosing spondylitis can also provide insights into the lifestyle of the person. Due to the fusion of the entire spinal column, the normal mobility of the person will be affected which may lead to the secondary complication of osteoporosis. In some cases, skeletal lesions of osteoporosis may also be observed in human remains along with lesions of ankylosing spondylitis (Cunha 2003).

2.3.1.3 Diffuse Idiopathic Skeletal Hyperostosis (DISH)

The major manifestation of DISH is ossification of the ligaments and ankylosis lesions of the spinal column, but no involvement of the cartilage, synovium, or intervertebral discs. This can be characteristically identified by ossification lesions forming a flowing pattern along at least four vertebral bodies on the anterior side. Other identifying features include lack of apophyseal fusion, and enthesopathies in the posterior surface of the heel, superior surface of the patella, and oleocranon at the elbow. Several skeletal remains of older males have shown the presence of these lesions that have helped in their identification (Resnick 1998).

2.3.2 Infectious Disorders

One of the important infectious disorders that cause characteristic skeletal lesions is periostitis, which is formation of new tissue in the periosteum. Rather than a specific disorder, it is a consequence of other trauma or diseases that may trigger the formation of layers of new bone tissues. According to Pinheiro et al. (2004), analysis of periostitic lesions on the skeleton can give major insights into the specific cause of the disease. In one case study of the examination of skeletal remains of an old woman, extensive reactive periostitic ossification lesions were identified that manifested as periostitic thickening in the tibia and the fibula. This eventually led

to the diagnosis of venous chronic insufficiency which was confirmed by the family of the deceased (Pineiro et al. 2004).

Periostitis is a non-specific indicator of skeletal stress and so, it may hold value in identifying physical abuse caused to a victim (Hunter et al. 2001). If, along with periosteum, the cortical lining and the bone marrow are also affected, it is indicative of osteomyelitis (Ortner 2003). Even bacterial infections such as tuberculosis, leprosy, brucellosis, and treponematosi leave characteristic skeletal lesions that can aid identification of deceased individuals. As brucellosis is transmitted from animals to people, it may give indicate contact of the person with animal and/or animal products, either through direct physical contact, air, or consumption of raw and unpasteurized dairy products. It is commonly identified by lytic cavitations on the vertebral column and lesions in the sacroiliac joint (Roberts 1996).

2.3.3 Metabolic Disturbances

The most prevalent metabolic disorders that affect the human skeleton include scurvy and rickets that leave a characteristic trail of skeletal lesions on the body. They mostly occur due to deficiencies of vitamins C and D respectively and are important indicators of malnutrition in the person. In contrast to developed countries, people in developing regions such as Africa are often malnourished and metabolic skeletal lesions in these populations are a common find. On the other hand, skeletal lesions of vitamin deficiencies, such as osteoporosis, that are widely prevalent globally are not very valuable in the identification of human remains and such lesions cannot provide a lot of information about the age, gender, or location of the person (Cunha 2006).

Fluorosis is a metabolic condition that can be identified by its characteristic lesions on the human skeletal system. When a person

undergoes intoxication due to accidental or deliberate consumption of large quantities of fluoride there are several irregular spurts of new bone formation throughout the skeleton system. These growth foci can be used to diagnose fluorosis and uncover information relating to the general health and well being of the person.

Deficiency of iron also has consequences for the human skeletal system. Some of the important pathological changes brought about by iron deficiency include porotic hyperostosis, cribraorbitalia, and pitting of the orbit roof. A secondary consequence of iron deficiency includes bone marrow hyperplasia which can lead to increase in size of the diploe and the occurrence of a pitting pattern on the bones (Aufderheide and Rodriguez-Martin 1998).

2.3.4 Neoplasias

Neoplasias of the bones may be benign or malignant and their characteristic appearance in the skeletal remains depends on several factors such as location, severity, and extent of spread from the site of origin (Manuel et al. 2004). Benign neoplasias are known as osteomas and they have limiting margins often surrounded by a capsule. They have the characteristic appearance of a button and can be easily used for identification of the deceased individual if X-ray records of the individual before death are available. On the other hand, malignant neoplasias are more widespread and its lesions can have varied and irregular shapes and sizes (Manuel et al. 2004). An example of a malignant neoplasia is femur osteosarcoma and its characteristic pattern of lesions can provide confirmative evidence of the person's identity. The most affected parts of the body in malignant tumors are bones of the skull, ribs, pelvis, and the vertebral column. Lesions caused due to prostate cancer may be osteoblastic or may show spiculae formation. Lesions caused due to lung and breast cancers may be either osteoblastic or osteolytic in nature (Manuel et al. 2004).

2.3.5 Enthesopathies

Enthesopathies are ligament disorders that result in pathological changes at sites of attachment of tendons to bones. Pathological lesions at these sites provide valuable information regarding the type and amount of physical activity done by the individual, which may result in the hyperdevelopment of a lesion at a particular location. Enthesopathies are usually indicative of occupational stress and they commonly include hypertrophies, lytic lesions, and additional bone facets. For example, a lesion on the tendon insertion site of the Achilles heel may indicate overuse of this site or a lesion on sites of attachment of the upper limbs point towards handedness in the person. Apart from use-related stress, the causes for enthesopathies may also include spondyloarthropathies and diffuse idiopathic skeletal hyperostosis (DISH) (Kennedy 2000).

2.3.6 Congenital Disorders

A considerable percentage of these disorders manifests as skeletal pathologies in people before they are born while they are in the uterus (Aufderheide and Rodriguez-Martin 1998). Some common examples of congenital disorders that affect the human skeleton include hyperplasia, hip dislocation, defects of the skull and the spine, cleft palate, and craniosynostosis. An important lesion of the spinal column is the congenital fusion of two or more cervical segments of the vertebral column known as the Klippel-Feil syndrome, which provides an important identification characteristic for human skeletal remains (Aufderheide and Rodriguez-Martin 1998). Congenital hip dislocation can indicate problems with gait and can be another important indicator for identification of the deceased (Cunha 2001). In one case study, the remains of a male skeleton showed numerous malformations and lesions on the spine, an important one being the fusion of the first two ribs along with asymmetry of the bone and scoliosis. These features eventually led

to the identification of the person by means of macroscopic analysis and radiographic techniques (Cunha and Mendonca 2000).

2.4 Case Studies from the Literature

Until the early 1960s, there were just a handful of published studies outlining the parameters of characteristic lesions in human skeletal remains and use of these lesions to identify the deceased individuals (Austin and King 2016). Given below are a few significant case studies that have highlighted the methods used to study, classify, and identify bone pathologies in skeletal remains and use these to accurately identify the deceased persons.

2.4.1 Estimation of Biological Sex

In 1963, the Crime Laboratory Director of the Fort Worth Police Department came across skeletal remains of a deceased person. Using the available knowledge and resources at that time, he measured the maximum diameters of the heads of the humerus and femur bones, and deduced that the skeleton belonged to a male person. The lengths of the femur, humerus, and tibia were accurately measured to deduce the stature of the person. Analysis of the pelvic bones would have strengthened this deduction; however, this analysis was not performed due to extensive fragmentation of this region. Despite the fact that the measurements clearly pointed towards the skeleton belonging to a male person, the records indicated that the skeleton had been classified as belonging to a female person. The case was reopened in 2004 in order to identify the deceased person and a facial reconstruction based on the skeletal remains was circulated via news channels. The person was identified as Kenneth Glaze, a 35-year old man, who lived in Texas and who was reported missing since August 1963 after acquaintances came forward with the deceased's personal information and earlier photographs (Austin and King 2016). This

case study highlights the fact that modern technologies are better equipped to estimate age, gender, and pathology of skeletal remains. It also throws light on the importance of gathering all possible evidence before coming to a conclusion.

2.4.2 Identification of Extensively Damaged and Mutilated Remains

Waghmare et al. (2015) studied 51 skeletal remains that were burned either due to fire-related accidents or intentional burning. Out of these skeletons, four of them revealed skull fractures indicating that their death was caused due to trauma to the head before they were burnt. From these four skeletons, two of them revealed additional fractures to the limbs indicating that they had sustained injuries to multiple parts of their body prior to their death. Out of the initial 51 skeletons that were analyzed, four of them were highly mutilated having only legs, arms, a head, or a trunk. These skeletons along with a 2 others did not provide conclusive evidence of sex or age and hence, these could not be deduced accurately. All human remains, except two, were positively identified using DNA fingerprinting analysis (Waghmare et al. 2015).

In five cases, forensic odontology was used for identification such as silver fillings, artificial or missing teeth, presence of artificial crowns, tooth deformities, or other peculiar dental features. In cases where the skeleton had sustained one or more injuries, the type and degree of trauma could be deduced to a large extent along with the detection of accidental or intentional death of the person. Overall, the authors identified that DNA fingerprinting was the most useful technique for tracing the identity of a person followed by forensic odontology analysis. In a lesser number of cases, clothes and personal belongings provided sufficient information regarding the identity of the person (Waghmare et al. 2015). This report throws light on the challenges involved in deducing the age and sex of

skeletal remains, identifying the cause and mode of death, and determining without doubt the identity of the persons.

2.4.3 Exhumation and Identity Determination

Exhumation refers to the process of digging up corpses from their places of burial in order to establish their identity and for crime investigation. In one such case report, a 10-year old girl's body was exhumed on the insistence of her mother two years after she went missing. This child was kidnapped, sexually assaulted, strangled, and then buried so that her remains would not be discovered and traced back to the kidnappers. The accused persons admitted to their crimes and revealed the location where they had buried the girl's body two years after the crime. Once the girl's body was exhumed, personal belongings on the body helped in the initial identification by the girl's mother. The skull was fractured and the mandible was present in two pieces along with two clavicles, two scapulae, manubrium, and twenty rib bones. All the other bones were also present as expected with some of the bones being present in two or three fragments. A thorough examination of these bones revealed the age and sex of the skeleton to be around 10 to 12 years and female, which was later confirmed by DNA analysis. The height of the child was determined to be around 138 to 140 cm. DNA profiling analyses was used to provide a confirmative conclusion regarding the identity of the child (Rani et al. 2012).

2.4.4 Use of Bone Pathology in Homicide Cases

Despite the fact that soft tissues provide the most accurate diagnostic evidence in deducing the time and cause of death, skeletal remains can also provide interesting insights regarding the trauma inflicted on the person and the shape of the weapon that caused it. In 2001, a woman was reported missing in Rome and several years later, her partner confessed to killing and burying her in

his garden (Donato et al. 2016). He also stated that he had hit the woman causing her to fall and hit her head on a fireplace built with crude stone. Once the skeletal remains of the woman were recovered, a lesion was noticed on one of her thoracic vertebra, and a small bone fragment was found covering the lesion. This bone fragment had a different colour compared to the rest of the bone as there were still some soft tissues present on the bone which protected it from decomposition. The soil composition and pressure also had an effect on the colour of the bone fragment as the soil particles couldn't gain access to the lesion and its corresponding piece of bone. Sex was determined by identifying the characteristic size and shape of bones and the conclusion that the skeleton belonged to a female was given without doubt. Other lesions included osteoarthritis of thoracic vertebrae and several small lesions that showed complete healing. The peri-mortem lesion that led to the woman's death was found on the mandible indicating a compound fracture and demonstrating chromatic homogeneity around the fractured edges. In this way, the skeletal remains were identified to belong to a female of 54 years of age, with lesions as confirmed from her partner that had led to her death (Donato et al. 2016).

Chapter 3

METHODS

3.1 Search Strategy

In order to find relevant journal articles for addressing the objectives stated earlier, a well-defined search strategy was used to search for original research articles in accordance with the quality guidelines defined by PRISMA (Moher et al. 2009). The PubMed database was used for the search and the search string entered in this database was (Bone Pathology OR Paleopathological Evidence) AND (Human Skeletal Remains OR Human Identification) AND (Forensic OR Paleopathology OR Crime Scene Investigation). Articles that were in English and provided access to the full text of the article were included.

3.2 Inclusion and Exclusion Criteria

Studies that specifically used pathological conditions of the bone or pathological changes in the bone due to other tissue disorders as a means for human identification or even age and sex determination were included. Both original research articles as well as case reports were included in the review. Articles that used bone pathologies to deduce the lifestyle of people living in a specific era and geographical location were included in the study. Articles that implied usefulness in human identification by studying specific skeletal pathological features were also included.

Studies that described extraction methods and environmental conditions in relation to analysis of human skeletal remains were eliminated from the study. Also, articles that only described morphological features of bones for human identification rather than pathological features were also excluded from the study. Studies conducted before the year 2000 were excluded as recent developments have made the analysis and description of bone pathologies more extensive. Articles that were in the form of

editorials, letters, short reports, comments, systematic reviews, literature reviews, and discussions were excluded.

3.3 Study Screening and Selection

The pre-defined inclusion and exclusion criteria were applied to the search results to finalize the list of studies to be included in the present review. The titles and abstracts of all the articles were analyzed to look for relevance for this study. In instances where a conclusive decision could not be taken on the title and abstract alone, the full text of the article was scanned to see if it met the inclusion criteria. The reference lists of the studies were also checked to identify other relevant studies by means of backward citation tracking.

3.4 Data Extraction and Synthesis

Once the studies to be included in the review were finalized, a data extraction spreadsheet was developed where important details were extracted and organized for each study. These details included type of study, geographical location, sample size, age of specimen(s), specific parts of the skeleton studied, pathological condition(s) reviewed, characteristic pathological features identified, technique(s) used for study, and the degree of success in human identification. The studies were categorized based on the type of trauma/pathological condition that was being studied in the skeletal remains and studies investigating similar types of trauma / pathological condition were grouped together under the same category.

3.5 Critical Appraisal

Critical appraisal of the studies was conducted using the CASP (Critical Appraisal Skills Programme) guidelines (given in Appendix 1). This sheet comprises of 10 questions that evaluate the

study objectives, research design, methodologies, data collection, data analysis, discussion of findings, and consideration of ethical issues. Using the CASP guidelines, the studies were classified as: (+++) satisfying 91% or more of the criteria, (++) satisfying 51% to 90% of the criteria, and (+) satisfying 1% to 50% of the criteria. Apart from the CASP guidelines, the studies were also analyzed to see if limitations and/or weaknesses of the studies have been considered and if the usefulness of the findings could be scaled up to a global level.

Chapter 4

RESULTS

4.1 Features of Studies Included in the Review

Using the PubMed database, 81 studies were identified initially which were then carefully screened to remove multiple studies by the same group and duplicates. Further, the studies were subjected to a rigorous screening process based on the inclusion and exclusion criteria defined in the Methods section. Finally, 23 studies remained which were included in the review. During the search procedure, it was seen that there were several articles that either studied or invented new extraction and analysis methods for studying bone pathologies in human skeletal remains. Comparatively, there were fewer articles that focused on using bone pathologies as a means to identify humans by estimating their age, gender, and other defining characteristics. Another notable fact was that the specified years were a limiting factor during the search procedure as most research on paleopathological human identification has been conducted before the year 2000. Several studies also described morphological patterns and alterations of bones as a means to identify humans. However, these were not included in the review as the objective here was to study pathological features of bones in relation to human identification.

The characteristic features of individual studies are presented in Table 1. These studies were highly heterogeneous with regards to location of excavation sites, geographical time periods, and specific pathologies investigated, which provided a rich diversity in the information obtained for the review. Three of these studies analysed the skeletal remains of famous people and 8 of the studies analysed the skeletal remains of more than one person. The various pathologies studied in these articles included osteoporosis, osteoarthritis, ankylosis, tuberculosis, cancers, rotator cuff disease, Paget's disease, dental anomalies, and Diffuse Idiopathic Skeletal Hyperostosis (DISH). Most of the studies used macroscopic analysis

and radiographic techniques to analyse skeletal lesions, whereas a few used advanced histological and molecular techniques to uncover further information about the pathologies identified in the skeletal remains.

4.2 Studies Used for Human Identification

Only three of the studies were case reports where skeletal lesions positively confirmed the identity of a person. One of these was an unusual case where a famous singer, Farinelli, had castrated himself before he hit puberty so that he could preserve the softness in his voice for his musical success. Investigation of his skeletal remains led to the identification of Hyperostosis Frontalis Interna (HFI), degenerative joint disease, and osteoporosis secondary to castration which helped confirm that the skeletal remains belonged to him (Belcastro et al. 2011). In another report, the presence of unilateral lumbosacral transitional vertebra helped confirm the identity of the deceased (Kanchan et al. 2009) and in yet another report, the identification was confirmed through an anomaly of a tooth position (Tinoco et al. 2010).

4.3 Studies Used for Evaluation of Trauma

Several studies have evaluated the effects of different types of trauma on the human skeleton. For instance, a study by Petrone et al. (2018) studied the skeletal remains of 80 individuals to characterize the effects of extreme burning trauma on the skeleton due to the volcanic eruption of Mount Vesuvius. Two other studies (Rahimi et al. 2015; de Almeida Prado et al. 2019) evaluated lesions caused due to bullet wounds in the skull. In yet another study conducted by Reinhard et al. (2013), the mutilation and murder of a young female was evaluated, and extensive fragmentation and fractures in her bones was studied.

Study	Geographical Location	Aim of the Study	Sample Size	Pathological Conditions Studied	Techniques used for Study	Results	Conclusion
Afzal et al., 2017	Ayub and Khyber Medical College, Pakistan	To observe diaphyseal nutrient foramina in long bones of the lower limb	90 long bones - 30 femora, 30 tibiae, 30 fibulae	Diaphyseal nutrient foramina	Macroscopic, counting of foraminae that were present 1mm away from the borders	Nutrient foraminae found in all three types of bones studied, most of them directed distally	Number and direction of nutrient foraminae in long bones of the lower limbs can potentially be used for human identification
Bartsiokas et al., 2015	Royal Tombs in the Great Tumulus in Vergina	To study the remains of a male, female, and neonate in Tomb 1	3	Flexional Ankylosis of left knee of male, osteoarthritis and laterocollis in male due to knee injury	Direct analysis, CT, scanning with 16-slice scanner, radiography, macrophotography, surface scanning	Identification of ankylosis in male as a result of severe wound to the knee causing lameness and uneven gait, persons identified as King Philip II,	Estimation of age, sex, stature, ethnicity, lesions on leg of male

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						wife and new born child	
Belcastro et al., 2011	Bologna	To study the effects of castration in the skeletal remains of famous singer Farinelli	1	Osteoporosis, degenerative joint disease of vertebral column, Hyperostosis frontalis interna (HFI)	Direct analysis	Characterisation of HFI in a castrated male	Osteobiography confirmed the skeletal remains to belong to Farinelli
de Almeida Prado et al., 2019	Brazil	To study a gunshot wound lesion in a skull preserved in the Cranium Museum, UNIFESP, Brazil	1	Gunshot wound to the head	Macroscopic analysis, CT scan	Characterisation of the path of the bullet wound through the skull	Data not used for human identification but has strong potential for similar cases
Holloway et al., 2013	Galler collection, Switzerland	To study skeletal lesions of tuberculosis	29	Skeletal lesions in tuberculosis	Macroscopic analysis	Characterisation of spinal and non-spinal lesions as a	Studying tuberculosis skeletal lesions may

		and their healing process in skeletal remains from a collection				result of tuberculosis and their healing status	be used for human identification
Kacki et al., 2018	Prague	To investigate the cause of death of the astronomer	1	Diffuse Idiopathic Skeletal Hyperostosis (DISH)	Macroscopic analysis, CT scan, X-ray, stable isotope analysis	Characterisation of lesions related to DISH	Diagnosis of DISH in skeletal remains may aid human identification
Kanchan et al., 2009	Coastal Karnataka, India	To study skeletal lesions of congenital abnormalities - transitional vertebra	1	Unilateral lumbosacral transitional vertebra	Macroscopic analysis, radiography	Characterisation of congenital abnormality	Skeletal pathology studied helped in confirmed identification
Lieverse et al., 2014	Cis-Baikal region of Siberia	To study skeletal lesions of metastatic carcinoma	1	Malignant Neoplasia, Metastatic Carcinoma	Macroscopic analysis, radiography	Characterisation of 34 metastatic lesions	Data not used for human identification but has strong potential for

		from the early time periods					similar cases
Masson et al., 2013	Southern part of Hungary	To study skeletal lesions from an individual suspected to have had tuberculosis	1	Tuberculosis, hypertrophic pulmonary osteopathy	Macroscopic analysis, biomolecular analysis	Confirmation of HPO secondary to tuberculosis	Data not used for human identification but has strong potential for similar cases
Minozzi et al., 2018	Necropolis of Casal Bertone, Rome (Italy)	To study skeletal lesions of metastatic carcinoma from the early time periods	1	Advanced prostate cancer	Macroscopic, microscopic, radiographic, histologic analysis	Characterisation of skeletal lesions in prostate cancer	Data not used for human identification but has strong potential for similar cases
Moghaddam et al., 2013	Canton of Lucerne, Switzerland	To study an unusual bony alteration of the skull and obtain a	1	Multiple osteosclerotic lesions, possibly benign tumor	Macroscopic, X-ray, CT, histology	Characterisation of osteosclerotic lesions of benign tumor	Data not used for human identification but has strong potential for similar cases

		differential diagnosis					
Panzer et al., 2014	Peruvian/Northern Chilean coastline, South America	To reconstruct the life story of a female mummy	1	Massive skull trauma, Chagas disease	Macroscopy, CT scan, histology	The origin, life, and diseases of the mummy were studied and characterised	Estimation of geographical origin, ethnicity, age, sex, and characterisation of Chagas disease and skull trauma pointing to ritual homicide
Petrone et al., 2018	Herculaneum, Pompeii, and Stabiae	To study the effects of the volcanic eruption of Mount Vesuvius on its victims	80	Burning trauma	Macroscopy, microspectroscopy, mass spectrometry	Characterisation of multiple fractures associated with exposure to extreme heat due to volcanic eruption	Data not used for human identification, only for characterisation purposes
Rahimi et al., 2015	Selangor, Malaysia	To characterise the lesions of a bullet	1	Trauma	X-ray	Characterisation of lesions found in fire victims	Data not used for human identification but has strong

		wound and burns					potential for similar cases
Reichart et al., 2006	Tbilisi, Georgia	To investigate skeletal manifestation of the presence of a metallic nail in the dentition	1	Presence of metallic nail in dentition	Radiography	Characterisation and analysis of reasons for nail adaptation in dentition	Data not used for human identification, only for characterisation purposes
Reinhard et al., 2013	Nebraska	To use a homicide case report for commenting on the Depravity Standard as a measure of criminal depravity	1	Trauma	X-ray, macrophotography	Characterisation of multiple trauma types and successful identification of the victim	Estimation of age, sex, race, stature, and skull reconstruction that aided human identification from a missing report three years back
Robbins et al., 2009	India	To characterise lesions of	1	Leprosy	Microscopy	Characterisation of leprosy lesions in	Estimation of age, sex, and burial rituals

		leprosy for estimating the spread of the disease globally				skeletal remains from 2000 BC	
Roberts et al., 2007	Britain	To characterise the range of lesions associated with rotator cuff disease	86	Rotator cuff disease	Direct analysis, statistical methods for recording frequencies of lesions	Characterisation of type, frequency, and pattern of lesions related to rotator cuff disease	Data not used for human identification but has strong potential for similar cases
Shaw et al., 2019	Norton Priory, North West of England	To characterise lesions of Paget's disease of bone	18	Paget's disease	Macroscopy, radiography	Characterisation of lesions associated with Paget's disease	Data not used for human identification but has strong potential for similar cases
Shepstone et al., 2000	Barton-on-Humber, north of England	To study distribution of osteophytes around femur in cases with	121	Osteoarthritis of the knee	Direct analysis, statistical analysis	Characterisation of presence and quantity of osteophytes around femur in presence and absence	Data not used for human identification, only for characterisation purposes

		and without osteoarthritis				of osteoarthritis	
Tinoco et al., 2010	Sao Paolo, Brazil	To use dental anomalies for human identification	1	Dental anomalies	Photography, skull-photo superimposition, craniofacial analysis	Use of dental anomalies to positively identify missing person	Anomaly in tooth position used for positive identification
Vairamuthu and Pfeiffer, 2018	Southwestern Ontario, North America	To characterize bone wasting in a juvenile skeleton	1	Bone wasting and stunting - Osteogenesis imperfecta type IV	Direct analysis, radiography, osteometry	Characterisation of skeletal lesions in osteogenesis imperfecta	Data not used for human identification but has strong potential for similar cases
Verlaan et al., 2007	Abbey court (Pandhof), Maastricht, Netherlands	To study skeletal lesions associated with DISH	51	Characterisation of lesions of DISH in three skeletons			Data not used for human identification, only for characterisation purposes

Table 1: Summary table of studies included in literature review

Chapter 5

DISCUSSION

5.1 Introduction

This literature review was undertaken in order to acquire, analyze, and evaluate evidence for the use of skeletal pathological lesions for construction of a unique biological profile that can aid human identification. The availability of 23 studies and case reports that have used skeletal lesions to aid positive identification of deceased victims shows that skeletal pathologies are extremely useful in forensic anthropology for providing a basis for the identity of a person. One of the reasons for this may be availability of sophisticated technologies for macroscopic and microscopic analyses of human remains. During the process of literature search, several articles were obtained which focused on different techniques for analysing pathological lesions in skeletal remains. This is evident in the available literature where relevant methodologies based on specific cases are used to provide a strong basis for identifying the remains of the deceased.

The remaining 20 articles that were included in the review characterised various diseases that caused pathological lesions in the skeleton. Such studies add to the knowledge base of possible skeletal lesions and their characteristic features for different diseases. Eventually, this type of information can aid human identification in forensic anthropology cases.

Based on the type of disease condition and/or trauma evaluated in the article, each article has been placed under a specific category. Each of these categories has been elaborated below in order to enhance our understanding of possible skeletal lesions that may point towards the presence of specific disease conditions in the human body.

5.2 Trauma

There were four studies that evaluated the lesions associated with application of trauma to the human body. Two of them studied the effects of fire on the human skeleton, one study evaluated the effects of a bullet wound to the skull, and the fourth study analyzed a homicide case where the body had been fragmented post-murder. The pathological lesions identified in each of these studies are presented below.

5.2.1 Thermal Damage

Petrone et al. (2018) conducted a very interesting study where they attempted to characterise the effects of a volcanic eruption on human skeletons. Mount Vesuvius, located about 12 kilometres away from Naples, is considered to be one of the most dangerous volcanoes undergoing a massively destructive explosion once every 2000 years. The last volcanic eruption to have occurred is dated back to 79 AD which claimed the lives of thousands of people living in nearby towns and cities. Another case report that studied the effects of burns on a human skeleton was investigated by Rahimi et al. (2015) where a young male was subjected to a bullet in the skull and his body was burned in an oil palm plantation in Malaysia. Their analysis brought to light a unique finding which was the presence of small black particles within the blood vessels of the organs. Both these studies have extensively characterised the effects of extreme heat on the human skeleton, the significant findings of which are discussed below.

The study by Petrone et al. (2018) identified different effects of heat on the skeleton based on the proximity of the individuals to the volcanic eruption. Some of the effects observed were charring and cracking of bones, contraction of hand and foot due to the flexor reflex mechanism, and hyperextension of the spinal column. Thermal

fractures typically appear as polygonal and linear cracks, which was evident in the analysis of skeletal remains in these sites. Extreme heat had resulted in an explosion of the skull leading to fractures with a stellate appearance as in fracture lines radiating from a common centre. These fractures were clear-cut with sharp and distinct margins coupled with darkly stained regions as the areas exposed due to fractures were charred as a result of thermal exposure (Petrone et al. 2018).

As this study investigated the effects of unimaginably high temperatures on the human skeleton, several unique occurrences were identified. Due to thermal fractures, the contents of the brain were exposed causing the evaporation of the organic liquids present in the brain tissues. This space was replaced by ash, which is an indicator of the extreme temperatures caused due to the volcanic eruption leading to the penetration of the intracranial cavity following the loss of organic fluids and soft tissues (Petrone et al. 2018).

In contrast to the study conducted by Petrone et al. (2018), Rahimi et al. 2015 investigated a case where a person had been recently killed and burned in an oil palm plantation in Malaysia. The person's death had presumably occurred due to a bullet wound to the head as evidenced by the presence of a foreign object within a fractured skull. A comprehensive skeletal analysis uncovered information such as the person's sex, height, and weight. The person's face was charred beyond recognition, and exposure to heat had caused flexion of the upper extremities and loss of soft tissue exposing several bones throughout the body. Thermal fractures were marked by a spider-web pattern similar to the pattern observed by Petrone et al. (2018) in volcanic eruption victims. The condition of the body in this case proves that fire is a challenging murder weapon as it destroys several important indicators of a person's identity. Fire usually affects the skull first causing multiple fractures and

eliminating the possibility of facial recognition followed by DNA analysis (Rahimi et al. 2015). Therefore, it is no wonder that the identity of the person could not be determined in this case investigation.

5.2.2 Ballistic Trauma

Rahimi et al.'s (2015) case investigation (described above) did not reveal many details about the effects of the bullet wound as the body had been heavily engulfed by the fire. They found a 1-cm entry point of the bullet on the left part of the mandible with the bullet still lodged in the skull proving the occurrence of a gunshot wound (Rahimi et al. 2015).

One other study in this review that investigated the effect of a gunshot wound on the skull was conducted by de Almeida Prado et al. (2018) who used a skull specimen from the Cranium Museum at UNIFESP, Brazil. This specimen was unique as the bullet wound had not caused the death of the person, but showed a remarkable healing pattern in the skull. It belonged to a 42-year old male who worked in a factory and who had died due to heart failure (de Almeida Prado et al. 2018). The wound lesion was present on the anterior-lateral part of the frontal bone of the skull with a piece of metal attached to it. The authors hypothesized that upon entry into the skull, the bullet underwent fragmentation lowering its energy intensity and causing a piece of it to remain within the skull. As the bullet's energy was lowered upon entry, it did not result in any life-threatening fractures as a result of which the individual could survive the gunshot wound. Healing of the wound was observed in the form of bending, a linear line of fracture, and bone remodelling signs (de Almeida Prado et al. 2018). This case report was not used for human identification; rather, the details of the individual to whom the skull belonged were already available in the museum. However, this case report is unique as the type of wound caused by the bullet allowed

the authors to speculate the type of weapon that could have fired a low-energy and low-intensity bullet that did not take the life of the person. This type of information can be useful in forensic anthropology cases where the cause of death of a person is under speculation.

5.2.3 Blunt Force and Sharp Force Trauma

Several articles in the news provide reports on the extremely disturbing ways in which people commit homicide; however, few provide an in-depth analysis of the victim's skeletal remains. Reinhard et al. (2013) described a homicide case in Nebraska where the body had been brutally fragmented and only the bone fragments of the individual were available for analysis. A superficial examination of these bones revealed several cut marks on them indicating that the persons responsible for the homicide had made several unsuccessful attempts to cut the bones presumably after the death of the individual. Once all the bones had been recovered from the site and analyzed, it was found that several of the bones were missing and the ones that were recovered were extremely fragmented. Despite the nature of the obtained skeletal remains, the researchers were able to determine the race, sex, and age of the individual. These were consistent with the reports of a missing person that was circulated three years prior to the discovery of the body, and by correlating this data, the identity of the deceased individual was confirmed through DNA analysis (Reinhard et al. 2013).

This case report represents three types of trauma applied to the skeleton: sharp force trauma, blunt force trauma, and patterned injury using a cylindrical weapon. The analysis of traumatic skeletal lesions revealed the extent and nature of struggle that may have taken place between the victim and the perpetrators. For instance, a circular lesion over the right eye orbit indicated that the perpetrator may have tried to attack the eye and the victim would have moved

her head causing an injury to her forehead. Lesions on the vertebrae were indicative of repeated attacks to the abdomen causing several deep fractures. In this way, several other lesions were described that helped the authors establish the details of how the criminal acts against the victim had taken place (Reinhard et al. 2013). Although the person had already been identified, the description of the types of wounds inflicted on the victim is important to track individuals who have a particular pattern of attacking their victims. This type of information can help the police identify the criminals thereby preventing these types of incidents in the future.

5.3 Bone Diseases

Diseases that cause pathological lesions in the bones that were described in studies included in this review were Diffuse Idiopathic Skeletal Hyperostosis (DISH), metastatic carcinoma, osteoarthritis, Paget's disease of the bone, and rotator cuff disease. Studies that have thrown light on the specific skeletal lesions of these disorders are described below.

5.3.1 Diffuse Idiopathic Skeletal Hyperostosis (DISH)

DISH is a systemic disorder that mainly affects the vertebral column resulting in the ossification of the anterolateral aspect of the individual vertebra. When the lesions appear on extraspinal surfaces, they appear as bony spurs causing peripheral enthesal ossification. Several studies have characterised the age-related, gender-related, and race-related ratios of DISH in the population to understand its global prevalence. A study conducted by Verlaan et al. (2007) characterised the lesions of DISH in 51 skeletons that belonged to ancient clergymen in order to understand the prevalence of the disorder in this group of people. They have extensively described the lesions of DISH for three skeletons which throw light on the specific bone pathology that represents this disorder.

The first skeleton showed the presence of osteoarthritis in the vertebral column and healed fractures in the ribs. Ossification was observed at several locations along the anterior longitudinal aspect, using which a confirmed diagnosis of DISH was made. An interesting observation was that ossification was absent in the part where the aorta was located proving that aorta pulsation prevents the occurrence of ossification in the neighbouring ligaments. Osteophytosis was present on the anterior margins of the vertebrae and extraspinal ossification was noted on the region of insertion of the subscapular muscle into the humerus. Apart from this, ossification of muscle insertions was also observed in the axial, peripheral, and cranial regions of the skeleton (Verlaan et al. 2007).

In the second skeleton, ossification was observed in the anterior longitudinal portion and the supraspinal ligaments of the vertebral column. Apart from this, ankylosis and kyphosis were also present in the spinal column and bilateral osteomyelitis was present in the tibia and fibula. The third skeleton too showed a similar ossification pattern in the vertebral column as the previous two cases thereby providing a confirmed diagnosis of DISH in the deceased individual. Apart from this, the skeleton showed signs of sacralization in the fifth lumbar vertebra and the left glenohumeral joint showed signs of osteochondritis dissecans. Initially thought to be a spinal condition, the presence of extraspinal manifestations in both living and deceased individuals have broadened our understanding of the disease pathology. As it does not specifically affect joints, it can be easily differentiated from other conditions similar to ankylosis (Verlaan et al. 2007). Therefore, its specific pathology that has been observed and analysed in skeletons has helped form a characteristic pattern of lesions for this disorder, the presence of which can be used to confirm the diagnosis of DISH in a deceased individual.

5.3.2 Metastatic Carcinoma

Metastatic carcinoma has been around since time immemorial and several groups of researchers have sought to understand its pathological journey through scores of generations starting from the early ages until recent times. Two studies included in this review have characterised skeletal lesions of metastatic carcinoma in the Early Bronze Age (Lieverse et al. 2014) and the Imperial Age (Minozzi et al. 2018). Lieverse et al.'s (2014) analysis of skeletal remains from the Early Bronze Age sought to study osteoblastic and osteolytic lesions of metastatic carcinoma in people who lived in the Cis-Baikal region of Siberia. The investigation focused on a particular hunter-gatherer cemetery in the region in order to characterise the lesions in people who lived during one of the earliest time periods. One of the skeletons that was recovered demonstrated the presence of four perforating lesions on the cranium, two of which were on the frontal bone, one was on the sphenoid process, and the fourth was on the occipital bone. The nature of these lesions was lytic and circular with irregular and jagged margins. Two of these lesions showed the presence of osteoblastic reactive bone around their peripheries and at the internal margins. Apart from these, there were 55 lesions found on the entire skeleton, half of which were lytic in nature and the other half were osteoblastic in nature (Lieverse et al. 2014).

On the other hand, Minozzi et al. (2018) studied skeletal samples of metastatic prostate cancer belonging to the comparatively more recent Imperial Age from the Casal Bertone region in Rome, Italy. The lesions that were identified in the skeletal remains were a mixture of lytic, osteoblastic, and osteoclastic types similar to the ones observed by Lieverse et al. (2014). Most of the lesions had irregular jagged edges and the peripheries showed signs of osteoblastic bone formation processes. A new finding that was not

seen in the earlier study was the presence of cavitations of new bone formation areas that appeared as radiolucent regions in radiographic images. Numerous such pits of regions where bone resorption and new bone formation had taken place were observed throughout the skeleton throwing light on the extensive skeletal involvement of metastatic prostate cancer (Minozzi et al. 2018). These types of studies may be important in characterising the extent of disease in a deceased individual and conducting genetic susceptibility tests to understand the risk profiles of other surviving family members.

5.3.3 Osteoarthritis

Osteoarthritis is an age-related condition that mostly affects people over the age of 60 resulting in extreme pain and physical limitations. It is distinguished from rheumatoid arthritis by the presence of osteophytes which are indicative of bone remodeling processes. However, osteophytes are not specific to cases of osteoarthritis and may be found in other disease conditions as well. Therefore, Shepstone et al. (2000) sought to understand the occurrence of osteophytes in cases of osteoarthritis in order to use them to provide a confirmed diagnosis of the condition. They were able to identify a characteristic pattern of the presence of osteophytes around the femur in cases of osteoarthritis (Shepstone et al. 2000). This information can help in classifying the disorder into sub-categories which, when matched with previous radiographs, can help in the identification of a person.

5.3.4 Paget's Disease of the Bone

Paget's disease of the bone is a metabolic bone disorder that occurs due to problems in one or more steps of the bone remodeling process. Although modern cases of the disease are well characterised, investigated, and analysed, Shaw et al. (2019) decided to investigate the lesions of the disease in skeletal remains

belonging to the medieval times. Based on their findings, Paget's disease in earlier times was found to be extensive affecting around 40% or more of the entire skeleton. The typical features of the lesions of Paget's disease in their study seem to be specific to skeletal remains obtained from Norton Priory in the North West part of England, proving that a specific sub-type of this condition was prevalent in this region (Shaw et al. 2019). This characterisation of different bone conditions can help trace the geographical location and/or ethnicity of the deceased individual based on the specific identifying features of the disease.

5.3.5 Rotator Cuff Disease

Rotator cuff disease is a condition of the shoulders which affects the range of motion of the shoulder joint causing pain and limiting mobility. This disease has not been actively investigated for the presence of lesions in skeletal remains and therefore, Roberts et al. (2007) decided to look for the presence of lesions of rotator cuff disease in skeletal remains in various locations in England obtained from different time periods. They were able to find characteristic lesions in the acromioclavicular and sternoclavicular joints with degenerative changes of the shoulder joint found in a significant number of cases. The typical characteristic features of the degenerative changes included pitting, presence of osteophytes, and signs of new bone formation and remodeling processes. Overall, the authors described subacromial impingement, subcoracoid impingement, and bicipital sulcus degenerative changes (Roberts et al. 2007). Studies like this add to the existing knowledge base of major bone pathologies and their characteristic lesions in skeletal remains. In certain cases, knowledge of these pathologies may be paramount in performing a positive identification of a deceased individual.

5.3.6 Other Bone Pathologies

Apart from the major bone pathologies described above, there were several other pathological features which the researchers stumbled upon while analysing skeletal remains. For instance, Tinoco et al. (2010) used dental anomalies for the positive identification of a deceased person in Sao Paolo, Brazil. In this particular case report, an anomaly in the position of a tooth of a young girl helped in her identification. Her body which was recovered from the banks of a river had five missing teeth apart from the positional anomaly of the upper left canine. Her family members described this anomaly as a tooth which was displaced forward, which piqued the attention of the investigators who had recovered the remains of the missing individual. By comparing previous photographs with pictures of the dentition of the discovered remains, the researchers were able to provide confirmed identity of the person. In general, dentition is sufficiently unique for an individual and smiling photographs of a person can help in analysing tooth position, shape, colour, and dental wear in post-mortem photographs. In certain applicable cases, characteristic features of the dentition may help in providing a confirmed identity of a deceased person as in this case report (Tinoco et al. 2010).

In another study, lesions of osteogenesis were identified and analysed in a juvenile skeleton belonging to the Late Archaic period obtained from North America to understand the pattern of bone wasting in the individual. Typical features observed in the skeletal remains include thin cranial vault, partially edentulous jaws, worn teeth with adherent calculus, signs of *cribra orbitalia*, weakly developed cortical and trabecular tissues, porous spongy bone tissues, anteriorly compressed vertebrae, weakly developed vertebral trabecular lattices, and very little surface development of muscle attachment sites. All these features are consistent with a diagnosis of

type IV osteogenesis imperfecta that typically leads to bone wasting and several other symptoms in children (Vairamuthu and Pfeiffer 2018). Identification of these features in skeletal remains can not only give insights into the life of the person but also aid identification in forensic anthropology cases.

5.4 Infectious Diseases

The two main infectious diseases that often come up in paleopathological studies are leprosy and tuberculosis, each of which is described in detail below.

5.4.1 Leprosy

Leprosy is a chronic and infectious disease caused by the microorganism, *Mycobacterium leprae*. The history of this disease dates back to the beginning of civilization and studying its global prevalence can give important insights into the spread of population around the world. Robbins et al. (2009) sought to investigate the pathological lesions of leprosy in skeletal remains of people belonging to the era of 2000 BC in India. Several pathological features that were found all over the skeleton included erosion, remodeling, atrophy, osteolytic lesions, infection, and bone resorption. Tooth loss and alveolar resorption is also noticed which is characteristic of people with leprosy (Robbins et al. 2009). These types of studies add information value to the existing research on the spread and prevalence of leprosy globally and also help identify skeletal remains of people from the earlier time periods.

5.4.2 Tuberculosis

Tuberculosis is caused by the microorganism, *Mycobacterium tuberculosis*, and is primarily a lung disease with minimal skeletal involvement. However, in around 5% of all cases of tuberculosis, osteolytic lesions develop mainly on the vertebral column leading to

its subsequent collapse. Holloway et al. (2013) investigated the skeletal remains obtained from the Galler collection in Switzerland to characterise the bone pathologies specific to tuberculosis.

Characteristic lesions include spondylitis, bone erosion, vertebral destruction, spinal angulation, kyphosis, fusion, and abscesses (Holloway et al. 2013). In contrast, tuberculosis lesions identified in skeletal remains from Neolithic Hungary showed the presence of hypertrophic pulmonary osteopathy that had developed secondary to tuberculosis (Masson et al. 2013). These studies provide insights into the skeletal lesions observed in infectious diseases of the lung which can be used for both, characterisation of the disease as well as identification in forensic anthropology cases.

5.5 Analysis of Skeletal Remains of Famous People

Three studies included in this review investigated specific lesions in famous people to acquire deeper insights about their lives and diseases. One study aimed to study the skeletal effects of castration in the famous singer, Carlo Broschi aka Farinelli, in Bologna. This was a unique case as the singer had voluntarily undergone castration before he hit puberty so that the treble pitch of his voice in adolescence could be preserved throughout his life. As a result of this, although his voice remained sweet and powerful for his entire singing career, he faced several health problems that were evident in his skeletal remains. Some of the conditions that were identified included long bones of the limb, osteoporosis, and persistent epiphyseal lines. Also, the frontal bone showed signs of hyperostosis frontalis interna (HFI) which causes a symmetrical thickening of the inner margins of the bone. This condition usually affects post-menopausal women and, in this case, can be attributed to his castration before puberty (Belcastro et al. 2011).

A study by Kacki et al. (2018) sought to analyse the skeletal remains of the famous Danish astronomer, Tycho Brahe, and found

evidence of DISH lesions in his skeletal remains. This investigation helped clear the controversy surrounding his death as several speculations indicated that he had died due to poisoning. As it turned out, he had suffered from DISH during his lifetime, which eventually led to his death (Kacki et al. 2018). Another study was conducted by Bartsiokas et al. (2015) to study the skeletal remains in three royal tombs located in the Great Tumulus in Vergina, Macedonia, to identify the remains of King Philip II. A wound had left him lame in his left leg and this feature was used to positively identify the skeletal remains that belonged to him (Bartsiokas et al. 2015).

Chapter 6

CONCLUSION

This work was undertaken to understand the available literature regarding pathological conditions of the human skeleton that can aid in the construction of a unique biological profile, which can eventually lead to positive identification in forensic anthropology cases. We found that the literature available in this regard is extremely extensive with detailed descriptions of skeletal lesions along with their differential diagnoses. There are several studies that have been undertaken to characterise different skeletal conditions in different geographical locations and belonging to different time periods. These studies provide a basis of identification of different skeletal pathologies and may be used by forensic anthropologists to understand different skeletal patterns when studying human remains. The literature also has a large number of case reports where skeletal remains of crime victims found in different parts of the world have been accidentally found and later, positively identified.

Despite the breadth and depth of the available literature, there are many cases where, due to extreme trauma inflicted on the victim and extensive fragmentation of the skeletal structures, construction of an accurate biological profile is not always possible. Also, it has been well documented that study of dentition is very important in forensic anthropology cases, and there is a need for more extensive information regarding dental anomalies in the literature. Future studies should aim at understanding the likelihood of forensic anthropologists in using skeletal pathologies as a basis for identification. These studies should also try to acquire first-hand information on the types of skeletal lesions that are more easily identifiable in human remains, and those lesions where multiple differential diagnoses are possible. This information will help in creating a standard protocol that can be followed by forensic anthropologists all over the world for analyzing skeletal pathologies in human remains.

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