Human Osteology and Forensic Science in Archaeology: A Case Study of Human Skeletal Remains Recovered from a Gandhara Grave Site

by

Abdul Basit

Taxila Institute of Asian Civilizations

Quaid-i-Azam University

Islamabad

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Human Osteology and Forensic Science in Archaeology: A Case Study of Human Skeletal Remains Recovered from a Gandhara Grave Site

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Master of Philosophy

Supervisor

Dr. Ghani-ur-Rahman

By

Abdul Basit

Taxila Institute of Asian Civilizations

Quaid-i-Azam University

Islamabad

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Candidate's Declaration

I hereby declare that this thesis is the result of my individual research, and that it has not been submitted concurrently to any other university for any degree.

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Abdul Basit

Supervisor's Declaration

I hereby declare that the MPhil. candidate **Mr. Abdul Basit** has completed his thesis **"Human Osteology and Forensic Science in Archaeology: A Case Study of Human Skeletal Remains Recovered from a Gandhara Grave Site"** under my supervision. I recommend it for submission in candidacy for the degree of Master of Philosophy in Asian Studies.

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Supervisor

Dr. Ghani-ur-Rahman

Associate Professor

TIAC, QAU

Quaid-i-Azam University, Islamabad

Taxila Institute of Asian Civilizations

Dated: ___________________

FINAL APPROVAL

This to be certify that we read the thesis submitted by **Mr. Abdul Basit** and it is our judgment that this thesis is of sufficient standard to warrant its acceptance by the Quaid-i-Azam University, Islamabad, for the award of degree of Master of Philosophy in Asian Studies.

Supervisor: ________________

Dr. Ghani-ur-Rahman

Examiner: _________________

Chairman: _________________

Dr. Ghani-ur-Rahman

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I have no words to pay my humble gratitude and thanks to Dr. Naeem and Ms. Hajira Batool for their guidance in the experimental work of Ancient DNA Extraction.

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Dedicated to my parents and grandfather Raza Khan

Contents

Introduction

Gandhara Graves are widespread throughout the Gandhara region, from Bajaur to the Indus. The graves are only in widespread throughout Gandhara but other regions (the areas east-of the Indus River) as well (99). Dani (1968: 108) has divided Gandhara Graves into three distinct groups belong to three distinct periods.

According to Dani,

> Period I Represented by the complete burial: bronze hairpins, two varieties and distinctive pottery types carinated, narrow-waisted, tall drinking vases and offering stands with straight-sided bowls. Period II Represented by the complete burial plus cremation and urn burials. Bronze, silver, and gold are found. Two more varieties of bronze hairpins are known. Pottery forms show remarkable evolution. The most important forms are the urns of different sizes, cooking vessels on stands, and narrow-necked, bottle-shaped vases. Period III Represented by the complete burial, cremation and urn burials, plus fractional burials after exposure. The important new finds are iron tools and weapons. Among other discoveries, beads of precious stones and terracotta figurines are important innovations (Dani 1968: 108).

Human osteology and forensic science are very valuable in order to study the human skeletal remains of these graves. These two scientific field are very advance. Human osteology is the study, recovery and interpretation of human bones. The science of human osteology is applied in medical and anatomical sciences. Human osteology is also applicable to other sciences like forensic science, paleontology and archaeology. Forensic osteology is often applied in legal investigations while paleontological investigations are carried out to study ancient pre-cultural human skeletal remains. In cultural contexts human osteology has a close relationship with archaeology. Human skeletal remains are important archaeological evidence to reconstruct the past. Osteological analysis of human skeletal remains from ancient cultures provides great information to archaeological science.

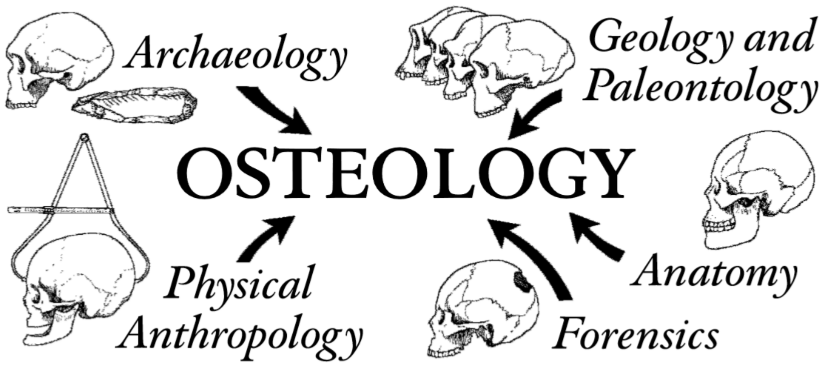


Figure 1 Osteology and associated scientific disciplines (White et al. 2012: 2).

According to White et al. (2012: 1),

> It has recently become fashionable to refer to the study of human remains from archaeological contexts as "bioarchaeology." However, human skeletal parts are only a small part of the biological remains characterizing most archaeological sites—indeed, most archaeological sites lack human remains altogether.

Human skeletal remains are importance because they are long lasting and show great resistance to decay. Bones and teeth are important for the estimation of age and sex of an individual. Furthermore,

> Study of the skeleton often makes it possible to discern a variety of pathologies from which the individual may have suffered. Analysis of groups of individuals may offer insights into prehistoric population structure, biological affinities, cultural behaviors, and patterns of disease. The evolutionary history of humanity itself is being read from the fossil record—a record comprising mostly teeth and bones ((White et al. 2012: 2).

Human osteology and forensic science have a close relationship with archaeology. Physical remains in the form of ancient human bones and teeth are important component of archaeological investigations. Osteological analyses have a variety of forensic applications starting from the identification of human skeletal remains to the investigation of crimes.

Osteology and archaeology combine to form a new field called osteoarchaeology. On the other hand forensic science and archaeology combines to form a new field called forensic archaeology. Hunter (2002: xxv–xxxii) defines forensic archaeology as:

> Forensic archaeology involves applying archaeological techniques to the crime scene. It is important to recognize that there are fundamental differences between traditional academic archaeology and forensic archaeology.

Therefore, both human osteology and forensic science are applicable to archaeology in the investigation of ancient human skeletal remains to acquire important archaeological information.

The present research is about the scientific study of ancient human skeletal remains recovered from Gandhara Grave sites with the application of human osteology and forensic science. The human skeletal remains in the form of two human skulls' fragments were recovered from two Gandharan Grave sites 'Juga Khat' and 'Warokay Gujjar Banr', Tehsil Kabal District Swat, Pakistan. These skeletal remains were kindly provided and recovered by Ikram Qayum[1] during the archaeology survey carried out by Taxila Institute of Asian Civilizations, Quaid-i-Azam University, Islamabad, Pakistan and the license for the archaeological survey was provided by the Directorate of Archaeology and Museums, KPK, Pakistan. These two sites have been destroyed by the local people due to agricultural activities. The present research focuses on the following aspects for the analysis of human skeletal remains recovered from the Gandhara Grave sites:

1. Identification and labelling
2. XRD (X-Ray Diffraction)
3. Odontology
4. Determination of occupation, medical conditions, habits and addictions and abnormalities of tooth formation and eruption
5. Dental radiography
6. Determination of age and sex
7. Ancient DNA Extraction

Statement of Problem

The main problem is that how to investigate damaged and fragmented ancient human skeletal remains with scientific approaches in Pakistan. In Pakistan during archaeological excavations these important ancient human skeletal remains are mostly ignored by Pakistani archaeologists because of the lack of scientific knowledge. In Pakistan there are a lot of weaknesses in traditional process of human past study and due to which numerous significant archaeological information could not be obtained from the ancient human skeletal remains. Published work about osteoarchaeology or forensic archaeology is very rare in Pakistan therefore a lot of work remains

[1] Ikram Qayum MPhil. Research scholar, Taxila Institute of Asian Civilizations, Quaid-i-Azam University, Islamabad, Pakistan.

to be done. The application of human osteology and forensic science to archaeology requires a remarkable degree of interaction between human osteologists, forensic scientists and archaeologists. In fact, at present in Pakistan there are very few human osteologists or forensic scientists who have a complete background in archaeology. On the other hand, most professionals involved with archaeology have a purely archaeological or artistic background, but often with no knowledge of the principles and techniques of human osteology and forensic science. The traditional academic separation between human osteology, forensic science and archaeology in higher education has restricted the mutual collaboration of these three disciplines. In Pakistan there are no laboratories associated with museums and archaeological institutions, or institutions dedicated to osteoarchaeology or forensic archaeology. To overcome these problems a mutual overlap of human osteology and forensic science with archaeology is required to place archaeology as a modern science in Pakistan.

There is a gradual development in the number of osteology articles in American Journal of Physical Anthropology since 1930-84 and 1996-2000. But unfortunatly in the field of archaeology Pakistani journals lack osteology articles due to rare reseach on this topic. Similarly there is no single article related to forensic science in any Pakistani journal related to archaeology.

	Osteology %	Analytical %	Descriptive %
1930–1939	36.8	13.5	86.5
1940–1949	33.7	21.1	79.0
1950–1959	42.0	29.7	70.3
1960–1969	44.3	35.4	64.6
1970–1979	51.3	44.1	55.9
1980–1984	55.0	43.0	57.0
1996–2000	56.0	43.0	57.0

Table 1

Content Analysis of Osteology Articles in the American Journal of Physical Anthropology, 1930-84 and 1996-2000 (modified and extended from Lovejoy et al. 1982).

Purpose and Scope of the Study

Ancient human skeletal remains are important evidence in archaeological investigations. The purpose of this research is to show the varieties of important information which can be gained from the scientific study of these ancient human skeletal remains. Furthermore, the most important point is that how to acquire information from the damaged or fragmented ancient human skeletal remains. The researcher has used these damaged or fragmented ancient human skeletal remains to acquire a variety of information by using different scientific techniques. By using valid scientific techniques and methodologies, such fragmentary ancient human skeletal remains are valuable for learning

Precisely, the main purpose of the present research deals with the:

a. Interdisciplinary relationship of human osteology and archaeology, and forensic science and archaeology along with the importance of human skeletal remains.
b. Identification and labelling of Skull-A and Skull-B fragments.
c. Application of XRD (X-Ray Diffraction) for the analysis of structural features of Skull-A and Skull-B and for the identification of the oldest skull between Skull-A and Skull-B.
d. Odontological Examination including dental radiography, determination of age and sex, occupation, medical conditions, habits and addictions and abnormalities of tooth formation and eruption.
e. Ancient DNA Extraction from the fragments of Skull-A and Skull-B.

As mentioned in the statement of problem there are a lot of weaknesses in Pakistan Archaeology due to the lack of scientific approaches for the analysis of ancient human skeletal remains. Therefore, the present research has a great purpose and scope in this scenario and it is also important to mention that the researcher has depended totally on Pakistani resources to carry out the research. This research is the initial step to introduce osteoarchaeology and forensic archaeology in Pakistan. The research will act as a template for the future research in osteoarchaeology and forensic archaeology in Pakistan.

Hypothesis

The application of human osteology and forensic science in archaeology can give essential information about the ancient human skeletal remains.

Method and Theory

Theoretical Framework

In the 1950s archaeology and skeletal biology were combined to make a new discipline. It was due to the "new archeology" that converted traditional archeology into a scientific discipline. The new approach was introduced by Binford (Binford 1962, 1964, 1977; Binford and Binford 1968) in which hypothesis testing by the application of scientific methodology became essential. Skeletal biologists were also influenced by the "new approach" that led to the development of biocultural approach to the analysis of skeletal remains. It was the reason that made archaeology an interdisciplinary field. Therefore Skeletal biology shared its methodology with processual archeology to make a new field called bioarcheology (Buikstra 1977; Larsen 1987, 1997). The new approach has also found its relation in other sciences like in paleopathology (Armelagos 1997) and this relationship is so strong bioarcheology is a vital part of paleopathology for most skeletal biologists (Armelagos and Dannis 2003: 58). Furthermore, due to the new approach forensic science also found its place in archaeology in the form of forensic archaeology. Similarly the present research is related to the new approach that depends on scientific methodology. The present research is scientific in which the hopothesis testing depends totally on scientific methodology of human osteology and forensic science for the analysis of ancient human skeletal remains.

Documentary Research

This research benefited greatly from archival and documentary research. Some books and articles suggested by the supervisor Ghani-ur-Rahman[2] were acquired from the central library of Quaid-i-Azam University, Islamabad, Pakistan. For further research the following websites were also used for documentary and archival research:

www.archive.org, www.sciencedirect.com, www.pnas.org, www.indjst.org, www.jstor.org

Field and Laboratory Methods

Collection of Ancient Human Skeletal Remain from Gandhara Grave Sites

The two fragmentary skulls (A and B) are damaged and are available in the form of fragments because both sites were destroyed for agricultural purpose and most of the ancient human skeletons were badly damaged. The skulls under research are named as Skull-A (recovered from Warokay Gujjar Banr) and Skull-B (recovered from Juga Khat). Skull-A is in the form of cranium fragments while Skull-B is in the form of cranium, cranium fragments and right mandible with two incisors, one canine, two premolars and one molar.

[2] Associate Professor and Acting Director Taxila Institute of Asian Civilizations, Quaid-i-Azam University, Islamabad, Pakistan.

Photography

Both fragmentary skulls A and B were properly photographed by the researcher. The photographs were then edited and highlighted.

Sampling and Cleaning

Small fragments from both ancient Skulls A and B were properly washed with ethyl alcohol and then converted to powder form for DNA extraction and XRD.

DNA Extraction

DNA extraction was done in the laboratory of Medical Genetics, Quaid-i-Azam University Islamabad, Pakistan. A phenol/chloroform method for DNA extraction from the ancient Skulls A and B was followed by me and Hajira Batool[3]under the guidance of Dr. Naeem[4] and Dr. Ghani-ur-Rahman.

XRD

XRD of both ancient Skulls A and B was done in XRD Laboratory, Department of Chemistry, Quaid-i-Azam University, Islamabad, Pakistan.

Odontological Examination

For the odontological examination of the Skull-B dental radiography (right mandible) was done in CDA Hospital, Islamabad, Pakistan.

Organization of the Research

The research is divided into the following chapters:

Chapter 1: Archaeology, Human Osteology and Forensic Science: An Interdisciplinary Relationship

Chapter 2: Identification and Labelling of the Human Skeletal Remains Recovered from Gandhara Grave Sites

Chapter 3: Determination of Age and Sex from Ancient Bones: A Case Study of Human Skeletal Remains Recovered from Gandhara Grave Sites

Chapter 4:Odontological Examination of the Human Skeletal Remains Recovered from Gandhara Grave Sites

Chapter 5: X-Ray Diffraction for the Analysis of Structural Features in the Human Skeletal

[3] PhD research scholar, Department of Medical Genetics, Quaid-i-Azam University, Islamabad, Pakistan.

[4] Associate Professor, Department of Medical Genetics, Quaid-i-Azam University, Islamabad, Pakistan.

Remains Recovered from Gandhara Grave Sites

Chapter 6: Ancient DNA Extraction from the Human Skeletal Remains Recovered from Gandhara Grave Sites

Literature Review

Forensic Odontology: An Essential Guide is written by Adamset et al. (2014). In thsi book the writer has given many useful methods for againg teeth and the abnormalities related to teeth. The book helped me to found out the abnormalities of the teeth under research. Furthermore, the book also provides information like habit, occupation and ancestry that can be determined from the teeth.

Scientific Methods and Cultural Heritage: an Introduction to the Application of Materials Science to Archaeometry and Conservation Science is written by Artioli (2010). The book is related to the new scientific methods that can be applied to the archaeological investigations. The book is helpful to know the mechanism of XRD.

Predictive accuracy of sexing the mandible by ramus flexure is written by Balci et al. (2005). The article is very beneficial in order to understand the sexing method from the mandible when there are no other methods available.

Characterization and Dating of Recent and Fossil Bone by X-Ray Diffraction is written by Bartsiokas and Middleton (1992). Through the idea of this article the present researcher has compared the dates of the two skull. The article deals with the methods that can enable us to determine the dates of skeletal materials whether they are recent or ancient.

Archaeology: A Very Short Introduction is written by Bahn (1996). The book is about some new concepts in archaeology. The writer has defined archaeology is different perspectives.

Determination of age in humans from root dentin transparency is written by Bang and Ramm (1970). This is the best and easy method to determine the age of human from teeth transparency measurements.

Archaeology as Anthropology is written by Binford (1962). The article is about how archaeology is anthropology or how archaeology is a science. The article helped to oeganize the theoretical framework.

The relationship of tooth wear to aging is written by Brothwell (1989). The method discussed in the article related to the age estimation of an individual from the patterns of tooth wear. The present researcher has applied this method in my research for aging the human skeletal reamins available for my research.

Forensic Chemistry is written by Collins (2007). The book discusses some essential concepts of forensic science. The concepts are also applicable to archaeology. Throughout the thesis the present researcher has used these concepts in order to analyze the human skeletal remains.

Aging Adults from the Skeleton is written by Collins et al. (2000). The book is a collection of different types of research articles related to forensic science, osteology and archaeology.

Age determination of adult human remains by teeth examination is written by Dalitz (1962). The writer has discribed aging methods by tooth examination of human skeletal remains. The method is very effective in age determination.

Archaeological Field Methods: An Introduction is written by Dancey (1981). The writer has discribed field methods of archaeology. These methods are very helpful for the recovery of skeletal remains from archaeological sites.

Human Osteology and Archaeology is written by Eakins (1980). The author has made a relationship of human osteology and archaeology. The article is very important in order to understand the concepts of osteology that can be applied in arcaheology.

Human and Nonhuman Bone Identification: A Color Atlas is written by France (2009). Boca Raton: CRC Press. The book describes some key methods to distinguish between human and nonhuman bones. The book is very useful from archaeological perspective.

Chemical dating of bones and fossils is written by Goffer (1980). The author has desrcribed different types of dating methods related to chemistry that can be applied to archaeology. These methods are very important for archaeologists.

Brief communication: A study of the predictive accuracy of mandibular ramus flexure as a single morphologic indicator of sex in an archaeological sample is written by Haun (2000). The author discribes sexing method from mandibular ramus. The mthod is useful when human skeletal remains are available in parts instead of a whole skeleton.

Forensic Radiology is written by Lee (1998). The radiographs can be used to interpret many essential point related to human skeletal remains.

Ancient Bone DNA Amplified Hagelberg, E., Sykes, B. & Hedges, R. 1989.. *Nature.* 342: 485. Ancient DNA amplification is very important in the modern archaeological research. The author describes some approaches that can be applied for DNA amplification.

Age estimation of adults from dental radiographs is written by Kvaal (1995). The author has described nondestructive method for age estimation from teeth. The method can be applied easily to the dental radiographs.

Bioarchaeology: Interpreting Behavior from the Human Skeleton is written by Larsen (1997). The writer has realted biology to archaeology in terms of the human skeletal analysis.

Human Osteology and Skeletal Radiology: An Atlas and Guide is written by Matshes et al. (2005). The book contains high quality photos of human skeletal system.

The Archaeology of Human Bones is written by Mays (1998). The book contains some important topics realted to the role of human bones in archaeology. Human bones are very important evidence for archaeological interpretation. The book is helpful in order to get archaeological knowledge from human skeletal remains.

Dentition in the estimation of age is written by Miles (1963). The author has described different types of age estimation techniques from the dentition of human.

Analytical Chemistry in Archaeology is written by Pollard et al. (2007). The book is about the analytical techniques used in archaeology. The writer has described many chemical methods that are very useful in archaeological analysis. Through these methods archaeologists can know much more about the archaeological materials.

Application of Lamendin's adult ageing technique to a diverse skeletal sample is written by Prince (2002). The author has expalined age estimation technique developed by Lamendin. The present researcher has also applied this technique in the research for the estimation of age of the skeletal remains.

Manual of Forensic Odontology is written by Senn (2013). The author has explained forensic methods that can be applied to the interpretation of teeth. From archaeological perspective these methods are very important for the analysis of archaeological teeth.

Sex Determination of Ancient Human Skeletons Using DNA is written by Stone (1996). The author has described DNA for sex determination in ancient human skeletons. This method is costly but useful when there is no other choice for sex determination.

Chemical weathering of bone in archaeological soils is written by White (1983). In this article the author has described the chemical weathering process that effect archaeological bones. This is important to know these chemical weathering process for applying any scientific method for the analysis of archaeological samples.

Human Osteology is written by White et al. (2012). The author has described human anatomy with high resolution photos of the skeletal parts of human. These photos are helpful for the identification of archaeological human bones.

Chapter No. 1

Archaeology, Human Osteology and Forensic Science: An Interdisciplinary Relationship

This chapter describes archaeology as a scientific discipline, the relationship of archaeology with other sciences and the relationship of archaeology with human osteology and forensic science.

Archaeology

Archaeology is basically about three things: objects, landscapes and what we make of them. It is quite simply the study of the past through material remains (Gamble 2001: 15). The word comes from the Greek (arkhaiologia, 'discourse about ancient things'), but today it has come to mean the study of the human past through the material traces of it that have survived (Bahn 1996: 2). Most archaeologists define archaeology as a sub-discipline of anthropology involving the study of the past through material remains. But archaeological methodology, theory and aims are different from anthropology and history but it solves the problems of these disciplines by providing data (Drewett 1999: 1). Archaeology has its own methodology, theory and aims by dealing with the remains of past peoples, societies and cultures (Drewett 1999: 2).

Archaeology is a field of study that seeks answers to question about the nature of human culture and society as it existed in particular times in the past, and about cultural and social change. From the earliest presence of human life on earth, the products and by-products of human activity have accumulated on and in the ground. These traces provide a link to past life ways and can reflect changes in behavioral patterns. Archaeology is the body of theory, method, and technique that guides the systematic recovery and analysis of the traces of past human activity. It enables scholars to bridge the gap between present and past cultures (Dancy 1981: 1).

Archaeology as a scientific discipline came into existence in the last part of the 20th century that is broadly defined as the application of scientific principles and methods to the characterization of materials that are related to cultural heritage. The field is so vast that there is no general agreement on the extent or even the definition of this discipline. The widely used term for the discipline is archaeometry, which has been in use since the founding of the journal *Archaeometry,* in Oxford in 1958. Before that Christopher Hawkes suggested using "archaeological science" to describe the discipline concerning the quantitative characterization of ancient objects and processes. Archaeometry is nowadays largely used as an alternative term for "archaeological science", or "science in archaeology". The application of scientific methods to archaeology requires a remarkable degree of interaction between specialists dealing directly with the different aspects of cultural heritage materials, such as archaeologists, curators, conservation scientists, art historians, and artists on one side, and scientists of different disciplines on the other. Most professionals involved with cultural heritage have a purely humanistic or artistic background but often they have no knowledge of scientific principles and techniques. The traditional academic separation between humanistic and scientific curricula in higher education has hindered the broad exchange of competences between art and archaeology, and science. The interface between disciplines is a fertile field where methodologies, analytical techniques, and especially the ideas developed in each specific scientific area may be enhanced, optimized, and ultimately applied with some ingenuity to diverse interdisciplinary environments (Artioli 2010: 1-3).

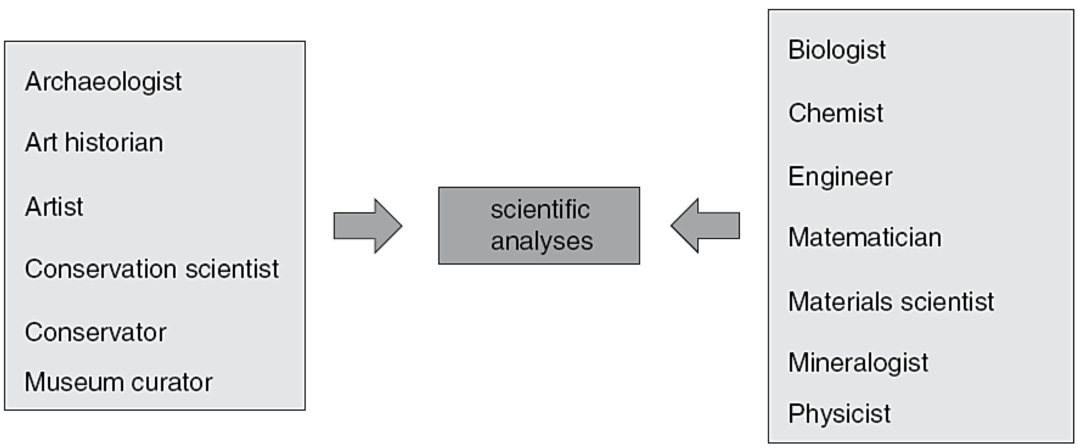


Figure 2 Science for the cultural heritage at the crossroad of a number of different professions and disciplines (Artioli 2010: 3)

It is a discipline that cannot exist without the interaction and information exchange with a wide range of natural and human sciences fields, including physics, chemistry, biology, physical science, engineering, agriculture, medicine, geology, ecology and forensic science. Recently it has also included modern material culture. Archaeology is therefore a culture historical discipline with a special source material and methodology and a dual objective; to study and to preserve past material remains. Archaeology is the only discipline that, as its main objective, documents, classifies, and interprets material culture in order to reconstruct societies past and present from individual farmsteads, roads, and cemeteries to whole societies, their environment, and their history; from local history to global history (Kristiansen 2009: 2).

Archaeology is very wide and enriched area, it can be as given below.

1) Archaeology is the study of material cultures in its relationship to human behaviour the physical manifestations of man's activities, his rubbish and his treasure, his building and his graves (Rahtz 1985: 1).

2) Archaeology is the special concern of a certain type of anthropologist. We cannot define archaeology except with reference to anthropology, the discipline of which it is part. Archaeology concerns itself with man in the past; it has been called the anthropology of extinct peoples (Deetz 1967: 3).

3) Archaeology is the study of our human past, combining the themes of time and change, using the material remains that have survived (Price 2006: 6).

The above has been written in different ways, however the fundamental principal stays the same as it is the study of human past. It is noteworthy that no definition includes preservation of the archaeological heritage, despite the fact that the collection and preservation of archaeological objects in museums antedates the development of archaeological research, as does the protection of monuments and sites (Kristiansen 2009: 23).

Human Osteology

Human osteology is the study of human bones. Scientists apply their knowledge to interpret and investigate these bones. Human osteology has a close relationship with other sciences like forensic science, anthropology, archaeology and paleontology etc. Osteological analysis plays a vital role in the analysis of archaeological materials such as ancient human skeletal remains (White et al. 2012: 2).

Human Osteology in Archaeology

Human osteology and archaeology have a close relationship because human skeletal remains play an important role in the study of human past. Human osteoarchaeology is the study of human skeletons from archaeological sites. That can provide information on demography, diet, health, activity patterns, genetic aspects and physique of past populations. Furthermore osteology in archaeology can contribute to the study of ancient warfare, migrations of peoples, and the study of human health and lifestyles. Therefore human osteology and archaeology are closely related to each other (White et al. 2012: 2).

Human Bones in Archaeology

Human skeletal remains contribute to many fields of research in the study of man's past. Specialists use human bones for different perspectives (Eakins 1980: 89). Human skeletons answer the questions of osteology. Ostelogy frames these questions. With the analysis of human skeletal remains many valuable answers are acquired. Osteologists are using advance chmical techniques, imaging and computer technologies. New techniques in osteology has led to the answering of new questions. Osteology as a science is very related to the study of past by putting a simple method to answer the question. (Armelagos and Dannis 2003: 53).

In archaeology bone identification is very important and it is the first step in osteoarchaeological investigation. The first step is to identify whether or not a bone is human or the object is bone or not. There are many materials that look like bones because bones take color of its environment (France 2009: 1).

Figure 3 Human femur (top), wood (middle), very weathered bone (bottom) (France 2009: 1).

Figure 4 Bleached white vertebra that has been in the elements (left), vertebra that was discovered in reddish soil (middle), vertebra that has been cleaned and preserved (right) (France 2009: 1).

Figure 5 Copper-stained bone (on left) from contact with copper while decomposing (France 2009: 2).

Figure 6 Human femoral neck. Note external textural differences between the femoral head and the neck (France 2009: 2).

The knowledge of morphology, texture and different components of bones is very important for the identification of bones (France 2009: 2).

Working with Human Bones

Excavation of bone is an important and time-consuming process. The skeletal material must be treated as important as other archaeological materials. Initial training is required to the beginners to make efficient excavations and recover skeletal material. Furthermore the knowledge of human skeletal anatomy is very essential for archaeologist (Eakins 1980: 91).

The recovery of human skeletal remains can be described in the following steps:

- The upper soil is removed with a trowel in a scraping motion of the edge of trowel.
- Frequent brushing for cleaning purpose
- Using dental picks, small soft brushes and spoons when required.
- A syring with rubber bulb can be used to clean the fragile area of the skeleton with puffs of air.
- Teeth and bones of the fingers and toes are loose in the soil therefore they must be treated with care.
- After exposing the skeleton the obserbale data is carefully noted and recorded (Eakins 1980: 91).

Forensic Science and Archaeology

Forensic Science is used to predict not the future but the past (Lee 1998: 280). In modern definition forensic science is the application of scientific knowledge to legal problems (Fisher et al. 2009: 3). In other words, Forensic Science is the scientific method of gathering and examining information about the past. The word forensic comes from the Latin *forensis*, meaning "of or before the forum." Forensic science applies scientific principles, techniques, and methods to the investigation of crime. Other related definitions of forensic may include the use of science to aid in the resolution of legal matters and a scientific analysis for the purpose of judicial resolve. Recently the term forensic is used to describe many scientific investigations and some these investigations are of historical importance. For example, a forensic scientist may work on the discovery of the composition of ancient pottery or the identification of ancient human remains (Collins 2007: 1-3). In the past, the primary tools in Forensic Science were observation and interpretation of physical evidence. In the second half of the nineteenth century for the first time science was applied to the forensic investigation (Erckert 1997: 1). Forensic Science is a multidisciplinary science closely related to other sciences like biology, chemistry, anthropology and archaeology etc. It is interesting to compare forensic science and archaeology because of the close relationship (Drewett 1999: 2). In the case of archaeology, scientific archaeology (also known as processual archaeology) is a theoretical movement rooted in the 1960s–1970s. Scientific archaeology represented a radical break from the then-dominant culture, the historical and antiquarian approaches to archaeology and resulted into Archaeology as a science (oxfordbibliographies.com). With the application of science archaeology has made a place in the sciences.

Archaeology also predicts the past, like Forensic Archaeology. Most archaeologists define archaeology as the study of the past through material remains. Archaeology has its own methodology and theory applicable to the remains of past peoples, societies and cultures (Drewett 1999: 2). Like Forensic Science, Archaeology is also a multidisciplinary science and has a close relationship with other sciences e.g biology, chemistry and forensic science.

Analytical knowledge is important both for archaeology and Forensic Science. The analytical knowledge of forensic investigators and archaeologists has a close relationship. Both fields of research emerged during the nineteenth century. Both disciplines were concerned with the proper identification of materials studied during investigation. Work of archaeologists and the work of forensic investigators are very similar. Both attempt to understand the nature, sequence, and fundamental reasons for certain events in the past. Their final goals may differ, but their philosophy is very identical. Both disciplines use and present evidence in order to prove their cases (Dupras et al. 2006: 103).

There are some basic principles of archaeology that can be applied to forensic science. These principals include provenience, context, features, stratigraphy, superposition and taphonomy etc. (Dupras et al. 2006: 105).

An archaeological excavation and a crime scene are similar in many ways. Both field investigates a past event. Both fields preserves past event by documenting, gathering, preserving, and interpreting physical evidence. Techniques are used to excavate archaeological burials with careful measurements and documentation. The exact location of all items *in situ* provide the basis for recreating the crime scene (Haglund et al. 2002: 96).

Due to the great similarities between archaeology and Forensic Science, archaeological methods can be applied to Forensic Science. The mutual relation of Archaeology and Forensic Science results into a new field called, Forensic Archaeology.

Forensic archeology is the application of archeological methods to forensic science. Forensic archeologists perform the controlled recovery of human remains and other evidence at forensic scenes (Nawarocki 1996: 1). In other words, forensic archaeology is the application of archaeological theory and methods to crime scene excavation and recovery of physical evidence/forensic evidence. It can also be defined as data collection activities carried out during the field recovery (Dupras et al. 2006: 3).

Forensic archaeology involves applying archaeological methods/techniques to the crime scene. Careful archaeological techniques can help in recreating the past scene (Dupras et al. 2006: 3). Following are some skills or knowledge associated with forensic archaeologists (Dupras et al. 2006: 4).

- Ground search methods (environmental changes associated with burials)
- Survey techniques (compass, theodolite, total station)
- Geophysical search methods (GPR, electromagnetic survey, metal detector)
- Site formation analysis and description
- Mapping techniques
- Spatial controls (establishing datum points, GPS, establishing grids)
- Excavation techniques

- Basic identification of human and nonhuman skeletal anatomy
- Artifact collection, documentation and preservation
- Site recording (casting of features, digital and still photography, documentation)
- Field sample collection (soil, botanical, entomological)

Collection and preservation of skeletal remains and associated evidence.

Chapter No. 2

Identification and Labelling of the Human Skeletal Remains Recovered from Gandhara Grave Sites

The study of bones, osteology, is an important part of human anatomy. Bones have serve multiple complex purposes, including protection, support, movement etc. and each part of the human skeleton has its unique shape and characteristics. It is very important to identify human skeletal remains in archaeological research. It is the first step of the human skeletal investigation in archaeology. By identifying different parts of the human skeletal remains an archaeologist can easily acquire a lot of important information like sex and age of the individual.

In this chapter the researcher has identified and labelled the human skeletal remains recovered from Gandhara Grave sites that include two fragmentary skulls named as Skull-A and Skull-B. Skull-A is in the form of cranium fragments while Skull-B is in the form of cranium, cranium fragments and right mandible with two incisors, one canine, two premolars and one molar. For the purpose of identification and labelling the researcher has used an atlas of human osteology 'Human osteology and Skeletal Radiology: An Atlas and Guide' written by Matshes et al. (2005) and 'Human Osteology' written by White et al. (2012). Higher power photographs of the atlas helped the researcher to compare and identify the human skeletal remains under the present research.

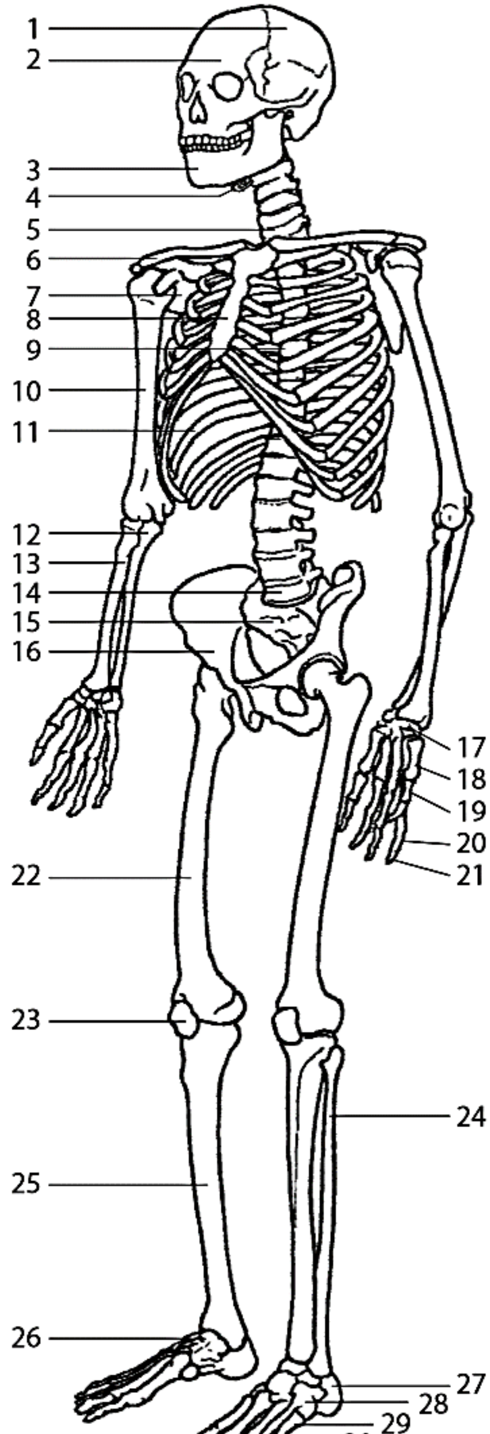


Figure 7 Human Skeleton (White et al. 2012: 12)

1. Paired cranial elements (22)	5. Cervical vertebrae (7)	9. Thoracic vertebrae (12; var*)	13. Radius (2)	17. Carpals (16)	21. Distal hand phalanges (10)	25. Tibia (2)	29. Metatarsals (10)
2. Single cranial elements (5)	6. Clavicle (2)	10. Humerus (2)	14. Lumbar vertebrae (5; var*)	18. Metacarpals (10)	22. Femur (2)	26. Talus (2)	30. Proximal foot phalanges (10)
3. Mandible (1)	7. Scapula (2)	11. Ribs (24; var*)	15. Sacrum (1)	19. Proximal hand phalanges (10)	23. Patella (2)	27. Calcaneus (2)	31. Intermediate foot phalanges (8)
4. Hyoid (1)	8. Sternum (1)	12. Ulna (2)	16. Os coxae (2)	20. Intermediate hand phalanges (8)	24. Fibula (2)	28. Other tarsals (10)	32. Distal foot phalanges (10) Coccyx (1; not visible)

Table 2 Human Skeletal System. (Only2nd and 3rd skeletal bones are available for my research)

TOTAL Bones: 206 Adult

* Commonly variable elements indicated by (var*)

(White et al. 2012: 12; Table by the researcher)

Skull-A

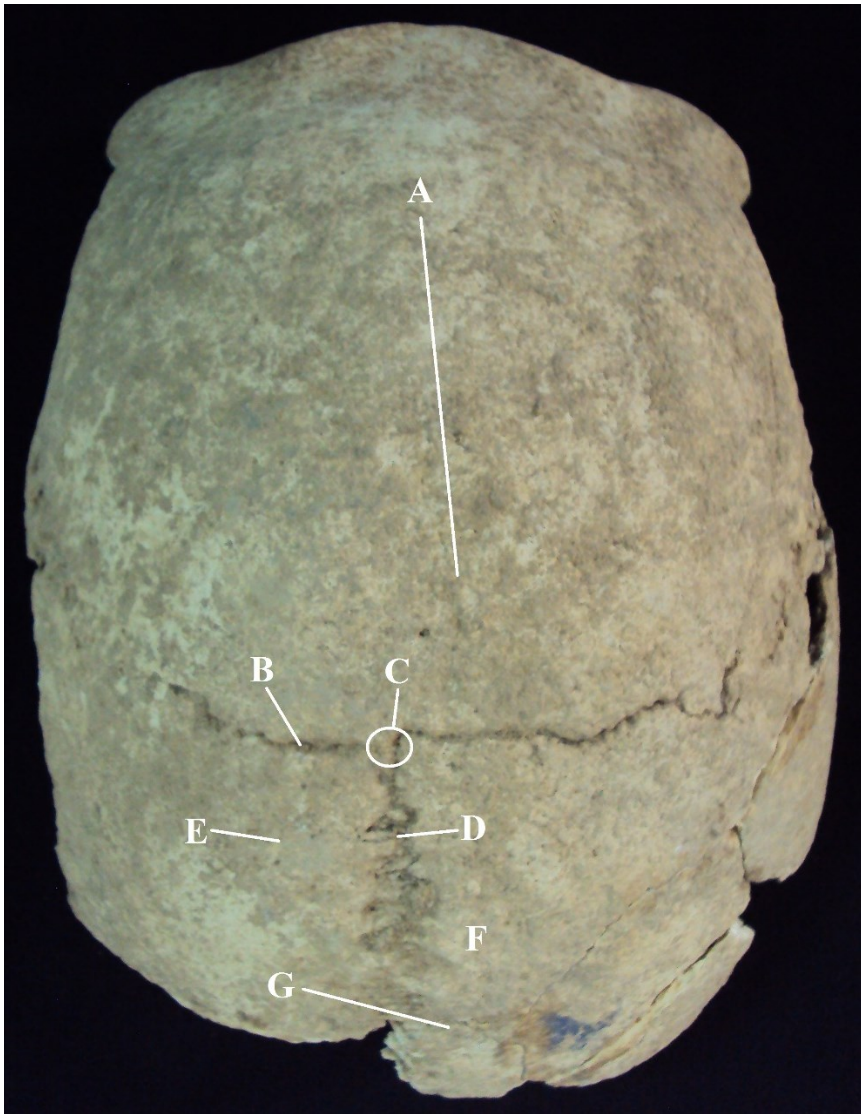


Figure 8 Skull-A, vertex view (superior ectocranial surface) (by researcher).

Figure 36:

A - Frontal bone

B - Coronal suture

C - Bregma (site of union of coronal and sagittal sutures)

D - Sagittal suture

E - Parietal bone

F – Parietal foramen (for emissary vein) (not visible)

G - Lambdoid suture

Figure 9 Skull-A, Skull cap, intracranial view.

A - Frontal crest

B - Coronal suture

C - Groove for branch of middle meningeal artery (not visible)

D - Granular foveola

E - Sagittal suture

F - Lambdoid suture (broken)

Skull-B

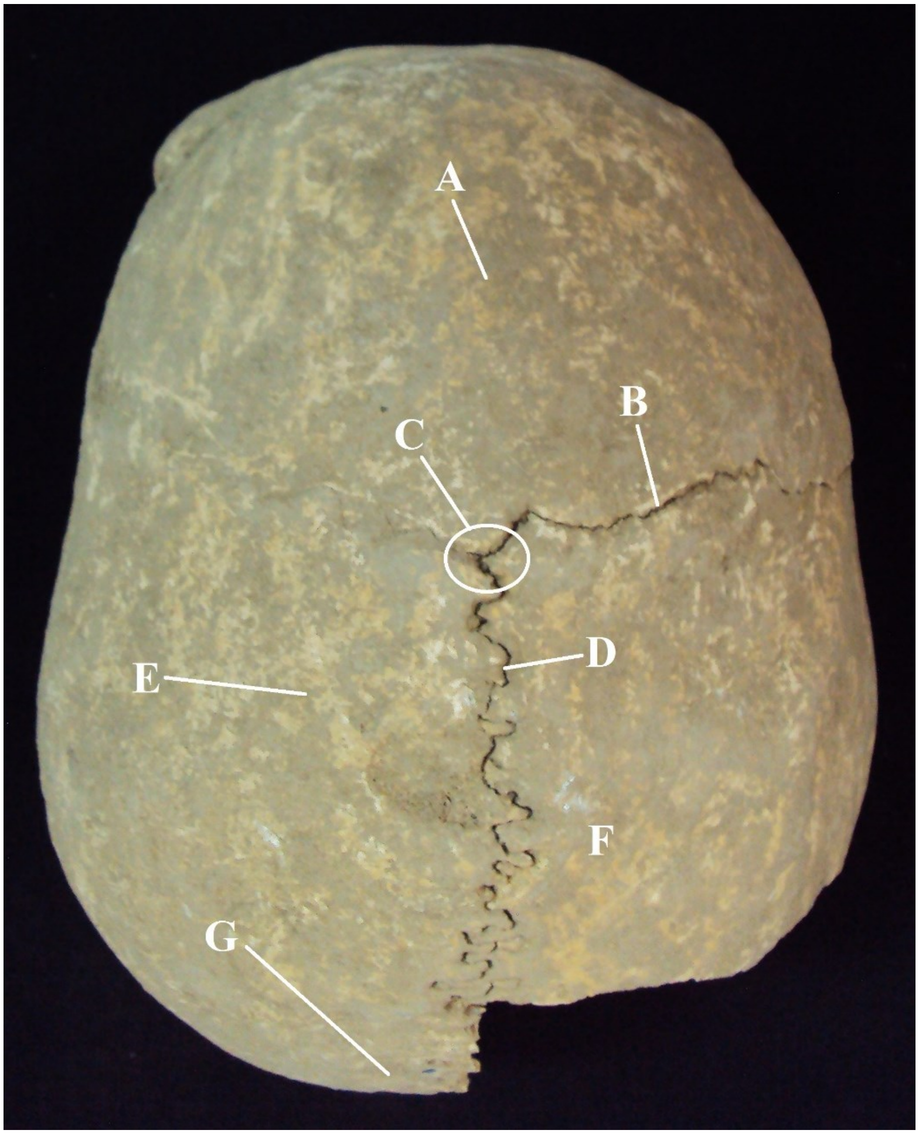


Figure 10 Skull-B, vertex view (superior ectocranial surface).

A - Frontal bone

B - Coronal suture

C - Bregma (site of union of coronal and sagittal sutures)

D - Sagittal suture

E - Parietal bone

F - Parietal foramen (for emissary vein) (missing)

G - Lambdoid suture (broken)

Figure 11 Skull-B, Skull cap, intracranial view.

Figure 39:

A - Frontal crest

B - Coronal suture

C - Groove for branch of middle meningeal artery

D - Granular foveola

E - Sagittal suture

F - Lambdoid suture (broken)

Mandible of Skull-B

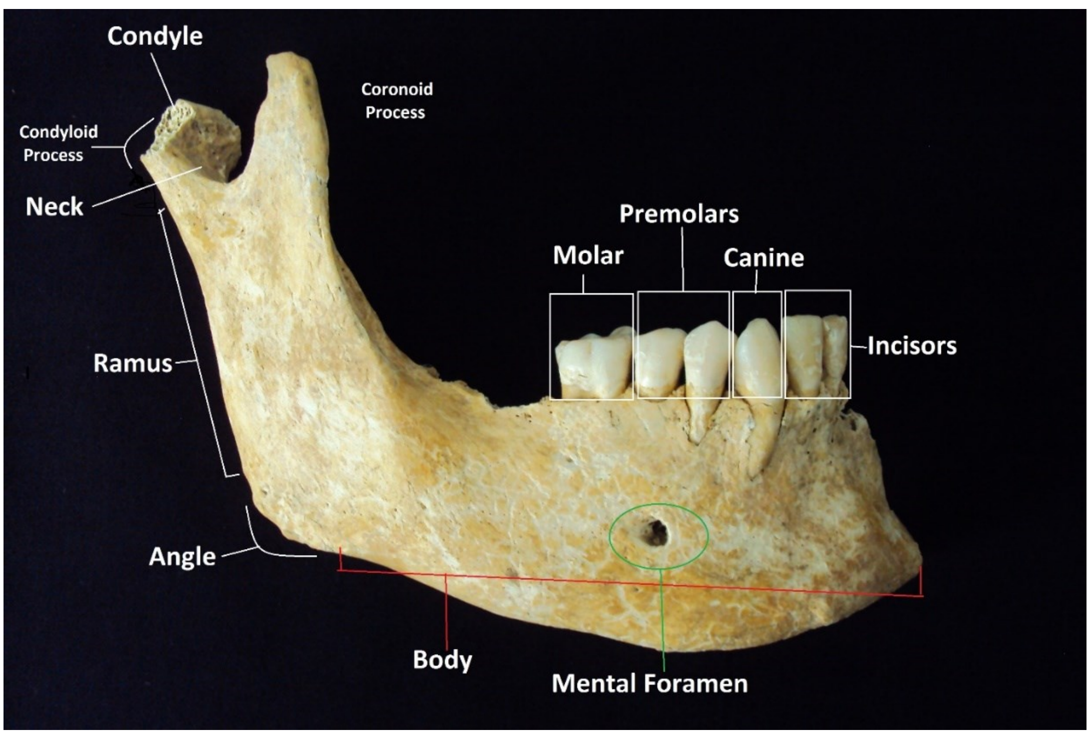


Figure 12 Mandible of Skull-B (by researcher).

Chapter No. 3

Determination of Age and Sex from Ancient Bones: A Case Study of Human Skeletal Remains Recovered from Gandhara Grave Sites

The chapter describes different types of methods used for age and sex determination from ancient bones. In the present research, for the age determination of Skull-B with right mandible having two incisors, one canine, two premolars and one molar, the researcher has used some forensic odontological techniques of adult dental age estimation. The researcher has applied Lamendin et al. (1992) method on 'Skull-B' for age estimation. The same method as mentioned by Lamendin et al. (1992) but with a little modification. In the method the researcher has used dental radiographs instead of sectioning the teeth. In the present research the modification in Lamendin et al. (1992) method is close to Kvaal et al. (1995) method in some aspects. The second method the researcher has used for the age estimation of 'Skull-B' is from tooth-wear patterns. In case of 'Skull-A' only cranium is available in fragments therefore, it is not possible to apply these methods on 'Skull-A'. For sex determination the researcher has examined robusticity of both skulls 'A and B'. Skull-B have a right mandible therefore, the researcher has also used sex determination method for the mandible.

According to Cox (2000: 61-62) determination of age from skeletal remains is important component of scientific archaeology, palaeodemography and biological anthropology. Age and sex of skeletons are very important in case of cemetery data for investigating whether certain grave goods are associated with one sex or other or with particular age groups. By analyzing age and sex data from human skeletons we can know the demographic compositions and past mortality patterns of earlier human communities. By analyzing age data with aspects of bone size in child skeletons we can investigate growth rates in the past (Mays 1998: 66).

There are many techniques for adult dental age estimation. Dalitz (1962) has historical significance and is still in use. Kvall and Solheim (1994) is important in archeological situations. Others techniques of dental age estimation includes color estimation of root dentin, root surface roughness and crown pulp area (Solheim 1993). Some techniques includes new technology (Thevissen et al. 2009). Following are some important techniques used for adult age estimation.

Lamendin et al. (1992)

Lamendin's method is based on root transparency (T) and periodontal recession (P) requiring three physical measurements made from the labial aspect of the tooth. The following measurements are required in millimeters

- measured periodontal recession height
- measured root transparence height
- measured root height

Lamendin's formula for age assessment is: Age= (0.18xP) + (0.42xT) + 25.53

P = (measured periodontal recession height ×100)/measured root height

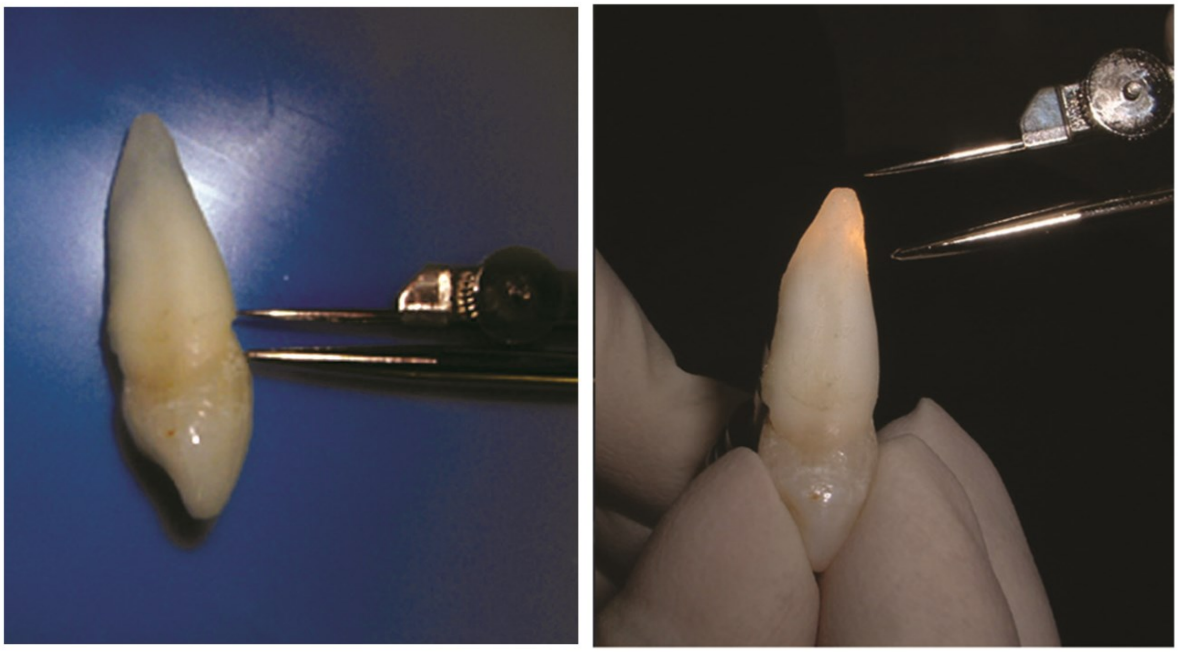
T = (measured root transparence height×100)/measured root height (Lamendin et al. 1992: 1373-1379).

Sample error rates of Lamendin et al. in mean error (ME) is given in the following table:

Age Interval (Years)		25–29	30–39	40–49	50–59	60–69	70–79	80–89	90–99	Overall
ME (Years)	Lamendin et al.	24.8	15.5	9.9	7.3	6.3	11.6	18.9	—	10
	Prince and Ubelaker	13.2	9.0	5.6	5.2	7.2	12.3	20.3	32.6	8.2

Figure 13 Mean Error (ME) Comparison of Lamendin et al. and Prince and Ubelaker Results (Senn and Weems 2013: 239).

However, age estimation using Lamendin et al. is important between the ages of 40 and 70 when the maxillary incisors, specially the central incisors are examined. Due to the unavailability of maxillary canine the present researcher has applied Lamendin's method on right mandibular canine of Skull-B. The right mandibular teeth are fully developed will long dental roots.



(a) (b)

Figure 14 Maxillary canine: (a) measurement of periodontal recession height, (b) measurement of root transparency (Senn and Weems 2013: 238).

Figure 15 right mandible of Skull-B recovered from Gandhara grave site (by researcher)

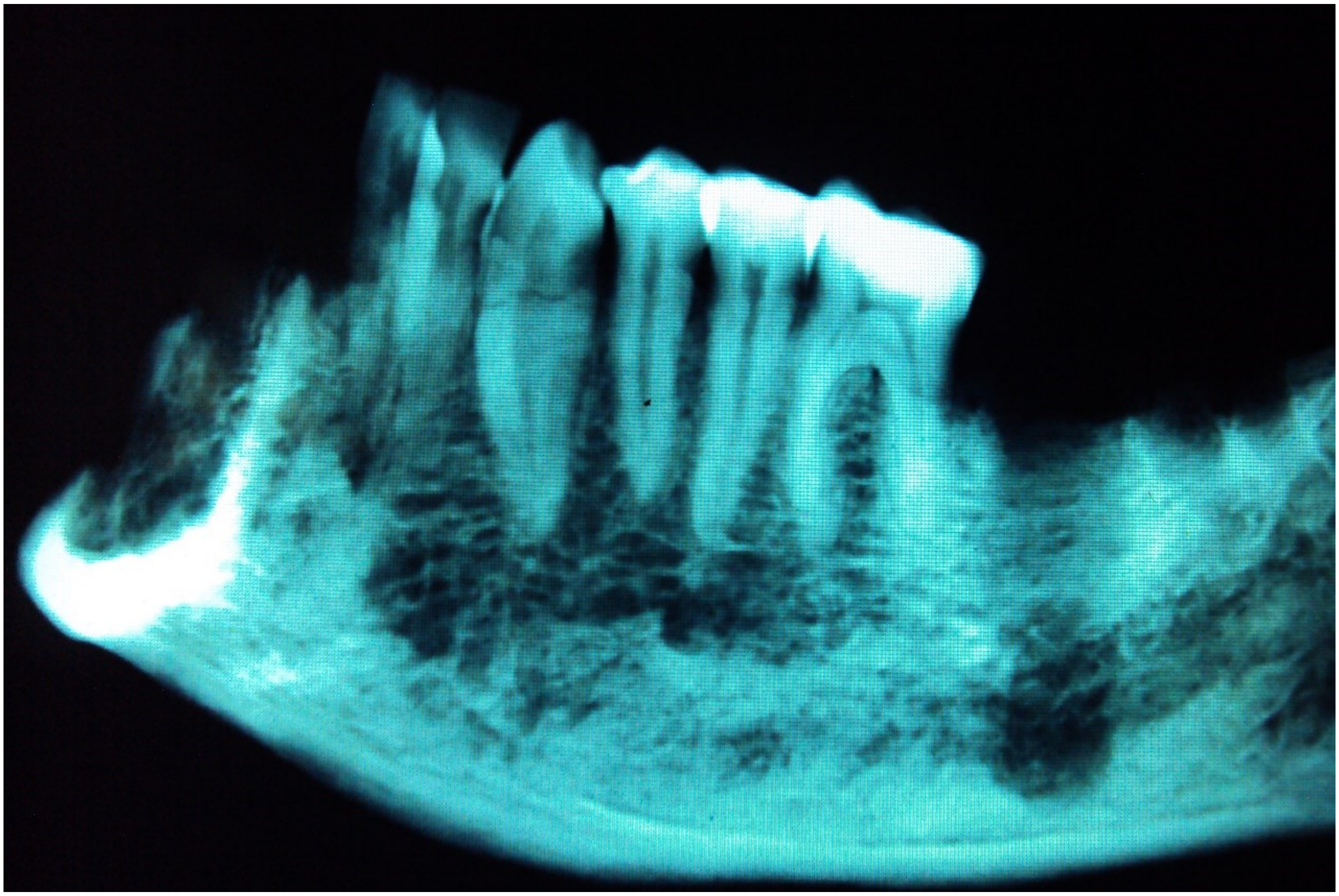


Figure 16 Radiograph of the right mandibular teeth of Skull-B recovered from Gandhara grave site (by researcher)

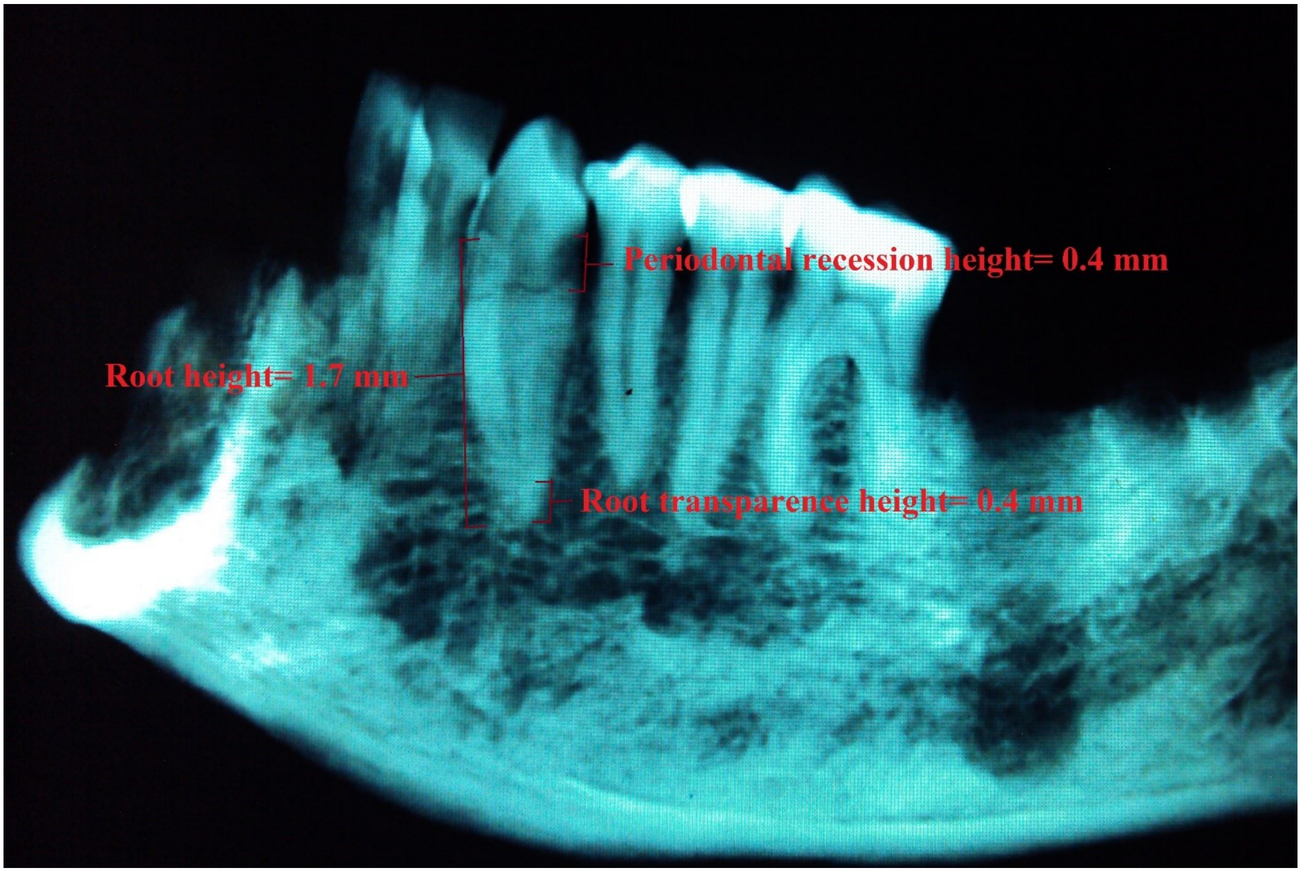


Figure 17 Measurement (in mm) of the right mandibular canine of Skull-B recovered from Gandhara grave site (by researcher)

The calculations are given as follow:

Measured root height= 1.7 mm

Measured periodontal recession height= 0.4 mm

Measured root transparence height= 0.4 mm

P = (0.4×100)/1.7= 23.52

T = (0.4×100)/1.7= 23.52

Age= (0.18x23.52) + (0.42x23.52) + 25.53= 39.63 years

The age of the person is approximately 39 years.

Johanson Sectioning (1971)

The Johanson sectioning technique is a modified form of Gustafson method. In this method the dental occlusion is examined. The teeth are sliced along the widest portion of the dental pulp and sectioned in a bucco-lingual plane to.25 mm. The following formula is used to estimate the age.

Age= 11.02+5.14A+2.30S+4.14P+ 3.71C+5.57R+8.98T (Johanson 1971: 1-126).

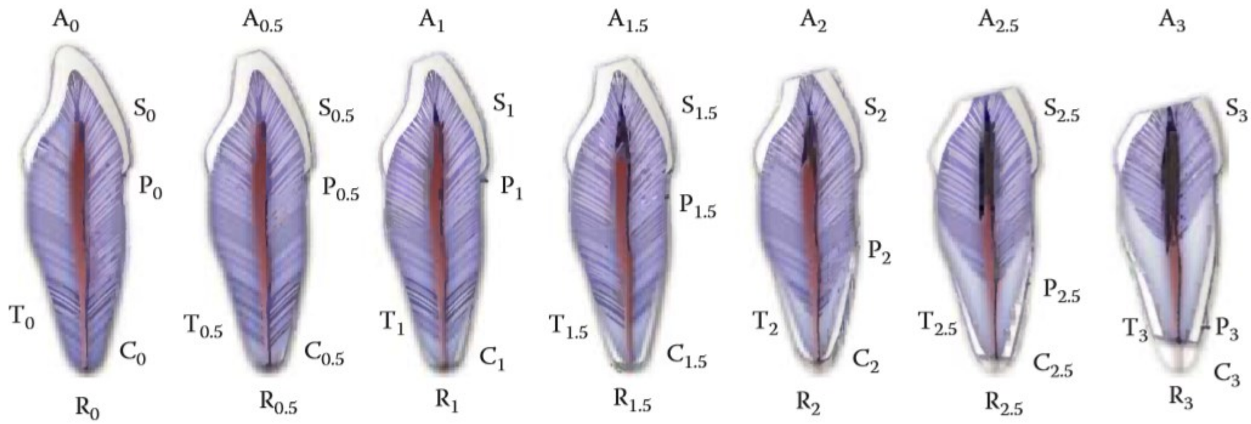


Figure 18 the Gustafson seven-stage post-formation change grading system, illustrated by Lewis (Senn and Weems 2013: 237).

Maples (1978)

In 1978 Maples developed this method that is based on five of the six Gustafson criteria. The following table shows Maples' formulas for root translucency and secondary dentin formation by tooth position (Maples 1978: 764-770).

Position Formulas for S and T		
	Estimated Age	SE (Years)
Central incisor	3.89S + 14.23T + 15.28	9.1
Lateral incisor	6.51S + 12.55T + 25.16	9.6
Cuspid	18.67S + 11.72T + 21.94	11.0
First bicuspid	2.82S + 15.25T + 19.65	12.2
Second bicuspid	4.79S + 15.53T + 17.99	7.6
First molar	11.28S + 5.32T + 10.86	11.1
Second molar	6.99S + 10.86T + 19.31	6.8
Third molar	4.71S + 12.30T + 24.57	12.0

Figure 19 Regression Formulas for the Variables of Secondary Dentin Formation (S) and Root Translucency (T) (Senn and Weems 2013: 237).

Prince and Ubelaker (2002)

Prince and Ubelaker used the same Lamendin et al. method for age assessment to the skeletal collections of Terry collected between 1900 and 1965. The collection contained skeletal remains of known gender, ancestry, age at death, and the reason of death to know the accuracy and precision of the method to a non-French population. A mean error of 8.23 years and a standard deviation of

6.87 years came out in this method when the Lamendin formula was used. Prince and Ubelaker used following formulas:

Male African Ancestry

$$\text{Age} = 1.04(\text{RH}) + 0.31(\text{P}) + 0.47(\text{T}) + 1.70 \quad 1 \text{ standard deviation} = 4.97 \text{ years}$$

Male European Ancestry

$$\text{Age} = 0.15(\text{RH}) + 0.29(\text{P}) + 0.39(\text{T}) + 23.17 \quad 1 \text{ standard deviation} = 5.92 \text{ years}$$

Female African Ancestry

$$\text{Age} = 1.63(\text{RH}) + 0.48(\text{P}) + 0.48(\text{T}) + (-8.41) \quad 1 \text{ standard deviation} = 7.17 \text{ years}$$

Female European Ancestry

$$\text{Age} = 1.10(\text{RH}) + 0.31(\text{P}) + 0.39(\text{T}) + 11.82 \quad 1 \text{ standard deviation} = 6.21 \text{ years}$$

The formulas include root height (RH) and the variables “P” and “T” same as Lamendin et al. (Prince and Ubelaker 2002: 107-116).

Bang and Ramm (1970)

Bang and Ramm included only root translucency in their age estimation technique as a variable. Because root translucency has not shown any variations related to ancestry or gender The Bang and Ramm method calculates age estimation data for whole and sectioned teeth. The tooth must be sectioned in the labio-lingual direction. In this method only length of root translucency in required in millimeters. If there the root translucency is not even the average length is calculated. The following calculations are for the root translucency if that are 9 mm or more or less than 9 mm (Bang and Ramm 1970: 238-244).

$$\text{Translucency zone} \leq 9\,\text{mm: Age} = B_0 + B_1X + B_2X^2$$

$$\text{Translucency zone} > 9\,\text{mm: Age} = B_0 + B_1X$$

X= measured length of root translucency in millimeters

B0, B1, and B2= tooth-specific regression coefficients

(Harms-Paschal and Schmidt 2010: 31).

Kvaal et al. (1995)

As previously mentioned adult dental age estimation methods requires extraction or sectioning of teeth. Kvaal et al. developed a new technique that did not sacrifice tooth. In this method he used radiographic method to estimate the change in pulp size due to the apposition secondary dentin. He used pulp width as the indicator of age because earlier studies had shown that pulp width was a best technique of age than pulp length (Kambe et al. 1991; Prapanpoch et al. 1992; Kvaal and Solheim 1994). Radiographic technique is best to calculate pulp width. Kvaal formulas for age assessment are for a) mandibular lateral incisors b) canines, and first bicuspids c) maxillary central and lateral incisors and d) second bicuspids. The technique would be more valuable by utilizing computer imaging software for accurate measurements and to minimize any error. Age can be known from the Kvaal et al. technique by calculating nine measurements and nine mathematical calculations (Kvaal et al. 1995: 175-185).

Preliminary Calculations	
Ratios	Regression Coefficients
P = p/r	M = mean value of all ratios
R = p/t	
A = a′/a	W = mean value of B and C
B = b′/b	
C = c′/c	L = mean value of P and R

Figure 20 Preliminary Ratio and Regression Coefficient Calculations from Radiographic Tooth Measurements P is the ratio between pulp (p) and root (r) length; R is the ratio between pulp (p) and tooth (t) length; A is the ratio between pulp width (a') and root width (a) at the CEJ level; B is the ratio between pulp width (b') and root width (b) midway between levels A and C; C is the ratio between pulp width (c') and root width (c) at the mid-root level. (Senn and Weems 2013: 242).

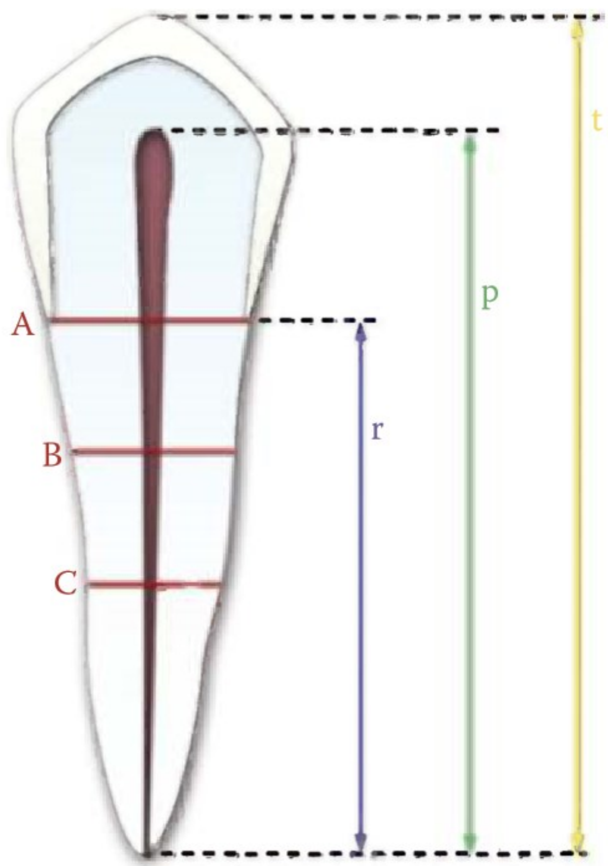


Figure 21 Illustration demonstrating the Kvaal (1995) measurements made on each tooth: t, maximum tooth length; p, maximum pulp length; r, root length from the mesial surface; A, root and pulp width at the CEJ; C, root and pulp width at mid-root (midpoint between CEJ and root apex); B, root and pulp width midway between levels A and C (Senn and Weems 2013: 242).

Table 8.12 Regression Formulas for Estimation of Age Using Radiographs

	Regression Formula	SEE (Years)
Six (6) teeth from both maxillary and mandibular arches	Age = 129.8 – 316.4(M) – 66.8(W-L)	8.6
Three (3) maxillary teeth	Age = 120.0 – 256.6(M) – 45.3(W-L)	8.9
Three (3) mandibular teeth	Age = 135.3 – 356.8(M) – 82.5(W-L)	9.4
Individual tooth #'s		
8 or 9	Age = 110.2 – 201.4(M) – 31.3(W-L)	9.5
7 or 10	Age = 103.5 – 216.6(M) – 46.6(W-L)	10.0
4 or 13	Age = 125.3 – 288.5(M) – 46.3(W-L)	11.0
21 or 28	Age = 133.0 – 318.3(M) – 65.0(W-L)	10.5
22 or 27	Age = 158.8 – 255.7(M)	11.5
23 or 26	Age = 106.6 – 251.7(M) – 61.2(W-L) – 6.0(G)	11.5

Figure 22 Regression Formulas for Estimation of Age Using Radiographs (Senn and Weems 2013: 242).

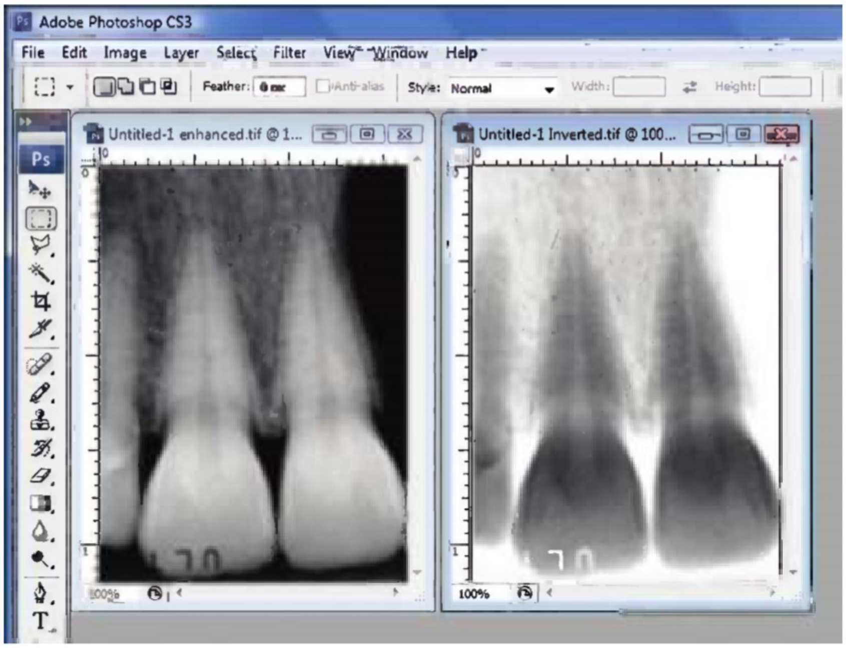


Figure 23 Kvaal (1995) technique: A Radiograph of tooth number 8 on the left and its inverted image on the right (Senn and Weems 2013: 243).

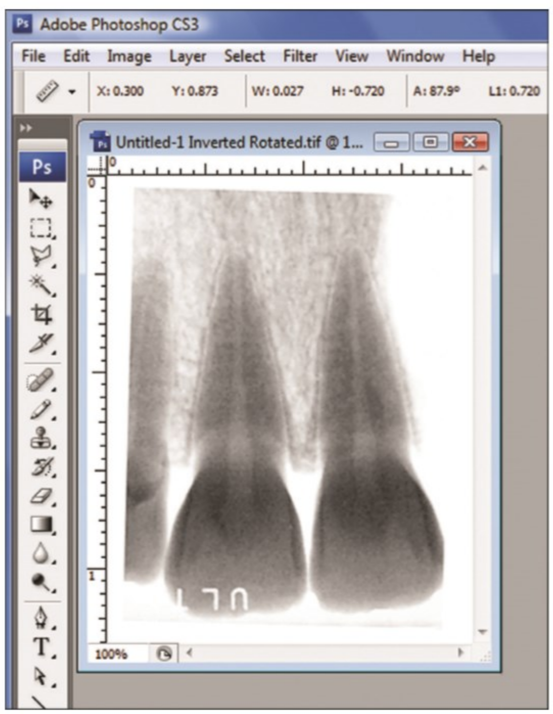


Figure 24 Kvaal (1995) technique: Inverted image of tooth number 8 rotated with the long axis of the tooth vertical (Senn and Weems 2013: 243).

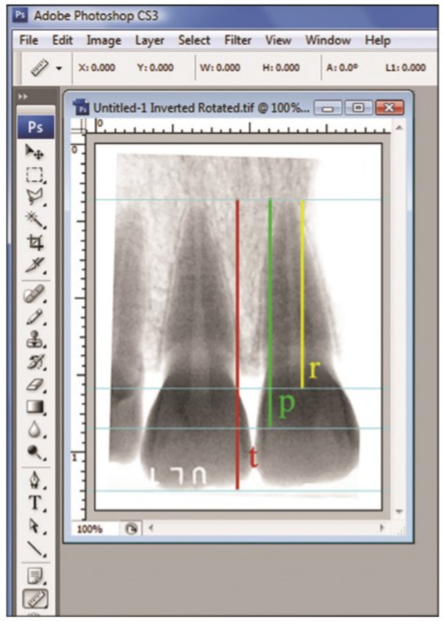


Figure 25 Kvaal (1995) technique: Guidelines (blue) are placed at the root apex, CEJ, coronal end of the pulp and incisal edge of the incisor to facilitate measurement of variables t, p, and r. (Senn and Weems 2013: 244).

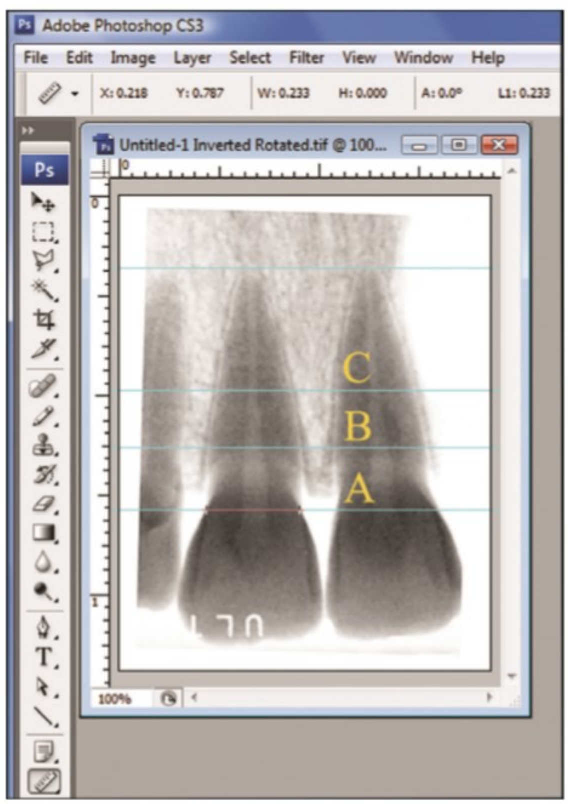


Figure 26 Kvaal (1995) technique: Guidelines (blue) are placed at level A, B, and C to facilitate the measurement of root width and pulpal width at each level (Senn and Weems 2013: 244).

Age Estimation from the patterns of tooth wear

A permanent tooth begins to wear after eruption. Patterns and intensity of wear depends on tooth developmental pattern, tooth size, internal crown structure and tooth morphology, and tooth angulation, the biomechanics of chewing, diet and nondietary tooth use (McKee and Molnar, 1988; Walker et al., 1991). If the degree of wear in a population is homogeneous then it means wear is a part of age. Therefore it can be used for age estimation. The osteologist should be careful in case if the teeth is used as a tool or infected due to pathological reason (Milner and Larsen, 1991). Miles (1963) for the first time established a scale of attrition based on the development of teeth. The technique is not valid for the individuals over 50 years of age (Miles, 2001). A variety of other techniques are also available that estimates age from the pattern of tooth wear (Molnar, 1971; Scott, 1979; Brothwell, 1989; Walker et al., 1991; Dreier, 1994; Mayhall and Kageyamu, 1997). Lovejoy (1985) has worked on the prehistoric Libben skeletal population and he observed that dental wear was very steady in the form and rate of that population. According to Lovejoy the estimation of age of a single individual in a forensic case on the basis of dental wear is only a approximation of age but in case of a large population the result will be more accurate. According to Lovejoy and colleagues (1985a) dental wear is the best technique for estimating the age of death in skeletal populations. According to Mays (2002) dental wear is a reliable indicator as he studied a very different historical Dutch skeletal collection. Hillson (2005) and Whittaker (2000) thought that this is the best choice if examined with the microscopic analysis of the permanent teeth. Drusini et al. (1997) used radiographic technique for age estimation of adult individuals to ±5 years on 78% of teeth assessed.

		di1	di2	dc	dm1	dm2	I1	I2	C	P3	P4	M1	M2	M3
A. MALES														
Ci	Cusp initiation	—	—	—	—	—	—	—	0.6	2.1	3.2	0.1	3.8	9.5
Cco	Cusp coalescence	—	—	—	—	—	—	—	1.0	2.6	3.9	0.4	4.3	10.0
Coc	Crown outline complete	—	—	—	—	—	—	—	1.7	3.3	4.5	0.8	4.9	10.6
Cr ½	Crown one half	—	—	—	—	—	—	—	2.5	4.1	5.0	1.3	5.4	11.3
Cr ¾	Crown three-fourths	—	—	—	—	—	—	—	3.4	4.9	5.8	1.9	6.1	11.8
Crc	Crown complete	0.15	0.2	0.7	0.4	0.7	—	—	4.4	5.6	6.6	2.5	6.8	12.4
Ri	Root initiated	—	—	—	—	—	—	—	5.2	6.4	7.3	3.2	7.6	13.2
Rcl	Root cleft present	—	—	—	—	—	—	—	—	—	—	4.1	8.7	14.1
R ¼	Root one-fourth	—	—	—	—	—	—	5.8	6.9	7.8	8.6	4.9	9.8	14.8
R ½	Root one-half	—	—	—	—	—	5.6	6.6	8.8	9.3	10.1	5.5	10.6	15.6
R ⅔	Root two-thirds	—	—	—	—	—	6.2	7.2	—	—	—	—	—	—
R ¾	Root three-fourths	—	—	—	—	—	6.7	7.7	9.9	10.2	11.2	6.1	11.4	16.4
Rc	Root complete	1.5	1.75	3.1	2.0	3.1	7.3	8.3	11.0	11.2	12.2	7.0	12.3	17.5
A ½	Root apex half closed	—	—	—	—	—	7.9	8.9	12.4	12.7	13.5	8.5	13.9	19.1

		di1	di2	dc	dm1	dm2	I1	I2	C	P3	P4	M1	M2	M3
B. FEMALES														
Ci	Cusp initiation	—	—	—	—	—	—	—	0.6	2.0	3.3	0.2	3.6	9.9
Cco	Cusp coalescence	—	—	—	—	—	—	—	1.0	2.5	3.9	0.5	4.0	10.4
Coc	Crown outline complete	—	—	—	—	—	—	—	1.6	3.2	4.5	0.9	4.5	11.0
Cr ½	Crown one half	—	—	—	—	—	—	—	2.5	4.0	5.1	1.3	5.1	11.5
Cr ¾	Crown three-fourths	—	—	—	—	—	—	—	3.5	4.7	5.8	1.8	5.8	12.0
Crc	Crown complete	0.15	0.2	0.7	0.3	0.7	—	—	4.3	5.4	6.5	2.4	6.6	12.6
Ri	Root initiated	—	—	—	—	—	—	—	5.0	6.1	7.2	3.1	7.3	13.2
Rcl	Root cleft present	—	—	—	—	—	—	—	—	—	—	4.0	8.4	14.1
R ¼	Root one-fourth	—	—	—	—	—	4.8	5.0	6.2	7.4	8.2	4.8	9.5	15.2
R ½	Root one-half	—	—	—	—	—	5.4	5.6	7.7	8.7	9.4	5.4	10.3	16.2
R ⅔	Root two-thirds	—	—	—	—	—	5.9	6.2	—	—	—	—	—	—
R ¾	Root three-fourths	—	—	—	—	—	6.4	7.0	8.6	9.6	10.3	5.8	11.0	16.9
Rc	Root complete	1.5	1.75	3.0	1.8	2.8	7.0	7.9	9.4	10.5	11.3	6.5	11.8	17.7
A ½	Root apex half closed	—	—	—	—	—	7.5	8.3	10.6	11.6	12.8	7.9	13.5	19.5

[a] The data are from Smith's (1991) compilation of published studies.

Figure 27 Average age (in years) of a skeletal individual based on an assessment of dental development at each crown position (White et al. 2012: 388).

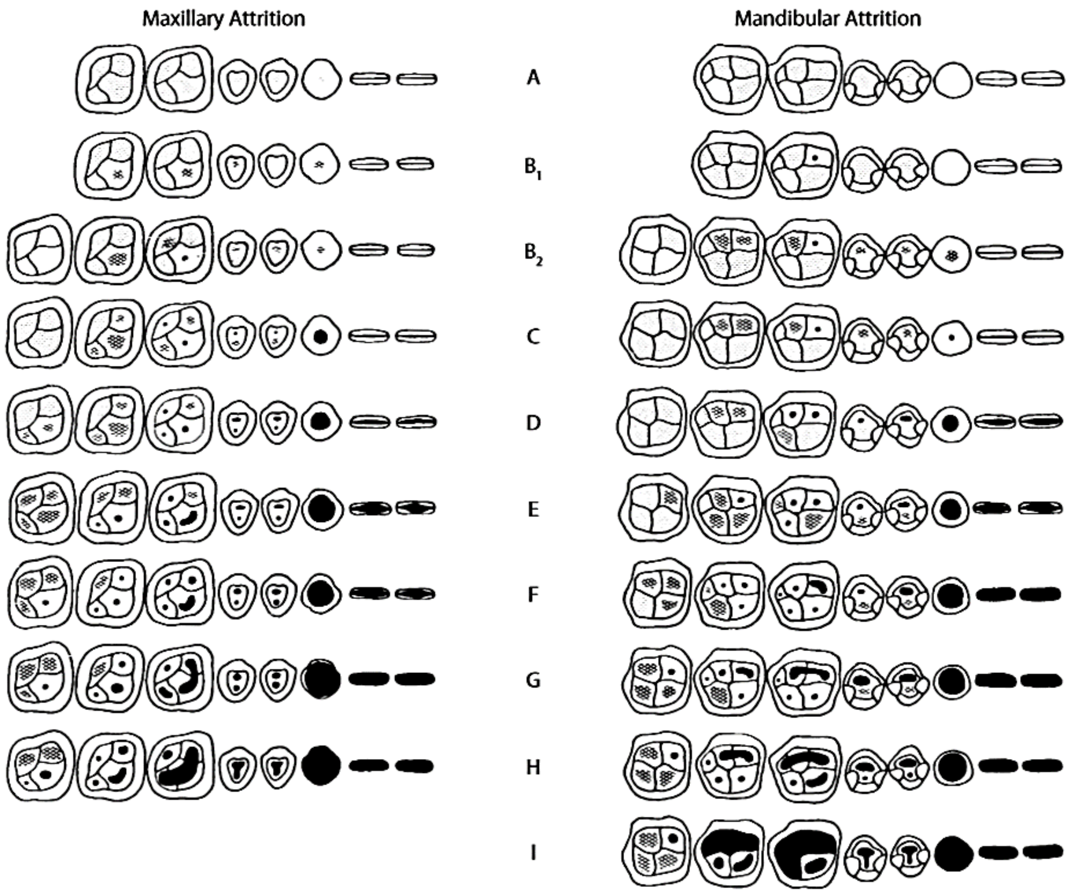


Figure 28 Modal tooth-wear patterns of a prehistoric Native American population from Libben, Ohio. Wear is divided into phases for right maxillary (left) and left mandibular (right) dentitions. Exposed dentine is shown in black. Age in years for the various phases are as follows: A, 12–18; B1, 16–20; B2, 16–20; C, 18–22; D, 20–24; E, 24–30; F, 30–35; G, 35–40; H (maxillary), 40–50; H (mandibular), 40–45; I, 45–55. See Lovejoy (1985) for a full description (White et al. 2012: 389).

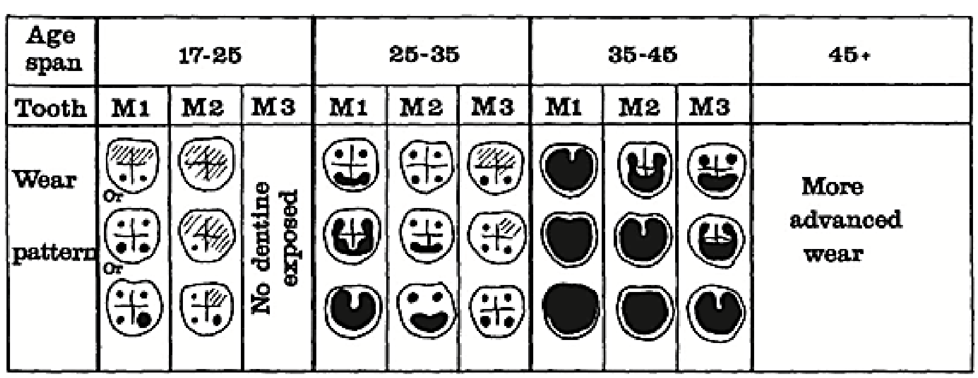


Figure 29 Estimated correspondence between adult age at death and molar wear phases for British material from Neolithic to medieval periods Source: After Brothwell (1981: Figure 3.9) (Mays 1998: 63).

The present researcher has followed the tooth-wear patterns developed by Lovejoy (1985) of a prehistoric Native American population from Libben, Ohio that is published by White et al. (2012) in his book 'Human Osteology'. It is clear from the Fig. 28, that the wear is divided into phases for right maxillary (left) and left mandibular (right) dentitions. Exposed dentine is shown in black. Age in years for the various phases are shown in the following table:

A	**12–18**
B1	**16–20**
B2	**16–20**
C	**18–22**
D	**20–24**
E	**24–30**
F	**30–35**
G	**35–40**
H (maxillary)	**40–50**
H (mandibular)	**40–45**
I	**45–55**

Table 3

For the present research only right mandibular dentitions (two incisors, one canine, two premolars and one molar) of 'Skull-B' are available. Two incisors, one canine, two premolars and one molar are enough to study their tooth wear pattern for age estimation.

Figure 30 Right mandibular teeth of Skull-B showing tooth-wear patterns (by researcher)

Figure 31 by enlarging the figure it is very clear to observe the tooth-wear patterns (by researcher)

Figure 32 Tooth-wear pattern of incisors (by researcher)

Figure 33 Incisors with 40x magnification showing tooth-wear patterns (by researcher)

Figure 34 Canine with 40x magnification showing tooth-wear patterns (by researcher)

Figure 35 Premolars with 40x magnification showing tooth-wear patterns (by researcher)

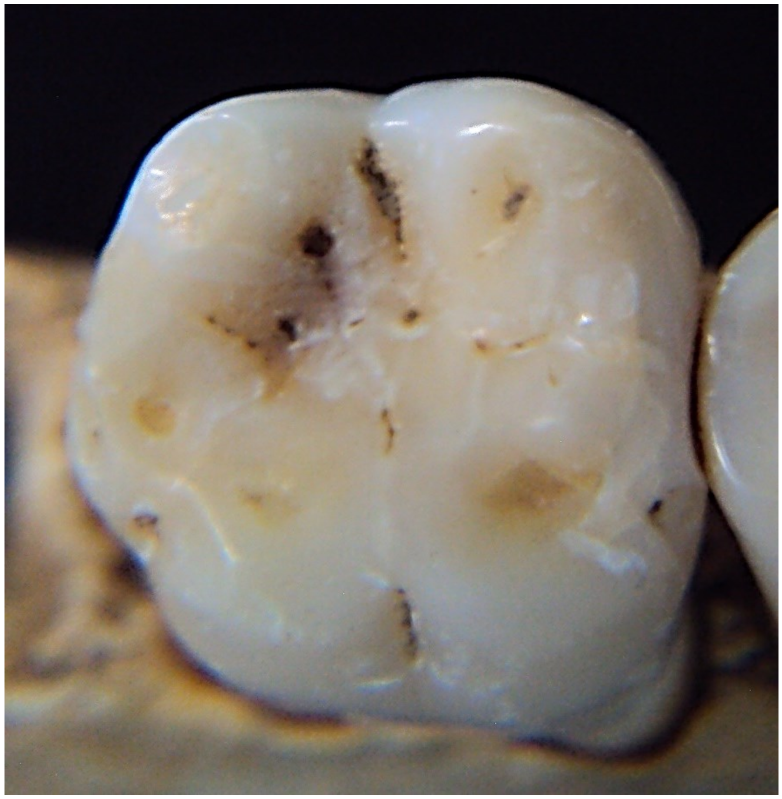


Figure 36 Molar with 40x magnification showing tooth-wear patterns (by researcher)

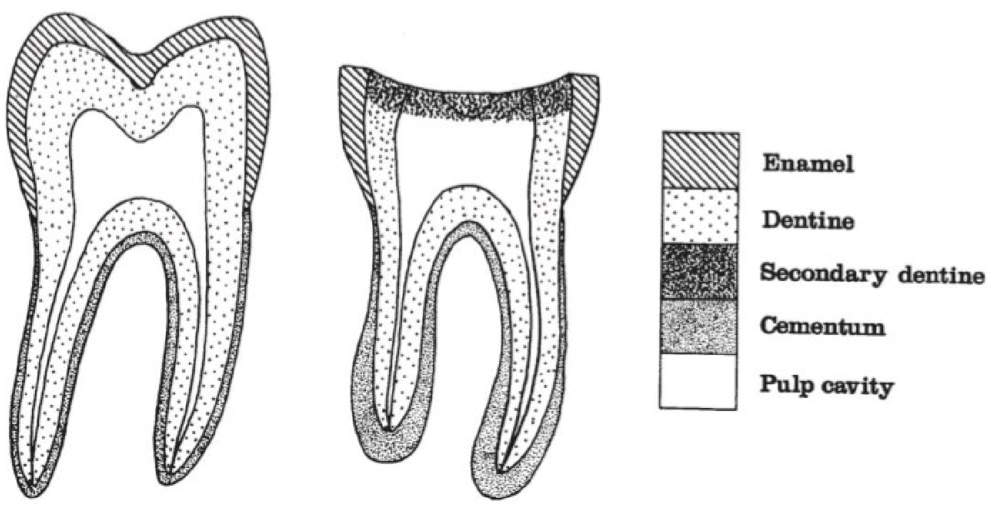


Figure 37 Diagrammatic sections through an unworn and a heavily worn molar (Mays 1998: 58).

After examining the tooth-wear patterns of mandibular teeth of 'Skull-B' with a 40x hand microscope the researcher has found these tooth-wear patterns similar to the 'G' phase of Lovejoy (1985) model of tooth-wear patterns. On the basis of these observations the estimated age of the 'Skull-B' is approximately 35-40 years.

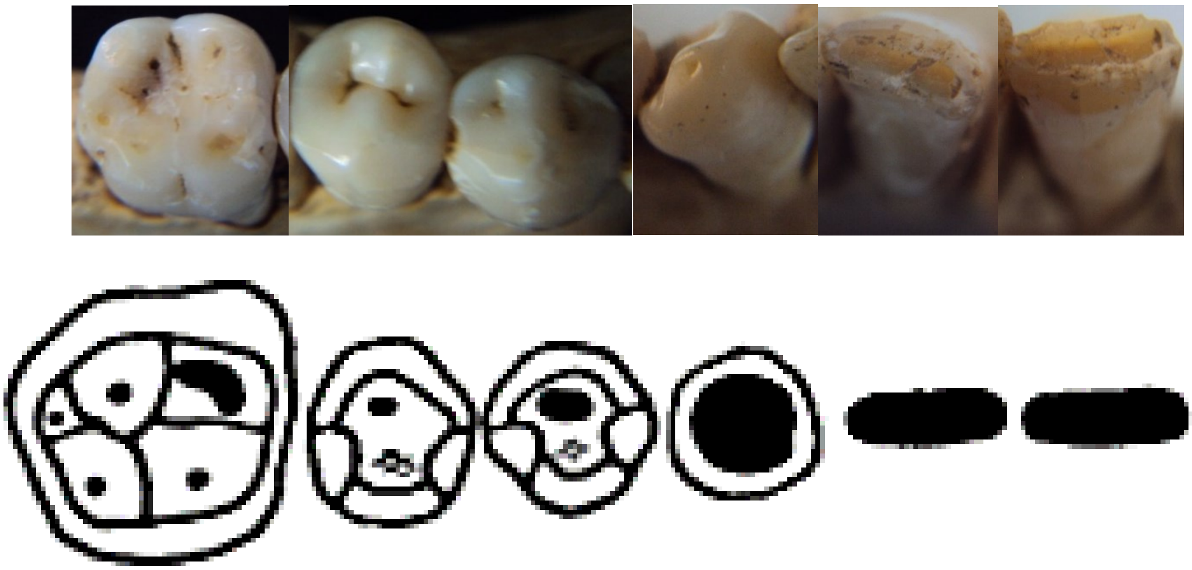


Figure 38 the tooth-wear patterns of the right mandibular teeth (two incisors, one canine, two premolars and one molar) of Skull-B compared to the Lovejoy (1985) model tooth-wear patterns (by researcher)

DETERMINATION OF SEX

Sex Indicators in the Adult Skeleton

Male and female adult skeletons are different in some aspects that help to determine their sex. Skull and pelvis are most important parts in human skeletons for the determination of sex. When we observe the pelvis then it can be seen that the female pelvis is broader than the male. The female pelvis is broader it forms the birth canal. The greater width of the female bony pelvis shows itself in a number of ways. The sub-pubic angle is important part that is formed between the left and right inferior pubic rami, the lower parts of the pubic bones. In females it have a tendency to be wider and more U-shaped. In male it have a tendency to be narrower (generally less than 90 degrees) and more V-shaped. There are also other features of the pubic bone that are useful in sex determination (Phenice 1969). The ventral arc is generally developed in females and in case of males they lack the ventral arc. The formation of the ventral arc in females is associated with the broadening of the female pelvis (Budinoff and Tague 1990). Generally the male skeleton is larger and has thicker bones with more developed areas for muscle attachment than the female (Mays 1998: 33).

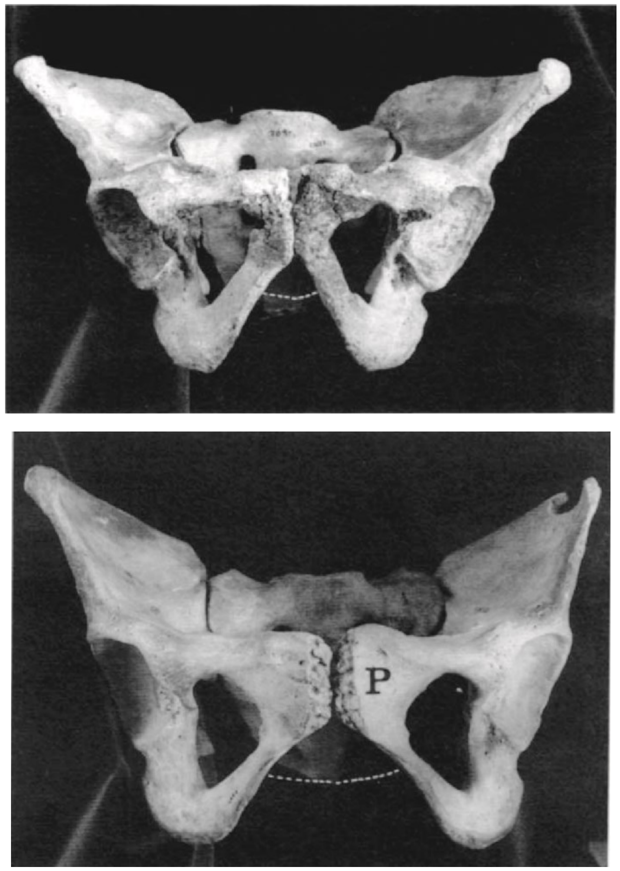
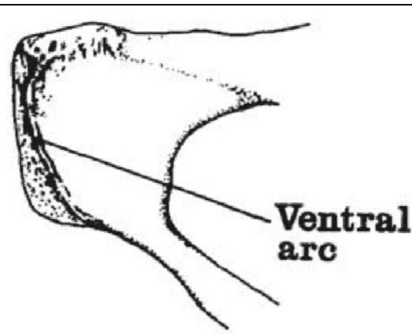


Figure 39 Adult female (above) and male (below) pelvic girdles. 'P' denotes the pubic bone. The sub-pubic angle is indicated by the dotted line (Mays 1998: 34).

Female	**Male**
	

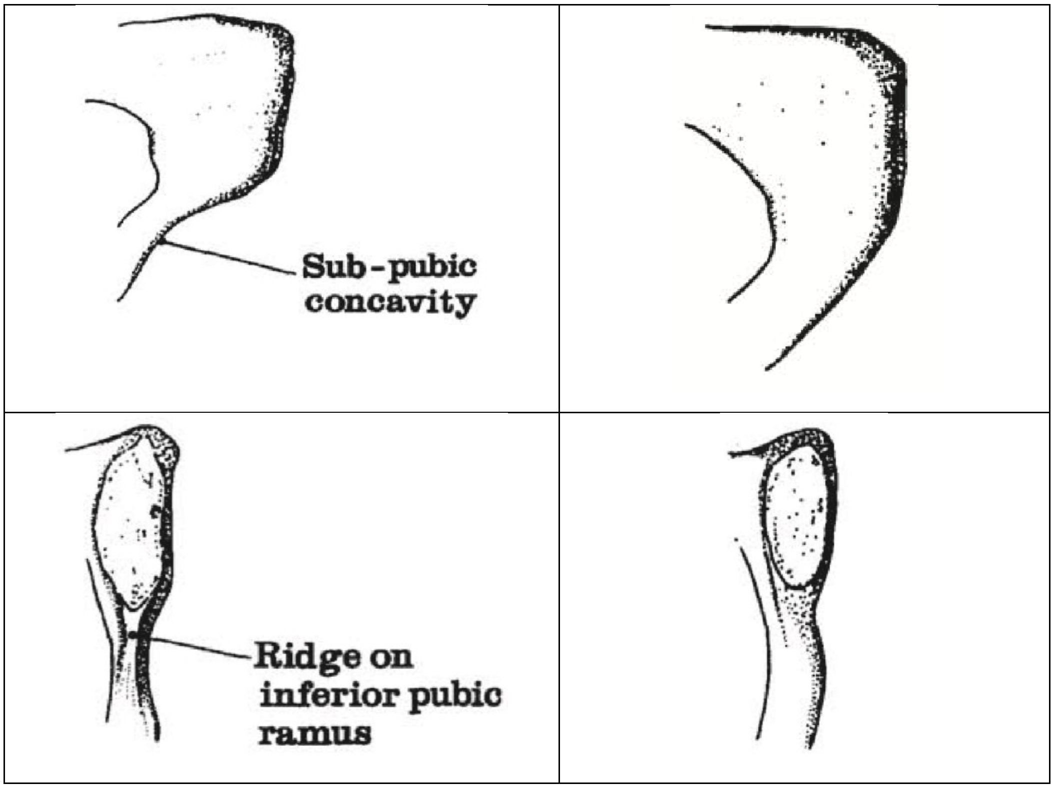


Table 4 (edited and created by the researcher) *Sex differences in the adult pubic bone. The anterior surface of the female pubic bone generally shows development of the ventral arc; this is usually lacking in the male, although slight ridging may occur. Seen from the dorsal aspect, the female pubic bone often shows a concavity in the inferior pubic ramus. The female pubic bone often shows a ridge on the medial surface of the inferior pubic ramus; in the male this area is generally rather flatter (Mays 1998: 35).*

Figure 40 Left pelvic bones of an adult female (above) and an adult male (below). The sciatic notch is indicated on each bone by the arrows (Mays 1998: 36).

Most of the important thing for sex determination is the skull. Male skull shows great robusticity. Specific characteristics of the cranium are useful for sex determination. These aspects are the brow ridge, the nuchal crest and the mastoid processes that characterize the male (Mays 1998: 33).

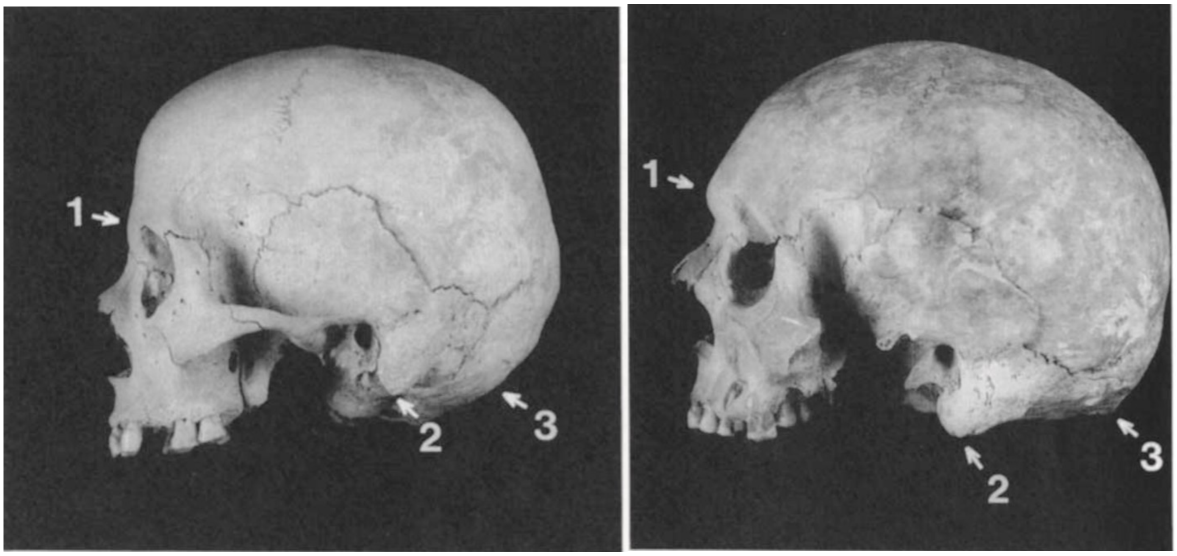


Figure 41 Adult skulls, female on left, male on right. The brow ridge (1) is more developed in the male and the forehead is more sloping. In the female the brow ridge is smaller and the forehead more vertical. The mastoid processes (2) and the nuchal crest (3) are more developed in the male (Mays 1998: 37).

Sex Determination from the Mandible

Males have gonial angles that are rugose and often everted (Acsádi and Nemeskeri, 1970; Novotný, et al., 1993; Kemkes-Grottenthaler et al., 2002). In case of females and immature individuals of both sexes have more gracile gonial angles that are not everted. According to the research of Loth and Henneberg (1996, 1998) the posterior border of the mandibular ramus could be used to sex unknowns with a predictive accuracy of about 90.6%–99.0%. They proposed that mandibles of adult males have a distinguishable angulation of the posterior border of the mandibular ramus at the level of the occlusal surface of the molars. In case of females they lacked flexure at that level. The technique was found by Koski (1996), Donnelly et al. (1998), Haun (2000), Hill (2000), and Kemkes-Grottenthaler (2002) with much lower accuracy (59%–80.4%). But recently, Balci et al. (2005) reexamined the method and found it with 90.6% accuracy that was higher in males (95.6%) than in females (70.6%). Balci et al. examined 120 mandibles in which sex could be known to only 69 or 57.5% (males: 67.4%, females: 20.0%). Loth and Henneberg (2001) also proposed that their posterior ramal flexure method could be used to determine sex in juveniles with 81% accuracy. But Scheuer (2002) found the accuracy to only be 64%.

The present researcher is agreed with the results of Baclci et al. (2005) that the sex determination from mandible has an accuracy of 90.6%. For their research they have examined 120 modern mandibles that are not archaeological. In the present research this method can be applied to the archaeological mandibles that are well preserved. The present researcher has applied this method for the determination of sex from the mandible of Skull-B. The mandible of Skull-B is not fully damaged and flexure of the posterior border of the mandibular ramus can be observed clearly. By examining the ramal flexion of the mandible of Skull-B it is clear that the individual is male. It is better to use radiographs of the mandible that will make the results more accurate.

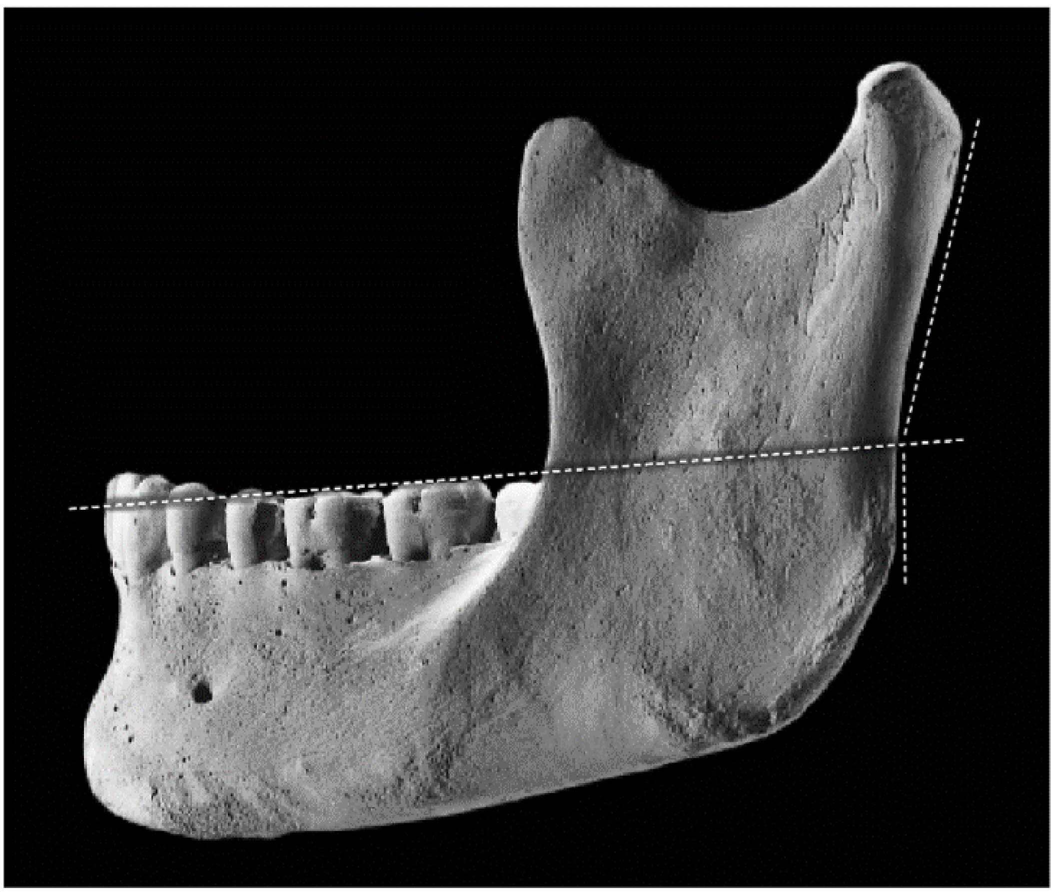


Figure 42 Ramal flexion. Flexure of the posterior border of the mandibular ramus in a young male individual. In contrast to males, who have a pronounced fl exure of the posterior ramal border at the level of the occlusal surface of the molars, females tend to have either a straight posterior ramal (White et al. 2012:414).

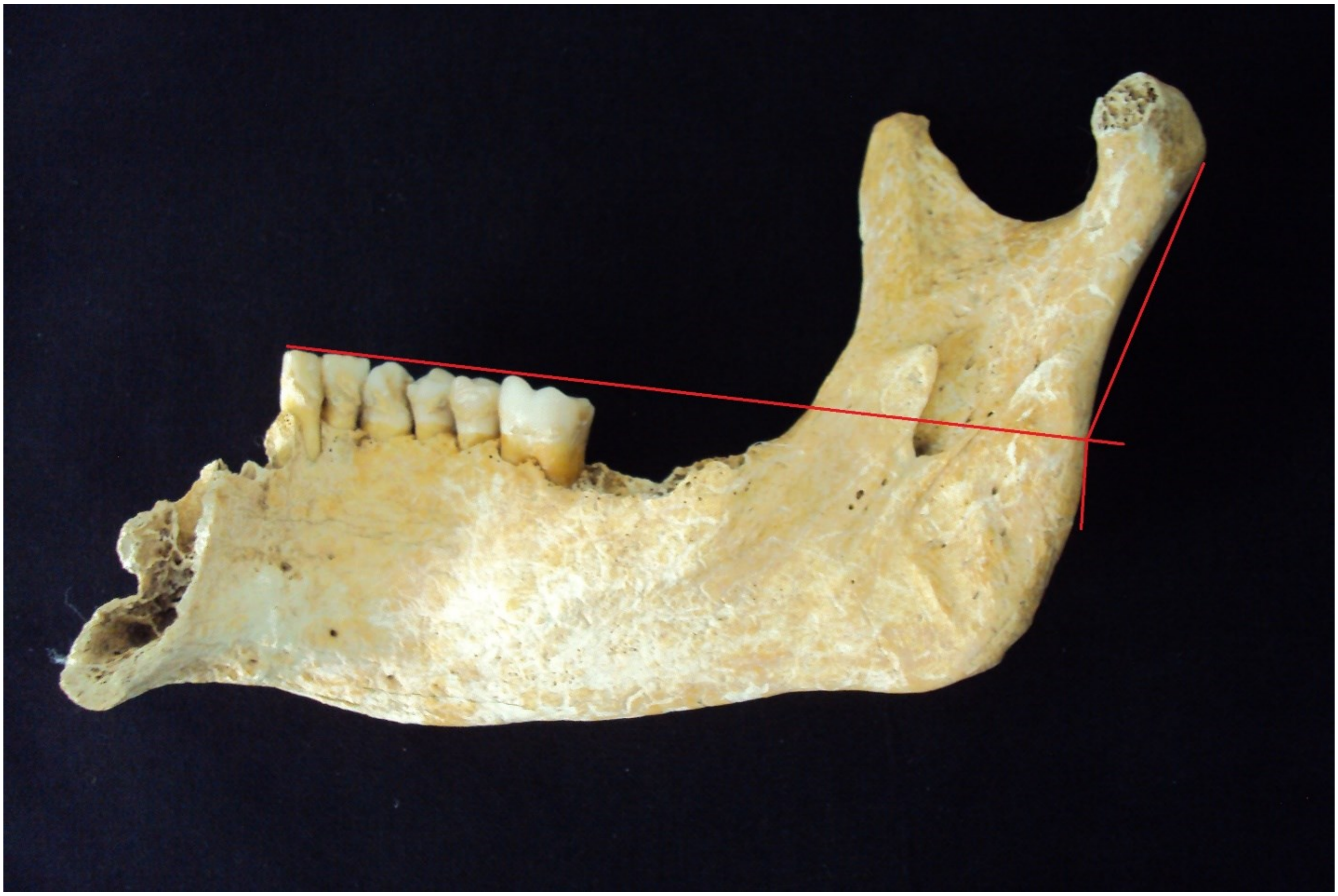


Figure 43 Ramal flexion. Flexure of the posterior border of the mandibular ramus of the Skull-B (by researcher).

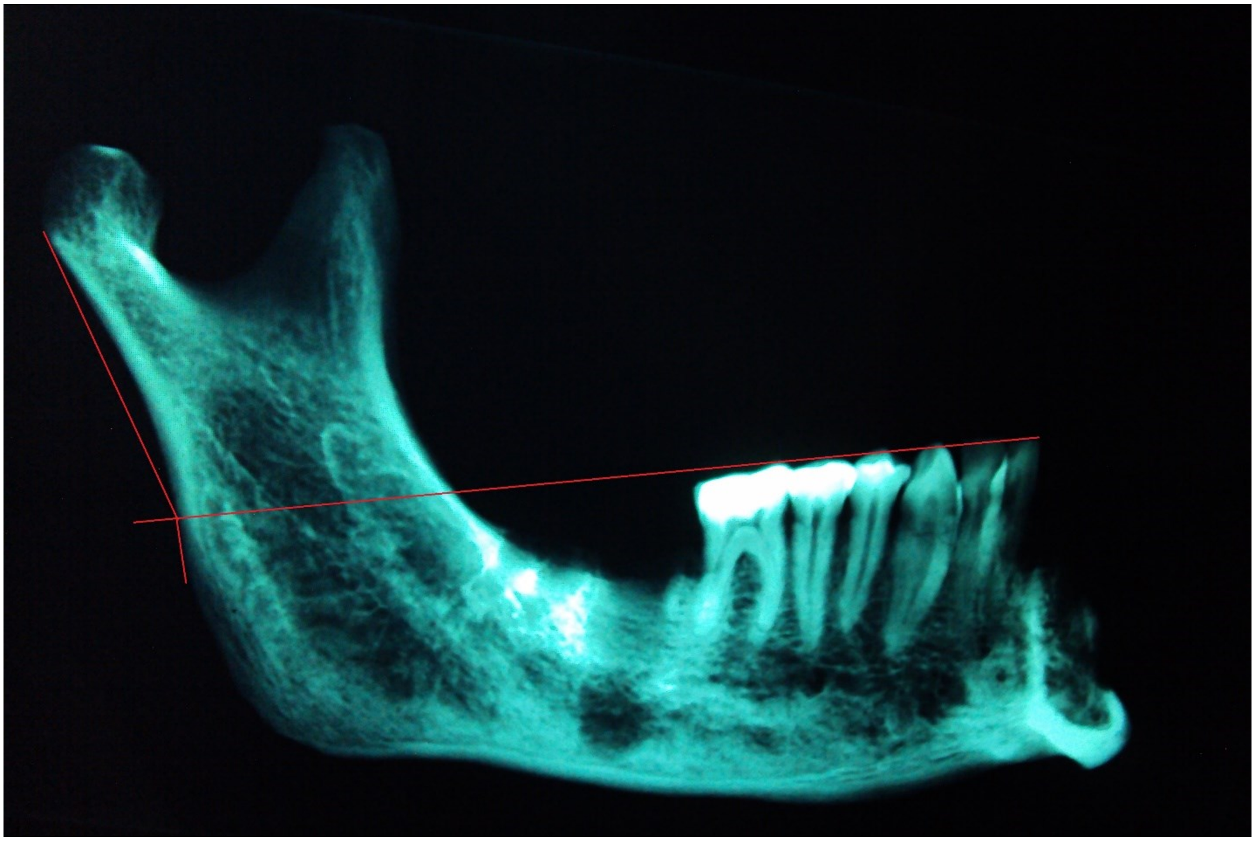


Figure 44 Ramal flexion. The radiograph showing flexure of the posterior border of the mandibular ramus of the Skull-B (by researcher).

Determination of Sex of Skull-A

It can be observed that parts of the skull in males tend to be larger and more robust than females. It provides useful indications for determining the sex of skulls. These traditional characteristics are used by osteologists. In male crania are characterized by greater robusticity as compared to female. Male crania display more prominent supraorbital ridges, a prominent glabellar region, and heavier temporal and nuchal lines. Male frontals and parietals tend to be less bossed as compared to female. Males tend to have relatively large, broad palates, larger mastoid processes, larger occipital condyles, squarer orbits and larger sinusesas compared to females. Male mandibles are characterized by more gonial eversion, more rugose muscle attachments, squarer chins and deeper mandibular rami (Gülekon and Turgut 2003). Using Chi-squared automatic interaction detection (CHAID) provides classification trees that are easy to use and result in a probabilistic determination of sex (White et al. 2012: 412-414).

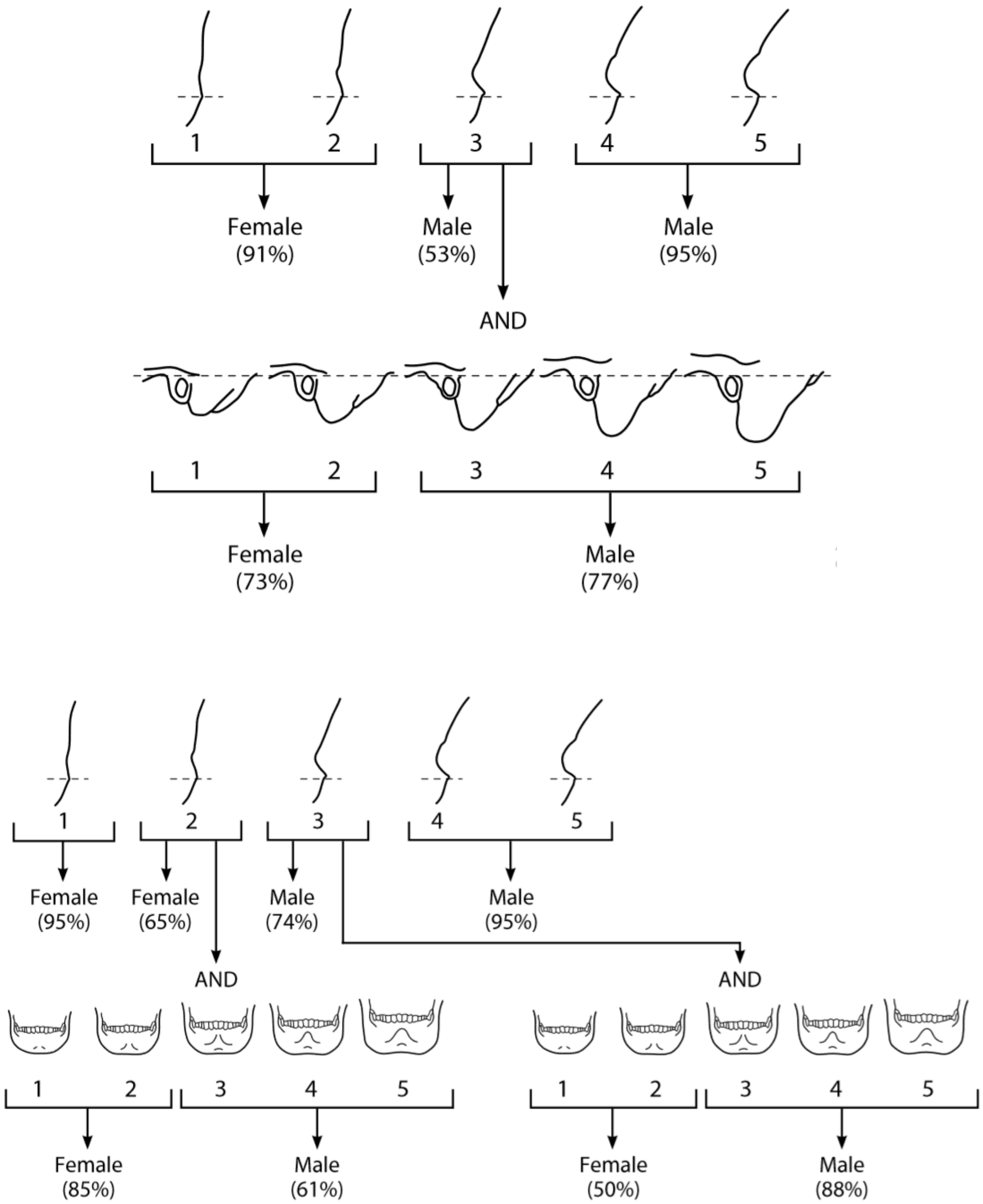


Figure 45 CHAID analysis decision tree. Two examples demonstrating the use of decision analyses in probabilistic sex determination. Top: the classification tree shown on the top is for European Americans crania which preserve both glabella (above) and mastoid process (below). Bottom: the classification tree on the bottom is for a mixed sample of Europeans, European Americans, and African Americans which preserve both glabella (above) and the mental eminence (below) (White et al. 2012: 414).

Figure 46 In Skull-A the brow ridge (highlighted by the line) is more developed and the forehead is more sloping.

By observing Skull-A the brow edge is more developed and the forehead looks like sloping therefore, it can be concluded that the individual is male.

Chapter No. 4

Odontological Examination of the Human Skeletal Remains Recovered from Gandhara Grave Sites

Teeth are very importance in osteological work in archaeology, forensics and paleontology. It is important to identify isolated teeth accurately. With a little organization and an appropriate analytical framework, it is very easy to identify any teeth. Due to morphological variation human teeth are more difficult to identify as compared to the other species. This chapter deals with the complete odontological examination of the human skeletal remains recovered from Gandhara Grave sites. For this purpose only right mandible of Skull-B is available.

Tooth Identification of the Right Mandible of Skull-B

First the researcher has identified mandible of Skull-B by comparing it with the following figure.

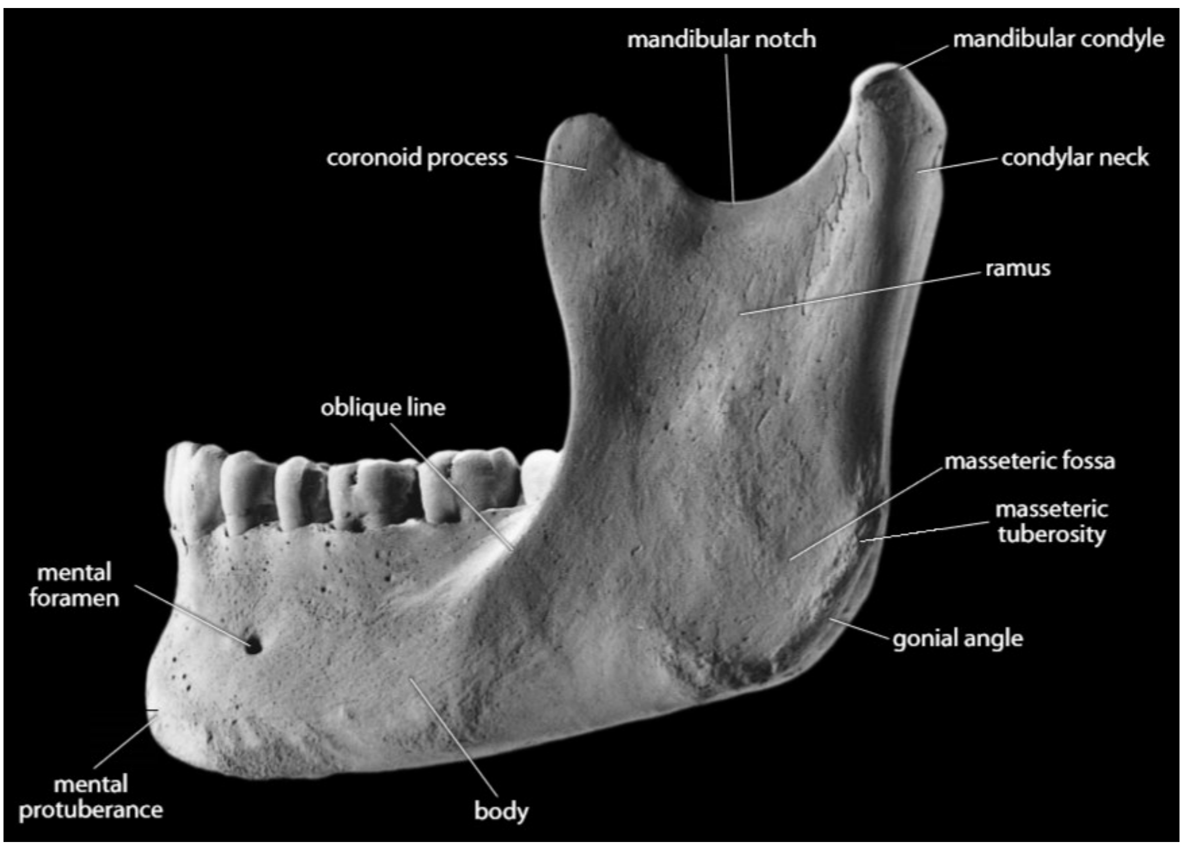


Figure 47 Mandible, lateral (White et al. 2012: 94)

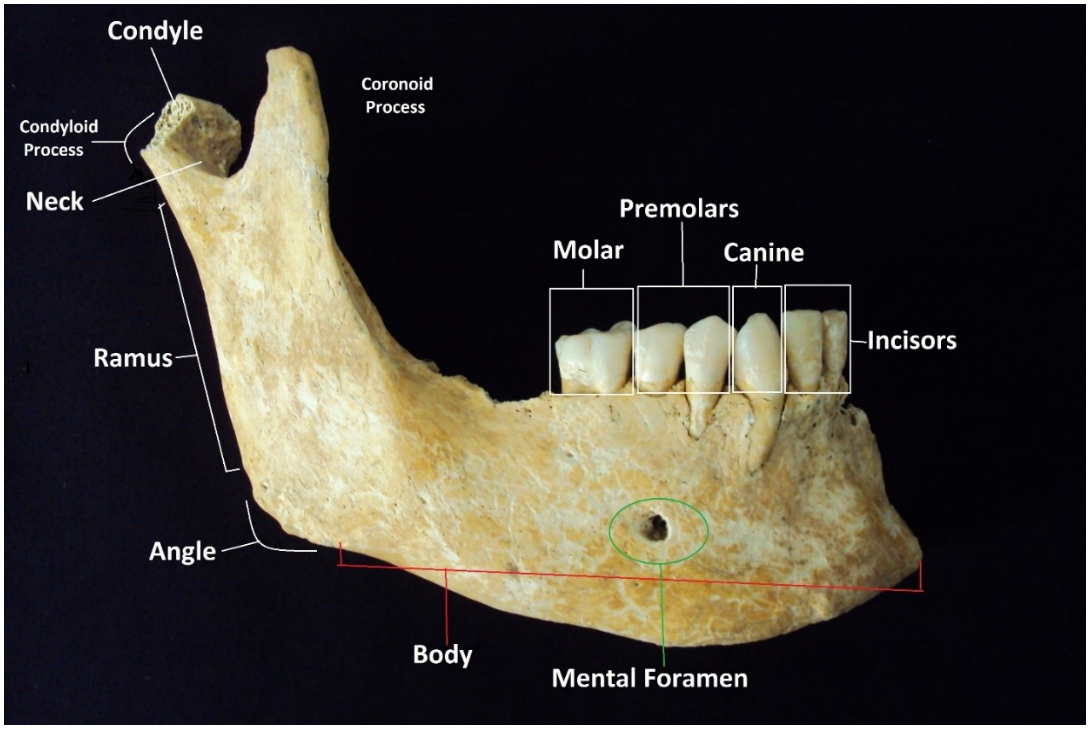


Figure 48 mandibular right half arcades with permanent dentitions of Skull-B

After identification of the half mandibular arcade of Skull-B, the researcher has compared it with the mandibular dentitions of. After comparing all the mandibular dentitions are identified. There are two incisors, one canine, two premolar and on molar in half mandibular arcade of Skull-B. All these teeth are permament and fully developed. Two molars are missing.

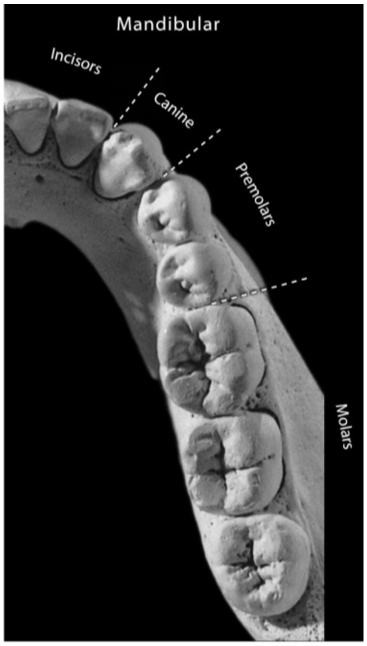


Figure 49 mandibular right half arcade with permanent dentitions (White et al. 2012: 111)

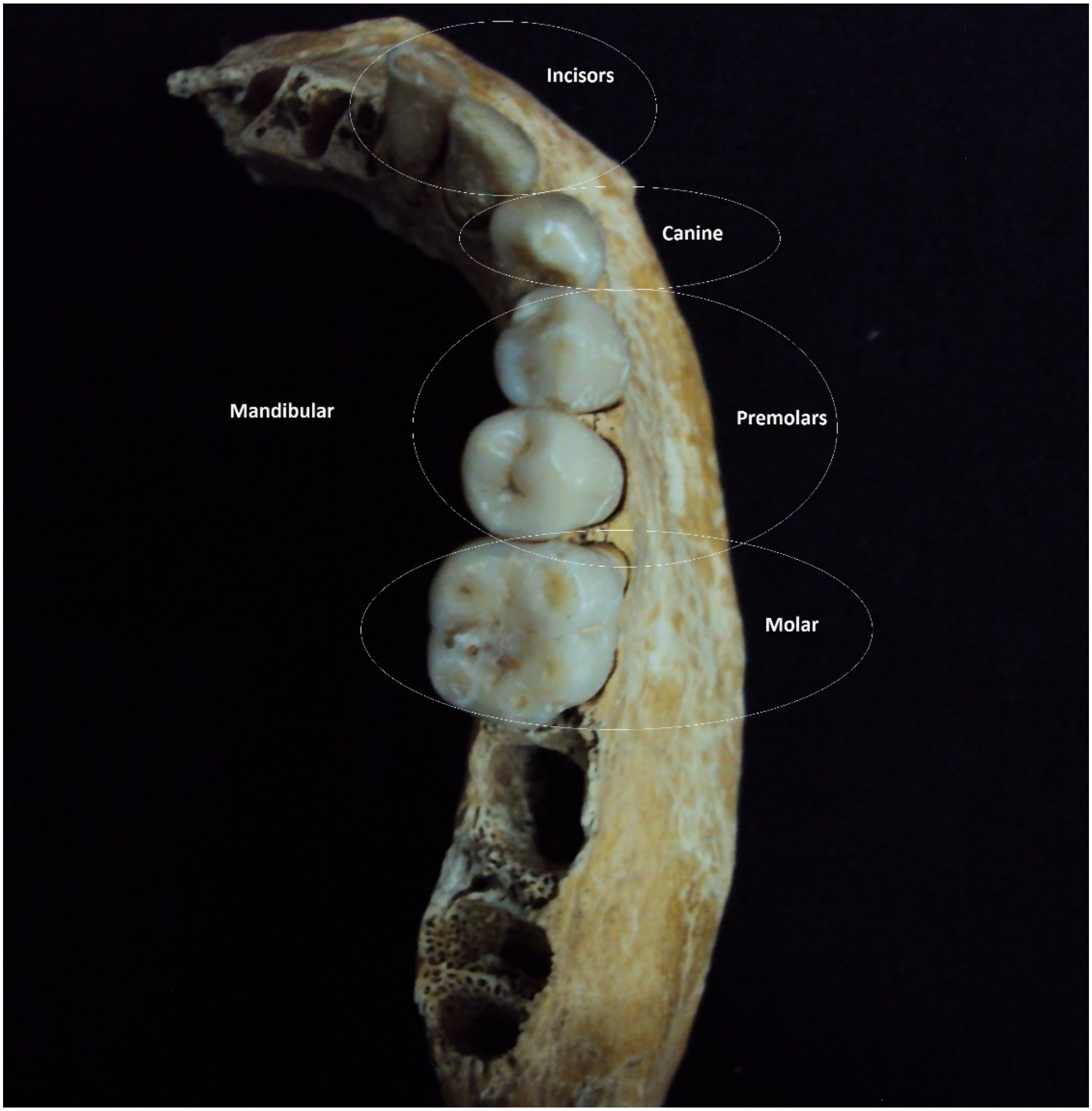


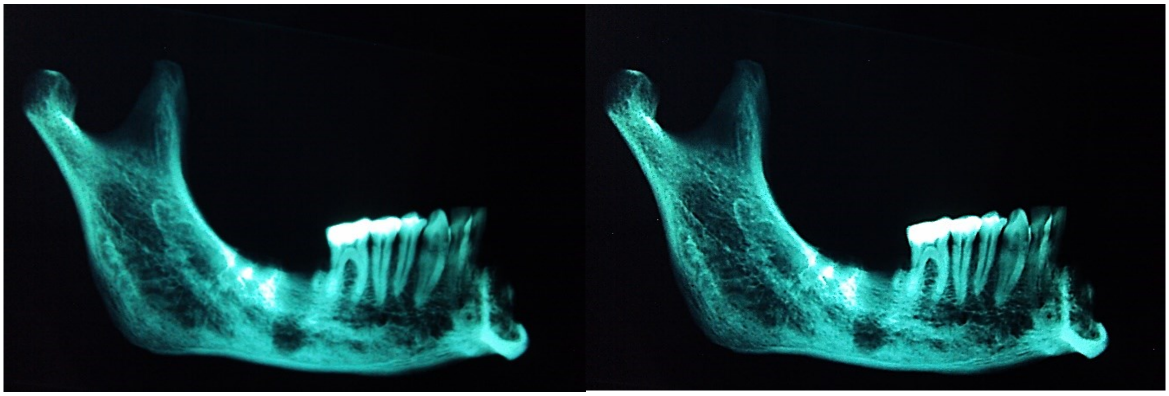
Figure 50 Mandibular right half arcade with permanent dentitions of the skull-B recovered from Gandhara Grave Site (by researcher).

Dental Radiography of the Human Skeletal Remains Recovered from Gandhara Site

Dental radiography is very important in dental identification that cannot be over-emphasized. Clearly visible dental anatomical structures in dental radiograph have many advantages. Denatl radiographs can be sufficient to provide a comprehensive understanding of the dental status (Adams et al. 2014: 87-89). Following are the features visible in dental radiographs which assist dental identification:

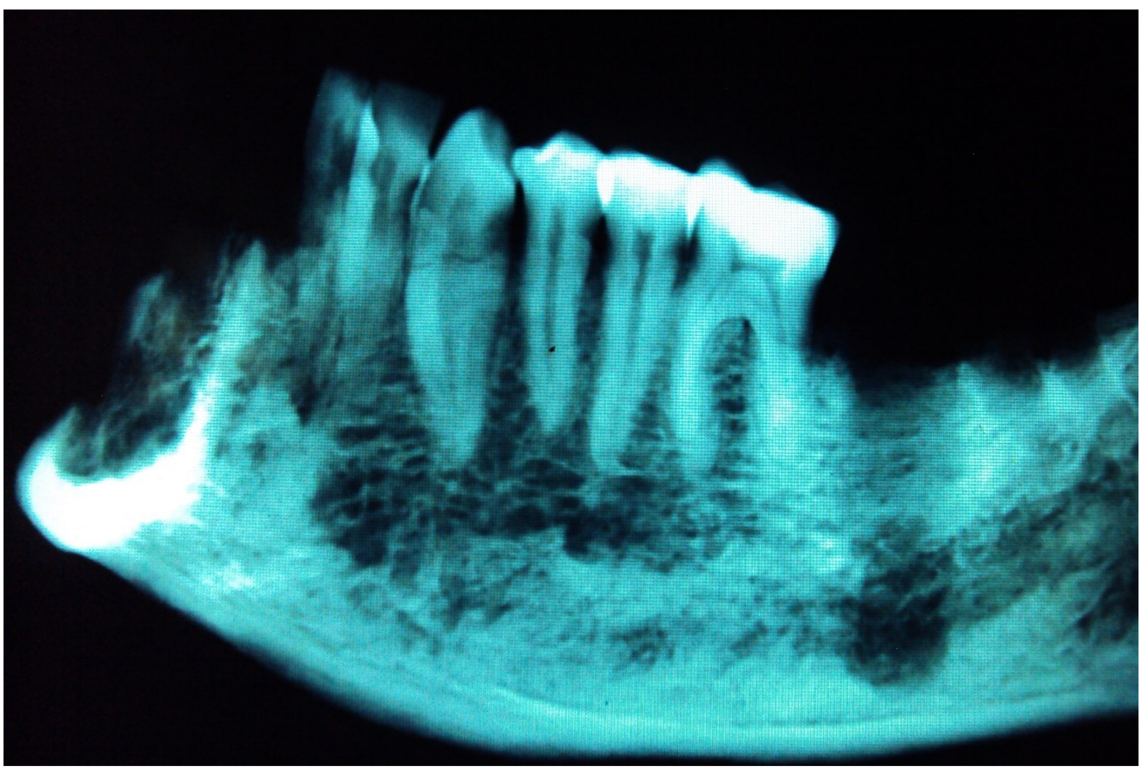
- Outline of restorations
- Root fillings
- Metal posts/pins
- Implants
- Root anatomy
- Pulp/root canal anatomy
- Inferior alveolar nerve canal
- Mental foramen
- Maxillary sinus floor
- Supernumerary teeth
- Unerupted teeth
- Plates and screws (Adams et al. 2014: 89).

The researcher has applied radiography on the mandible of Skull-B. The illustration of figure 53 is clearly demonstrating the Kvaal (1995) measurements in which: t, maximum tooth length; p, maximum pulp length; r, root length from the mesial surface; A, root and pulp width at the CEJ; C, root and pulp width at mid-root (midpoint between CEJ and root apex); B, root and pulp width midway between levels A and C. Dental radiographs are very useful in dental identification and age estimation. Without dental radiography archaeologist cannot draw a full profile of an individual.



(Original) (Edited to highlight dentition more clearly)

Figure 51 Radiograph of the Mandibular right half arcade with permanent dentitions of the skull-B recovered from Gandhara Grave Site (by researcher).



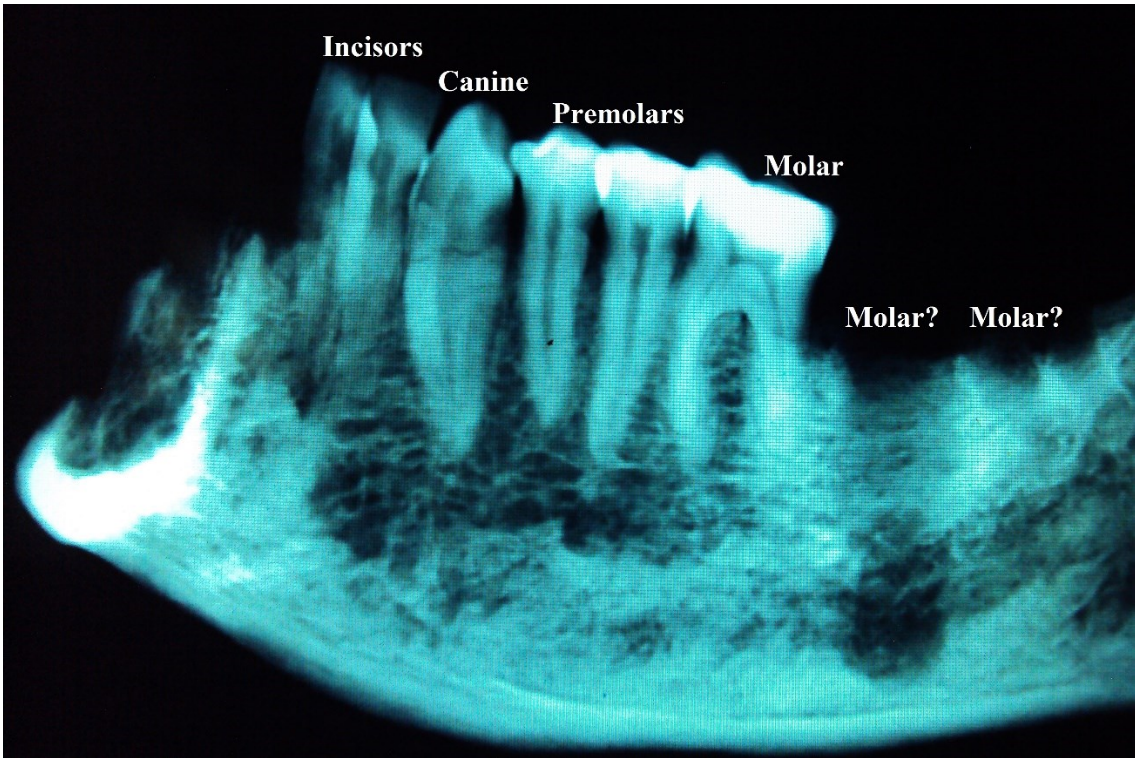
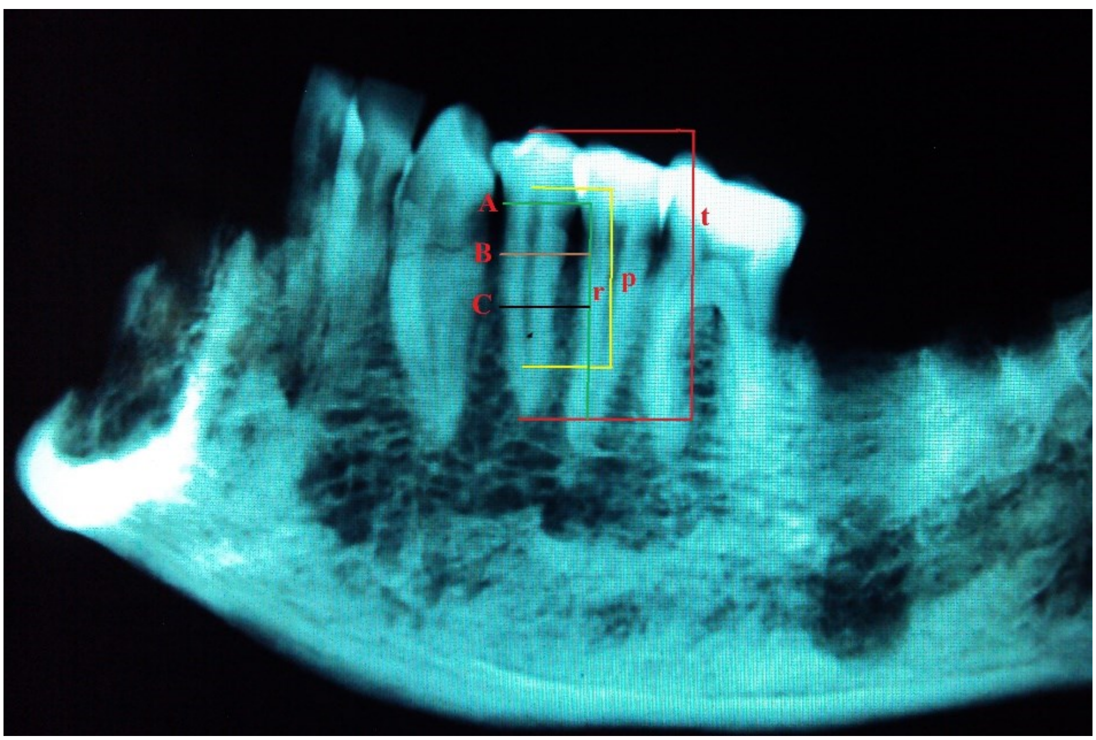


Figure 52 Radiograph of the Mandibular right half arcade with permanent dentitions of the skull-B recovered from Gandhara Grave Site. The Radiograph clearly shows dental anatomical structures and two missing molars (by researcher).



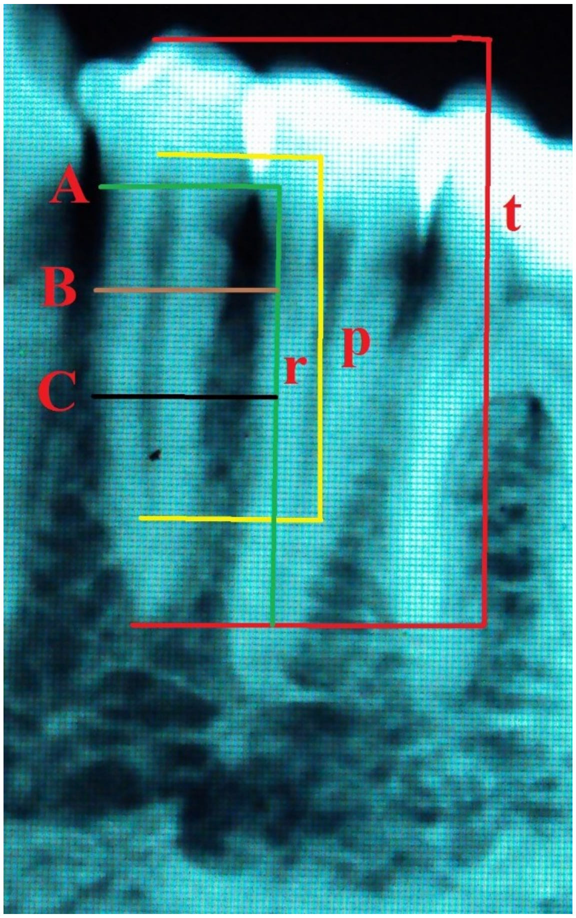


Figure 53 Radiography of the Mandibular right half arcade premolar demonstrating the Kvaal (1995) measurements of skull-B recovered from Gandhara Grave Site (by researcher).

Dental Profiling of the Human Skeletal Remains Recovered from Gandhara Site

Dental profiling is also known as 'reconstructive dental identification.' It can be defined as, 'the process of extracting information from the teeth in an attempt to piece together a profile of the deceased individual.' There are very important information that can be acquired from dental profiling. Gender, ethnicity, geographical provenance, habits, nutritional deficiencies and indicators of past severe illness are some of these information (Adams et al. 2014: 101). This is forensic perspective but I think dental profiling is very important in archaeology or forensic archaeology. Gender, ethnicity, geographical provenance, habits, nutritional deficiencies and indicators of past severe illness are key information that can help to reconstruct the past of an entire population.

Gender

Pelvis and the skull and mandible show high level of sexual dimorphism. Similary teeth also show sexual dimorphism in their mesiodistal, buccolingual and diagonal crown dimensions, as well as in cuspal diameter. Using the teeth to determine gender in skeletal remains should be used with caution, if at all. (Adams et al. 2014: 101). Teeth of male are larger as compared to the female.

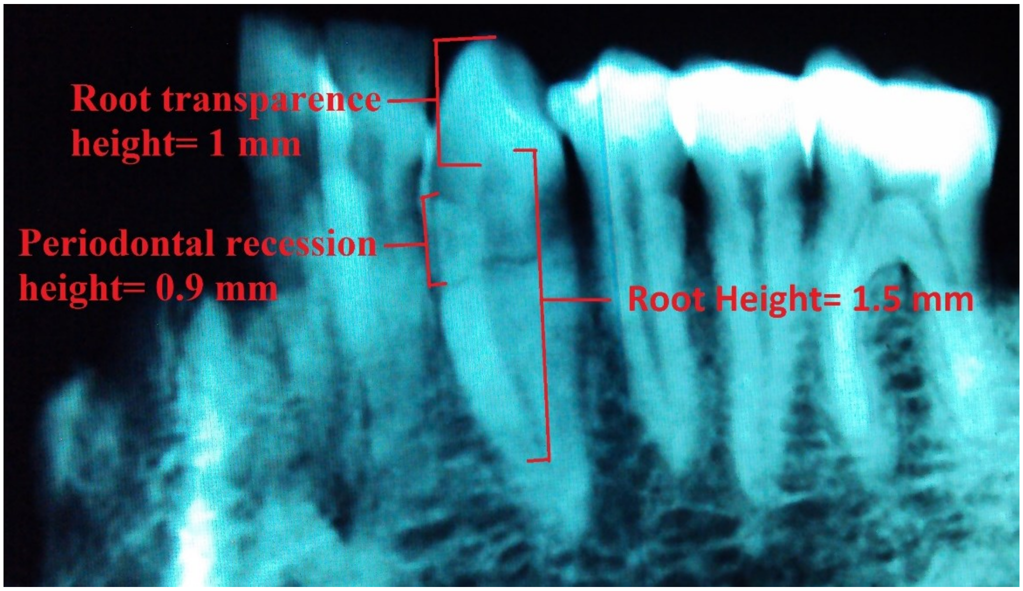


Figure 54 the mandibular right half arcade dentition of Skull-B. The large size of dentition shows that the individual is male (by researcher)

Ethnicity

Determination of ethnicity is puzzling because no 'pure' race actually exists due to gene flow. Only anthropologists (including archaeologists) or odontologists are likely to give the best indications of ethnicity. For example the shape of the upper is more horseshoe shaped in Asians and U-shaped in Caucasians. Similarly teeth may also provide indicators of ethnicity. Morphometric variations in teeth have a history of description for more than 100 years. The most widely used scheme, and the standard recommended by anthropologists working in the field, is the Arizona State University dental anthropology system which provides plaques for scoring the level of variation of a particular trait.

Some examples are:

- Shovelling of upper incisors are found among Asians and Native Americans
- Enamel extensions on the facial surfaces of molars are found in Asians
- Carabelli's trait is frequently found in Europeans
- Prominent canine mesial ridge are found in Bushman Africans
- Uto-Aztecan premolar – where the buccal cusp of upper first premolars bulges out to the buccal – frequently found only in Native Americans, with the highest frequency in Arizona.

Most of these variations present differently in different populations in which only a few present in one population (Adams et al. 2014: 102**;** Senn and Weems 2013: 81).

Figure 55 Cusp of Carabelli on UL first molar (*Senn and Weems 2013: 81).*

Figure 56 Two supernumerary mandibular left premolars (Senn and Weems 2013: 82).

Figure 57 Shoveled maxillary incisors. (Senn and Weems 2013: 82)

In the present research the only available mandibular right half arcade dentition of Skull-B are very helpful to identify the ethnicity of the individual. By examining the dentition the researcher has found enamel extensions on the facial surfaces of molars. According to Adams et al. (2014: 102) enamel extensions on the facial surfaces of molars are found in Asians. Therefore the Skull-B belongs to an Asian individual.

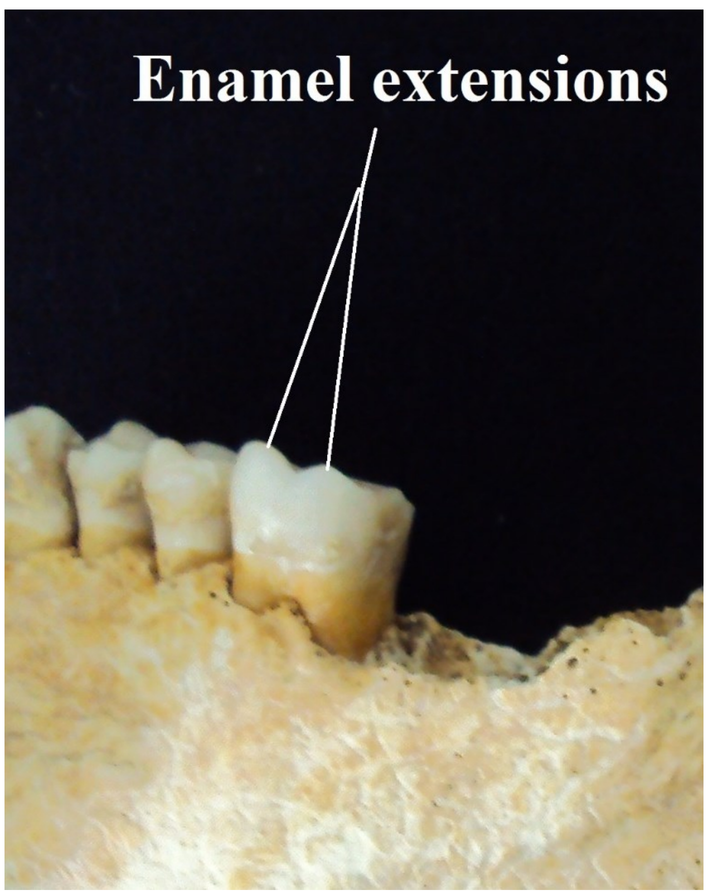


Figure 58 mandibular right half arcade of Skull-B. The molar showing enamel extensions (by researcher).

Age assessment

Age estimation is the most useful information that can be gained from the teeth. Dental age estimation is the most reliable age estimation method (Adams et al. 2014: 102). The researcher also applied dental age estimation method of Lamendin et al. (1992) (see chapter 'Determination of Age and Sex from Ancient Bones: A Case Study of Human Skeletal Remains Recovered from Gandhara Grave Sites').

Habits

Dietary, social and Occupational habits can be observed in the appearance of the teeth. Extrinsic staining of the teeth is caused by tobacco smoking or drinking of red wine or tea and/or coffee. Tooth surface loss due to erosion can suggest excessive consumption of acidic drinks and foods or conditions like bulimia or anorexia. Specific areas of abrasion can give clues of occupation-related habits (Adams et al. 2014: 102).

Habit	Appearance	Cause
Coffee, tea, red wine drinkers	Brown or black staining of teeth. More obvious when calculus is present on teeth	An extrinsic stain caused from drinking the liquids
Pipe smoking	Unusual tooth wear patterns and staining	Unilateral attrition
Mastication of leather to soften for making garments	Extreme attrition of teeth	Some Native North American habits
Betel leaf and nut chewing	Black or brown stains	Extreme extrinsic stains
Tooth mutilation	Various sharpened or flattened teeth	Some African and Australian tribes
Tooth blackening—Ohaguro	Black teeth	Japan and Southeast Asia custom
Alcoholism	Acidic erosion commonly on the lingual surfaces of teeth	Regurgitation and acid reflux
Drugs of abuse—methamphetamine, cocaine, heroin, methadone	High caries rate, attrition, acidic erosion, tooth fracture advanced periodontal disease	Oral neglect, dry mouth, high sugar consumption, regurgitation, bruxism
Mouth rinses including chlorhexidine, scope, cepacol	Exogenous staining from chlorhexideine and cetylpyridinium chloride	Extrinsic stains from rinsing mouth

Figure 59 Habits and Customs That May Affect the Teeth (Senn and Weems 2013: 83).

By analyzing dentition of Skull-B the researcher has observed black and brown stains on mandibular molar that shows that the person may be involved in chewing nuts and the generalized tooth abrasions the mandible show that the person was sandblaster, grain mill or saw mill worker by occupation.

Occupation	Appearance	Cause
Musicians, traffic officer Carpenters, electricians Seamstresses, hairdressers Shoe maker/repairer Upholsterer, glassblower	Unusual abrasion or wear patterns on teeth	Stripping wires, holding nails, brads, pins, needles with teeth, biting on reed/mouthpiece, whistle, etc.
Sandblasters, grain mill, saw mill workers	Generalized tooth abrasion	Abrasive dust and particulate matter causing abrasion
Miners, cement and stone cutters, jackhammer operator		
Chemical, galvanizing and battery workers making or using acids	Eroded labial surfaces of anterior teeth, often smooth	Decalcification of enamel and dentin from acidic fumes
Bakers, candy makers, sugar refinery workers	Dental caries on facial surfaces of teeth	Sucrose exposure
Metal workers using copper, nickel, tin, iron	Green, yellow, black stains	Dust and fumes from exposure to the metals
Wine tasters	Erosion of labial surfaces of maxillary anterior teeth	Wine tasting of more than 20 samples per day
Competitive/professional athletes	Acidic erosion, dental caries	Increased consumption of sports drinks, gels; decreased pH of swimming pool water

Figure 60 Occupational and Environmental Conditions That May Adversely Affect the Teeth and Soft Tissues of the Mouth (Senn and Weems 2013: 87).

Figure 61 Molar of Skull-B. The black and brown stains show that the person may be involved in chewing nuts (by researcher).

Figure 62 Mandibular dentition of Skull-B. Generalized tooth abrasions show that the person was sandblaster, grain mill or saw mill worker (by researcher).

Dental Anomalies

Development of the teeth can be affected in different ways and the resultant appearance of a tooth may be of value in constructing the profile of a person. Amelogenesis imperfecta or dentinogenesis imperfect are inherited traits that can be made visible on radiographs and can link an individual to his relatives who will also present with similar tooth anomalies. Scurvy is caused by vitamin C deficiency are less commonly seen but should also be kept in mind. Severe illness disturbances during enamel formation of the permanent teeth will affect ameloblasts and result in hypoplasias. These disturbances remain visible in the teeth throughout lifetime. Hutchinson's insicors or barrel-shaped incisors are a specific developmental anomaly that is associated with congenital syphilis (Adams et al. 2014: 103).

In the present research, the researcher has examined the tooth anomalies of Skull-B. The enamel defects show that the individual was suffered by congenital syphilis, small erosion of lingual surfaces of mandibular incisors from acid dissolution. The individual also had *tetracycline staining*. But there is no amelogenesis, hypodontia or fluorosis. The absence of fluorosis shows that the person belonged to an area where there was balance fluoride in water.

Condition	Appearance	Cause
Congenital syphilis	Mulberry molars and Hutchinson's incisors	Enamel defects
Cleidocranial dysplasia	Multiple supernumerary teeth, small or missing clavicles, forehead bossing	Hereditary congenital disorder (syndrome)
Dentinogenisis imperfecta	Endogenous brownish-blue discoloration of dentin. Opalescent appearance; Sclerotic pulps	Hereditary; abnormal development of odontoblasts
Amelogenesis imperfecta	Endogenous brown yellow, white "snow capped" discoloration of enamel	Hereditary; abnormal development of ameloblasts
Ectodermal dysplasia	Congenitally missing teeth, peg teeth, sparse hair	Hereditary x-linked, abnormal development of ectodermally derived structures
Anorexia, bulimia, alcoholism, GERD, hiatal hernia	Erosion of lingual surfaces of maxillary anterior teeth	Acidic erosion of teeth from regurgitation of gastric contents
Generalized enamel hypoplasia	Hypoplastic enamel of multiple teeth, horizontal discoloration, malformations	Febrile illness, malnutrition hypoxia, trauma leading to ameloblast damage during tooth development
Tetracycline staining	Endogenous discoloration of the dentin which appears as yellow/ brown/green bands	Ingestion of tetracycline family of antibiotics during tooth formation

Figure 63 Past and Present Systemic Disease, Hereditable Maladies, and Medications That May Affect the Teeth (Senn and Weems 2013: 84).

Figure 64 Mulberry molar resulting from congenital syphilis (Senn and Weems 2013: 84).

Figure 65 Mandibular dentition of Skull-B. The enamel defects show that the individual was suffered by congenital syphilis (by researcher)

Figure 67 Hypodontia as a result of ectodermal dysplasia (Senn and Weems 2013: 85).

Figure 68 Amelogenesis imperfect (Senn and Weems 2013: 85).

Figure 66 Erosion of lingual surfaces of maxillary incisors from acid dissolution (Senn and Weems 2013: 86).

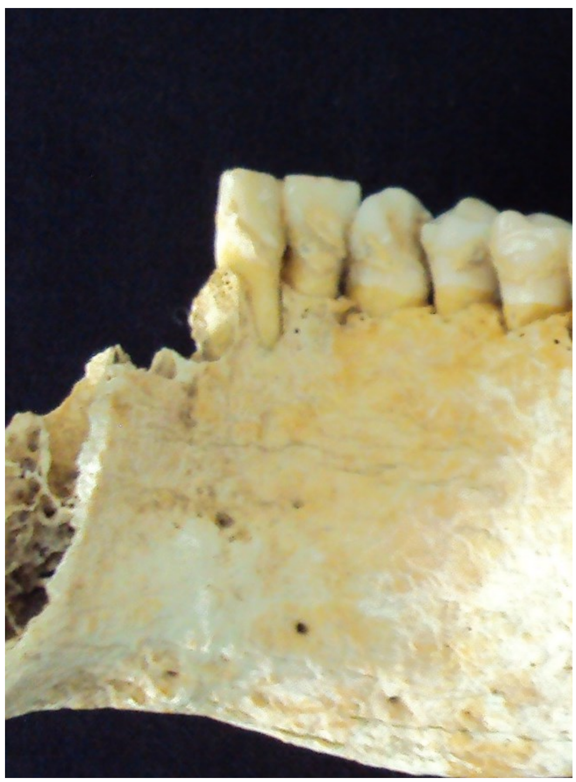


Figure 67 Small erosion of lingual surfaces of mandibular incisors of Skull-B from acid dissolution (by researcher)

Figure 68 Tetracycline staining (Senn and Weems 2013: 86).

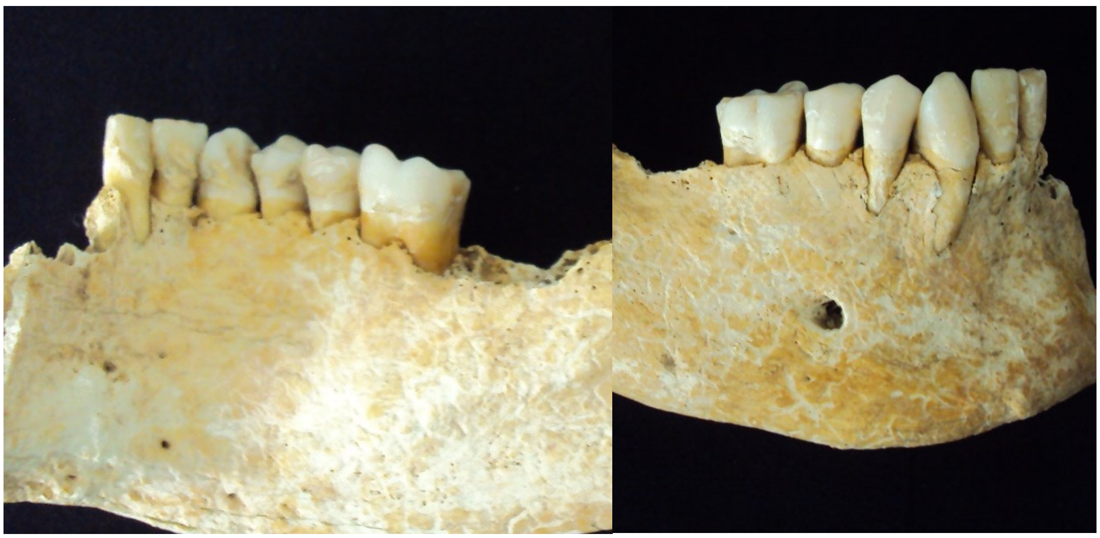


Figure 69 Tetracycline staining of the mandibular teeth of Skull-B (by researcher).

Fluorosis

Fluoride concentrations in excess of 2 parts per million (ppm) during dental development can cause a degrees of fluorosis. That characterizes small opaque patches to enamel hypoplasias and dark staining. (Adams et al. 2014: 103).

Figure 70 Moderate and mild fluorosis with distinctive discoloration of the anterior teeth (Adams et al. 2014: 103).

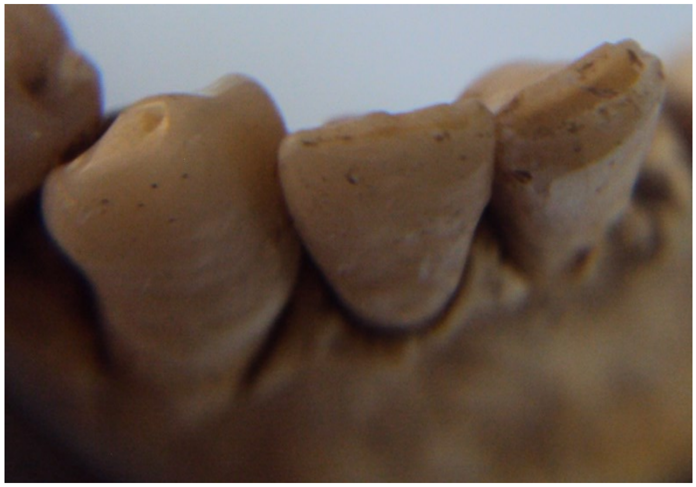


Figure 71 mandibular dentition of Skull-B show no fluorosis (by researcher).

Tetracycline staining

Tetracycline staining causes a band of discoloration on all or a number of teeth. The number of teeth affected and the location of the band related to the stage of tooth development at the time of tetracycline administration are very important to determine the age at which the individual suffered a serious illness.

Figure 72 Tetracycline staining in deciduous and adult dentition ((Adams et al. 2014:105)

Figure 73 Tetracycline staining (Senn and Weems 2013: 86).

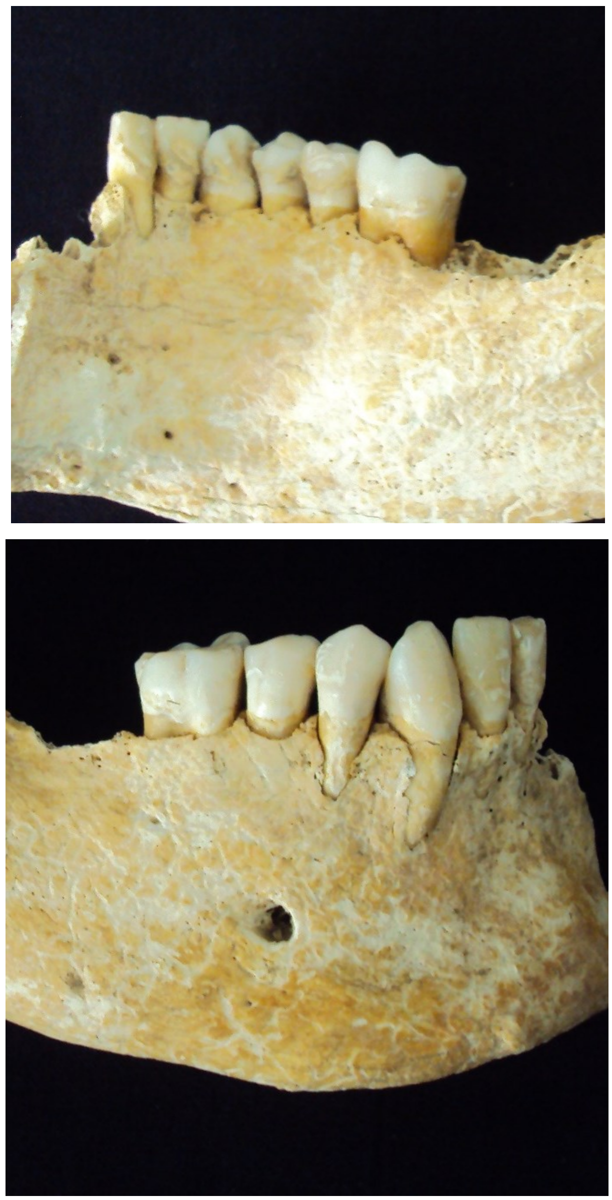


Figure 74 Tetracycline staining of the mandibular teeth of Skull-B (by researcher)

Results Summery

By examining the mandibular right half arcade dentition of Skull-B the researcher has found Enamel extensions on the facial surfaces of molars. According to Adams et al. (2014: 102) enamel extensions on the facial surfaces of molars are found in Asians. Therefore the Skull-B belongs to an Asian individual.

By analyzing dentition of Skull-B there are black and brown stains on mandibular molar that shows that the person may be involved in chewing nuts and the generalized tooth abrasions the mandible show that the person was sandblaster, grain mill or saw mill worker by occupation.

By examining the mandibular teeth of Skull-B it can be observed that enamel is defected that shows that the individual was suffered by congenital syphilis that caused small erosion of lingual surfaces of mandibular incisors by acid dissolution. The individual also had *tetracycline staining.* But there is no amelogenesis, hypodontia or fluorosis. The absence of fluorosis shows that the person belonged to an area where there was balance fluoride in water.

Chapter No. 5

X-Ray Diffraction for the Analysis of Structural Features in the Human Skeletal Remains Recovered from Gandhara Grave Sites

X-rays were discovered in 1913. After the discovery of X-rays William Henry Bragg and his son, William Lawrence Bragg examined patterns made by X-rays as they passed through different types of crystals. They found that the patterns were a response to the regular arrangements of atoms within the crystals. They also developed mathematics from which one could determine the arrangements of atoms from the X-ray patterns. For this contribution they were awarded the Noble Prize (Price and Burton 2011: 119).

In XRD a beam of X-rays is directed on a mineral and the angle at which the beam is reflected onto a detector is recorded. X-rays reflects from a layer of atoms in a crystal but when X-rays penetrate deeper into the crystal then they travel a farther distance. The distance between the crystal layers and the angle of the incident is such that the extra distance traveled is exactly equal to an integral multiple of the wavelength of the X-ray, the wave heights will then coincide; constructive interference occurs, and an X-ray reflection is observed at the detector. The wavelength, l, is equal to an integral multiple, n, of twice the crystal spacing, d, times the sine of the incident angle, θ, one observes an X-ray at the detector. This is called the Braggs' equation. Braggs: nl= 2d sin (θ) (Price and Burton 2011: 119).

In case wavelength l, it is chosen in advance and remains always constant and known. One only needs to know the angles θ at which X-rays are diffracted to calculate the crystal lattice distances, d. From that the identity of the unknown mineral can be determined. Modern diffractometers use databases to match the pattern of an unknown sample to specific minerals (Price and Burton 2011: 120).

For XRD a small sample of 5–10 mg or more is taken from an artifact and crushed to a powder and then it is placed in the middle of the diffractometer. The detector rotates around the sample, the computer counts the intensity of the X-rays at each angle and reports them in a graph as shown. The angles at which the sample diffracts X-rays are then listed with their relative intensities and then they are compared to the searchable database. The computer then presents the closest matches to know minerals (Price and Burton 2011: 120).

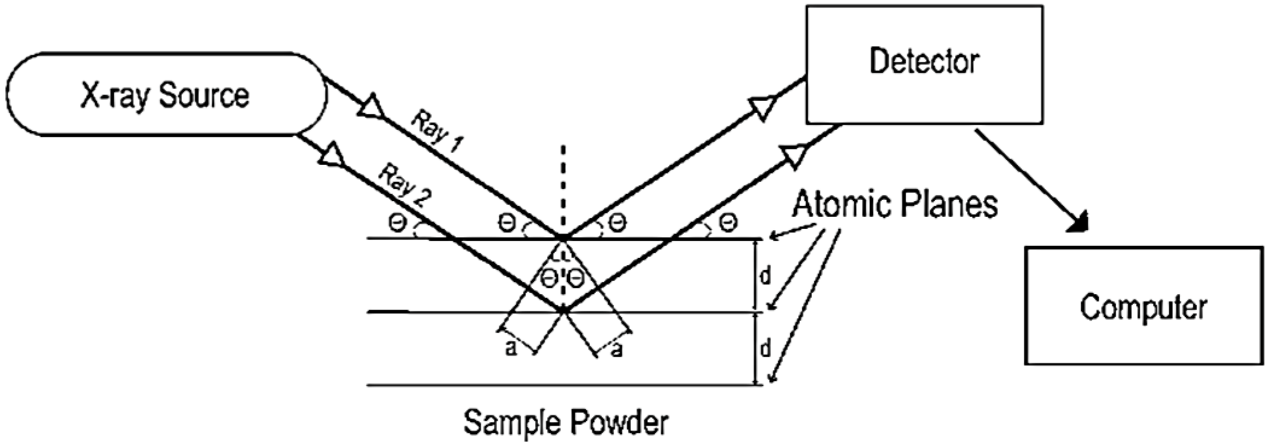


Figure 75 The X-ray diffractometer beam path and detector (Price and Burton 2011: 119).

A major problem challenging analysts of archaeological or fossil bone is the calculation of its biological reliability. It is difficult to determine the preservation of the organic or inorganic portion of bone based on external morphology. The burial and fossilizing environments have variety of conditions that make it difficult to predict patterns of interaction among environment and external morphology and internal preservation of bone. This difficulty is faced by anthropologists in order to attempt to reconstruct an individual's diet or health status from the chemical or isotopic composition of the skeleton (Schoeninger et al. 1989: 281). In such cases X-Ray Diffraction is the best method to know the chemical composition of the skeletons. X-ray diffraction has been used to determine components and crystallinity of bone and other associated materials (Robles 2002; Meneghini et al. 2003; Reiche et al. 2003; Fantner et al. 2004). The main aim of using XRD in this study is to measure the crystallinity index of bone. Sillen (1989) proposed that bone mineral is generally described with poor "crystallinity".

Bone mineral is a significance biomaterial with a used is different fields like biomedicine, archaeology, forensic sciences, anthropology and paleontology. Bone has a hierarchiacal structure composed of different structural units with different size and scales (Weiner and Traub 1992). These units work to perform different functions with good mechanical properties to the bone (Rho et al. 1998; Weiner and Wagner 1998; Currey 2002).

Bone is composed of two structural components: inorganic mineral and organic material. The inorganic mineral is called calcium hydroxyapatite Ca10 (PO4)6(OH) 2 and organic material is made up of collagen, non-collageneous protein, lipids, mucopolysaccharides and other carbohydrates (Goffer 1980; Child 1995). The mineral and organic component of bone differ in their response to taphonomic processes. Corrosion of archaeological bones begins directly between death and final burial. Physical, chemical and biological processes that alter bone is called diagenesis of bones. It also depends on the characteristics of the soil environment that play an important role in the deterioration or preservation of bone (Cook and Heizer 1953; Ezra and Cook 1957; White and Hannus 1983; Price et al. 1992; Child 1995; Tuross and Dillehay 1995; Wilson and Pollard 2002; Collins et al. 2002).

A number of factors can alert the mineral composition of bones. One of the more significant changes to the microstructure of bone occurs when this material is heated. Heated bone is one of the most challenging osteological materials to study. The process of heating produces a many complicated changes within the material. Changes involving the structure and chemical composition of bones may be caused during the fossilizations process.

X-ray Diffraction of the Powdered Samples of Skull-A and Skull-B

Small fragments from both skulls were taken and washed with ethyl alcohol. The fragments were then dried in open air. After drying the fragments were grinded and converted to powder form. For X-ray diffraction the powdered samples were taken to the XRD laborarory, Department of Chemistry, Quaid-i-Azam University, Islamabad, Pakistan. X-ray diffraction patterns were recorded using a diffractometer (sample size 5 mg). Both samples show crystallinity in the form of peaks. The only mineral phase which is apparent in both samples is apatite

Measurement conditions and XRD patterns for both samples are given bellow.

XRD Results of the Powdered Sample of Skull-A

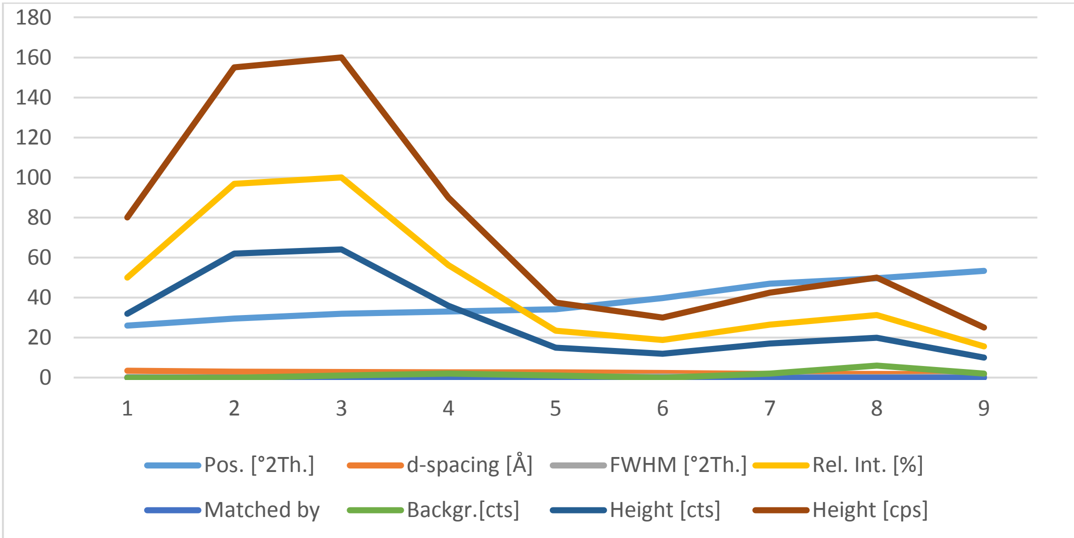
[Measurement conditions]		
Sample identification		
Comment		
Anode material	Cu	
K-Alpha1 wavelength	1.540598	
K-Alpha2 wavelength	1.544426	
Ratio K-Alpha2/K-Alpha1	0.5	
Divergence slit	Fixed	1.52mm
Receiving slit	0.1	
Monochromator used	NO	
Generator voltage	40	

Tube current	30	
File date and time	########	
Unit cell		
h k l	0 0 0	
Scan axis	Gonio	
Scan range	10	80
Scan step size	0.02	
No. of points	3500	
Scan type	CONTINUOUS	
Time per step	0.4	
[Scan points]		

No.	Pos. [°2Th.]	d-spacing [Å]	FWHM [°2Th.]	Rel. Int. [%]	Matched by	Backgr.[cts]	Height [cts]	Height [cps]
1	26.0094	3.42591	0.1968	50		0	32	80
2	29.5625	3.02175	0.059	96.88		0	62	155
3	31.9298	2.80292	0.3149	100		1	64	160
4	33.0632	2.70937	0.3149	56.25		2	36	90
5	34.1883	2.62275	0.4723	23.44		1	15	37.5
6	39.8036	2.26474	0.6298	18.75		0	12	30
7	46.9178	1.93658	0.4723	26.56		2	17	42.5
8	49.6188	1.83731	0.3149	31.25		6	20	50
9	53.3288	1.71649	0.576	15.63		2	10	25

Table 5 XRD Results (by researcher).

Table 6 XRD Results in the form of a chart (by researcher)



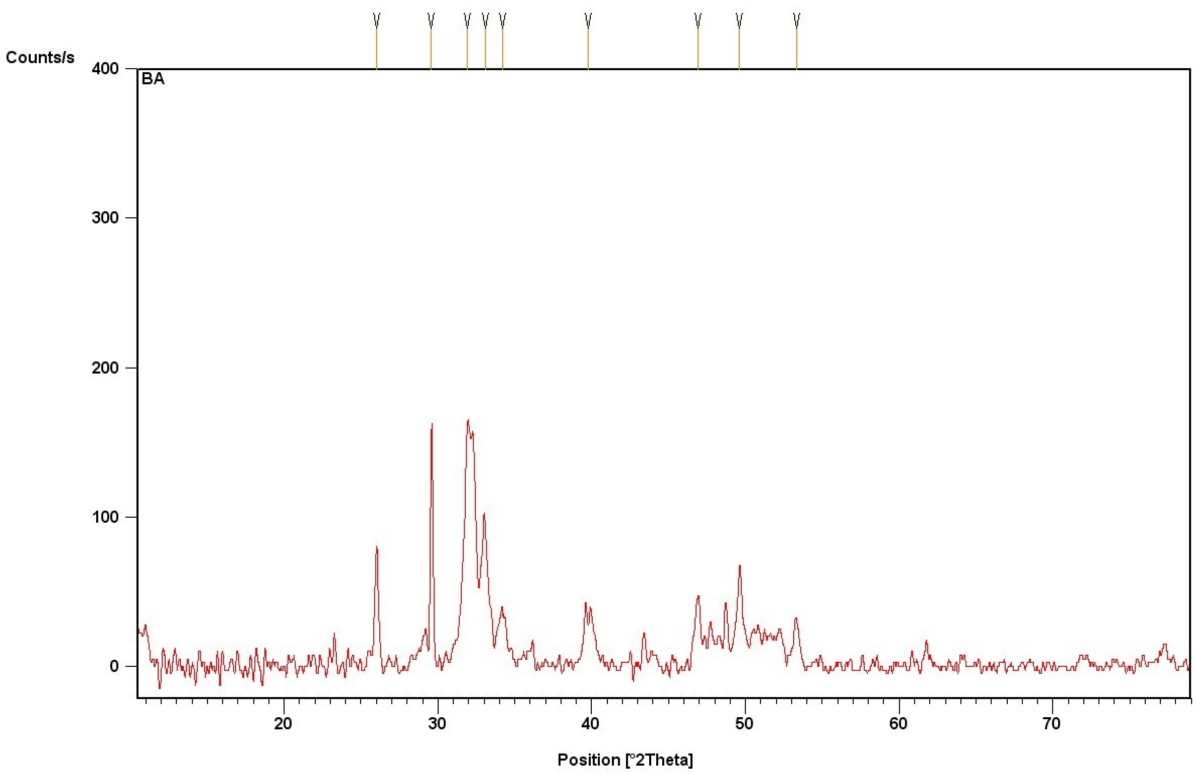


Figure 76 XRD Graph (by researcher).

XRD Results of the Powdered Sample of Skull-B

Table 7 Measurement Conditions for XRD of the Powdered Sample of Skull-B (by researcher).

[Measurement conditions]		
Sample identification		
Comment		
Anode material	Cu	
K-Alpha1 wavelength	1.540598	
K-Alpha2 wavelength	1.544426	
Ratio K-Alpha2/K-Alpha1	0.5	
Divergence slit	Fixed	1.52mm
Receiving slit	0.1	
Monochromator used	NO	
Generator voltage	40	
Tube current	30	
File date and time	########	
Unit cell		
h k l	0 0 0	
Scan axis	Gonio	
Scan range	10	80
Scan step size	0.02	
No. of points	3500	
Scan type	CONTINUOUS	
Time per step	0.4	
[Scan points]		

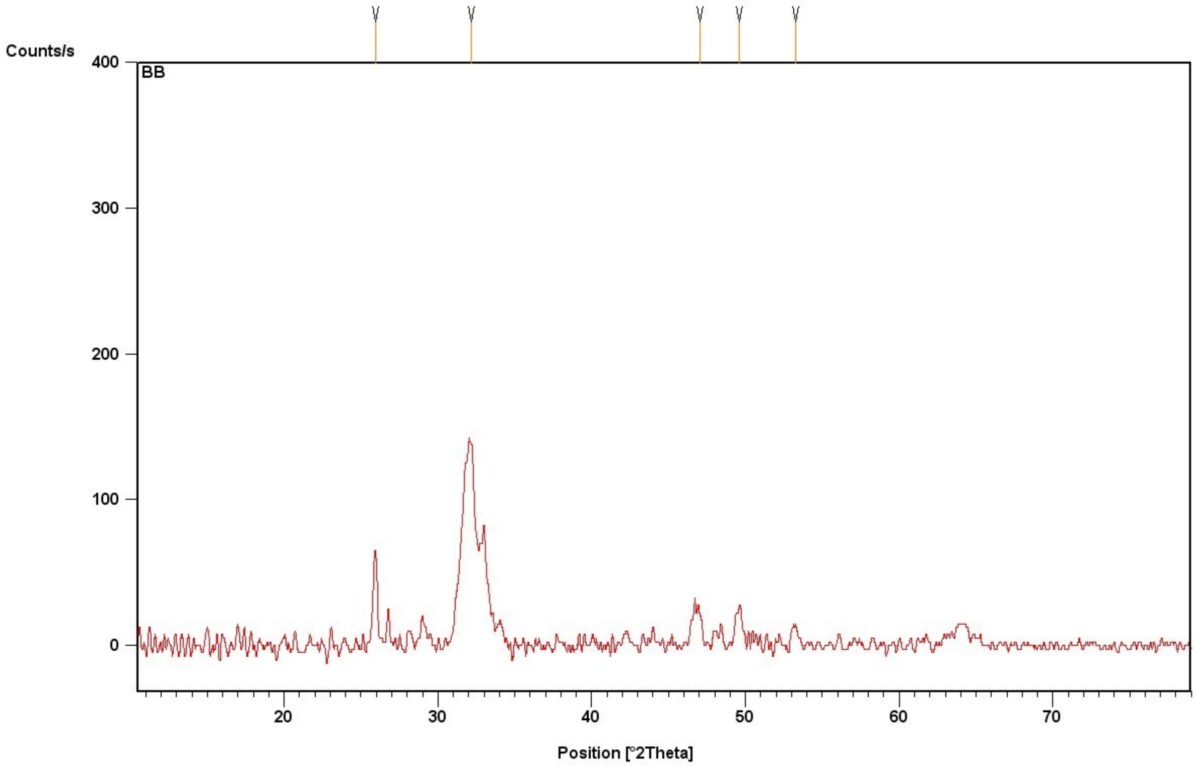


Figure 77 XRD Graph

No.	Pos. [°2Th.]	d-spacing [Å]	FWHM [°2Th.]	Rel. Int. [%]	Matched by	Backgr.[cts]	Height [cts]	Height [cps]
1	25.9778	3.43001	0.2362	50		0	25	62.5
2	32.1639	2.78304	0.7085	100		5	50	125
3	47.0631	1.93094	0.6298	16		0	8	20
4	49.5695	1.8375	0.672	20		0	10	25
5	53.302	1.71871	0.9817	33.2		-10	16.6	41.49

Figure 78 XRD Results (by researcher).

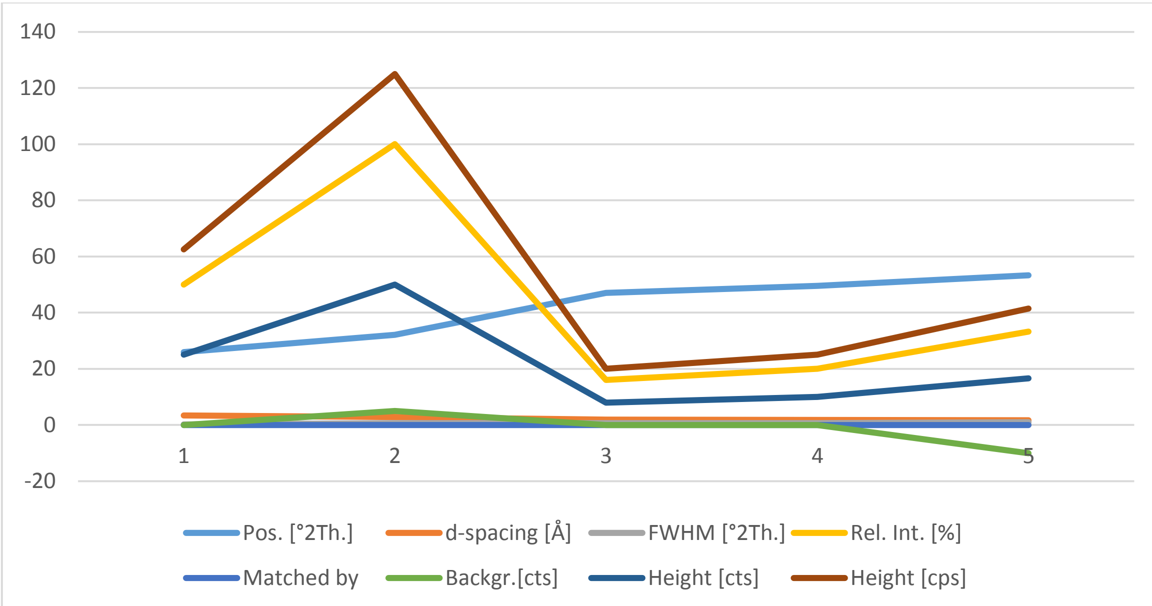


Figure 79 XRD Results Chart (by researcher).

Comparison of the Degree of Crystallization of Skull-A and Skull-B by X-Ray Diffraction Patterns

Bone chemistry is important to reconstruct the habitat of ancient populations. It also helps to know about the post-mortem changes that are occurred to a bone. Bone crystallinity and fossilization changes over time. According to Reiche et al. (2003), this might be caused by the degradation of the organic matter and its leaching that induce an increase in bone porosity, which allow the pore water to dissolve chemical species and to penetrate more deeply into the bone structure and to be in contact with a large available surface of apatite crystals. The older bone shows higher crystallinity and therefore sharper and high peaks in XRD patterns. By this method it is possible to compare the crystallinity of two bones.

Modern bones show poor crystallinity and less sharp peaks in XRD patterns while archaeological bones show maximum peaks in XRD patterns. The increase in the crystallinity of archaeological bone is due the addition of geological apatite. Other reasons may be influence of water, dissolution of bone or recrystallization (Bartsiokas and Middleton 1992: 63).

Similarly by comparing the XRD patterns of the powdered samples of Skull-A and Skull-B. By comparing the researcher has found XRD patterns of Skull-A different from that of Skull-B. XRD patterns of Skull-A show more crystallinity with sharper peaks. It means that Skull-A is older as compared to Skull-B. This may be due to the increase crystallinity over time in Skull-A.

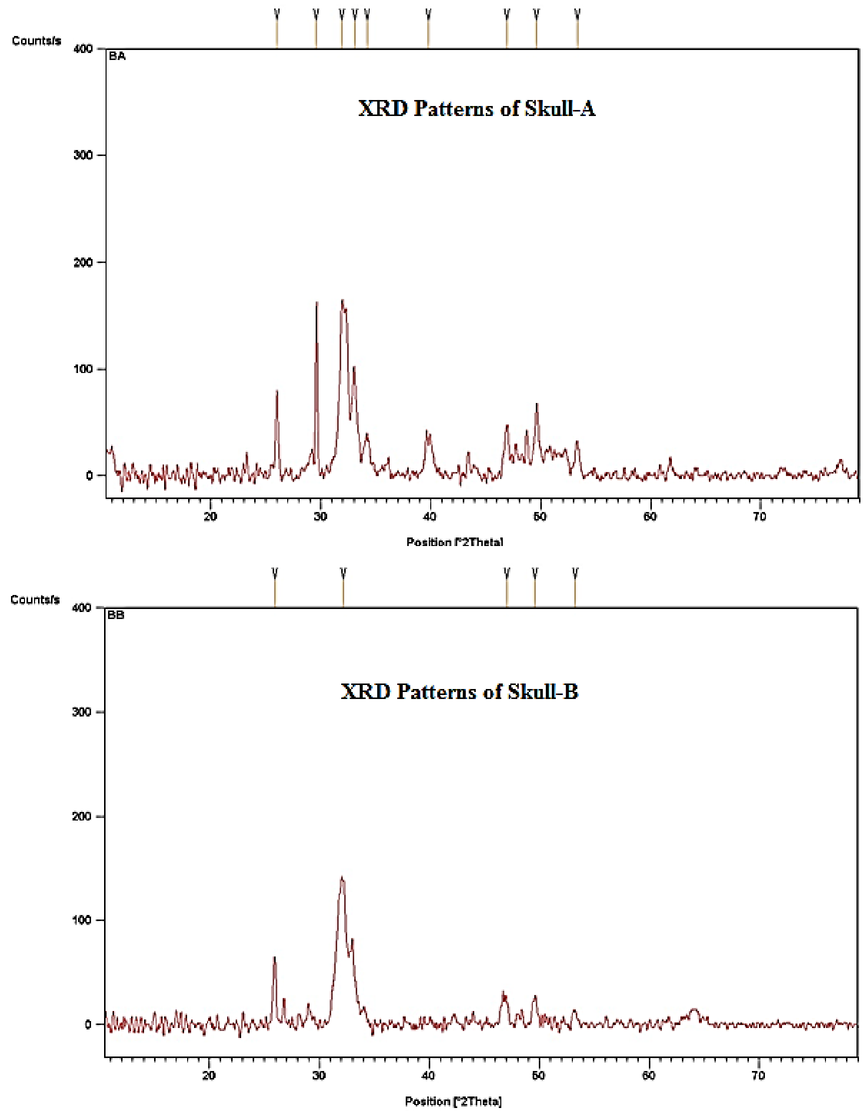


Figure 80 XRD Patterns of Skull-A and Skull-B Powdered Samples. The sharper peaks show that Skull-A is older as compared to Skull-B (by researcher).

Furthermore, the researcher has compared XRD patterns of Skull-A and Skull-B with the XRD patterns of unheated, powdered modern human bone and two unheated, powdered archaeological human that were studied by Schoeninger et al. (1989).

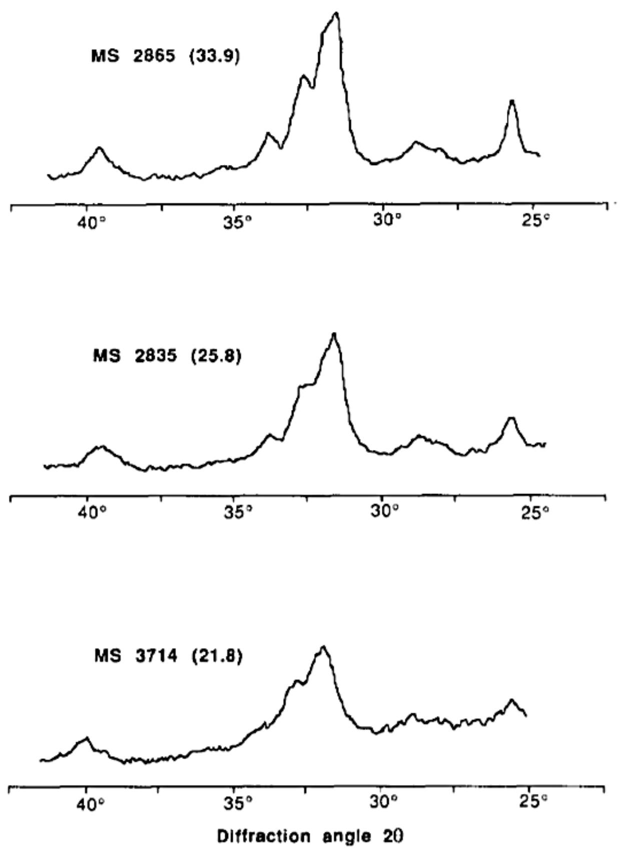


Figure 81 X-ray diffraction patterns of unheated, powdered human bone. The pattern at the bottom is that from the same cadaver specimen. The blunted, diffuse peaks indicate a material with extremely small crystals. The other two patterns were made from archaeological bone from the coast of Georgia. The pattern at the top are more clearly defined peaks, indicating a more crystalline material than is present in the modern bone sample. The middle pattern is a sample from the Georgia coast which had more histological structure in the thin section than the other sample. The X-ray diffraction pattern is intermediate between the modern and the most degraded sample. The only mineral phase which is apparent in these three specimens is apatite. (Schoeninger et al. 1989: 286).

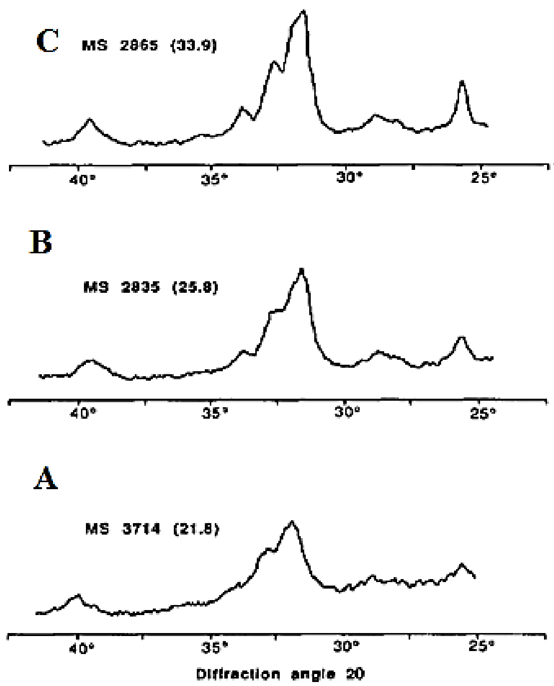


Figure 82 XRD Patterns, A. Modern Human Bone, B and C. Archaeological Human Bones. As mentioned by Schoeninger et al. (1989) (figure reproduced by the researcher).

The above figure show that the XRD patterns of unburnt modern human bone (A) has smoother and lower peaks as compared to the XRD patterns of the unburnt archaeological bones (B and C) that show higher and sharper peaks. This is because archaeological bones show more crystallinity.

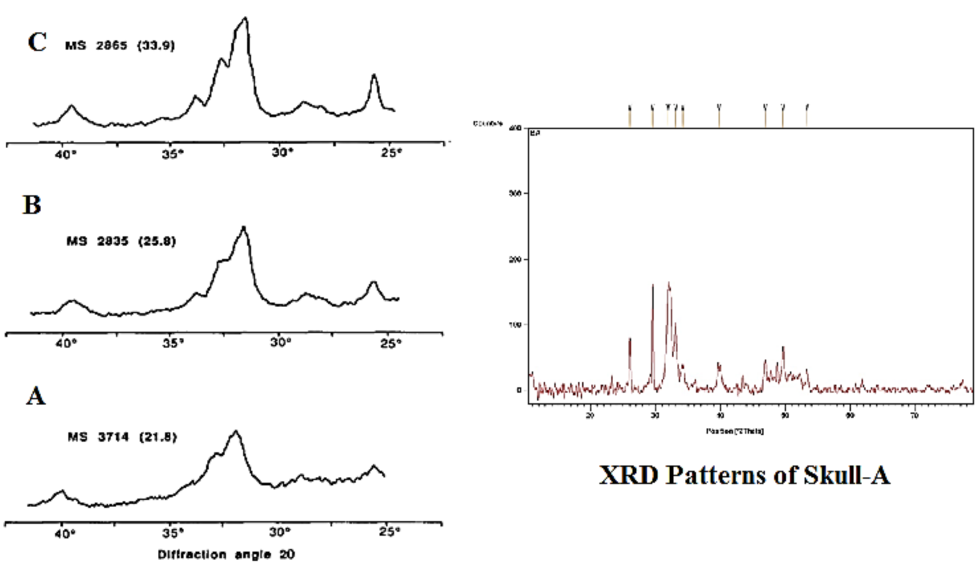
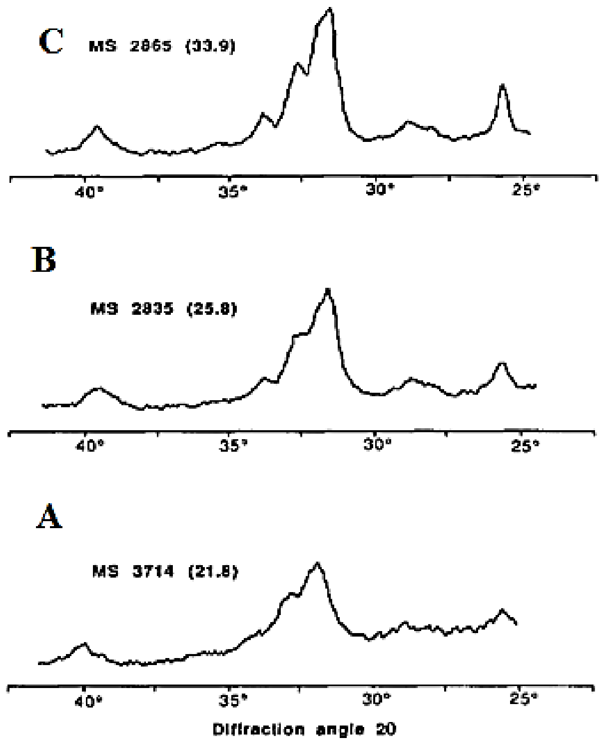
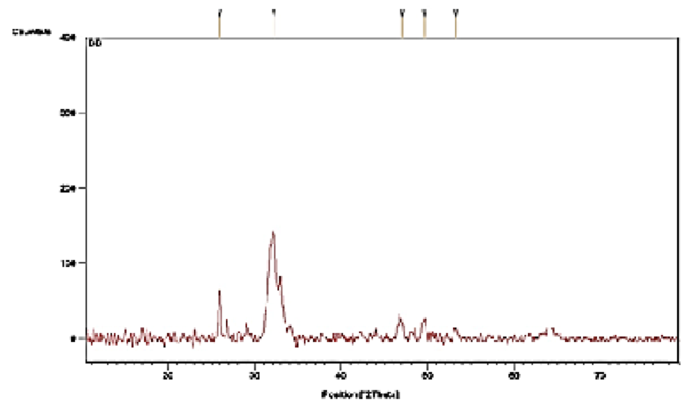


Figure 83 XRD Patterns of Skull-A compared to unburnt modern human bone A and unburnt archaeological bones B and C (by researcher).

XRD patterns of Skull-A are more close to the XRD patterns of the archaeological human bone 'C'. This suggests that Skull-A is more ancient an archaeological as compared to Skull-B.





XRD Patterns of Skull-B

XRD patterns of Skull-B are close to the XRD patterns of unburnt archaeological human bone (B). That suggests that Skull-B is less ancient as compared to Skull-A. Furthermore, XRD patterns of heated bone samples show higher and sharp peaks due to the changes in crystalline structure of the bone. As shown in the following figure.

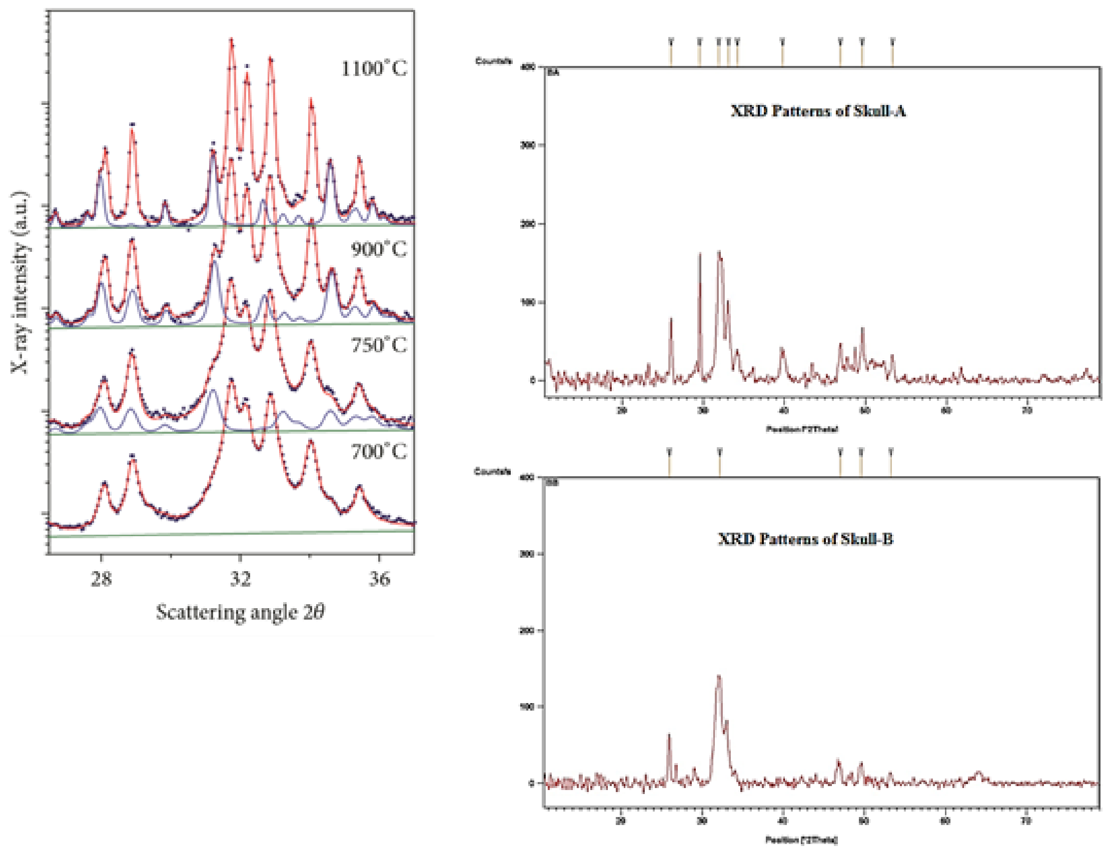


Figure 84 The series of XRD patterns for a tooth heat-treated at 700–1100° (Piga et al. 2016: 7) compared to the series of XRD patterns of unburnt samples of Skull-A and Skull-B (by researcher).

Results Summery

The XRD patterns of Skull-A and Skull-B show that Skull-A is more ancient than Skull-B. This is due to the more crystallization in Skull-A over time.

Chapter No. 6

Ancient DNA Extraction from the Human Skeletal Remains Recovered from Gandhara Grave Sites

DNA Structure

DNA is the genetic material of an individual. DNA is present in every cell cell's nucleus, cytoplasm, and mitochondria. It is important to note that DNA in every cell of the individual's body is identical. Therefore DNA can be taken from blood, sweat, saliva, skin cells, hair or bone cells for the identification of a person (Kobilinsky et al. 2007: 11).

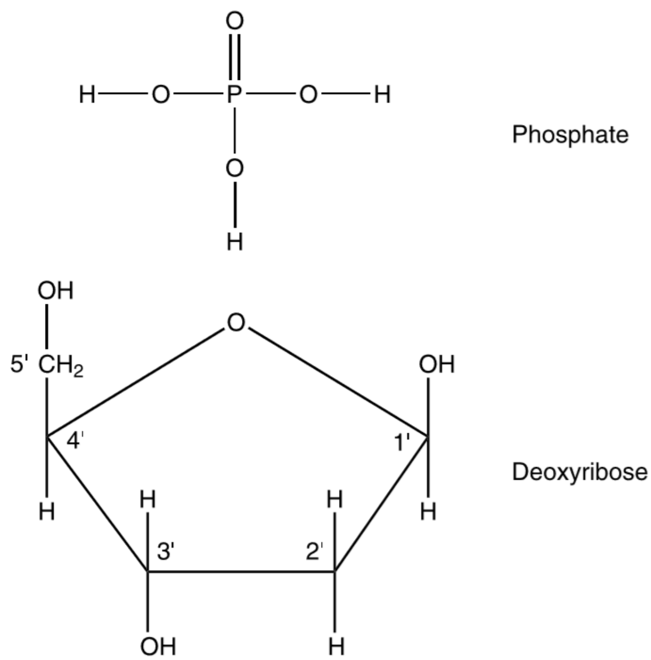


Figure 85 the chemical structure of a phosphate molecule and the structure of the sugar deoxyribose, two components of DNA, are shown here (Kobilinsky et al. 2007: 12).

DNA consists of two spiral strands. These two spiral strands are parallel and form a doublehelix. Each strand of the doublehelix is a linked chain in which the links consist of a very large number of units called nucleotides. Thre smaller chemical compounds (1) a phosphate, (2) a sugar, and (3) a base made Every nucleotide. There are four different bases, A (adenine), T (thymine), G (guanine), and C (cytosine). A and G are double-ringed nitrogen-containing compounds, called purines. T and C are single-ringed nitrogencontaining compounds, called pyrimidines. The base is the identifying part of a nucleotide. Each phosphate group is linked to a sugar molecule and is also attached to one of the four nitrogen-containing bases. The phosphate group of each nucleotide is, except one in each strand, also chemically bonded to the sugar molecule of the adjacent nucleotide, and the whole cahin formed is called the polynucleotide chain. The uppermost phosphate molecule of the “a” (left) strand and the lowermost phosphate molecule of the “b” (right) strand are attached to only one sugar molecule. The location of the singly attached phosphate molecule of each chain formes a linear orientation. The linear orientation is called polarity on that polynucleotide strand. The DNA molecule consists of two spiraling strands. They are oriented in antiparallel fashion. All chemical reactions involve in the nucleotides of the left chain will continue from top to bottom. The reverse is for the right-hand strand. The bases in the two strands of DNA have fixed positions. An A in one chain is always attached to a T in the other chain, a G is always attached to a C, forming complementary base pairs. The base of one nucleotide is attached to the base of its complement through hydrogen bonds. Two hydrogen bonds connect an A with a T, and three hydrogen bonds connect a G with a C. The arrangement by which a purine is always linked to a pyrimidine results in a double helix with a uniform diameter. This cylindrical molecule contains major and minor grooves that may be related to its function (Kobilinsky et al. 2007: 12-15).

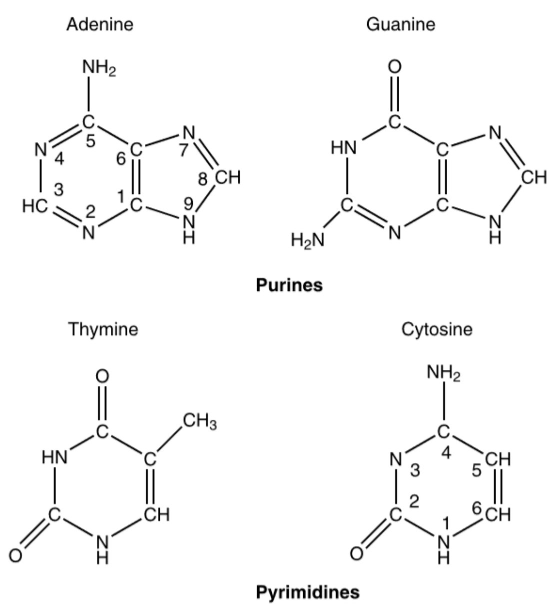


Figure 86 The chemical structures of the bases in DNA are shown here (Kobilinsky et al. 2007: 13).

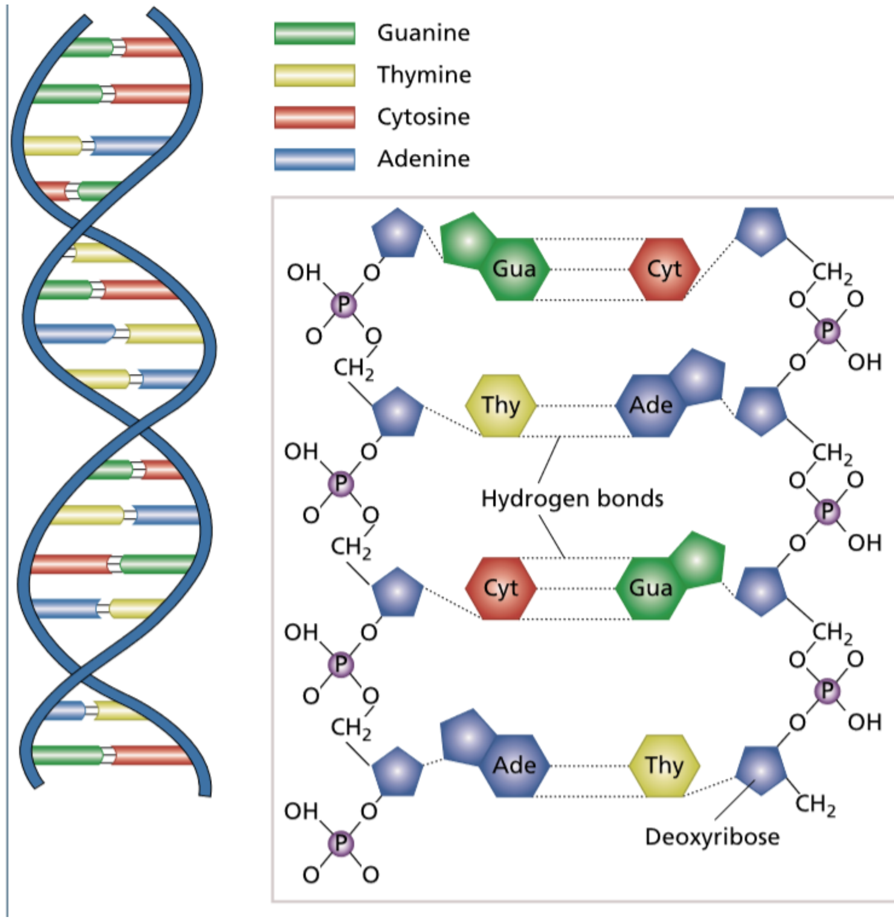


Figure 87 the structure of DNA in resemblance is same to a ladder that has been twisted around itself. The rungs of the ladder are composed of the four nucleotide bases: guanine (G), cytosine (C), adenine (A), and thymine (T). Each nucleotide always bonds with its complementary base pair; guanine bonds with cytosine, while adenine bonds with thymine (Kobilinsky et al. 2007: 14).

The Study of DNA from Ancient Bones

For the first time Chinese scientists extracted DNA from a rib cartilage of a 2,000-year-old mummy from Hunan province (Herrmann and Hummel 1994). In 1980s it had become clear that ancient bones are a source of DNA extraction (Hagelberg et al. 1989; Horai et al. 1989). The genetic information in ancient DNA recovered from human skeletons can provide important information of the relationships between populations. It is also a source of sexing ancient skeletons. In palaeopathology ancient DNA can help us to detect inherited diseases (Mays 1998: 187).

Some Factors Inflencing DNA Survival in Ancient Bone

Höss and Pääbo extracted DNA from an 25,000 years old Alaskan horse bone (Höss and Pääbo 1993). But the survival of DNA in ancient bone is often poor. Survival of DNA in a bone depends on burial conditions. The conditions are different for each site. Shinoda and Kunisada (1994) researched on the bones recovered from the cemetery of Kuma-Nishioda, Kyushu, Japan, dating to about 100 BC-AD 200. A 213 base-pair mitochondrial DNA sequence could be amplified in 55 out of 100 burials. Differences in soil conditions were likely responsible for the difference in DNA survival. Bone preservation in a cemetry is not always a good indicator of DNA survival (Faerman et al. 1995). Chemical reactions are slower at lower temperatures therefore material buried in cold environments preserve DNA very better as also mentioned by Hoss et al. (1996). Poinar et al. (1996) proposed that in warm climates such as Egypt, DNA survival may be limited to a few thousand years. Richards et al. (1995) worked on bone up to two thousand years old excavated from warm sites in temperate latitudes. He used a number of primers to amplify DNA segments of between 149 and 359 base pairs in length. He became successful in about 50 per cent of samples.

Applications of DNA Analysis to Archaeological Problems

Investigation of Genetic Relationships

DNA analysis is important to solve problems related to genetic relationships in archaeology. Shinoda and Kimisada (1994) found an attachement between mitochondrial DNA markers and mode of burial. They suggested that an individual's genetic background play a part in determining place of burial. During the Yayoi period in northern Kyushu genetic relationships played and important role in determining burial treatment.

Sex Determination

Females have two X sex chromosomes while males have an X and a Y sex chromosomes. Analysis of chromosomal DNA is a source of determining sex it is not clear from skeletal form. Stone et al. (1996) amplified a small fragment of the amelogenin gene that was extracted from 20 archaeological skeletons from the site Illinois, USA. The site dated to about AD 1300. Faerman et al. (1995) were also successful in determining sex in archaeological skeletons. In the result six out of seven specimens were sexed correctly, in the remaining case sex could not be determined due to poor DNA survival.

Ancient DNA and Palaeopathology

DNA from ancient bones can give clues of ancient diseases. Ancient disease caused by infecting microorganisms may leave some evidence of DNA. But such studies may face some pitfalls (Hummel and Herrmann 1995). DNA from infectious agents are mostly present in minute quantities. Some studies have shown successful isolation of bacterial DNA from archaeological bone.

Ethanol/Chloroform Precipitation-based PCR Amplifiable Method for DNA Extraction from the Human Skeletal Remains Recovered from Gandhara Grave Sites

Introduction

The experiment has been performed in the Department of Medical Genetics, Quaid-i-Azam University, Islamabad, Pakistan, by the present researcher and Hajira Batool under the guidance of Dr. Naeem and Dr. Ghani-ur-Rahman. The researcher has followed the method of Kalmar et al. (2000) because all the equipments/chemicals were available, in the lab of Medical Genetics, mentioned in the method.

The method used for the DNA extraction from the fragments of Skull-A and Skull-B is ethanol/chloroform precipitation-based method for DNA extraction from ancient bones that is published by Kalmar et al. (2000) for the extraction of DNA from ten 500-1200 years old human bones from four different Hungarian burial sites. They succesfully extracted DNA and amplifialated it in PCR.

Materials and Methods

Samples

Small fragments were separated from Skull-A and Skull-B. The sex of both skulls I have already determined.

Contamination Precausion

In order to prevent contamination all stages were carried out under sterile conditions, using latex gloves and mouth masks.

DNA Extraction

We have extracted DNA in the following steps

1. 0.1 g bone powder+ 1 ml lysis buffer+ Incubate overnight at 37^{o}C+ centrifuge

2. Add 1 ml lysis buffer+ incubation 55°C for 1 hour+ centrifuge
3. Add nuclei lysis+ incubate overnight 37°C
4. Centrifuge+ take upper layer
5. Phenol/chloroform step
6. Precipitation step
7. Ethanol washing+Dehydration

Surface material was removed from the samples of Skull-A and Skull-B. The samples were washed with ethyl alcohol and distilled water. The surface of samples was removed and only the inner part of the sample was ground into a sterile agate mortar and converted into powder form.

Powdered sample was suspended in 1.6 ml extraction buffer (0.1 M EDTA, 0.5% N-laurylsarcosine-Na salt, 100 mg/ml proteinase K), vortexed and incubated overnight at 37°C with continuous vertical rotation. After phase separation by centrifugation at room temperature 12000 r.p.m for 10 min, 250μl supernatant was transferred to a 1.5 ml Eppendorf tube and 3.5μl 1 μg/μl Dextran Blue, 250 μl 4 M NH4-acetate and 500 μl 96% EtOH were added and mixed by vortexing . PCR is inhibited in a dose-dependent manner at concentrations of Dextran Blue only >125μg/ml. The DNA was precipitated at 70°C for 7 min and centrifuged at 14000 r.p.m at 4°C for 15 min. The pellet was redesolved in 20-30 μl deionised, distilled water. The remaining extract was stored at 20°C.

Amplification

A typical amplification reaction contained 2-7 μl of bone extract, 1 U *Taq* DNA polymerase, 160 μg/ml BSA, 200 μM each of dNTP, 20 pmol each of mtDNA-specific primers, 1xPCR Buffer in 25 μl total volume. Denaturation was at 93°C for 5 min, followed by 35 cycles of denaturation at 93°C for 1 min, annealing at 58°C for 1 min and extension at 72°C for 1 min. The last cycle was followed by an extra extension step at 72°C for 5 min. Amplification reactions were prepared in a PCR box using dedicated pipettes that had never been in contact with amplified DNA.

In order to check the fragment size and quality of amplification one-fifth of the PCR product was run on a 5% native polyacrylamide gel.

Results

Ethanol/chloroform precipitation-based PCR amplifiable method is efficient and easy for DNA extraction from ancient bones. Chemicals and equipments mentioned in the method are available in Pakistan. Therefore ancient DNA extraction is possible in Pakistan.

Conclusion

Human osteology and forensic science have a close relationship with archaeology. Human skeletal remains are important archaeological evidence to reconstruct the past. Human osteology and forensic science are essential in archaeology for the analysis of human skeletal remains. Osteological and forensic analysis of human skeletal remains from ancient cultures provides great information to archaeological science. These important information include determination of age and sex from ancient bones. Odontological examination is important for the determination of habits, ancestry, dental abnormalities and many other important clues about an individual through the analysis of skeletal remains. X-ray diffraction of the human skeletal remains reveals information about crystallinity and age. The most advance technique is DNA extraction from the ancient human skeletal remains that enables us to know about the genetic relationship, paleopathology, sex and genetic disorders from the ancient skeletal remains. By applying all these osteological and forensic methods to the human skeletal remains recovered from Gandhara Grave sites, it made it possible to reconstruct the archaeological profile of the individuals of Skull-A and Skull-B. In the future, the application of human osteology and forensic science to Gandhara Grave Sites will open new horizons in the field of archaeology.

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