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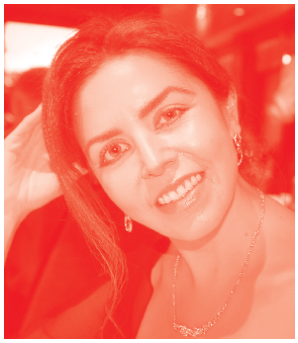

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Meet the editor

Alessio Vovlas received a Ph.D. in Science and Technology from Turin University, Italy, in 2014 with a thesis on "Evolutionary Biology and Biodiversity Conservation," and an MS in Natural Science from Bari University, Italy, with a thesis on "Mitochondrial DNA Variability of Indonesian Populations." He is an effective member of A.P.S. Polyxena, an NGO that is part of the Butterfly Conservation Europe, European Citizen Science Association (ECSA), and Societas Europaea Lepidopterologica (SEL). He is also a member of the Society for Conservation Biology and part of the membership committee of the International Union for Conservation of Nature (IUCN) Commission on Education and Communication (CEC). Dr. Vovlas' main research interests are molecular biology, ecology, zoology, science communication, and education. He has published several scientific, national, and international publications and was a coordinator of some nature conservation and citizen science projects in natural park areas.

Contents

Preface

This book presents a well-defined picture of the current state of the art in the field of biological anthropology. The issues presented in this volume comprise five sections, each focusing on a certain aspect of biological anthropology. Each chapter provides an overview of background information on some aspect of biological anthropology as well as presents relevant case studies.

This book will serve principally as a handbook for graduate and Ph.D. students. It is also useful for a broader audience, including academic researchers and practitioners in biological anthropology. Although this volume was written by several hands, the approach given by the authors managed to give the book a more cohesive form. For that, I am deeply grateful to Dolores Kuzelj at IntechOpen for her guidance in the preparation and publication of this book.

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Section 1

Introduction: General Statement of Scope and Problems

Chapter 1

Introductory Chapter: Biological Anthropology

Alessio Vovlas

1. Introduction

Biological anthropology, or physical anthropology, is a discipline divided into several different branches [1]. In this book, remarkable experts in anthropology have dedicated illustrative chapters on different sectors of the discipline. Each chapter presents a case study and the applications of the most interesting specific techniques. Each chapter offers a widespread diversity of topics covering the broader subjects of biological anthropology, providing an extensive sample of the applications of several methods in anthropology.

This volume intends to provide to the reader an overview of the contemporary state-of-the-art in some different aspects of biological anthropology, in specific in bioarchaeology and paleopathology and forensic anthropology. It could be an important resource for the scientific community that belongs to this discipline, such as evolutionary biologists, ecologists, medical researchers or a starting point for exploring these practices by students.

2. Bioarchaeology

Bioarchaeology is the study of human and animal remains. The term was first proposed by Grahame Clark to designate the studies of animal bones from archaeological sites [2]. Since the end of the '70s the term bioarchaeology is used within the meaning of human osteoarchaeology. The bioarcheological investigation is focused on the study of human variation and evolution in earlier society, using specific methods and techniques in osteological analysis from the mortuary context in archaeological records [3]. Skeletal and dental remains offer an important source of biological information offer for interpreting lifeway of past peoples. Skeletons can provide insight into living conditions, health status, disease, dietary history, lifestyle, violence, trauma, ancestry and demography [4] from individual to population perspective.

Bioarchaeology manifest itself the strict connection between culture and biology [5] and, as Goodman said, one would not exist without the other [6]. This integrative and interdisciplinary research mixed traditional macroscopic skeletal analysis [7, 8] (for estimating biological profile i.e. age, sex, ancestry, stature), bone chemistry [4, 9], ancient DNA [10] with grave contexts, local legends, sagas and other historical information [11] with which to address questions about past populations. In its book "Skeletons in our closet", C.S. Larsen said: "*If only the dead could talk [...] they could tell us about their lives, the food they ate, the disease they experienced and the stress they have encountered [...] bones and teeth bear the physical signs of a person's diet, disease, stress and lifestyle - the skeleton is the "voice" from the past*" [12].

Over the past few decades, new methods and technological innovation providing remarkable strategies to exploring past human relationship and activities [13]. For example, computational and statistical methods [14], computer-based methodologies (e.g. 3D face reconstruction) [15] or analytical procedures for identifying isotopes and biomolecules [13] in biological material excavated in archaeological sites have become very popular in the studies of this discipline. The use isotopic analysis of bones and teeth has exploded over the past few decades to the point where it is now an established tool that is routinely used to investigate questions relating to diet and mobility [16] and for radiocarbon dating, climate and habitat reconstruction [9, 17].

The chapter "Anthropological and paleodietary analysis of human temains: a case study from the teutonic settlement of Torre Alemanna in Puglia (Cerignola, FG, Italy)" illustrates how craniological and craniometric analysis, study of nutritional and occupational stress markers allows to understand the interaction between man and environment, and how we can use this knowledge to reconstruct the *modus vivendi* of people who lived in the past.

3. Paleopathology

Paleopathology is strictly connected with bioarchaeology. Paleopathology entails the study of ancient human disease [18] from human bones and mummies. The paleopathological investigation provides fundamental understanding concerning the antiquity of some diseases and their dissemination in past human groups [19]. Human lives changed drastically over the last hundred years and these temporal changes in human lifestyles are detectable in the skeletal records. Osteological and biological markers highlight to the paleopathologists the pathological condition of past populations, affected by consistent anatomical alteration like trauma, dental disease, activity or mechanical stress, osteoarthritis, tumours [20–23]. Paleopathology need a profoundly interdisciplinary endeavour, encompassing aspects of the biomedical science, the humanities, and the social sciences [24]. Understanding ancient diseases is strictly connected with clinical science, with its process of paleopathological problem solving, the practice of differential diagnosis and the construction of diagnostic arguments [25, 26].

Diagnostic criteria can be applied to evaluate inflammation, surgical intervention (e.g. cranial trepanation) or infections of pathogenic microorganisms in the past, like Tuberculosis [27, 28], Leprosy [29, 30], Treponematosis [31], Brucellosis [32].

Paleopathology requires also a consistent biochemistry approach. The use of isotope provides evidence of metabolic disease and nutritional deficiencies, like vitamin deficiencies in a disease like rickets [33], scurvy [34, 35] and pellagra [36].

The chapters "Cranial trepanation: case studies between the IV century BC and VI century in southern Italy" and "Anthropological and palaeopathological study of a skeleton from late Roman Sicily" explore ancient evidences of human disease. Furthermore, examining osteological alteration in human remains could indicate a crucial element in finding ways to address the world's major health problems of the 21st century.

4. Forensic anthropology

The study of human remains is not limited only to ancient finds. In recent years *forensic anthropology* emerged and assumed the configuration of an important

applied subfield within biological anthropology, by the application of studies on the modern human skeleton to the legal process. Data and interpretations offered by forensic anthropologists have contributed in critical ways to the solutions of many medicolegal problems [37]. Forensic anthropologists operate with their know-how in laboratory-based analysis and episodic involvement in forensic cases [38] or to address legal issues to establish personal identification by human identifying.

Technological advances represent a key potential for improved capability in scientific identification of human remains recovered in a medicolegal context [39].

In addition to age, sex, stature, ancestry determination is it possible to carry out analysis for facial, dental and soft tissues identification. Scientific progress allowed the application of these technologies in crime context as homicide, mass-fatality disasters, genocide. Forensic anthropologists can thus provide Technical services to medical examiners, like cranial and post-cranial evidence, anatomical and biological profile based on *ante-*, *peri-* and *post-mortem* conditions [40].

In recent years DNA analysis, radiographic evaluations of skeletal details, computerized tomography (CT) are becoming increasingly used in the field of forensic anthropology. Also, DNA sequencing, blood genetics, fingerprints provide invaluable assistance in the identification of victims and perpetrators of crimes [41].

The chapter "Forensic anthropology" presented here focus on applied aspects of forensic anthropology analysis and how forensic anthropologist can contribute to an investigation.

Moving from Mirko Grmek naturalistic approach to the history of medicine [42], is possible to link his concept of "pathocenosis" to the evolutionary perspective. In the broad area of study that anthropology covers, a multidisciplinary approach can provide us with a wealth of information on various issues. Biological issues, faced by comparing, for example, how pathologies have coexisted with humans over time, can provide us with useful information. We can therefore study not only how the daily life of *Homo sapiens* has changed over time or understand how the environment has influenced human beings, but also catalogue and make available all the information on the relationship between natural landscape and man that is so much taken into consideration today [43]. We hope this book will be of support not only to experts in the field but also to curious young people eager to undertake these fascinating and fundamental disciplines of study.

Acknowledgements

At last, I would like to thank all of the authors for their contribution, encouragement, time and patience. This volume would not be possible without their perseverance. I want to thank all the colleagues and collaborators who have supported that project, spent time exchanging ideas or even just provided general encouragement. I am deeply grateful for all of you.

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Section 2

Anthropological and Paleodietary Analysis of Human Remains

Chapter 2

Anthropological and Paleodietary Analysis of Human Remains: A Case Study from the Teutonic Settlement of Torre Alemanna in Puglia (Cerignola, FG, Italy)

Fabrizia Andriani, Francesca Baldassarre, Grazia Sassanelli, Sandro Sublimi Saponetti, Cristina Valdiosera and Ilaria Vigliarolo

Abstract

The main object of this paper is to reconstruct the presence of the knights of the Teutonic Order in the archeological site of Torre Alemanna (Foggia, Italy), one of the best preserved Teutonic production sites. This is an interdisciplinary study that includes archeological and anthropological research combined with the paleonutritional results and radiocarbon dating. Specifically, for this study, the area 5 of the complex has been investigated. The cemetery is located in the northwest corner of the complex, and the burials are probably dated at the beginning of the Teutonic settlement. In order to draw conclusions about their presence, attention has been focused primarily on ergonomics activities, injuries, morphological and metric characters, diet, and dating.

Keywords: anthropology, archeology, Teutonic Order, paleodiet, rider syndrome, radiocarbon dating, injuries, Apulia

1. Introduction

This anthropological study has concerned with human bones from the archeological context of Torre Alemanna, and it has allowed to reconstruct a complete biological framework of the skeletons excavated (**Figure 1**). In this research, all the methodologies of surveys necessary to have a complete overview of the archeological site have been used. In general, craniological and craniometric analysis is especially useful for understanding the genetic characterization of populations and possible migration routes. The study of nutritional and occupational stress markers allows to understand the interaction between man and environment and to reconstruct the *modus vivendi*. Through paleonutritional studies, it is possible to reconstruct the type of power supply and the relative subsistence strategies of ancient human groups. In this particular case, the reconstruction of the biological and paleopathological profile of individuals combined with the data of the paleodiet allows to understand their *ante-mortem* status. It is possible to understand if the individuals belonged to a privileged social

Figure 1.
Cemetery area. Individual T1S4.

class and consequently if they had a good diet. The study of occupational stress markers and traumatic injuries let us know if they were warrior monks, through the presence of markers of riding and injuries from interpersonal violence. In addition, it is possible to realize if the individuals came from the north with the study of the skull through the analysis of the ancestry assessment, the shape distance, and PCA.

2. Archeological studies

2.1 The archeological site of Torre Alemanna

Since the Early Middle Ages, many northern European pilgrims had gone to the Holy Land to undertake long and dangerous devotional journeys [1].

In the last years of the third crusade (1182–1190), the Teutonic Order was born in San Giovanni d'Acri, Palestina [2]. Teutonic knights were to be German and from a noble family, they could join the Order after the fourteenth year of age. They were subject to rules, such as observing chastity and poverty. However, in case they wanted to keep their assets, what they obtained from the rents had to be given to the Order, in such a way as to help the poor, the sick, and for hospital assistance. Even the meals were regularized and were preceded by prayer. They could eat meat three times a week, on Friday fish, and the other 3 days dairy and eggs. There were no prohibitions for legumes, vegetables, and fruit. Everything left over from the meals was offered to charity. During the meals, it was necessary to respect the silence and in some established days fast. From the first half of the thirteenth century "baliati," complexes of the goods belonging to the Order were created, particularly in Germany, Bohemia, Moravia, Italy, Greece, Netherlands, and so on. In these places, the Teutons had churches, castles, villages, farms, and mills, but above all, hospitals and hospices were built everywhere.

The presence in Italy of the Teutonic Order should be attested since the end of the twelfth century because the knights have acquired some properties in Apulia and Sicily, for the purpose of providing assistance for Crusaders and Pilgrims who were headed to Holy Land [3].

Torre Alemanna is one of the best preserved Teutonic production sites [4] (**Figure 2** [5]). Since 1226, Teutonic Order settled in that site and in the near *castrum* of Corneto [6]. Corneto was a thriving village with ideal characteristics for

the agricultural production. Water was recoverable from the Carapelle and Ofanto rivers and from numerous canals and springs [7]. The proceeds were to join in part the supplies for the Holy Land, the place where the headquarters of the Order was located in the castle of Monfort [8]. The site of Torre Alemanna takes its name from the quadrangular tower, probably having the function of lookout (**Figure 3** [9]). The tower was 24 m tall and 10 × 10 m wide. The knights of the Teutonic Order built the tower, including a preexisting church with interesting paintings. The remains of the thirteenth-century medieval church are the quadrangular choir corresponding to the ground floor of the tower; some precious stone remains evoking a decoration of considerable value; and the frescoes of the late thirteenth to the early fourteenth century. The pictorial decorations show the images of holy bishops and popes, along with episodes of the Passion of Christ and a Tree of the Cross. It is not possible to know if in Torre Alemanna there was a religious community, as happened to St. Leonardo of Siponto (also occupied by the Teutonic Knights), but being

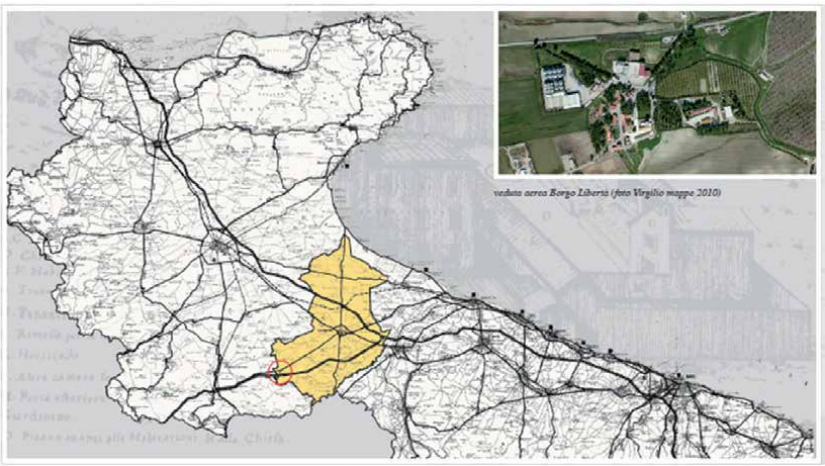

Figure 2.
Monumental complex of Torre Alemanna in the municipality of Cerignola [5].

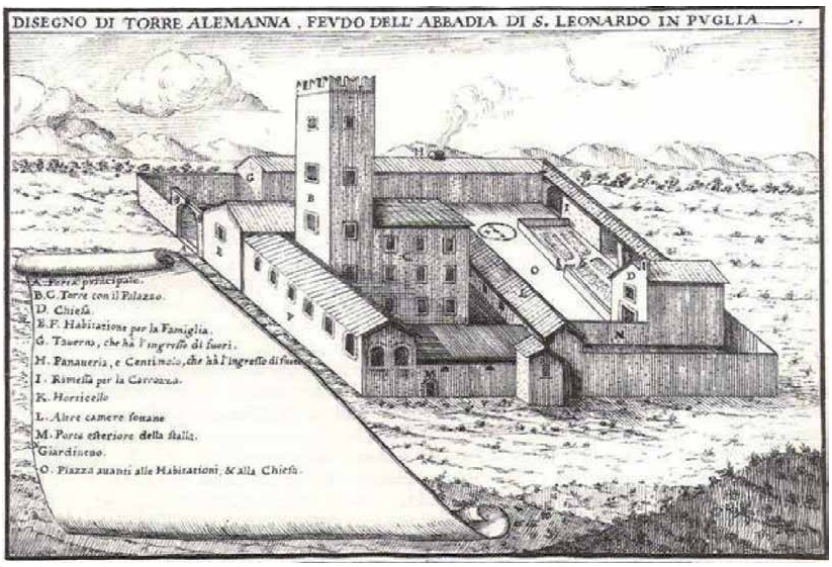

Figure 3.
Torre Alemanna on the seventeenth century manuscript [9].

an important fief with an agricultural-pastoral company, there had to be officers or intermediate ministers in addition to the lowly hand. From 1999 until 2008, archeological research focused on the extension of the investigation in the area of the church and its surrounding space [10]. We know about two other anthropological studies of individuals belonging to Knightly Orders: one archeological site is located in the courtyard of the House of the Teutonic Order of Vipiteno – Deutschordenhaus Sterzing (Bolzano, Italy) [11] and another in the Holy Trinity Church of Konstanz (Germany) where a knight of the Order of San Giovanni has been studied [12].

2.2 The Teutonic cemetery

The burial ground of the area 5 of the complex has been examined under the direction of Dott.ssa Giovanna Pacilio from the "Soprintendenza per i Beni Archeologici della Puglia" in 2007–2008.

The burials are located in the northwest corner of the complex, and it is probably dated at the beginning of the Teutonic settlement [13].

A first layer of sandy deposit was dated from the second half of the thirteenth to the early fourteenth century, on the basis of the material found, in particular on the discovery of proto-majolica ceramics. A second layer of ground coincides with the occupation of the cemetery dated with radiocarbon analysis. The layers, then, have been cut to build the foundations of the original fence wall in the north of the complex. The space inside the wall has been used as a cemetery connected to the church, located to the east.

Certainly, the burial ground follows the construction of the perimeter wall of the complex, and this is confirmed by the discovery of a burial covering the trench of the groundwork, and it leans on the structure of the wall.

The burial ground is therefore attributable to the first phase of the settlement, as well as to the initial phase of the Teutonic occupation.

Later, a part of the area 5 was cut after the construction of a hypogaeum that intercepted a series of burial. In fact, tibias, fibulas, and feet bones were under the wall.

2.3 The emblem of Torre Alemanna

Many ceramics found during the excavation of the site report an emblem characterized by a circumference with, drawn inside, diameter and, perpendicular to it a radius, thus forming the letter Tau "T" [14]. Among the types of ceramics on which the emblem is present, we report not only plates and bowls of majolica, plates and mugs of graffiti, and polychrome slipware pottery but also closed forms. It is also possible to find the same emblem on different stone artifacts and boundary markers. It was probably the symbol of the community of Torre Alemanna attested from the end of the fifteenth to the seventeenth and eighteenth centuries, period immediately following the presence of the Teutonic knights in the site. The oldest testimony dates back to the twelfth century. It is an exemplary stone, now walled inside the tower on the wall overlooking the entrance of the farm, where a bas-relief emblem has been found, indicated as "Ancient distinctive sign of the monks of the Teutonic Hospital of Jerusalem, still in use at the end of the twelfth century" [8]. Various interpretations have been given on the emblem of Torre Alemanna, and some scholars have identified the monogram of the Knights of the Teutonic Order (*Ordo fratrum domus hospitalis Sanctae Mariae Theutonicorum in Jerusalem*). On the left side of the main entrance to the monumental complex of Torre Alemanna, an ancient emblem of the Teutonic Knights has been found, dating back to the fourteenth century. On the right, there is a coat of arms that belonged to one of the Teutonic commanders of Torre Alemanna (**Figure 4**). This was supposed to be the commander Thile Dagicter

Figure 4.
Coat of arms on the main access portal.

von Lorich (Thyle von Lorch) because the coat of arms in Torre Alemanna was the same as that found on the diptych found in the castle of Marienburg, the main seat of the Teutonic Order in Prussia (currently in Poland). The same coat of arms is present in the graffiti on the frescoes inside the gothic church incorporated in the tower.

3. Anthropological studies

3.1 Anthropological material

The cemetery area is located in the northwest corner of the complex, which was called area 5.

All individuals are buried in earthly pit with an east-west orientation.

Six of the individuals examined (USS22, USS23, USS25, USS27, T1S3, and T1S4) were in primary deposition, in dorsal decubitus.

Decomposition took place in an empty space, and the bodies were probably laid inside wooden boxes or wrapped in a tight shrouds, as the joints did not maintain the connections and their original positions.

The individuals T1S1, T1S2, USS24, USS26, USS32, and USS33 are instead in secondary and reduction deposition that is the skeletal remains have been moved from the place where the decomposition took place.

Almost all individuals have fragmented and incomplete bones. For the purpose of a correct analysis of the conservation status, the bone quality index (I.Q.O.) has been applied, which was rather high only for a few individuals, with a healthy cortical surface exceeding three quarters, that is 75–99%.

The results of analysis of the skeletal sample from area 5 show a total of 12 individuals subdivided as follows: 2 subadults, including 5-year-old male and 12-year-old female, and 10 adult individuals, including 6 males between the age of 37 and 45 years and 4 females between the age of 25 and 35 years.

3.2 Methods of anthropological analysis

The biological and paleopathological profile of individuals is reconstructed on the basis of the following aspects: the identification of morphometric characters [15]; the determination of age and sex [16, 17]; nutritional and/or stress diseases and the detection of periodontal disease and caries [18, 19], tartar deposit evaluations [20], and the degree of dental [21]; the enamel hypoplasia lines [22]; markers of skeletal

biomechanical stress—syndesmosis injuries, enthesopathies, new articular surfaces, and degenerative joint diseases [23–27]; degenerative changes of the spine [28]; biomass body [29]; the investigation of the cross-sectional geometry of the humerus and femur [19, 30–32]; and the human typologies [33]. The degree of representation of the individual's skeleton is calculated as a percentage of the total theoretical number of human bones. Statistical analyses are based on the incidence and percentage frequencies of individual data. Unlike the other bones that change in relation to environmental influences, keeping a trace of stress, the skull is quite conservative of its original genetic characteristics as regards some absolute measures and morphological reliefs and is not affected by the environmental interferences to which it is subjected. On the basis of this characteristic, it is possible to start the study of the biogenetic dynamics affecting the territory in question, through constant integration with historiographical and archeological sources. Populations from different geographic regions of the world have apparently different skull features, especially the shapes of orbital cavity and nasion (**Figures 5–8**).

Craniometry involves quantifying characteristics on an objective scale in an attempt to define the ancestral identity of an individual. Measurements are continuous and may be subjected to multivariate analysis to provide estimates of distance and probabilities of affinity to the reference sample.

In total, 12 cranial measurements [15] were used: 5 to the vault, 1 to the base, and 6 to the face: glabello-occipital length (GOL), basion-nasion length (BNL), maximum cranial breadth (XCB), minimum frontal breadth (WFB), porion-bregma height (PBH), basion-prosthion length (BPL), bimaxillary breadth (ZMB), nasion prosthion length (NPH), orbital breadth (OBB), orbital height (OBH), nasal breadth (NLB), and nasal height (NLH).

Determination of the type of injury and weapon recognition was performed by comparing with study collections and data known in the literature [34, 35]. The definition of the blows—"downward cuts," "middle cuts," "upward cuts," "blow from left to right," and "blow from right to left"—follows the terminology noted in the medieval defensive treaties. As for the possible weapon identification, this is determined by the combined reading of the morphology of the lesion and the historical context of reference.

3.3 Paleodiet studies and laboratory procedure

Paleodiet is a discipline that has the objective of reconstructing the dietary habits of the populations of the past through investigations of chemical-physical

Figure 5.
Skull in norma frontalis *and* lateralis *of male individual T1S1.*

Figure 6.
Skull in norma frontalis *and* lateralis *of male individual T1S4.*

Figure 7.
Skull in norma frontalis *and* lateralis *of male individual USS25.*

Figure 8.
Skull in norma frontalis *and* lateralis *of male individual USS26.*

type. The information obtained through the study of the paleodiet together with the anthropological, archaeozoological, and archaeobotanic data turns out to be an important contribution for the archeology [36].

In the human body, there are two categories of elements. Majority elements have a concentration greater than 0.01%, whereas the trace elements have a concentration less than 0.01% of body mass [37]. In addition, it is necessary to distinguish the trace elements in essential for diet, potentially essential, nonessential, and toxic [38].

On the basis of this classification, the most considered food markers are strontium, magnesium, zinc, copper, calcium, lead, and iron.

Strontium and magnesium are indicators of a mainly vegetarian diet.

Strontium is mainly concentrated not only in plants but also in mollusks of marine and terrestrial origin and in small fish [39].

Magnesium is an indicator of a diet mainly based on cereals and legumes [40, 41]. Its presence may also indicate the intake of wheat germ, oatmeal, maize, beans, peas, lentils, and dried fruit. The refined flours used in more recent times contain a reduced quantity of magnesium, contrary to the whole grain flours usually employed in the oldest phases.

Zinc and copper are indicators of a predominantly protein diet. High concentrations of zinc are due to the intake of red meat: horse, veal, pig, and lamb [42]. It can be found to a modest extent in fish products, with the exception of oysters and crustaceans in general, and in some vegetables, particularly beans, peas, soya, and lentils.

The food with high content of copper is the ox's and calf's liver and the offal in general; it is very high in the mollusks, in the crustaceans, and in the greater part of the seafood [42].

Calcium, although not a trace element, is essential to evaluate the *intravitam* health status and the *postmortem* conservation status of the individual [43–45].

It is necessary to relate to calcium each element analyzed in order to compare the data and mitigate the influence of any diagenetic contamination. The relationship with nondiagenetic elements can be problematic because calcium is an element subject to frequent *postmortem* alterations [46]. An additional method of correction is generally applied, which consists in correlating the Sr/Ca and Zn/Ca ratios of the human sample to those of animals living in the same site at the same time as humans [47]. The greater compactness of the animal bone matrix leads to a less interchange of elements between the bone and the soil and consequently a less loss of calcium.

The selection of the sample is fundamentally important for the result of the paleonutritional analyses. In the course of this study, fragments of long bones were taken, the cortical component of which is less prone to diagenetic phenomena and allows to remove internal and external encrustations easily. In addition, in order to make the correction with the site, a sample of *bovis* was taken that was found in the same cemetery context.

The surface portion of the samples was removed using a scalpel and a probe. In order to eliminate the organic component, the samples were placed inside a muffle at 600°C. Once extracted, they were pulverized in a mortar and placed under a bell dryer to eliminate the hydration water present inside.

About 0.500 g were taken from the pulverized samples and then subjected to two consecutive acid attacks (HNO_3 in pure concentration and HCl in 1 M) on a plate with a temperature of approximately 140°C.

The liquid samples were made up to volume in 50 ml flasks with bidistillate water and, if necessary, filtered. Subsequently, the dilution of the nondirect elements, namely calcium, magnesium, and strontium, which are essential for reading in the atomic absorption spectrometer (AAS), was carried out.

In addition, standard calibration solutions have been prepared: white and calibrated for each element. White is a solution composed of bidistillate water and

acids with the same concentration as those present in the samples. It allows both to establish the relative zero of the calibration line and to provide the spectrometer with the exact composition of the solutions that will have to be read.

Calibrates are taken from a standard solution with a known concentration and are of three different qualities (useful for creating a calibration line). HNO_3 and HCl are added in the same quantity as in the samples and made up to volume with bidistillate water. For nondirect elements (Ca, Mg, and Sr), lanthanum (0.1% m/V) should be added to reduce interference during analysis in both analytical and blank samples and standards.

Finally, the samples were read using the atomic absorption spectrometer (AAS). The AAS is a widespread technique since it is easy to use and has low costs.

3.4 Radiocarbon dating

The sample was directly radiocarbon dated using accelerator mass spectrometry (AMS) at Beta Analytic Inc. (Miami, Florida). The date of this sample was calibrated to years cal BP using Oxcal v4.3.2 software based on the IntCal13 atmospheric curve.

4. Results and discussion

4.1 Results of anthropological analysis

4.1.1 The biological profile

The average stature for males is 1.72 m and for females is 1.56 m. Average body mass for males is 70 kg and for females is 60 kg.

Craniometric measurements and indices of all adult subjects are shown in **Tables 1** and **2**.

4.1.2 The shape distance and PCA analyses

The Penrose shape distance analysis cranial series used to provide a comparative basis for medieval remains includes 6 Apulian samples [48–53], 3 Italian

	GOL	BNL	XCB	WFB	PBH	BPL	ZMB	NPH	OBB	OBH	NLB	NLH
T1S1 M	165	87	140	93	106,2	87	125	64	39,5/37,9	31,6/31,6	22,0	43,0
T1S3 F	170	99	145	92	110,2	91,8	124	60,7	39,8/-	29,3/-	25,3	47,7
T1S4 M	176	93	150	106	109,7	91	137	66,7	39,9/41,8	35,8/36,6	23,8	55,9
USS22 F	-	-	150	102	-	99,4	133	-	-	-	22,7	-
USS23 M	181	93	142	-	112,9	85	-	61,6	41,5/-	33,7/-	25,8	52,3
USS25 M	181	94	152	93	119,8	86	128	72,3	41,4/40,7	33,3/34,2	23,4	53,7
USS26 M	-	108	-	-	-	104	-	67,3	38/-	30,6/-	-	44,3
USS32 F	-	95	-	92	-	90	125	63,8	37,0/38,9	33,0/32,4	23,6	45,4
USS33 M	180	98	131	95	113,2	97	129	67,2	39,7/40,3	30,5/30,9	24,8	49,7

Table 1.
Cranial measurement of adult individuals.

	8/1	9/8	20/1	20/8	40/5	48/45	52/51d	54/55
T1S1 M	84,85	66,43	64,35	75,48	100,00	51,20	80,00	51,16
T1S3 F	85,29	63,44	64,82	76,00	92,72	48,95	73,62	53,03
T1S4 M	85,22	70,67	62,32	73,13	97,85	48,69	89,72	42,58
USS22 F	-	68,00	-	-	-	-	-	-
USS23 M	78,45	-	62,36	79,48	91,39	-	81,20	49,33
USS25 M	83,98	61,19	66,19	78,82	87,23	56,48	80,43	43,57
USS26 M	-	-	-	-	96,24	-	80,53	-
USS32 F	-	-	-	-	94,70	51,04	89,19	51,98
USS33 M	72,78	72,52	62,91	85,14	98,98	52,09	76,83	50,00

Table 2.
Cranial indices of adult individuals.

samples [54–56], and 7 European samples [35, 57–60]. Penrose shape distances were calculated between each pair of compared populations. The shape distance actually measures the precision of the mean difference between two populations [61]. Accordingly, it is considered to be a reliable indicator of morphological difference based on body form, rather than difference based on absolute body size.

For each comparison, once the distance of Mahalanobis (d^2) and the distance of generalized form of Penrose (C^2_H) have been calculated, the shape distance (C^2_Z), that is the difference in proportions and the size distance (C^2_Q), which is the difference between dimensions, is examined [33].

The shape distance (C^2_Z) allows to obtain an estimate of the "genetic distance" between the individuals in question and the populations of comparison, so as to return a location ethnicity of the individuals themselves. Determination of ancestry has been based on the morphological and metrical examination of certain skeletal traits in the skull. In addition, considering that the individuals could be the result of the mixture of different, physically contrasting groups, according to historical data, a number of morphological and mathematical criteria were used to classify each skull into one of the so-called groups or subracial types (Baltic, Mediterranean, Dinaric, and Alpine) [62, 63] in order to recognize morphological affinities among them.

In terms of morphological features, the skull was typologically similar to the Northern European skeletal range, referred to as Borreby phenotype, short-skulled with East Baltid and Alpinid tendencies, characterized by wide and planoccipital skull with spheroid shape in superior view, face roundish, leptorrhine, and nasion depression.

Borreby was a Danish village where neolithic skeletons mixed with bell-beaker invaders at the end of the Copper Age.

Throughout history, Pomeranian Region was a meeting place for different populations such as Baltic, Slavic, and Germanic.

Particularly, four male skulls (T1S1, T1S4, USS25, and USS23) have metric features very close to the sample of the Late Middle Ages that came from Aleksandrów Kujawski and Radziejów Kujawski (Poland) [64], and it has been tested with a multivariate analysis using the *shape distance* [33] (**Tables 3**–**7**). Both sites were

Geogr. locations	Geographical coordinates	Chronological dating	Skeletal series	Amb.5 TA07 t1s1	Amb.5 TA07 t1s4	Amb.5 TA07 uss25	Amb.5 TA07 uss23
Puglia	40.922N 17.128E	XIV - XV	Conversano (Castiglione)	0,52	1,08	0,80	0,52
Puglia	41.130N 16.870E	XII - XIV	Bari	0,92	1,50	1,50	1,13
Puglia	41.223N 16.286E	1155-1285*	Andria	0,90	1,53	1,41	1,03
Puglia	40.954N 16.919E	884-1024*	Casamassima	0,97	1,34	1,55	0,96
Puglia	40.144N 18.494E	XV	Otranto	0,50	1,04	0,91	1,12
Puglia	40.954N 17.303E	XI - XIII	Monopoli	0,88	1,38	1,15	0,97
Abruzzo	42.821N 13.556E	VI - VII	Castel Trosino	1,27	1,76	2,37	1,29
Latium	42.500N 11.681E	VII	Ischia di Castro	1,24	2,19	1,87	0,99
Tuscany	43.310N 10.509E	V - VIII	Livorno Cecina	1,38	1,70	1,73	0,83
Ungary	47.158N 18.402E	X - XI	Szekesfehervar	0,99	2,11	2,38	2,47
Germany	54.311N 10.086E	XIV	Kiel	0,68	1,50	1,39	0,82
Poland	52.867N 18.699E	Late med.	Aleksandrow Kujawski	0,61	**0,70**	**0,50**	0,69
Poland	52.616N 18.533E	Late med.	Radziejow Kujawski	**0,38**	**0,70**	0,86	**0,41**
Poland	54.164N 19.402E	Late med.	Elblag	1,59	1,33	1,63	0,93
Croatia	45.497N 18.894E	Early med.	Croatia	1,46	1,92	1,72	1,46
Russia	60.004N 32.289E	XII-XIII	Staraya Ladoga	0,86	2,21	1,10	1,07

Table 3.
The shape distance (C2Z) of 16 medieval cranial series with four individuals of Torre Alemanna. Males only.

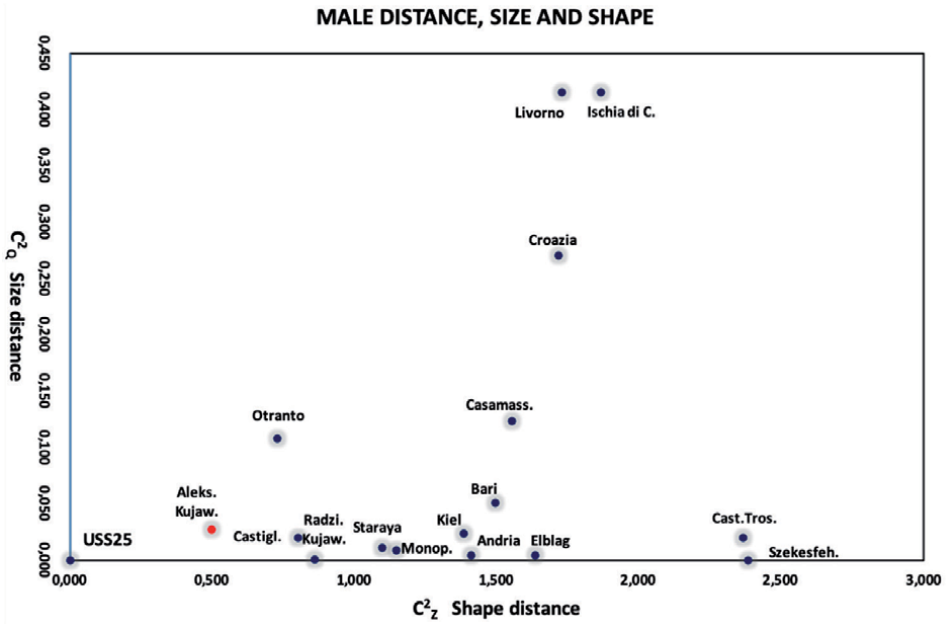

Table 4.
The affinities of 16 medieval people with the individual USS25, employing the "size" and "shape" method of 12 skull measurement. Males only.

located in ancient Pomeranian Region, near the town of Torun, and were taken over by Teutonic Knights in the first half of the fourteenth century.

Other subjects (USS32, USS33, and T1S2) show craniometric affinities with medieval samples of Puglia.

Male skeletal sampling as a whole tested with shape distance shows affinity with both Castiglione and Aleksandrów Kujawski and Radziejów Kujawski sites (**Table 8**).

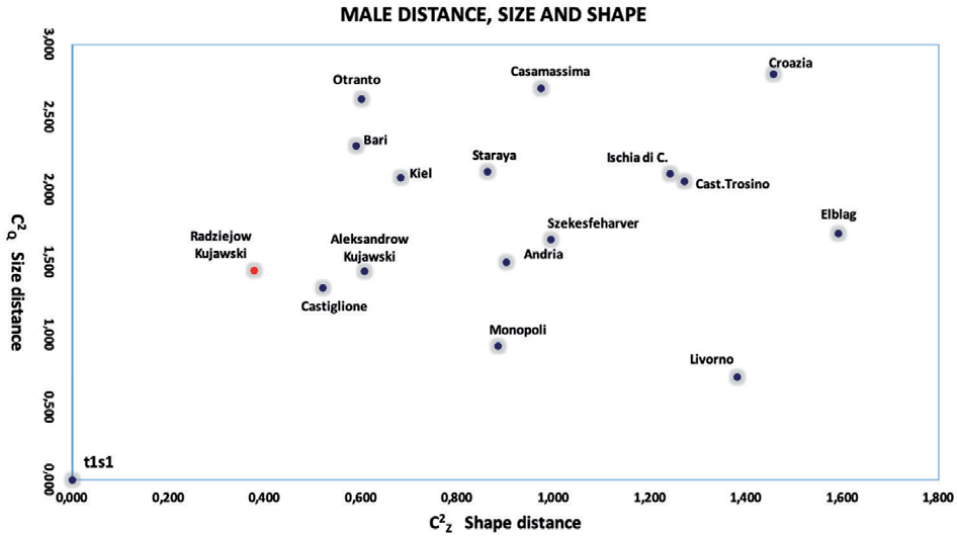

Table 5.
The affinities of 16 medieval people with the individual T1S1, employing the "size" and "shape" method of 12 skull measurement. Males only.

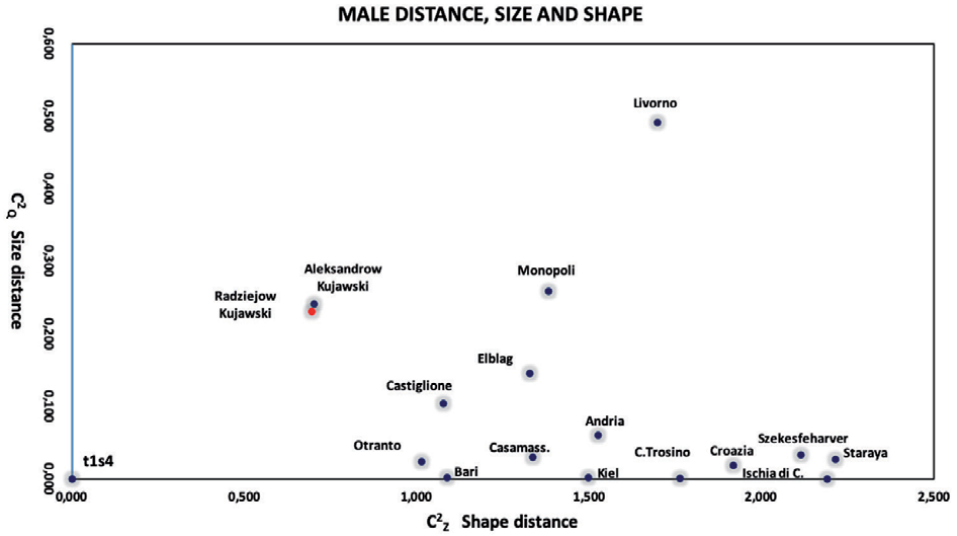

Table 6.
The affinities of 16 medieval people with the individual T1S4, employing the "size" and "shape" method of 12 skull measurement. Males only.

The diagonal matrix obtained through Penrose distance values was used as input for cluster analyses and PCA [64].

The principal component analysis (PCA) aims to reduce a high number of data (in this case, the main craniometric measurements) to a few representative values defined as "principal components" in order to highlight any intergroup correlations.

Two types of approach were used to analyze the Torre Alemanna sample.

The first type of approach compared the Torre Alemanna sample with the other medieval comparison samples.

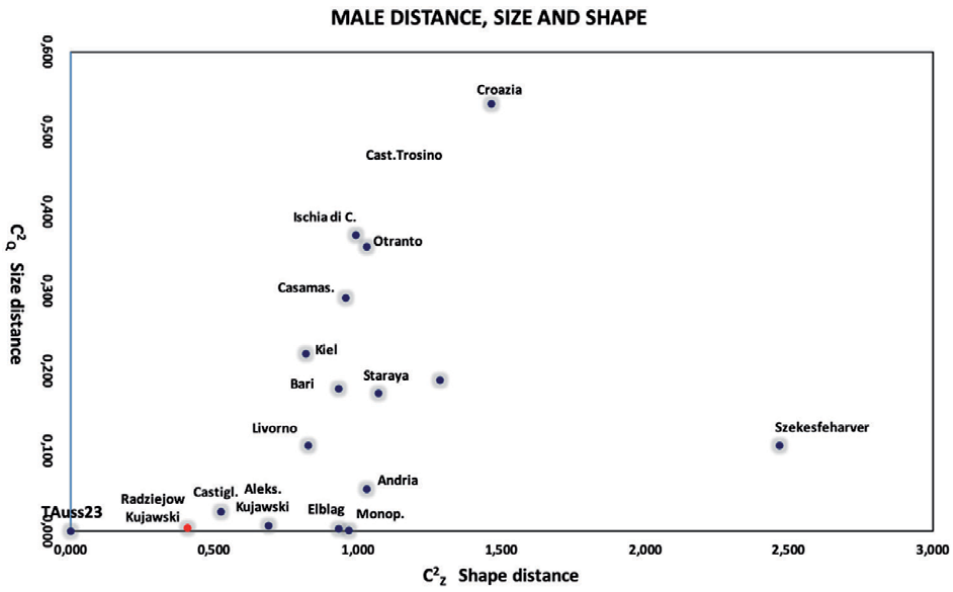

Table 7.
The affinities of 16 medieval people with the individual USS23, employing the "size" and "shape" method of 12 skull measurement. Males only.

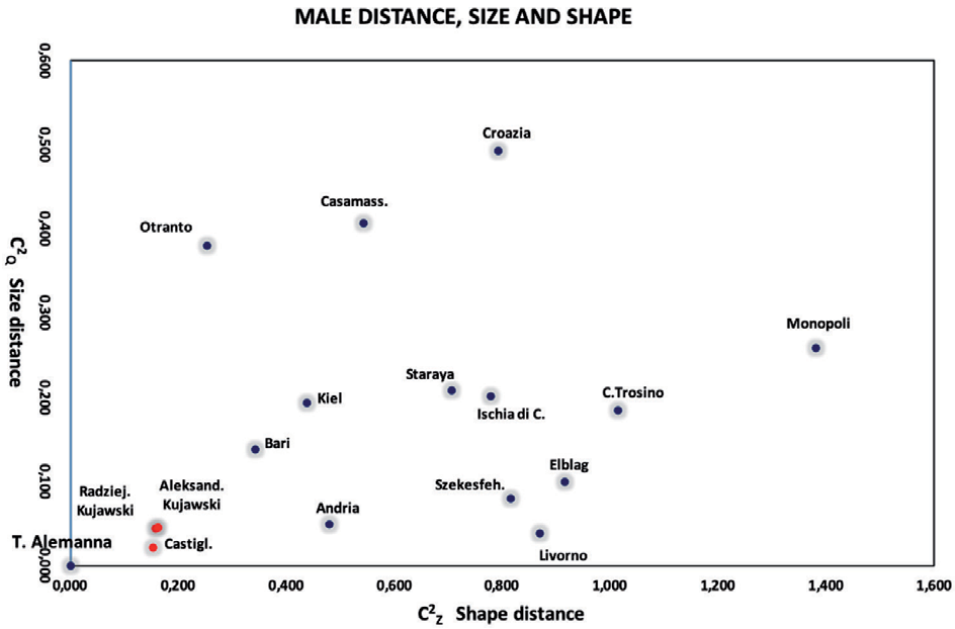

Table 8.
The affinities of 16 medieval people with Torre Alemanna sample, employing the "size" and "shape" method of 12 skull measurement. Males only.

The second one compared the individuals to investigate the behavior between each of them and the other medieval comparison samples.

Due to the absence of some data, craniometric measurements 5, 20, and 40 have been excluded from the PCA.

For the same reason, in order to obtain a more reliable result, the "USS23" individual was not considered.

Considering the first type of approach, it is possible to note that most of the variance is almost equally distributed among the first four principal components, underlining the homogeneity of the sample.

The first and third main components (respectively, 28.85 and 16.91% of the common variance) describe the sample in terms of face shape showing a certain closeness to the Apulian sampling of Andria and to the Pomeranian groups. The second and fourth main components (23.38 and 12.27% of common variance) describe the craniometric set in terms of shape and above all size of the skull, showing a strong affinity with Castiglione, Aleksandrów Kujawski, and Radziejów Kujawski sites, confirming what emerged from the other statistical investigations carried out (**Table 9**).

The second type of approach allows a targeted comparison taking into consideration the individuals "USS25," "T1S1," and "T1S4."

The variance is well distributed among the first four principal components also in this case, with a cumulative value of 80.43% (**Table 10**).

The first three principal components describe the set of individuals mainly in terms of face shape. The fourth component instead describes the size of the skull (**Table 11**). The behavior of individuals in relation to the principal components is investigated with the aim of assessing the affinity of each of them to the Pomeranian groups.

Through the first three principal components, and therefore in terms of face shape, the individuals "USS25," "T1S1," and "T1S4" show a certain closeness with the samples of Aleksandrów Kujawski and Radziejów Kujawski. Only with the fourth principal component, and therefore in terms of skull size, it is possible to describe the strongest affinity, confirming once again what has already been highlighted by the other multivariate statistical studies conducted.

Finally, the individual who most of all seems to be related to the Pomeranian groups turns out to be t1s1.

4.1.3 Occupational stress markers

Starting from the upper limbs, on the collarbones, the strength index was assessed, based on the percentage ratio between the circumference in the middle and the maximum length, and the data obtained showed a very high index for all individuals. The clavo-humeral index, based on the percentage ratio between the maximum length of the

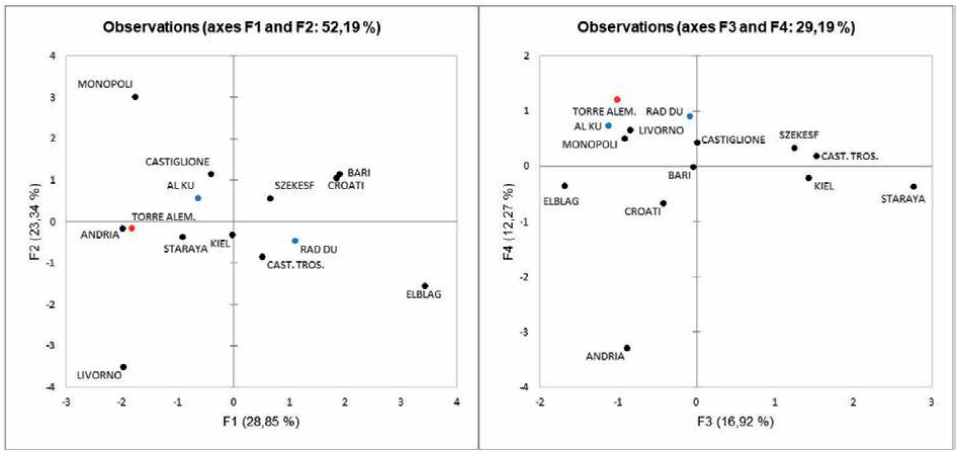

Table 9.
Principal component analysis of the Torre Alemanna sample.

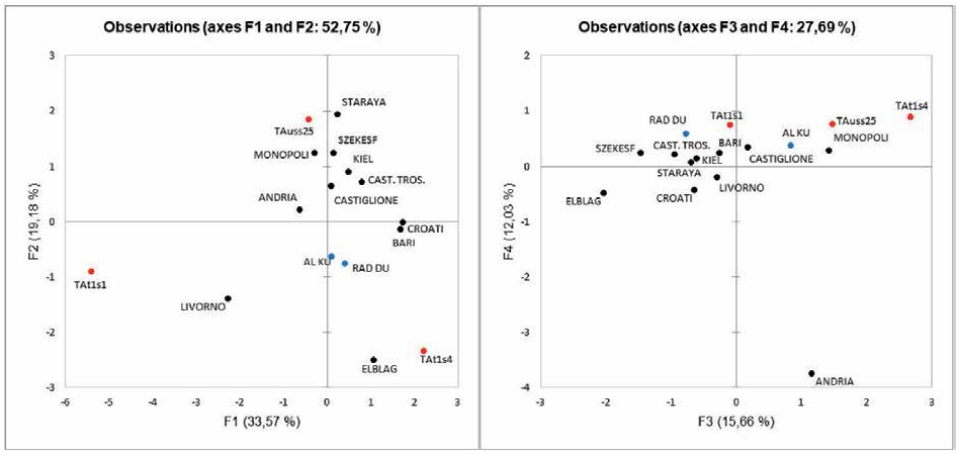

Table 10.
Principal component analysis of three male individuals (USS25, T1S1, and T1S4).

Acronym	*Sex, age*	*Bone/district*	*Wound*	*Timeline*
USS23	M Mat	Nasal bone	Sharp	Ante-mortem
USS25	M Ad	Skull, L. Parietal	Puncture	Ante-mortem
		Skull, Frontal	Sharp	Ante-mortem
		Skull, Maxillary	Blunt	Ante-mortem
		L. Clavicle	Sharp	Peri-mortem
		R. IX Rib	Fracture	Ante-mortem
USS26	M Ad	Cervical vertebrae	Fracture	Ante-mortem
		Rib	Puncture	Ante-mortem
T1S1	M Ad	Rib	Fracture (2)	Ante-mortem
		Fourth metacarpal	Fracture	Ante-mortem
		L. Femur	Sharp	Peri-mortem
T1S3	F Ad	L. Clavicle	Sharp	Peri-mortem
		L. Humerus	Sharp	Peri-mortem
		R. Coxal	Sharp (2)	Peri-mortem

Table 11.
List of individuals with injuries.

clavicle and the width of the lower distal epiphysis of the humerus, is characterized by a short clavicle. When it was possible, the index of platibrachy, or diaphyseal, was evaluated on the humerus of the above-mentioned individuals and showed a rounded diaphyseal section (euribrachy and hypereuribrachy), a symptom of poor development of the *Deltoid* muscle and the *Pectoralis major*. The radius has an average strength and diaphyseal index as found also in the ulna analysis. As far as the lower limbs are concerned, the situation of platymeria is common; therefore, morphological modification of the femur in the part located below the trochanters, which is flattened in the anteroposterior direction. The analysis of the tibial indices shows a condition of euricnemia with the absence of transverse flattening of the upper portion of the diaphysis, indicating a limited level of stress on the quadriceps and soleus muscles. Forces repeated over time and concentrated on a muscle or on a specific ligament can produce different types and grades of markers, detectable through the analysis of enthesopathies, changes in the insertion points of the tendons, and syndesmopathies, changes in ligament insertion points.

The main movements that our skeleton can do are abduction, movement lateral removal from the medial line of the trunk; adduction, movement toward the

midline of the trunk; flexion, bending resulting from the decrease in the angle of a joint; extension, increases the angle of a joint; external rotation, rotational movement around a longitudinal axis of a bone that moves away from the medial line of the body; and internal rotation, rotational movement around a longitudinal axis of a bone that approaches the midline of the body.

By analyzing the development and distribution of markers, it is possible to reconstruct the movements and actions most carried out in life by the individuals considered.

The data obtained revealed a good development of the ligaments and muscles in particular, a case of syndesmopathy of the costoclavicular ligament, a strong development of the *Teres minor* and *Teres major* of the scapula, and a case of enthesopathy of the *Gluteus maximus* of the femur were evaluated.

The acromioclavicular joint is responsible for the lifting movement of the arm above the head. It helps the movement of the scapula with consequent rotation of the arm. A common injury to the acromioclavicular joint is the dislocation, and this is different from the dislocation of the shoulder, which refers to the dislocation of the glenohumeral joint. Dislocation of the acromioclavicular joint is particularly common for those who practice swimming, horse riding, mountain biking, and cycling.

The accessory articular facets are small supplementary articular surfaces that have formed on the bone following load stress or taking specific postures. The individuals of Torre Alemanna have some accessory facets located on the femur, patella, and pelvis.

As regards the femur, the following were identified: in-depth articular fovea (dimples of the femoral head, in which the round ligament of the femur is inserted); Allen pits located bilaterally, due to compression of the neck of the femur by the edge of the acetabulum; and facets of Poiriers, located on the articular surface of the head of the left femur and indicating an extension of the coxofemoral joint at the hip level is given by the extension of the articular surface of the femoral head on the anterior surface of the femoral neck. The stressors that underlie this marker are two: the extreme flexion and abduction of the femur it produces contact between the head of the femur itself and the edge of the acetabulum.

The second factor, however, is due to the pressure exerted by the iliopsoas muscle on the medial edge of the femoral neck. It was originally described as a marker due to squatting; presence on the left femur of the third trochanter; strong alterations of the first trochanter; and important rough line.

On the patella, a slight fossa of the vast lateral was detected, portion of the muscle *Quadriceps*. With its action, it extends the leg and stabilizes the patella opposing its tendency to dislocate itself, and it also increases the effectiveness of the quadriceps by bringing forward its traction force and is the major producer of force during the movement of extension of the leg on the thigh.

The pelvis has rough edges along the margins of the acetabular cavity, which due to mechanical stress is ovalized and shows also the ischial bursitis (**Figure 9**).

Finally, on the front surface of the lower end of the tibia, kneeling facets have been identified, directed parallel to the front edge of the articular surface with the talus, which indicate a hyperflexion condition of the foot in the squatting position. On the basis of the results obtained from the analysis of the musculoskeletal markers and of the accessory joint facets, it is possible to argue that the individuals had the "rider's syndrome," that is individuals who frequently ride horses.

Specifically, the characteristics of the aforementioned syndrome and which are found in the analyzed subject are oval-shaped slab acetabulum; facets of Poiriers; head with deep fovea of the right femur; presence of the third trochanter of the left femur, and marked muscle pressures of the small and medium buttock.

This situation is particularly evident in the USS25 individual, in T1S4 and USS23. Furthermore, the presence of the important costoclavicular ligament in all

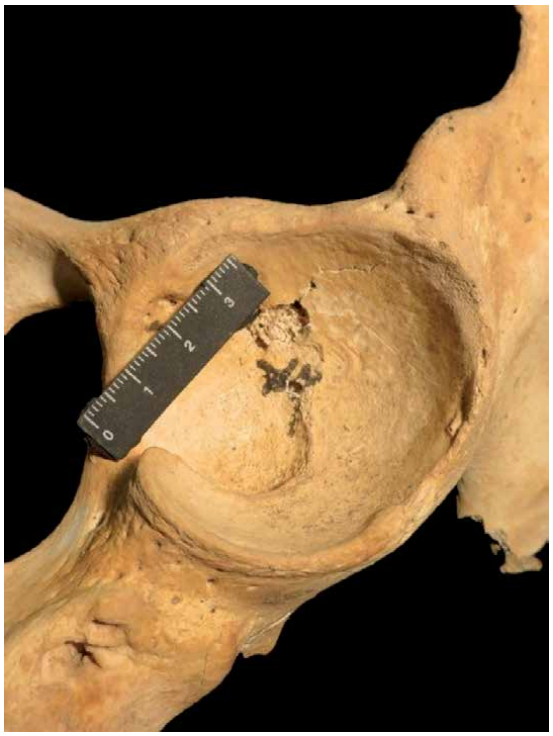

Figure 9.
USS25. Pelvis with rough edges along the margins of the acetabular cavity, oval due to mechanical stress.

individuals is related to stresses on the clavicles and ribs due to a weight overload such as, for example, the presence of an armor.

In conclusion, it can be said that the individuals in question had a first period of horse-riding activity followed by the habit, in recent years, of using a wagon as a means of transport.

Osteoarthrosis is a degenerative joint disease, noninflammatory, chronic, and progressive, characterized by the loss of articular cartilage, which leads to the formation of lesions deriving from direct interosseous contact.

Not only it can be caused by biomechanical stress due to heavy physical activities or small traumas, but also advancing age and genetic predisposition can be causes of appearance. In addition, it has been observed that the onset of osteoarthrosis is due to climatic factors; in fact, countries with colder temperatures show a high percentage of the pathology.

There is therefore a loss of the cartilage located in the diarthrosis joint, inside which the synovial membrane is located, which covers and nourishes the joint surface and lubricates it with the synovial fluid, which covers the central part of the epiphysis.

This results in interosseous contact and abrasion of the subchondral bone, which leads to bone remodeling due to bone neoformations at the joint margins, called osteophytes.

It is possible to distinguish different degrees of lesion of the surface, starting from the slightest shape characterized by thin barely perceptible bone edges, up to the most serious cases, which show evident spicules protruding from the bone surface. The destruction of the cartilage and the consequent interosseous contact cause the smoothing of the articular surface, so as to make the bone take on the appearance of ivory, eburnation.

The individuals under analysis have generally mild osteoarthrosis and, in most cases, characterized by the loosening of the margins of the joints with a rim that does not protrude more than 2 mm.

In some cases, a slight porosity has been detected, particularly in the sternal end of the clavicle, at the level of the femoral head.

4.1.4 Study of traumatic injuries

The study of traumatic injuries is very important in the paleopathological field since they are the most commonly encountered pathological conditions. It is possible to distinguish injuries in: accidental, they provide information about a lifestyle individual; intentional trauma, such as war, allows to obtain information about combat techniques and the type of weapons used; and surgical injury, produced by therapeutic interventions by ancient physicians.

Through the analysis of the fractures, it is possible to detect if it is a cause of death of the studied subject or if a scarring process has occurred. During the reparative phase, a good vascularization is necessary that allows a good supply of blood and nutrients, in order to facilitate bone neoformation.

The presence and location of traumatic injuries in an individual depend on factors related to lifestyle, age, sex, health conditions, employment, and social status within a group. The sample shows 16 lesions distributed over 5 adult subjects including 4 males and 1 female.

There are 8 sharp injuries, 1 puncture lesion, 1 blunt lesion, and 6 fractures: 6 of these, on 3 individuals, are perimortem wounds (**Table 11**).

The USS23 individual shows an healed sharp lesion on left nasal bone (**Figure 10**).

The USS25 individual has the following injuries:

- puncture lesion (4.7 × 3 mm) located on the left parietal bone, nearby of the sagittal suture. The hole is oval, and the margins are rounded. The remark endocranial revealed the presence of a bone scale anterior to the lesion for the penetration of the pointed body. Waiting for an X-ray report, it can be said that this is not a cause of death (**Figure 11**);

- perforation (16.4 × 13.2 mm) located on the frontal bone, 37.6 mm from the point bregma on the median sagittal plane. The color of the margins is clear. It is probably an inexperience in the archeological excavation;

- sharp lesion (12.1 × 7.7 mm) of elliptical shape on the frontal bone, located at 26.5 mm from the temporal crest and 29 mm from the top of the right orbit. It is antemortem injury since there are traces of bone repair in the form of osteosclerotic islands (**Figure 12**);

- localized lesion on the maxillary bone, which led to a sinking with subsequent probable loss of the right medial and incisive tooth bone neoformation of a ridge, on the medial sagittal plane in correspondence of the suture margin between the two maxillary bones, below the pyriform opening (**Figure 13**);

- there is an extensive area of inflammation consisting of several raised areas of osteosclerosis associated with porosity zones. The portion of the anterior face of the right maxilla shows a slight but different angle from the left, with the lower part tending to protrude anteriorly. Etiogenesis is not yet known, but it is possible to hypothesize a direct traumatic event or inflammation process following the odontogenic abscesses visible in the right portion of the maxillary bone;

- cutting lesion (5.8 × 1.8 mm) located on the medial third of the left clavicle, in north-south direction on the anterior diaphyseal arch. There are bone neoformations. It is probably a blow inflicted by an aggressor placed anteriorly and right-handed, through a backhand slash (**Figure 14**); and

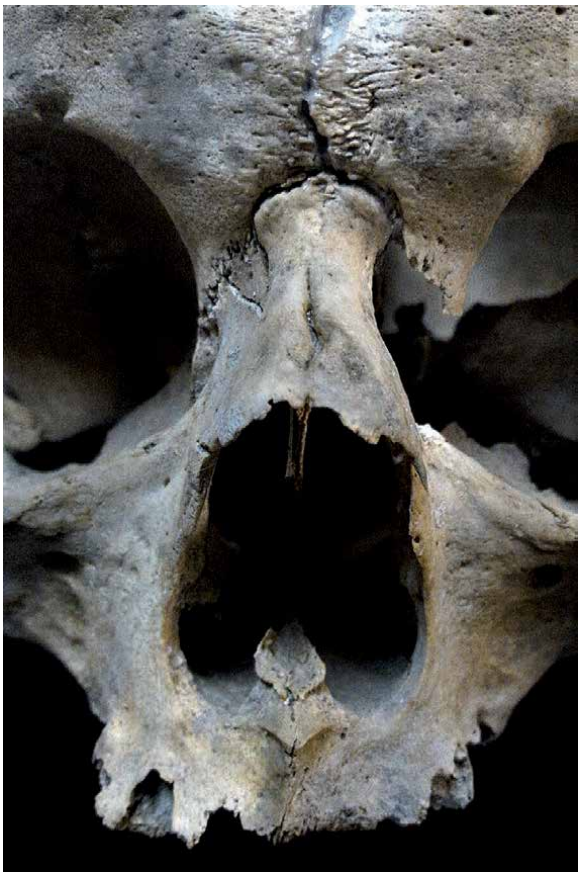

Figure 10.
USS23. Healed sharp lesion on left nasal bone.

Figure 11.
Puncture lesion located on the left parietal bone USS25.

Figure 12.
USS25. Sharp lesion located on the frontal bone.

Figure 13.
USS25. Lesion on maxillary bone.

- probable fracture with subsequent repair on the IX rib on the right.

The USS26 individual shows a half circular lesion on a rib, followed by inflammation of the bone (**Figure 15**). It seems to be related to an intentional trauma during an episode of interpersonal violence. Two cervical vertebrae are partially fused along the left margin of the bodies, probably as a result of a trauma (**Figure 16**).

The T1S1 individual presents:

- the fracture of two ribs, which, given their condition, could not be numbered (**Figure 17**);

Figure 14.
USS25. Cutting lesion located on the medial third of the left clavicle.

Figure 15.
USS26. Half circular lesion on a rib, followed by inflammation of the bone.

Figure 16.
USS26. Two cervical vertebrae partially fused.

- a healed fracture of the fourth metacarpal of the right hand (**Figure 18**); and
- a sharp lesion on the anterior surface of the diaphysis of the left femur, as perimortem injury.

The T1S3 individual presents four sharp lesions: one of these was on the acromial extremity of the left clavicle, one on the greater tuberosity of the left humerus, and two on the right coxal bone (**Figure 19**), of which one is localized on the iliac wing, the other

Figure 17.
T1S1. Fracture of the rib.

Figure 18.
T1S1. Healed fracture of the fourth metacarpal.

on the posterior edge of the acetabulum. They are all perimortem injuries; some of these show elastic bone reaction and are probably consequence of interpersonal violence.

4.1.5 Dental and bone markers of nutritional stress

Finally, from the analysis of the dental records, it appears that the dental wear is of a mild degree and basically due to chewing and not to work activities. Only a few individuals show caries but in a small number; however, the presence of tartar is more important, confirming the mainly meaty diet. Both of these situations suggest poor levels of dental hygiene.

Periodontal disease is an inflammatory disease affecting the gums and involves bone resorption of the alveolar margins with consequent exposure of the tooth root and its possible loss. The detection consists in measuring, using the caliper, the distance created between the alveolar margin and the dental crown. The categories of periodontal disease identified vary from mild, more frequent, to moderate.

Figure 19.
T1S3. Two sharp peri-mortem *lesions on the right coxal bone.*

Dental enamel hypoplasia is an indicator of stress and is caused by deficient enamel matrix formation. It manifests itself in the form of lines or wells on the surface of the teeth, following the slowdown or interruption of the production of enamel by the ameloblasts during the development phases.

Interruptions can be caused by malnutrition or disease that occurred during childhood; as a result, they can provide important information regarding the health and quality of life of a population.

The teeth most affected by hypoplasia are the incisors and canines, followed by the molars; it is found on their buccal surface. By examining the location of the defect hypoplastic, it is possible to go back to the moment of its formation, which in the studied subjects turns out to be between 2.5 and 5 years of age.

The percentage values of the Cortical Area (%CA) were evaluated on the diaphysary midsection of the humerus and are considered a bone indicator of nutrition.

The average %CA for males is 74% that means a good dietary condition.

4.2 Paleonutritional data

In order to evaluate possible diagenetic alterations of the test samples, the pH of the soil has been assessed. The result obtained is based on a basic pH indicating an essentially conservative soil. However, depletion may have occurred in the concentration levels of some elements (Ca^{2+}-Sr^{2+}-Mg^{2+}), due to the permanence of skeletal remains in the soil (**Table 12**).

All samples have an optimal level of calcium, except for the USS25 sample, probably due to a bad condition of *postmortem* preservation.

The magnesium intake differs considerably but remains within the reference standards. The sample data T1S1 shows a significant cereal intake, as opposed to the USS33 sample.

Zinc concentrations show good protein intake, particularly in T1S4 and USS23 samples.

The evaluation of copper data shows a minimal intake of mollusks and offal in general in the T1S4 and USS33 samples. However, they are below the minimum standard. Lead was only investigated in two samples, which show no significant contamination (**Table 13**).

For the analysis of strontium, it is in this case fundamental to relate the results of human osteological samples with that of the faunistic osteological sample of *Bovis* that lived in the site at the same time as the individuals under examination (**Table 14**).

It can be seen that both strontium results from the analysis of human and animal samples have low values (**Table 15**).

Despite the correction of the data, only the USS25 sample indicates a plant contribution.

Therefore, based on the evaluation of the data, it is possible to state that the only individual to have a diversified and balance diet is USS25.

4.3 Results of radiocarbon dating

A fragment of a rib (~500 mg) of the USS25 skeleton was used for radiometric analyses.

This radiocarbon date places this individual between 1342 and 1378 AD, meaning that he would have been active during the second half of the fourteenth century most likely died before the end of the century.

The date fully coincides with the Teutonic occupation of the settlement.

Chemical solution	*Level of acidity (pH)*	*Temperature (°C)*
Torre Alemanna	8,71	24,9°
Double-distilled water	5,38	24,3°

Table 12.
Level of acidity of the soil.

Samples of Torre Alemanna	**Calcium** (mg/g)	**Strontium** (ppm)	**Magnesium** (ppm)	**Zinc** (ppm)	**Copper** (ppm)	**Lead** (ppm)
T1S1	383	29	2929	134	9	/
T1S4	361	28	2525	189	20	/
USS23	429	26	2052	199	15	/
USS33	266	/	1580	131	23	18
USS25	80	50	1835	167	3	45
Standard	250-350	150-400	1500-2500	120-250	30-60	24-70

Table 13.
Concentrations of elements considered as nutritional markers found in the human osteological samples.

Sample	Calcium (mg/g)	Strontium (ppm)	Magnesium (ppm)	Zinc (ppm)	Copper (ppm)	Lead (ppm)
TA Fauna	99	70	3278	139	20	28
Standard	250-350	150-400	1500-2500	120-250	30-60	24-70

Table 14.
Concentrations of elements considered as nutritional markers found in the faunistic osteological sample.

Samples of Torre Alemanna	Strontium	Human strontium/ Animal Strontium
T1S1	29	0.41
T1S4	28	0,40
USS23	26	0,37
USS25	50	0,71

Table 15.
Correction of the data.

5. Conclusions

The taphonomic, anthropological, and paleopathological investigation of the osteological remains of the burials of area 5 of Torre Alemanna has allowed to reconstruct a complete biological stage of the individuals.

The burials belong to the cemeterial occupation's phase of area 5, situated in the northwestern corner of the complex, dated presumably to the initial phase of the Teutonic settlement, on the basis of the ceramic material found in the stratigraphy. Part of area 5 was cut after the construction of a semi-hypogeal environment, which intercepted a series of depositions. All individuals are buried in earthly pit with an east-west orientation. The results of analysis of the skeletal sample from area 5 show a total of 12 individuals subdivided as follows: 2 subadults, including 5-year-old male and 12-year-old female, and 10 adult individuals, including 6 males between the age of 37 and 45 years and 4 females between the age of 25 and 35 years.

The skulls have metrical characteristics very close to the sample of the Middle Ages coming from Radziejów Kujawski and Aleksandrów Kujawski (Poland), located in the ancient Pomerania, conquered by the Teutonic around 1309. From the morphological point of view, they show a typology similar to the northern European skeletal series indicated as Borreby. Borreby is a Danish village, where paleolithic skeletons were found with characteristics indicating a hybridization between the northern (Scandinavian) and the Baltic and Slavic populations.

The skulls of this type are found in the meeting areas between different populations, such as Pomerania, where historically Slavic, Baltic, and Germanic populations have had the opportunity to cross for centuries.

Particular syndromes related to riding horses and carrying weight overloads have been found, which suggest that the individuals examined have spent part of their lives in a very active way.

Several lesions were found, indicating episodes of interpersonal violence occurred *antemortem* and *perimortem* injuries, which some were the cause of death.

The data obtained from the paleonutritional analysis indicate a nutrition especially based on a good supply of cereals and protein. Only the individual USS25 has a balanced diet characterized also by a good supply of vegetable plant.

In the light of all these considerations, it is assumed that the individuals, presumably not autochthonous, participated actively in the military life and that they could therefore be part of the community of the monks-warriors who occupied the complex of Torre Alemanna for 200 years.

This publication is the first part of the project that will also include the manual facial reconstruction of the one of individuals found in area 5.

These were the results of a combination of humanistic and scientific studies.

It is a multidisciplinary project in which researchers of different training and competence have joined. The perspective is to enhance an extraordinary archeological site located in our region.

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Section 3

Forensic Anthropology: Skeletal Analysis and Techniques

Chapter 3

Forensic Anthropology

Purva Wagisha Upadhyay and Amarnath Mishra

Abstract

Physical anthropology has been making progress in the field of forensic science. Forensic anthropology is the study of identifying and establishing identity of the skeletal remains present at the crime scene. The purpose of the chapter is to throw a light on the field of forensic anthropology as it seeks data like age, sex, ethnic groups, and other characteristic features after the examination of the skeletal remains. Forensic anthropology helps in determining the manner and cause of death, and if the body is still in the decompositions stage, time since death can also be estimated. Advancement in forensic anthropology will not only help to solve the case but it will also increase the opportunity to work in this area. In this chapter, there is an explanation of some of the methods used in forensic anthropology for the analysis of identification and other purposes.

Keywords: anthropology, archeology, osteology, skeleton, skull

1. Introduction

Anthropology is the scientific discipline of all features of creature progress and interface. It studies tools, language, ethnicity and social interactions, and how we relay to other societies. Physical anthropology studies human differences, especially those by which we can be acknowledged.

Forensic anthropology studies this identifying distinctiveness on the remnants of a person. These distinctive characters can be used to exhibit the sex, ethnic group, height, and physical health of a victim from his or her remains. Forensic anthropology is the application of the science of physical anthropology to the lawful process. Forensic anthropologists apply standard scientific techniques developed in physical anthropology to categorize human remains and to aid in the recognition of crime [1]. Forensic anthropologists are skilled like physical anthropologists who apply their expertise to solve cases of homicides by identifying the ethnic group; sex; age; stature; antemortem, postmortem, and perimortem injury time since death; and facial reconstruction (2D and 3D). Their job with law enforcement personnel includes scene search, excavation and recovery, questions of recognition, cause of death, manner and mode of death, and evaluation of time since death. The practice scope of this order is the identification of skeletonized remains, badly decomposed cadaver, anonymous human remains, and aging of persons, mass disaster and homicide victims, and unfairness of commingled skeletons or bones. Forensic anthropology is divided into subfields such as forensic archeology, forensic osteology, and forensic taphonomy [2].

Forensic archeology is a division of forensic science in which the appliance of archeological method is used in the examination of a scene of crime to recognize evidence and for the crime scene reconstruction. The work of a forensic

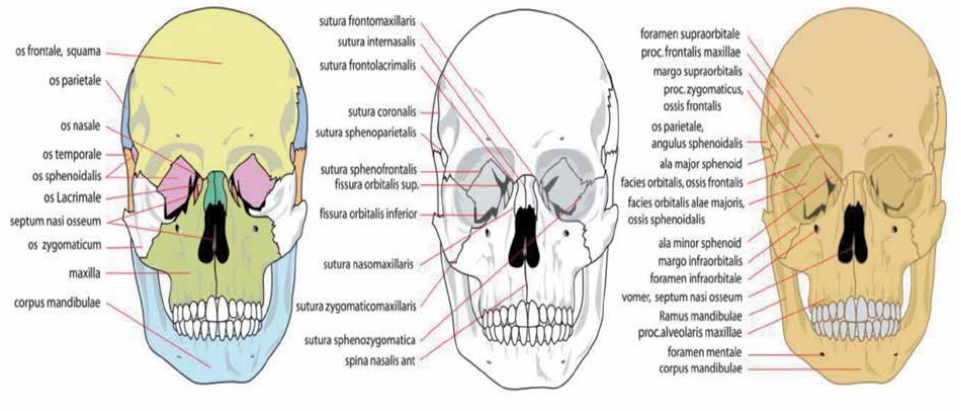

Figure 1.
The skull.

archeologist is to excavate and recover the human remains, weapons, etc. The procedure of forensic archeology is generally the same as that used by the conventional archeologist. In earlier 10 years, the fields of forensic archeology have developed as a key coordinator in the cases of mass death. In 1976, Morse, Crusoe, and Smith were the first to describe the theory of forensic archeology. As per the evolution in forensic archeology, the archeologist has transferred the technical approach for investigate and rescuing evidences to a forensic procedure. The archeological practices such as excavation, evidence identification, recovering the remains, and the study have followed a new approach to the particular type of scene of crime. Forensic archeologists apply their understanding of excavation steps just to be sure that the skeletal remains are collected in a particular manner and they are acceptable as evidence in the court of law. Forensic archeologists also establish the site of grave which might had been overlooked. The archeologist can situate the site of grave which can be seen in the soil's dissimilarity by the darker and looser organic soil in the region of those areas [3] **Figure 1**).

1.1 History

In Europe in the 1800s, the genesis of the ethnic groups of humans was intensely agitated. Scientists began with skull dimensions to make a distinction among those. The differences between male and female framework, and the evolution, aging, and fusing of bones were also examined, laying the structure for today's awareness. Jeffries Wyman (1814–1874) represents a key early pathfinder of forensic anthropology evidence. Wyman had a medical degree from Harvard University and was the first administrator of the Peabody Museum of American Archeology and Ethnology in 1866. His evidence in the trial of Harvard professor of chemistry John W. Webster, accused of the murder of Dr. George Parkman, captivated widespread media, and scholarly notice. At Harvard in 1894, Thomas Dwight gave his lecture about the study of human skeletal remains in a lawful setting (i.e., forensic anthropology). Thus, he was rightfully hailed as the "Father of Forensic Anthropology [4]."

Another nineteenth century murder trail brought well-known notice to anthropological testimony. In Chicago, Adolph Luetgert, a sausage producer, was hold responsible of assassinating his wife and disposing of the dead body by inserting it in a solution of potash in one of the factory vats. Investigation of the remains exposed minute remains that were bought to the notice of Dorsey of the Field

Columbian Museum in Chicago. In the Luetgert trial of 1897–1898, Dorsey testified that the small remains recovered from material connected with the sausage vat were matched from a human female. His testimony was cruelly blamed by defense expert who argued that such determinations could not be through with poise from such minor evidence. In 1932, the FBI declared the opening of its first crime lab. The Smithsonian Institute became a working partner, aiding in the recognition of human remains. In 1939, the FBI published' Guide to the Identification of Human Skeletal Material".

Anthropologist William Krogman made available his book, "of Guide to the Identification of Human Skeletal Material," in 1962. Forensic anthropologists use this textbook even today. "Skeletal age change sin young American males" was in print in 1954 by Tom Mckern and T. Dale Stewart. In this report, Mckern and Stewart recognized skeletal aging approach based on facts from skeletal remains of Korean War military. In the early 1960s, the computer was discovered; this enabled forensic anthropologists to use discriminant purpose study, a statistical system to categorize sex and origin of skeletal remains. More recently, a new practice in DNA originated in the mitochondria of cells has been used in detection. Thomas Wingate Todd was in charge for the compilation of human skeletons in 1912. In total, Todd got skulls and skeletons of humans, anthropoids and other mammals. Todd also developed age estimation based on physical individuality of the pubic symphysis. Though the principles have been bringing up to dated, these estimates a range of skeletonized remains.

2. Biological profiling of skeletal remains

2.1 Demography

All skeletal examinations start with Krogman's "big four"- age, sex, ethnic group, and stature. Each feature narrows the pool of possible "matches" considerably- sex alone cuts it by half. If a skeleton is absolute and unharmed, these aspects can be assessed with great correctness. Using the most recent systems, sex can be established with assurance, age estimated to within about 5 years, and stature approximated with a typical deviation of about 1.5″ (3.5 cm). However, forensic anthropologists are more likely to be dealing with incomplete, fragmented specimens so they must be equipped to glean as much information as probable from every bone. Demographic characteristics include ethnic group, age, sex, stature and build [5].

2.2 Ethnic group

Ethnic group may be defined as coarse classificatory machinery for biological characteristics. Caucasoid, Mongoloid, and Negroid are the three foremost ethnic groups. However, there will always be ambiguous cases because of admixture. Moreover, there is huge variation within each group, and skin color is one of the aspects in racial categorization. In the skeleton, cranio-facial morphology is the finest pointer of racial phenotype. An extended, low, slender skull exhibiting alveolar prognathism and a broad, flat nose with gentle sills is typically negroids. Racial differences can be found in other parts of the body. Caucasoids have forward curvature of the femur but negroids have straight femur bone. Negroids have narrow pelvis, but measurements tell better. Size differentials reproduce disparities in total body proportions. Negroids have proportionally longer limbs than Caucasoid; the reverse holds true for mongoloids (**Figure 2** and **Table 1**) [6, 7].

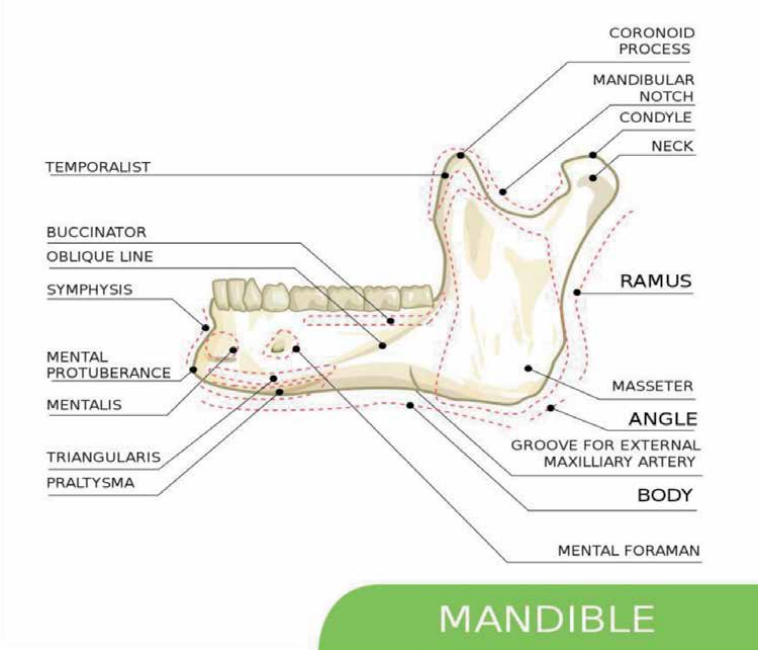

Figure 2.
Mandible.

Characteristics	Europeans origin	Asian origin	African origin
Protrusion of jaws [7]	Orthognathic	Prognathic	Prognathic
Nasal spine	Prominent, large, or long	Medium, tilted	Little or no nasal spine
Nasal aperture	Narrow	Medium	Wide
Nasal sill	Single and sharp	Single and sharp	Double or guttered
Zygoma	Single	Forward projecting (extra bone present)	Single
Dentition	More crowded	Less crowded	Less crowded
Palate	Narrow	Wide	Intermediate palate width
Dental row	Parabolic curve	Simple elliptical (rounded)	Hyperbolic curve (U-shape)
Incisors	Blade shape	Shovel shape	Blade shape
Palatal suture	Arched or jagged	Straight	Not straight
Maxillary molars	Carabelli's cusps	Four cusps	Four cusps
Cranial suture	Simple	Complex and/ or with sutural bones	Simple
Chin	Square and projecting	Blunt	Retreating
Cranium	High	Low and sloping	Low and with post-bregmatic depression
Anterior curvature of femur Bone	More curved	Straighter	Straighter

Table 1.
Differentiating features and points between ethnic groups.

2.3 Sex

If the adult skeleton is absolute or at least has an unbroken pelvis, the sex can usually be determined with 100% accuracy. However, as mentioned earlier, forensic skeletons are hardly ever complete, and the available bones may not be obviously dimorphic. There is no marker that helps in the distinction of primary sexual features. Although gender differences have been quantified in immature skeletons, they remain subtle until secondary sex characteristics begin increasing during adolescence. Attempt at sexing pre-pubescent bones have been completed by means of measurements of growth-based differences between males and females, but the outcome is far from reality. In adults, sex can be determined by the evaluation of pelvis [6].

A systematic data of cranial morphology can allow experts to come up to 90 to 95% accuracy. However, the observer must be well known with population-specific variants because sex-linked distinctiveness varies from one group to another. In adult males, it has been observed that the posterior ramus has a separate angulations or flexure at the level of the occlusal surface of the molars, whereas females maintain the straight, juvenile arrangement.

2.4 Age

The bony skeletal arrangement is not absolute at beginning, but somewhat begins with the configuration and development of centers of ossification. Till the commencement of teenage years, there are diaphysis (shaft) and epiphyses at both the ends of long bones. These are linked by cartilaginous metaphysis or emergent regions that are replaced with bone when development is absolute. Because development at every bony joint is finished at different ages and in a set order, following the succession of epiphyseal union will permit age estimation to within 1 year from about 13 through 18 years. If adolescent is between 13 and 18 years, merging of lower epiphysis and opening of proximal epiphysis in the humerus bone is observed. Age is then determined by fusion of joints in the body. Once development is inclusive, age estimation becomes a great deal and more difficult because post maturity age changes are subtle, uneven, and often extremely variable from one individual to the next because remodeling rates and patterns are sensitive to a many of internal and external factors. The sterna end of the rib is proving to be the mainly reliable form of age estimation. In evaluation of pubic symphysis and ribs from the similar persons indicated that the rib was two times as possible to imitate age accurately (**Figure 3** and **Table 2**) [8, 9].

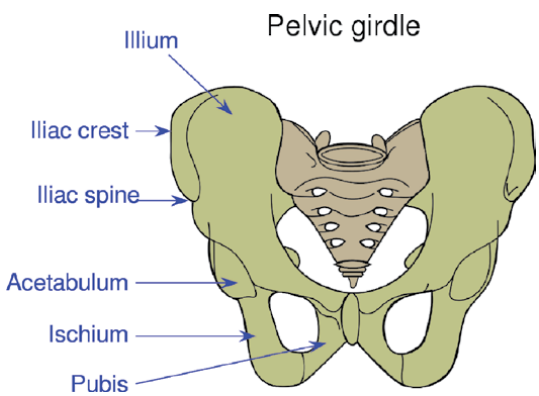

Figure 3.
The pelvic girdle.

Characteristics	**Male**	**Female**
Occipital protuberance [7]	More prominent	Less prominent
Mastoid process	Larger	Smaller
Supraorbital ridges	Prominent	Absent
Upper orbital margin	Rounded	Sharper
Ramus of mandible	Wide, sharply angled, flared	Narrow, chin less angled
Chin	Squared, protuberant	Rounded or pointed
Ossification of ribs	Marginal	Central
Ribs' ends' appearance	Crab claw-like	Rough planar end
Pelvis	Larger and more robust	Wider and larger pelvic inlet
Pubic body	Triangular	Rectangular
Subpubic angle	Narrow	Wide
Subpubic concavity	Absent	Developed
Parturition pits	Absent	Developed
Characteristics	**Male**	**Female**
Ilium [7]	Narrow	Wide
Sciatic notch	Narrow and deep	Wide and shallow
Sacrum	More curved, longer, and narrower	Flatter, broader, and smaller
Muscle attachment in pelvis	More	Less
Humerus head diameter	>47 mm	<43 mm
Radius head diameter	≥24 mm	≤21 mm
Femur head diameter	47.5 mm	42.5 mm
Q-angle in femur bone	Smaller (11.2 ± 3)	Larger (15.8 ± 4.5)
Knee	Larger	Smaller
Zygomatic arch	Broader	Narrower
Sternum	Body is more than twice as large the manubrium length	Body is less than twice the manubrium length

Table 2.
Differentiating features between male and female for sex determination.

Several methods are there for the estimation of age with their advantages and disadvantages. No aging technique is even close to 100% accuracy. There are two sources of fault: (1) individual difference as reported in the standard deviation of the way and (2) differences between the sample population and the population of origin. No aging process should be used without help unless there is no choice. Choice of method is, of course, limited when partial or fragmentary remains are the only material accessible. Always offer a range when estimating age. It is far better to include a 10- to 12-year age range, particularly in big individuals, and be successful in matching the lost person by other individuality than to give a 3- to 5-year range and miss the recognition entirely (**Table 3**) [10].

Characteristic	Age estimation
Metopic suture fusion [7]	Closure by 1–2 years, fusion by 7–8 years
Lateral portion of occipital bone fusion with squamous bone	1–3 years
Basioccipital fusion with occipital	5–7 years
Fusion of occipital with sphenoid	11–16 years (in females), 13–18 years (in males)
Mandibular symphysis fusion	6–8 months
Medial clavicular epiphysis	Mid-twenties or 15–35 years
Coracoid Process in scapula	15–17 years
Glenoid epiphysis	17–18 years
Acromial epiphysis	~20 years
Ribs' head epiphysis	17–25 years
Segment 3 and 4 of sternum	4–10 years
Segment 2 with 3–4 of sternum	11–16 years
Segment 1 with 2–3–4 of sternum	15–20 years
Xiphoid to body	40+ years
Xiphoid appearance	3–6 years
Transverse lines (S1 and S2) in sacrum	Mid-twenties or later
Humerus distal epiphysis	11–15 years female, 12–17 years male
Humerus proximal epiphysis	13–17 years female, 16–20 years males
Radial distal epiphysis	11–13 years female, 14–17 years male
Radial proximal epiphysis	14–17 years female, 16–20 years male
Ischiopubic ramus	5–8 years
Acetabulum	11–17 years
Ischial tuberosity	16–20 years
Femoral distal epiphysis	14–18 years female, 16–20 years male
Femoral head fusion	12–16 years female, 14–19 years male
Tibial distal epiphysis	14–16 years female, 15–18 years male
Tibial proximal epiphysis	13–17 years female, 15–19 years male

Table 3.
Age determination in male and female.

2.5 Stature

Usually and together, the femur and tibia are the majority significant main elements of height. Therefore, the greatest evaluation of height is acquired from regression formulae resultant from femoral and tibial lengths. These equations have been intended for all of the long bones and long bones give more preciseness than arm bones, it may be the only parts establish attempts have been made to amplify accurateness by using the combined assistance of multiple bones. Body proportions differ by both ethnic group and sex. Negroids, for instance, have long limb bones comparative to stature than Caucasoid. Thus, it is essential for determining sex and ethnic group in sort to make use of the correct regression formulae for the

evaluation of stature. Trotter standards are used for Caucasoid and negroids. Smooth bony surfaces and minute muscle origins are trait of a gracile or sedentary individual. It is significant to keep in mind that although males inherently have extra muscle mass than females, males will not have as well-developed attachment sites as female body builders. All small people do not necessarily have slender builds; conversely, huge height is not always linked to massiveness. Although standard weight can be approximated for a known height, there is no technique to establish obesity from the skeleton. The formulae for stature estimation differ by sex and ethnic group, so it is worthwhile to know the sex and ethnic group of the focus before commencement of stature analysis. Long bones are often measured on an osteometric board. The large sliding calipers used for measuring tree diameters are also extremely helpful. Most long bone measurements are easy utmost lengths. This includes the measurement of the humerus, radius, ulna, femur, and fibula. The tibia is a bit extra complicated. The femur is now and then measured with both condyles in contact with the osteometric board. This is called the bicondylar length or oblique length and is particularly helpful because it orients the femur in anatomical location. Bicondylar length provides information about sex as well as stature. After measuring each bone according to directives, insert the measurement into the suitable formulae (**Figure 4** and **Table 4**) [6, 10].

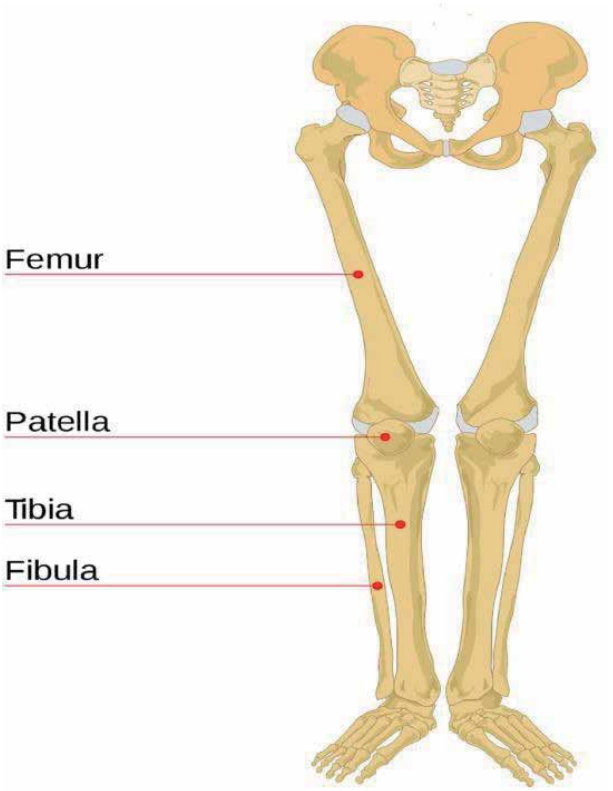

Figure 4.
Lower limb bones.

Ethnic group	Bone	Male	Female
European	Humerus	2.89 * L + 78.10 ± 4.75	3.36 * L + 57.97 ± 4.45
	Radius	3.79 * L + 79.42 ± 4.66	4.74 * L + 54.93 ± 4.24
	Ulna	3.76 * L + 75.55 ± 4.72	4.27 * L + 57.76 ± 4.30
	Femur	2.32 * L + 65.53 ± 3.94	2.47 * L + 54.10 ± 3.72
	Tibia	2.42 * L + 81.93 ± 4.00	2.90 * L + 61.53 ± 3.66
	Fibula	2.60 * L + 75.50 ± 3.86	2.93 * L + 59.61 ± 3.57

Table 4.
Stature estimation using Glesser formula.

3. Methods used in forensic anthropology investigation

In the field of forensic science, successful recovery and examination of DNA has a massive impact. In forensic anthropology, exact knowledge related to sex of the unidentified remains can be represent and offer to identity to it through molecular examination. The molecular analysis is also being used for establishing ancestry evaluation and for detection of species. Determining identification with the help of DNA has played the most important role in the field of analysis. Whether the tooth or the bone needs to be analyzed during the examination of skeletal remains. As DNA test is costly as well as destructive also, these are decisive and influences the outcome of the analysis. For Human Identification from the skull is generally classified as:

A. Reconstruction Method: Reconstruction method proposes only possibilities and probabilities without certainty of as many criterions of individualization from the skull, like ethnic group, age, sex, stature, etc., as possible. Types: (a) Modeling clay method (3D), (b) computer software programs (2D), and (c) manual sketching method (2D).

B. Comparative Method: Comparative method gives a high degree of reliability and precision where the photographs and radiographs are used for evaluation with the skull. Types: (a) Metric measurements and (b) superimposition: (i) Negative SI and (ii) Video SI. Evidential value of superimposition technique in the court of law is corroborative [10].

Radiography: Sex determination is one of the most confused puzzles, which needs to be solved by radiographic examination. Sexual dimorphism represented by the skeletal system determines the accuracy with which the skeleton can be identified. There are different morphological features between males and females. For example, in negroids, left side frontal sinuses gave accuracy in sex determination by 60% [11, 12].

Radiocarbon dating: It is one of the most used methods for determining the age of the skeleton. The amount of Carbon-14 obtained from the skeleton remains or any other part helps in determining the age of the remains [13, 14].

X-Ray fluorescence (XRF): X-Ray fluorescence is used for the determining of mineral content of bone such as Zn, Pb, and Fe. The presence of these elements at different quantity helps in determination of diet of an individual and the abundance

of these elements in the bones also helps in determining the area to which an individual belongs. The main advantage of this technique is that this technique takes very less time for examination [15].

4. Conclusion

Forensic anthropology has many disciplines, one of which is forensic osteology. Forensic anthropology is mainly for the study of basic identification like estimating age, ethnic group, sex, and stature from the skeletal remains. Anthropologists might get several evidences that may indicate the cause of death and manner of death, and if the flesh is found on the skeleton, which means the body is still under the decomposition stage, the time since death can be narrowed down easily by gathering all the necessary information from the skeletal remains; a biological profile of the individuals can tell us how they lived and the manner of their death [16]. Forensic anthropology has already made progress in its field. The advancement in forensic anthropology is only possible and dependent on the advancement of the older technique and by creating new techniques based on the type of cases. Forensic anthropology plays a major role in identifying and establishing the identity in an investigation. The main application of anthropology is to process the sight of crime, inspect the remains, and to generate a profile of the skeletal remains found at the scene of crime to testify it in the court of law.

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Section 4

Ancient Cranial Surgery: Cranial Trepanation

Chapter 4

Cranial Trepanation: Case Studies between the IV Century BC and VI Century in Southern Italy

Alessio Vovlas, Nunzio Di Nunno, Ginevra Panzarino and Sandro Sublimi Saponetti

Abstract

Cranial trepanation is among the oldest head surgical procedures, creating a hole through the skull. This procedure is the surgical removal of a portion of the skull as a medical treatment to cure a specific disease or as a religious or magical ritual. Cranial trepanation has been reported from very early times and widespread all over the world. This work is based on data from six trepanned skulls from the Hellenistic Age (IV century BC) to the Late Antiquity (VI century). The samples come from Puglia (Southern Italy), from the northern (the peninsula of Gargano) to the central area: three cases come from Vieste (Foggia), Ischitella (Foggia) and Alberona (Foggia), the others three from Gravina in Puglia (Bari) and Canosa (Barletta, Andria, Trani). All skulls have been evaluated via an anthropological, radiological and odontological approach. This study indicates the value of a methodology, which encompasses anthropology, radiology and imaging, and archeo-forensic investigations, highlighting the need for multidisciplinary teamwork in any assessment of human remains. The study supports the hypothesis of the presence of a long tradition of medical centres in the Mediterranean basin area.

Keywords: trapanation, skull, puglia, radiology, head surgery

1. Introduction

Cranial trepanation represents one of the earliest forms of head medical surgery [1] performed on the skull to remove a fragment of *calvarium* using sharp instruments or drill. Trepanation, or trephination, has been used frequently in the past in many cultures over the world [2]. This procedure implicates incision of the scalp, cutting through the soft tissue and removal of the bone [3] without damage to the underlying blood vessels, meninges and brain [4]. According to Verano [5] we use trepanation to describe ancient cranial surgery, supporting the Greek origin of the term *trypanon* (a drill or a borer), and trephination to the surgery performed with the "trephine", the most widely used tool for this procedure through the end of the 19th century [6].

This surgical procedure was first documented in Neolithic [7–9] and widely adopted in later times. The earliest written description is provided by Hippocrates of Cos in the *Corpus Hippocraticum* in the V centuries BC [10]. The author described scraping, incision and perforation techniques. Then Celso [11], Eliodoro [12] and

Galeno [13] described two instruments: the *modiolus* a cylindrical piece of iron used for small perforations, and the *terebra* for larger perforations. The diameter of the perforation was in direct proportion to the diameter of the instrumentation employed.

Although writings are a precious source of information, skeletal remains are crucial as they present a direct indication of these procedures allowing us to study them in detail. Ancient examples can be found throughout the world since prehistory and their numbers are in thousands [14, 15]. Its use declined during the mediaeval period [16] and some primitive communities still perform this procedure [17].

In 1867 French surgeon-anthropologist Paul Broca, after examining a pre-Columbian skull sent to him to Peru by Ephraim George Squier, surprised scientist and physician by suggesting that the cranial opening presented on the skull was the result of a trepanation [18]. Even nowadays, some societies still perform this form of intervention, practising the same techniques reported in ancient times [6].

Campillo [19] grouped the main techniques used for trepanation in three general procedures: grooving, scraping and incising (boring, chiseling or cutting). Sometimes these techniques can then be combined with each other [20].

Grooving is the most recurrent trepanation technique used in Europe and probably the easier to perform. In grooving, according to Aufderheide et al. [21] a pointed instrument etches a round or oval groove. It implicates drilling the bone with a sharp and hard stone or a metallic element (groove), repeatedly drawing and redrawing the groove through to the *diploe* while pressure is exerted. Scraping is the oldest trepanning technique [22] consists in thrusting with a forward and backward movement on the bone surface, until the vault wears away and perforation is complete. For this procedure multifaceted stone or later advanced instruments such as scrapers (raspatories) were also used [23]. Incising method is the most common technique recorded in South America [24] however has been scarcely used in Europe. Depending on the tools used, the hole, usually with serrated edges, can have different polygonal shapes, mainly rectangular if a knife was used, or circular [25, 26] if a pointed tool was used.

In Italy we know this practice even in prehistoric times and singular cases of cranial trepanation have been described by various authors [8, 27–34] also from apulian context [35–38].

The aim of the current study is to report and describe six cases of trepanations discovered in necropolis located in southern Italy. Furthermore discusses the survival rates, considers sex and age of the affected individuals, the localization of the trepanations and might provide explanations for the surgical operation.

2. Materials and methods

All the crania are undergone anthropological observation to establish a general biological profile. According to the commonly used methodology were determined gender and age [39–41] and morphometrical indicators [42]; also periodontal pathologies [2] and caries [2, 43], tartar deposits [44] and hypoplastic enamel [45] in order to establish possible nutritional stress and/or previous disease pathologies. The estimation of stature and body mass was carried out according to the methods of Trotter and Gleser [46, 47] and Ruff [48]. The investigation concerns the skeletal markers of biomechanical stress through evaluation and interpretation of any syndesmotic pathologies, enthesopathy, supernumerary articulatory facets or degenerative disease of the articulations [49–53]; finally, the criteria of Borgognini Tarli and Repetto [54] was used in order to evaluate any possible alterations to the spinal column. It was applied geometrical techniques concerning the transversal sections of the humerus and the femur [55–59]. The crania were examined by radiological investigation with Computerized Axial Tomography with 3D reconstruction (CAT, Siemens Somatom

Emotion 16 slice). The images were acquired with l mm slices and total radiation Kv 130 and mAs 240. Then the images were digitally elaborated with the Siemens Syngo workstation. The CT examination allowed evaluating the degree of bone remodeling of the cranial perforation. Furthermore, the examination of the CT scan images allowed for an evaluation regarding the degree of bone remodeling, or remodeling of the cranial perforation, which had taken place. It could be determined whether the perforation had been performed near death or whether the perforation had prolonged the subject's life. Also, it was used the method based which takes in consideration the relationship between the volume of the dental pulps (measured in pixels) concerning the entire volume of the dentition [60] on the canines to evaluate an approximate age.

3. Results

3.1 The trepanned crania

The findings are six and come from Apulia (South Italy), from the northern to the central area: three cases come from Vieste (Foggia), Ischitella (Foggia) and Alberona (Foggia); the others three from Canosa (Barletta, Andria, Trani) and Gravina in Puglia (Bari) (**Figure 1**). Three skulls (Gravina, Vieste and Ischitella) are dated to the Greek Classical period (IV-III centuries BC); the other three (Alberona and the two cases from Canosa) between the Late Roman period and the Late Antique period (III-VII centuries).

For each case study we reported: 1) Archeological information (archeological site, the number of the grave and period); 2) biological profile (gender, age at death, height and body mass); 3) presence of anatomical variations; 4) pathologies;

Figure 1.
Italy, Apulia (square): Geographical location of the graves.

5) Cranial characteristics; 6) characteristics of the lesion (location, shape, measurements, type of edges, healing); 7) reference. The findings date span from 1981 up until 2010: some skulls are incomplete and so do not have all the information.

3.2 Case 1: CSP 2

1. Archeological information: Canosa (Barletta-Andria-Trani); San Pietro archeological area (2001); II archeological dig; tomb n.2.; period VI-VII centuries.

2. Biological profile: male. Presumed age at death: 21–25 years old based on the centres of ossification and dental eruption. Estimated height: 178.5 cm. Body mass: 78 kg.

3. Anatomical variations: none.

4. Pathologies: cribra cranii, widespread porosity in correspondence to the greater wings of the sphenoid bone, cribra orbitalia type cribotica (grade 6 di Hengen [61]), vascular striae across the frontal bone, suprainiac fossa [62], occipital bone, severe periodontal disease, vascular perforations on periarticular regions of the long bones. Differential diagnosis for lesions observed on this individual can include: scurvy, trauma, trepanomatosis, non-specific osteomyelitis and possible osteoblastoma, large parietal foramina. We can exclude the possibility that these lesions may be the result of treponematosis, non-specific osteomyelitis or osteoblastoma because the framework of that lesions may be associated with scurvy in adults - as regards periostitis and periodontal disease [47] - and with scurvy in infant - as regards cribra orbitalia, the vascular impressions on the ectocranial surface of frontal bone [63], the diffuse porosity on the large wing of the sphenoid bone, the porosity on the hard palate and severe periodontal disease [45]. Association between periodontal disease and subdural hemorrhages, in particular, indicates scurvy, the sign of avitaminosis C.

5. Cranial characteristics: hyperdolicocrania, camecrania, acrocrania, hypereurimetopic, hyperleptoprosopia, leptorinia, hypsiconchia, mesorine. Cranial capacity: 1560 cc.

6. Characteristics of the lesion: thick, triangular-shaped, symmetrical lesions in correspondence to the parietal foramina. Each triangle has one side which is parallel to the parietal foramen of the sagittal suture with a sideways facing apex. Borders present signs of new bone formation, with obliterated diploe. The medial side of the lesion, on the left parietal bone, situated 12 mm from the parietal foramen of the sagittal suture. The measurements of the perforation are as follows; transversal diameter 14.7 mm, sagittal diameter 10.2 mm. The medial side of the perforation on the left parietal bone is 10 mm from the parietal foramen on the sagittal suture. The measurements of the perforation are as follows; transversal diameter 10.4 mm, sagittal diameter 6.1 mm. Both lesions have rounded margins and are sloping inwards. The smooth lanceolate depression of the edges indicates apposition of new cortical bone around the lesions in a person who survived to the procedure. This data suggests that the perforations were performed on a live subject who consequently survived the intervention and it may be a consequence of subdural hemorrhage for scurvy (**Figure 2**) [64].

7. Reference: [36].

3.3 Case 2: SST 25

1. Archeological information: Gravina in Puglia (Bari); Santo Stefano archeological area (1993); chamber tomb n.25; *kline* II; dep. IV; period: III century BC.

2. Biological profile: male; presumed age at death: 48–57 years old; estimated height: 174 cm; body mass: 89 kg.

3. Anatomical variations: evidence of metopic suture on the frontal bone. Cleaved acromion the left shoulder.

4. Pathology: hit by a pointed weapon (probably an arrow); calcifications in the right maxillary sinus.

5. Cranial characteristics: hyperdolicocrania, camecrania, acrocrania, hypereurimetopic, hyperleptoprosopia, leptorinia, hypsiconchia, hyperleptorinia; cranial capacity: 1593 cc.

6. Characteristics of the lesion: the lesion is located on the left parietal bone, near the parietal eminence. It has an elliptical, irregular shape 18 mm x 15 mm with a major vertical axis. The posterior margins of the lesion are 72 mm from the craniometric point, *lambda*, while the inferior angle is situated 45.3 mm from the *asterion*. The margins of the lesion are more irregular along the superior side, and decline inwards, rather like the sides of a volcano, up to the point when they meet a bony layer, which occupies the superior part of the perforation. On the external surface 6 mm from the lower-inferior margin of the lesion, there is a small elliptic perforation 4 mm x 2 mm in size, which obliquely penetrates the cranial cavity. The apposition of the osseous tissue seems to have occurred at a later time than the perforation. The remarkably rounded margins suggest that the subject consequently survived the cranial trepanation, living for a long time, perhaps more than a year (**Figure 3**). The small perforation near the trepanation was probably done to drain the wound.

7. Reference: [38].

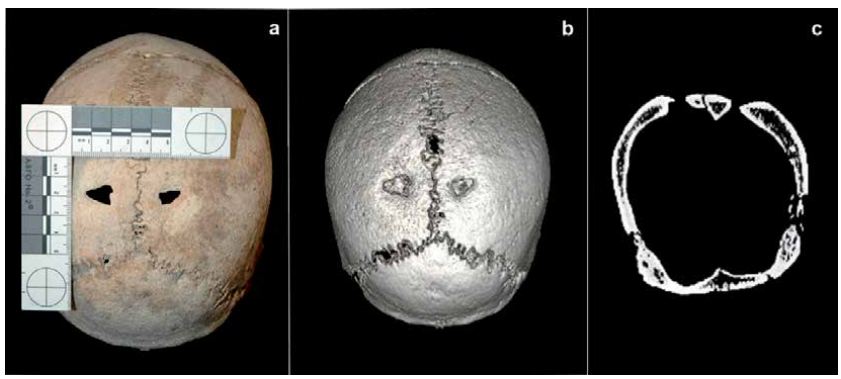

Figure 2.
"CSP 2": Photo report (a) and CT scan (b), superior view; X-ray (c), coronal view.

3.4 Case 3: ISCH 3.2

1. Archeological information: Ischitella (peninsula of Gargano, Foggia); Monte Civita archeological area (2010); tomb n.3; interred II; period: IV century BC.

2. Biological profile: male; presumed age at death: 36–44 years old; estimated height: 165.5 cm; body mass: 76 kg.

3. Anatomical variations: none.

4. Pathology: incised lesions with no sign of the formation of scar tissue on the left femoral diaphysis and the right tibia. Osteochondritis dissecans on the right femoral head.

5. Cranial characteristics: hyperdolicocrania, camecrania, hypertapeinocrania, hyper*eurimetopic*, mesenie, mesoconchia, leptorinia. Cranial capacity: 1291 cc.

6. Characteristics of the lesion: there is a smooth margined, trapezoidal shaped lesion present on the occipital squama of the cranium, along the right occipito-temporal suture and in correspondence to the mastoid process. The lesion is regular in shape and size. The perforation reveals, at an ectocranial level, that the greatest diameter, antero-posteriorly situated, measures 17 mm while the shorter side measures 8.6 mm. The radiological observation confirm that the lesions appear irregular contours and not rounded as to no bone remodeling post-intervention: the absence of apposition of osseous tissue suggests that either the subject did not survive trepanation or that the procedure was, in fact, carried out post mortem since the lesion did not undergo cicatrisation (**Figure 4**).

7. Reference: none.

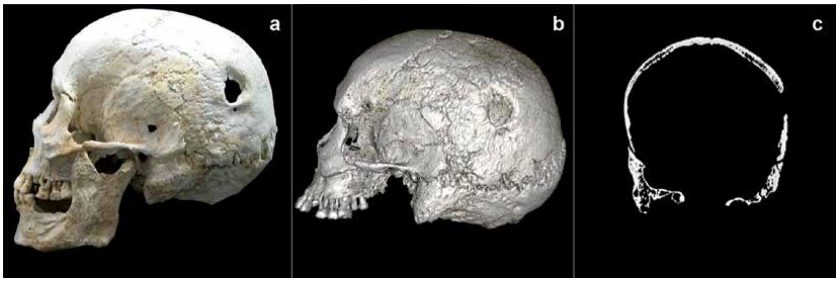

Figure 3.
"SST 25": Photo report (a) and CT scan (b), lateral view; X-ray (c), coronal view.

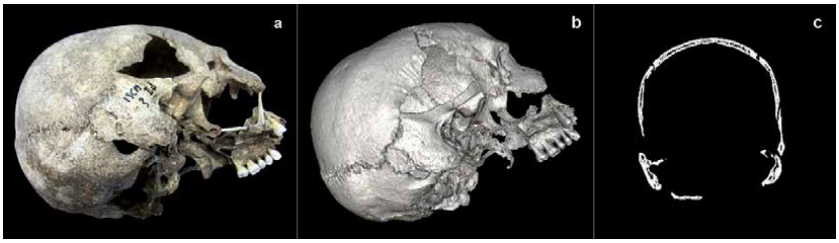

Figure 4.
"ISCH 3.2.": Photo report (a) and CT scan (b), occipital-lateral view; X-ray (c), coronal view.

3.5 Case 4: ALB 1

1. Archeological information: Alberona (Foggia); Church of the Nativity of the Virgin Mary (1995); period: III-IV century (according to radiocarbon dating).

2. Biological profile: male (gender grading =0,97); age at death: 40 years old; estimated height: 164 cm; body mass: 68 kg.

3. Anatomical variations: *torus acusticus*.

4. Pathology: none.

5. Cranial characteristics: hyperdolicocrania, ortocrania, metriocrania, stenometopic, mesenie, hypsiconchia, mesorine; cranial capacity: 1414 cc.

6. Characteristics of the lesion: on the left parietal bone and covering a part of the occipital bone a quadrilateral shaped lesion is present with the medial edge almost brushing the lambda craniometric point, while the inferior edge skirts past the lamboidal area of the lamboid suture. The lesion measures 29.9 mm in a cranial-caudal direction and 31.6 mm towards the biparietal area. The apposition of osseous tissue suggests that the subject consequently survived cranial trepanation (**Figure 5**).

7. Reference: [37].

3.6 Case 5: VIE r.s.18

1. Archeological information: Vieste (peninsula of Gargano, Foggia); Vieste, Via Spina (2008); period: III century BC.

2. Biological profile: male; generically adult. We cannot provide more detailed information because it's impossible to attribute its post-cranial skeleton.

3. Anatomical variations: none.

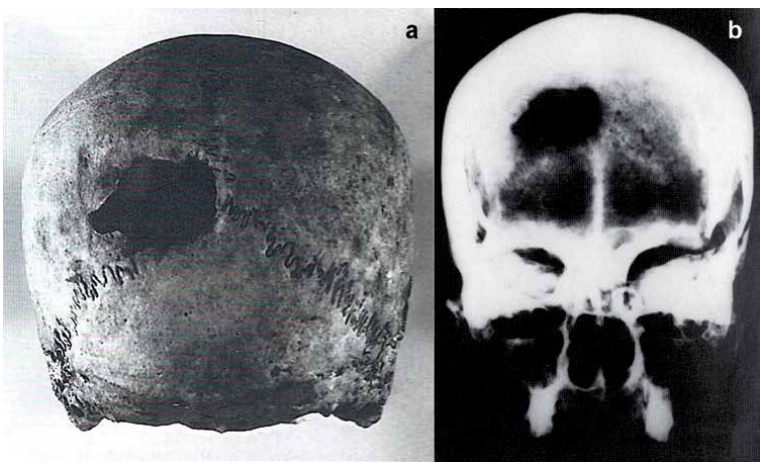

Figure 5.
"ALB 1": Photo report (a) and X-ray (b), occipital view.

4. Pathology: round shape blunt injury located on the left parietal bone at the coronal suture of the left *pars bregmatica*.

5. Cranial characteristics: undetectable.

6. Characteristics of the lesion: on the left of the squama occipitalis, a pyriform shaped lesion is present. Its base is placed medially and the apex laterally encompassing the medial aspect of the lamboid suture. The ectocranial dimensions are transversely 23.2. mm x 11 mm. The cranio-caudal dimensions, 19.2 mm. The ectocranial dimensions are transversely 21.4 mm x 11 mm, cranio-caudel 19.2 mm. The medial margin of the lesion slopes internally to form an inverted volcano shape. The margin appear moderately rounded. The apposition of osseous tissue suggests that the subject consequently survived cranial trepanation (**Figure 6**).

7. Reference: [36].

3.7 Case 6: the "Bologna skull"

1. Archeological information: Canosa (Barletta-Andria-Trani); unknown origin (recovered from Bologna in 1999); period: VI century (according to radiocarbon dating[)].

2. Biological profile: probably a female; young adult (according to the patency of the cranial sutures).

3. Anatomical variations: undetectable.

4. Pathology: undetectable.

5. Cranial characteristics: without the mandible.

6. Characteristics of the lesion: the semi-circular shaped lesion is situated on the right parietal bone. The margins of the perforation are fairly regular and the walls of the lesion are vertical and perpendicular to the parietal bone there is an evident loss of bone tissue around the hole with crateriform enlargement with the diploe visible on all sides of the perforation. The anterior margin of the perforation is 9 mm away from the bregma along the right coronal suture. The medial edge is 13.5 mm from the bregma along the sagittal suture. The ectocranial dimensions are 12.5 mm x 11 mm. The absence of apposition of

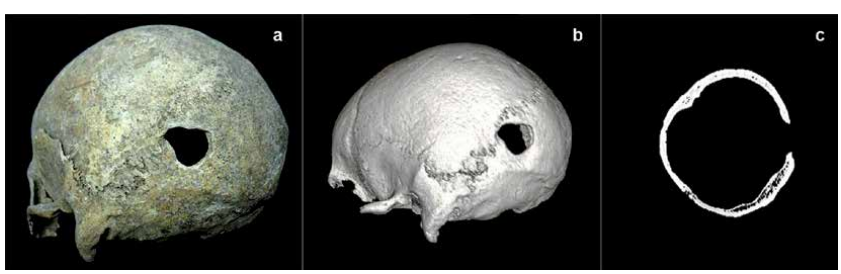

Figure 6.
"VIE r.s. 18": Photo report (a) and CT scan (b), occipital-lateral view; X-ray (c), coronal view.

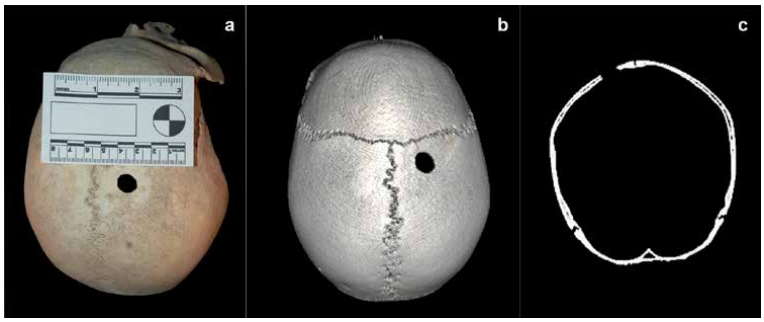

Figure 7.
The "Bologna skull": Photo report (a) and CT scan (b), superior view; X-ray (c), coronal view.

osseous tissue and not evident thickening marginal bone remodeling suggests that the subject did not survive trepanation as the lesion did not undergo cicatrisation (**Figure 7**).

7. Reference: [27, 36].

4. Discussion

About distribution of this evidence by gender and age on the sample, five subjects are male (CSP 2; SST 25; ISCH 3.2.; ALB 1; VIE r.s.18) and just one probably female (the "Bologna skull"). All the crania are from adult subjects as most found in the anthropological literature, except for rare cases [32, 64, 65]. Mariani-Costantini and colleagues ([32], analyzed the skull of a child from Imperial Rome, that showed evidence of hydrocephaly. The authors argue against hydrocephaly as the need for trepanation, performed to alleviate symptoms of this disease. The most common hypothesis surrounding trepanation is that it was performed as a surgical treatment in cases of severe disease, including scurvy [64], meningiomas and bone tumors [66], chronic ear infections [67], headaches and seizures [33]. In Roman time individuals were trepanned to alleviate intracranial pressure due to trauma or disease, treat possible cranial fractures [68]. In a case of cranial trepanation found in a Roman necropolis (Cassino, Italy, 3rd century BC) the surgical procedure seems to be referred to a treatment of a sword wound [31]. Five skulls have just one lesion (SST 25; ISCH 3.2.; ALB 1; VIE r.s.18; the "Bologna skull"); in one case (CSP 2) the result of the trepanation is a double hole. As also seen in a number of cases, the skull can be poly-trephined by two or more holes [8].

The site of cranial intervention is systematic: in four of the cases (CSP 2; SST 25; ALB 1; "Bologna skull") involves the parietal bone (two holes on the right side, three on the left); in three cases (ISCH 3.2.; ALB 1; VIE r.s.18) involves the occipital bone; finally in only one skull (ISCH 3.2.) the temporal bone, on the right. The shape of the lesion is regular and attributable to geometric shapes (triangle, trapezium, quadrilateral, piriform, semicircular) in five cases (CSP 2; ISCH 3.2.; ALB 1; VIE r.s.18; the "Bologna skull"), except one case (SST 25). Measures ranging from a minimum of 8.6 mm to a maximum of 31 mm. The edges of the lesions are rounded and sloping inward where the operation has been successful and the patient survived; otherwise, where the intervention did not have healing or was made *post mortem*, the edges are irregular and the diploe is visible on all sides of the perforation.

Analysis of skeletal features shows in four cases (CSP 2; SST 25; ALB 1; VIE r.s.18), convincing evidence that the cranial trepanations were carried out *ante mortem*: the hypothesis is confirmed both by radiographic and tomographic examination, which revealed evidence of remodeling of the cranial tables, indicative of the subjects having survived the procedures. Bone regrowth is representative of the individual's extended postoperative survival and his rehabilitation.

However, in two cases (ISCH 3.2.; the "Bologna skull"), are absent signs of osseous remodeling, suggesting that the subjects did not survive the trepanation or alternatively that the procedure was made *post mortem* on the corpse. As documented in both ancient and present times, trephination has also been reported to be adopted as a magic-ritual practice. In *post-mortem* symbolic trephinations, cranial bones were being used as religious objects that have curative powers in Medieval Europe and bone roundels were worn as ornaments or amulets [69, 70].

Different techniques of drilling are attested. In three cases (CSP 2; SST 25; ALB 1) the morphology of the trepanned apertures – especially the broad shallow bevel of the edges - indicates that was probably used the scraping technique, when a sharp-edged tool is repeatedly scraped over a designated portion of the bone until the vault was away and oval or round-shaped perforation is complete: the edges, in fact, are of this type - a broad shallow bevel, smooth and remodeled - while the diploe is not visible.

A double well healed skull trephination associated with a post-cranial traumatic event occurring *intra vitam* to a young male from the Early Chalcolithic cemetery of Pontecagnano (South Italy, ca. 4900–4500 cal BP). X-ray and 3D-CT scan skull-cap evaluation revealed that an orifice was probably produced by scraping with a sharp stone tool, obtained by clockwise rotation motion of a right-handed surgeon facing the patient [71]. A second trephination instead seems carried out by drilling with a stone point as a tool, which produced a round, cone-shaped hole, involving only the external cortical layer.

In two cases (the "Bologna skull"; VIE r.s.18) the trepanned apertures indicate that was probably used the incision/chiseling technique: edges are steeply beveled and the diploe is visible. The latter case (ISCH 3.2) is an unusual trepanation for anatomical location and it's difficult to recognize the precise technique. A metal or a hard instrument was employed, maybe drills and chisels.

Cranial trepanation is the most valid explanation for the presence of these holes on the skulls, excluding the possibility that they are anatomical variations, matches of trauma, secondary breaks after the deposition or diagenetic events of the soil. Concerning CSP 2, based on differential diagnosis cranial trepanation is the most valid hypothesis. For this study, we reconsidered the possibility that it can be caused by a pathology. Enlarged parietal foramina or "Catlin marks" are symmetrical, normally oval defects in the parietal bone situated on each side of the sagittal and involve the lack of ossification of the posterior membranous parietals suture. In the past, enlarged parietal foramina were confused with trepanations [72]. Concerning this finding, we do not totally exclude the possibility that the holes were caused by this pathology. But, based on the data obtained from the CT image, there appears to be a re-apposition of the bone after the trepanation. This further supports the hypothesis of medical intervention. In this case, it would be an operation performed by a person with in-depth anatomical knowledge.

From contemporaneous perspective, the motivation of trepanation events in the past can only be conjectured. Although in literature various motives are debated, grouped into two wide-ranging categories: therapeutic and ritual [73].

Explanations for ritual trepanations are the hardest to interpret, as their motive is culturally based [74]. Motivations for therapeutic trepanations are mainly

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CASE	SKULL	SITE	PERIOD	SEX	AGE	LOCATION	TECHNIQUE	HEALING
1	CSP 2	Canosa - S.Pietro	VI-VII century	M	Young adult (21–25)	Parietal (right side; left side)	Scraping	Present
2	SST 25	Gravina - S.Stefano	III century BC	M	Mature (48–57)	Parietal (left side)	Scraping	Present
3	ISCH 3.2.	Ischitella - Monte Civita	IV BC century	M	Adult (36–44)	Occipital; temporal (right side)	Chiseling	Absent
4	ALB 1	Alberona - Church of the Nativity of the Virgin Mary	II-III century	M	Adult (40)	Parietal (left side); occipital	Scraping	Present
5	VIE r.s. 18	Vieste - Vieste via Spina	III century BC	M	Generically adult	Occipital	Not determinable	Present
6	"Bologna skull"	Canosa - unknow origin	VI-VII century	F	Young adult (20–30)	Parietal (right side)	Chiseling	Absent

Table 1.
Summary view of the data.

twofold: were performed to alleviate trauma caused by various factors (e.g. open and closed fractures of the skull, remove foreign bodies) or treat injury and illness [75, 76].

In our case studies there are no specific evidences which can highlight the motives behind the trepanation. We cannot motivate the procedure, if for therapeutic, magic/ritual or both reason, based on the location, the technique, the shape, the success of the surgery or other cultural or archeological evidence. In one case (CSP 2) it's possible that the drilling is therapeutic, to rid the tissues of the head from subperiosteal hemorrhage, one of the main symptoms of scurvy, of which he was ill the subject [64]; in another case (SST 25) we hypothesize that the hole, caused by a pointed weapon, maybe an arrow, produced a penetrating wound with the formation of an epidural or subdural hematoma, which necessitated the surgical operation. The absence of signs of healing in two cases (ISCH 3.2.; the "Bologna skull") suggests two possible interpretations: therapeutic if the surgical operation is not successful and the patient died during or shortly after; ritual if these drillings have been made after the death of the subject. **Table 1** shows a summary view of the data.

However, only a scarce numbers of studies have underline the role of anesthesia [77] to minimize pain during operations performed. Mednikova [78] suggests that immersion in altered states of consciousness may have been a necessary part of the process of trepanation, along with shamanic practices, such as consumption of psychotropic substances or ecstatic dance. Inserting these elements in the Mediterranean and southern Italian context, it is possible to mention how Tarantism in Puglia has been known since ancient times as an ecstatic and therapeutic dance for other kinds of pathology. We have no direct evidence about the use of drugs or different methods of altering consciousness used. We can only speculate about the consumption of fungi [79] or the use of smoke from the burning leaves, such as the use of juniper (*Juniperus sibirica*) and thyme (*Thymus vulgaris*) in shamanic practices in some Siberia population [80], as reported in ethnographic materials collected worldwide. John Moyle in his work "Chirurgus marinus or the Sea surgeon" suggests administering alcohol, in specific Cordial, a liqueur obtained from various plants, before head surgery [81].

In some tombs in the vicinity of some of the findings analyzed, in Daunia, some anthropomorphic stone funerary monuments (VIII - VI century BC) were found at the end of the last century. Studying the figurative scenes drawings, Leone [82] hypothesized the use of *Papaverum somniferum* in medical practice.

5. Conclusion

The approach of this work is global, both in terms of geographic and chronological point of view. First, the burials are representative of the territory of Apulia, from the northern to the central area of the region. The findings also cover a long period (nine centuries) from the Greek Classical (IV-III centuries BC) (SST 25; ISCH 3.2.; VIE r.s. 18) to the Late Antique period (III-VII centuries for CSP 2; ALB 1; the "Bologna skull"). Therefore, the chronological and geographical distribution and some common characteristics of the trepanation, as described in the chapter of "Discussion", allow us to draw some historical considerations. First of all, it's attested a great number of scraping, the oldest trepanning technique [22] that provides the greatest control over the process and involves the lowest risk of damaging the brain, using, especially before the widespread use of metals, a sharp-edged oval stone but more advanced instruments such as metal scrapers (raspatories) [83]. Also the large geographical distribution and chronology of the

case studies allow us to support the hypothesis of the presence of a long tradition of medical centres in Apulia. We hypothesize the existence of two medico-surgical centres for craniotomy: the oldest is of probable Greek origin, maybe Taranto (Southern Italy), the most important Greek cities of South Italy and Sicily ("magna Grecia") where it circulates Greek culture, including the medical-surgical theories of Hippocrates. Moreover, the presence of a Greek medical school in Southern Italy could be hypothesized concerning the presence of other similar findings from Pontecagnano (Salerno, Italy) [84], Poggiardo [35] and Hymera [85]. Chronologically successive is a second centre that could be Canosa, the city of the miraculous Bishop Sabinus (514–566), connected to the Byzantine military, considering the events linked with the Greek-Gotica war (535–553) and the Longobardian invasions of Italy in 568.

The study of cranial perforation also allows us to underline the utmost importance of radiodiagnostic examination as well as a multidisciplinary approach to reach an optimum situation for a complete evaluation of human remains. The analysis of cranial osteological information should be multidisciplinary involving anthropology, forensic pathology, odontology and radiography. Human skeletal remains with pathological lesions of dubious origin should always undergo, not only direct observation with systems of magnification but also an in-depth multidisciplinary and structural radiological examination to create a correct diagnostic picture regarding the origin and characteristics of the pertinent lesions, applying forensic sciences standards. Such findings could have an important historical and cultural relevance regarding the methods and reasons for cranial perforation which would enable such lesions to be identified either as those executed as a therapeutic measure or those executed as part of a ritualistic magic cult.

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Section 5

DNA Fingerprinting

Chapter 5

DNA Finger-Printing: Current Scenario and Future

Sandeep Sitaram Kadu

Abstract

Linearly arranged chemical structure in chromosome is known as DNA. It is a double helix made up of two strands of genetic material spiraled around each other. Each strand has a sequence of bases. There are four types of basis namely adenine, guanine, cytosine and thiamine which are very unique to each individual just like their actual fingerprint. The nitrogen base adenine always binds with thymine and cytosine also always binds with guanine. Thus the DNA profiling unique to each individual is collectively known as DNA fingerprinting. DNA determines individuality or uniqueness of the each human being except in uniovular twins. The chances of complete similarity are one in 30 billion to 300 billion i.e. half the population of world. The technique of DNA fingerprinting was first developed by Dr. Alec Jeffery's from Britain in 1984. He discovered a minisatellite region close to the human myoglobin gene. He isolated this sequence and used it as a probe to investigate human DNA. He found that the minisatellite probe result was a complex band pattern for each individual. In India, initially it was done at CCMB, Hyderabad by Dr. Lalji Singh. Now there are various centers where DNA fingerprinting is carried out. In Maharashtra it is carried out at Sate Forensic Science Laboratory, Vidya Nagar, Kalina, Mumbai – 400 098 (Phone 022–26670755). Using this technique FBI formally concluded the participation of Mr. Bill Clinton in Monica Lewyninskey case. In India more than 79 cases have been solved by using this technique including important case of Dhanu and Shivarasan alleged assailant of Late Priminister Shr. Rajiv Gandhi, Tandori case, Madhumati murder case etc.

Keywords: DNA, restriction fragment length polymorphism (RFLP), polymerase chain reaction (PCR)

1. Introduction

Linearly arranged chemical structure in chromosome is known as DNA. It is a double helix made up of two strands of genetic material spiraled around each other. Each strand has a sequence of bases. There are four types of bases namely adenine, guanine, cytosine and thiamine which are very unique to each individual just like their actual fingerprint. The nitrogen base adenine always binds with thymine and cytosine also always binds with guanine. Thus, the DNA profiling unique to each individual is collectively known as DNA fingerprinting [1].

DNA determines individuality or uniqueness of each human being except in uniovular twins. The chances of complete similarity are one in 30 billion to 300 billion i.e., half the population of world [2].

"DNA fingerprinting", is a fingerprinting of exclusive type as there is no specific method to modify it known as it remains the same in all body parts. In every DNA of there is about 0.1% differences but among every individual person, 99.9% are identical but as the DNA sequence is just like a fingerprint [3]. It was once thought that there are many bases of nucleotide which were consuming time to detect in olden days but now scientist have introduced some techniques which were quicken in the identification process. This (DNA fingerprinting) technique is one of them it is like an individual's bar-code. DNA present in every cell of an individual person has histone proteins which are found tightly bound to the DNA present in chromosome [4]. DNA fingerprinting in Forensic Science had a tremendous impact. Forensic genetic science is an intersection between science and crime. It helps the police/judicial to aid justice. It is an important tool for court outcomes and all the serious and unsolved mystery cases. The police investigation progress clearly depends on forensic science services; Forensic genetic science in its own way requires understanding the importance, scope, and limitation of DNA fingerprinting. It made the court to accepts as pivot evidence [5–7]. When other methodologies failed, DNA fingerprinting was kept as the last resort and it played a supportive role when strong evidence in support is needed. In both at small-scale and large-scale disasters. In Criminals identification it provides an approach to the victim in an impressive way. For Victims and Criminals identification it became a gold standard. The National Laboratories of National Research Council of USA had issued on February 2009, in a Forensic Science major report showed with the exception of nuclear DNA analysis, no Forensic method thoroughly shown to have the capacity to consistently demonstrate a connection between evidence and a specific individual or source with a high degree of certainty [8–10]. Therefore, Variable Number Tandem Repeats (VNTRs) have been identified. These are non-coding sequences and do not have any regions with unravel information about genome. Absence of genetic material in these regions, hence helpful in identifying an individual person [11, 12].

DNA profiling is also known as DNA fingerprinting, Typing, DNA Genetic typing, Genetic Fingerprinting, Genotyping or Identity testing. It is DNA base pair sequence method of isolation and identification. The technique of DNA fingerprinting was first developed by Dr. Alec Jeffery's from Britain in 1984 [13–15]. He discovered a minisatellite region close to the human myoglobin gene. He noticed that these minisatellites are not useful for genetic transformation as genes and have repetition. Jeffreys also documented that minisatellites have unique pattern in each person [16–19]. He isolated this sequence and used it as a probe to investigate human DNA. He found that the minisatellite probe result was a complex band pattern for each individual [20].

In India, initially it was done at Centre for Cellular and Molecular Biology (CCMB), Hyderabad by Dr. Lalji Singh. Now there are various centers where DNA fingerprinting is carried out. In Maharashtra it is carried out at Sate Forensic Science Laboratory, Vidya Nagar, Kalina, Mumbai – 400 098 (Phone 022–2667075).

Using this technique FBI formally concluded the participation of Mr. Bill Clinton in Monica Lewyninskey case. In India more than 79 cases have been solved by using this technique including important case of Dhanu and Shivarasan alleged assailant of Late Prime Minister Shri. Rajiv Gandhi, Tandori case, Madhumati murder case etc.

2. Collection and preservation of material

1. In living persons:

   i. Blood: The blood should be collected from peripheral vein with EDTA as an anti-coagulant in two tubes and should be transported to the laboratory as early as possible at −20°C [21].

   ii. Vaginal swabs/smear: It is to be air-dry and put it in clean autoclave glass/plastic container.

2. In dead bodies:

On the basis of priority all required biological samples must be taken in order as mentioned below.

   i. Skeletal muscle/Tissue: On duty doctor should collect the least putrefied part of the muscle or tissue and must send it without any delay to avoid further putrefaction [22].

   ii. Hard (Tough) Tissue: Dead bodies in which the putrefaction has already present, collect hard tissues where putrefaction rate is comparatively less. Muscle tendons, foot, heel skin, scalp skin, palm skin, stomach wall is observed to have lesser degree of decomposition [23].

   iii. Tooth: On duty doctor must send complete set of teeth present in deceased body [24].

   iv. Scalp hairs with roots: Small quantity of scalp hairs along with roots should be sent. One important precaution must be taken i.e., hairs must not be cut but should be plucked [25].

   v. Blood of deceased should be taken.

   vi. Bones: In cases of completely skeletonized bodies and if no other tissues are present, send the longer bones such as humerus, femur etc. Occasionally portion of muscle or tendon may be present on long bones, do not remove them, send it with bones as it may contain the cells where DNA traces can be detected [26].

3. Scene of crime and other important evidences:

   i. Blood stains: In homicidal, accidental or suicidal manners, blood stains present at scene of crime, scrapings of blood stain on floor, blood-stained clothes, weapons and other relevant evidences must be collected and sent for further analysis [27].

   ii. Semen stains: In sexual assault cases all wearied garments especially undergarments of both survivor and suspect, also bed garments should be collected. In addition, collect condom and other important circumstantial evidences [28].

Type of biological evidence and accurate preservatives for particular evidence:

Biological evidence	Important biological and other evidences	Preservative
Blood	a. In living, blood samples should be collected in tubes supplied by FSL [29]. b. Post mortem blood collected in clean sterile glass vial. (Samples should be kept in ice)	4% EDTA solution
Tissue/muscle piece/scalp skin etc.	Sample must be kept in clean sterile plastic or glass container add the correct preservative as suggested. (Samples should be kept in ice)	DMSO or normal physiological saline or 4% ETDA solution or keep the tissue as it is in −20°C refrigerator
Teeth	Air dry all the teeth available. Keep samples in dry clean and sterile plastic or glass container and then handover it. Must Keep any tissue stuck up to teeth as it is.	No preservative
Scalp hair	Air dry the sample, put in dry clean and sterile plastic or glass container	No preservative
Bone	Air dry and rap in clean brown paper. Do not grind it or apply any chemical on it. If the issue or tendon is stuck up to the bone keep as it is. Do not separate or disturb it.	No preservative
Blood-stained clothes and scrapping, etc.	Clothes must be air dried and should be kept in clean brown paper. Avoid packing damp clothes. New sharp blade must be used for scrapping the blood stains from the wall or floor. Take care that paint on the wall should not get mixed with blood. Keep all scrapped blood stain material on clean white paper and then place it in the packet [30].	No preservative
Semen stains	Clothes must be air dried and should be kept in clean brown paper. Avoid packing damp clothes. Take sterile, clean and dry piece of cloth, make condom inside out put all the material on cloth. Condom also must be dried first and then to be packed it in brown packet.	No preservative
Vaginal swabs/ smear	Air dry and put it in clean autoclaved glass/plastic container.	No preservative

Apart from material mentioned above; perspiration, oil, urine (when concentrated) and feces are also can be used to analyze DNA; as all these contain nucleated cells.

3. Authentication and forwarding

1. All samples collected and preserved as indicated above should be delivered to laboratory without undue delay preferably within 48–72 hours after collection [31–33].

2. Blood samples in cases of paternity disputes and in cases where they are used as control samples for identification purpose should be collected in the presence of judicial officer.

3. The sample should be sealed and the specimen of seal on paper should be sent along the sample for verification.

4. The identification card and the forwarding note should be filled, certified and sent to the lab along with samples.

5. In person who had blood transfusion within 3 months preceding the date of collection. The samples are not useful.

6. Collected and preserved material can be forwarded to the laboratory by executive magistrate or senior inspector of police.

4. Technique of DNA fingerprinting

The technique essentially involves [34, 35] (**Figure 1**):

i. Isolation of DNA from nuclei.

ii. Fragmentation of DNA by treating with restriction endonucleases.

iii. Gel electrophoresis of the fragments after alkalinization.

iv. Blotted on to sartorious nitrocellulose filter.

v. P^{32} labeled probe hybridization.

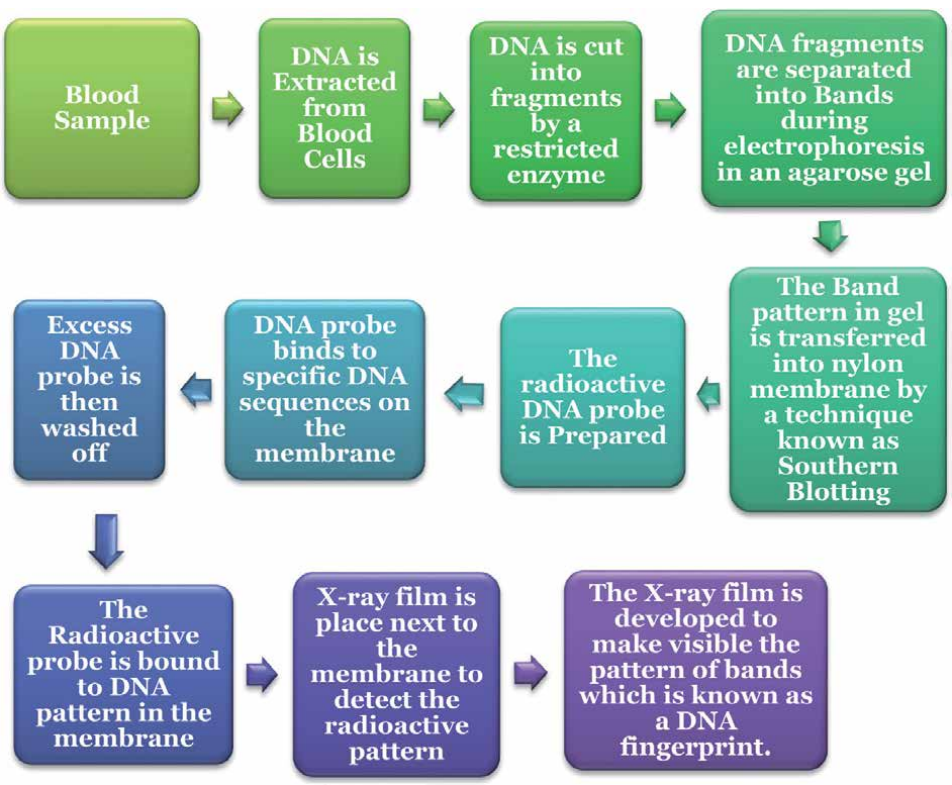

Figure 1.
Steps in DNA fingerprinting technique.

vi. Autoradiographed at room temperature on to an X-ray plate.

vii. Bands appearing on the X-ray plate as dark lines are the permanent Band Patterns where the probe had attached.

The various techniques in use at present are [36–40]:

1. Restriction fragment length polymorphism (RFLP):
   - Using Multi locus probes (MLPs)
   - Single locus probes (SLPs)
   - Variable Number Tandem Repeat (VNTR) sequences.
2. Polymerase chain reaction (PCR)

4.1 Restriction fragment length polymorphism (RFLP)

This was first Forensic DNA analysis technique adopted for analysis. This kind determines variation in the length of a defined DNA fragment. The pattern looks like a very simple super market bar code.

Basic requirement of DNA fingerprinting is nucleated cells. DNA is present in the nucleus of cell, so it can be only extracted from body fluid or tissues having nucleated cells. All the samples should be frozen at −20°C before use. Isolation or extraction varies according to the type of biological evidence present; the amount of evidence and the kinds of cells present.

DNA molecules are segregated by following steps:

a. Cell membranes are broken down and different cellular organelles are fragmented.

b. DNA molecules are detached by using soap and salt solution.

c. DNA molecules are separated from remaining proteins.

d. RNA and polysaccharide are detached with the help of enzymes.

The secluded DNA is passed through ultraviolet spectrophotometry for measuring its quantity.

DNA is completely broken-down by using enzymes named as restriction endonucleases. Restriction endonucleases identifies the unique sequence and cut the double stranded DNA at several fragments. It is known as 'restriction fragments length polymorphism' (RFLP). RFLP's are product of dissimilarities present in DNA molecule. This dissimilarity in restriction fragment length is because of variable number of tandem repeats (VNTR) [41].

Fragmented DNA molecules are run on agarose gel electrophoresis. The different restriction fragments are separated varying in length in between 0.5 to 25 kb which varies from one individual to another. The length of smaller fragments is less so naturally it moves for longer distance as compared with larger fragments.

The gel is later stained with ethidium bromide for 40 minutes which tightly binds to DNA and fluoresces under ultraviolet light.

By using capillary transfer technique of southern DNA is transferred from the agarose gel to nylon membrane. With this technique it creates mirror image replica of fragment distribution. Commonly vacuum blotting of transfer technique is applied because it takes less time. DNA is then fixed by hit at 80°C or cross-linked by the cation of UV irradiation [42].

With application of hybridization technique, pairing of two single stranded DNA is done to convert it double stranded DNA. It involves the addition of probe to the nylon membrane. DNA is created with specific technique hence it goes to specific programmed locus on an exact chromosome. Normally, probe is also labeled with radioactive marker like P^{32}. Firstly, probe identifies its matching sequence, then it hybridizes with it. Due to presence of radioactive marker, hybridized fragment turns into radioactive. Generally, four probes are used to analyze four different DNA regions at same time.

Loosely bound probes are removed by washing it with 0.05% SDS. Subsequently membrane is wrapped in the saran wrap and kept in the X ray cassette along with X ray film and exposed to 80°C. The probes are exposed depending on its specific activity and exposure time ranges from few hours to days (maximum 10 days). X ray films are then developed and fixed in the respective reagent and finely washed in water and dried. Finally developed autoradiograph is the DNA pattern of that particular individual seen as black bands. These black brands are radioactive hybridized unique fragmented sequences. X-ray film shows unique band pattern known as AUTOROD. This unique band pattern is nothing but the DNA fingerprint of that individual whose biological material is tested. It also serves as permanent unique record of that individual (**Figure 2**) [43, 44].

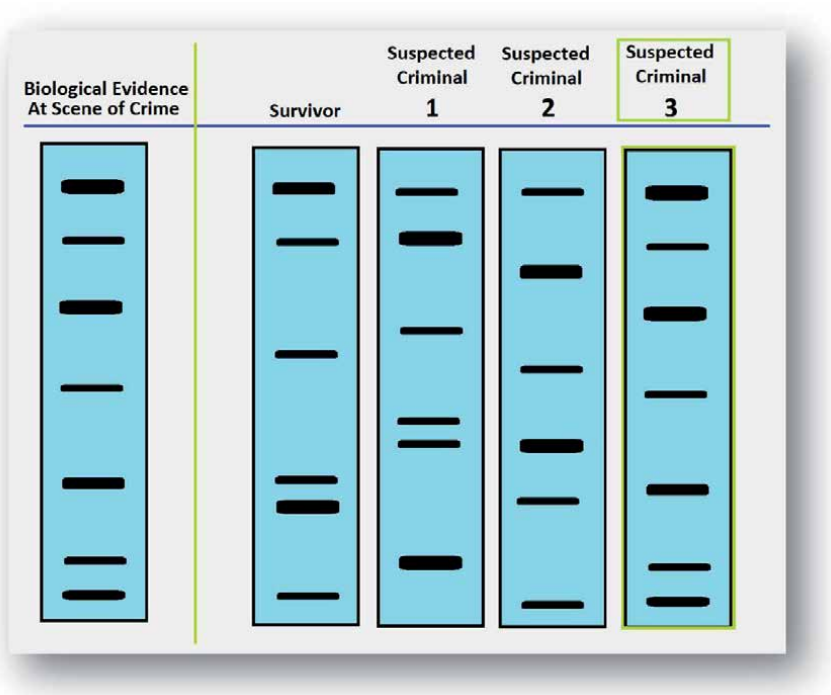

Figure 2.
DNA fingerprint band pattern.

4.1.1 Multi locus probe (MLP)

The probe used detects variations at several genetic regions simultaneously. The Band Pattern produced on X-ray plate produced a strip of 30–40 dark bands. The MLP Method was originally described by Sir Jefferys. He used three minisatellite regions turned 33.5, 33.6, 33.15 after previously cloning and characterizing them.

33.5 and 33.15 contain repeats of a similarly version of the core sequence and consequently produce similar but not identical DNA fingerprints. Probe 33.6 is comprised of a shortened derivative of the core and hybridizes to a new set of resolvable hypervariable fragments per individual in the 4–20 kbs range, 33.6 detects 6 additional and 33.5 detects two additional hypervariable fragments, 33.15 detects 15 fragments [45].

4.1.2 Single locus probe (SLP)

Further modification of this method led to the development of SLPs which analyze only single hypervariable location in human DNA. These play a very major role in Forensic practice as they have far greater detection sensitivity than the MLPs. Each SLP detects just two bands (One maternal and one paternal). It is so sensitive that it identifies even a single hair root. Results can also be obtained from degraded DNA, often found in Forensic samples as SLP detects the remaining, non-degraded alleles among the DNA fragments. As they detect only two bands/SLP, using single SLP reduces the probability to 1/10000 population as compared to 1 in 02 MLP. Using multiple SLPs is therefore the practice now a days. SLPs are human specific. MLPs detect DNA fingerprint in all vertebrates. 80% of Forensic work depends on SLPs [46].

4.1.3 VNTR

This method uses set of probes which detect specific variable number tandem repeats of a sequence. These also remember the minisatellite in that they consist of a repeated sequence with the number of copies of the sequence varying from one person to the other. However, where there are usually many minisatellites of a given type in a genome, there is only one VNTR of each type. These probes therefore produce simpler banding patterns. Several VNTR probes are used. Each of which recognizes one VNR site to characterize a DNA sample. After the frequencies of the various bands produced by each VNTR probe have been established for each ethnic group. This can be used to calculate the probability of any particular combination of patterns occurring in each individual [47].

4.2 Polymerase chain reaction (PCR)

This is general technique routinely used for increasing amount of a specific section of DNA in a sample. This is called DNA amplification. PCR more often referred to as molecular Xeroxing.

It was devised by Kary Mullis and his colleagues in 1985 in Henry Elrichs Laboratory at the Caus Corporation in California. It is test tube method of copying simultaneously the two complementary strands that make a gene sequence. In this method millions of similar DNA fragments can be synthesized within hours. Primers are used to amplify specific segment of DNA. Primers finds the DNA ends that can be duplicated. After heating DNA sample, there is detachment of two strands. Then two new strands which matches to the both original strands are produced by enzymatic action of DNA polymerase. DNA polymerase is originally

obtained from *E. coli*, but it is heat unstable. Hence original DNA polymerase gets inactivated at high temperature, recently new heat stable DNA polymerase is derived from bacteria *Thermus aquaticus*. By using heat stable DNA polymerase, PCR technique can amplify DNA sequence up to 1 million in 20 cycles and up to 1 billion in 30 cycles. This amplified DNA can be now explored by any of the standard molecular biological method [48, 49].

5. Methods

5.1 HLA DQa/HLA DQ A1

This was the first system among the PCR based variation in DNA sequence, is detected using specially designated to be complementary to and thus target, a particular sub region within this locus. The original probe detected 6 common DQ alpha alleles that in combination, determine 21 possible genotypes.

The final results are seen as series as blue dots on a paper like strip. A comparison of the pattern of the dots between typing strips indicates whether two samples may have originated from the same source [50].

5.2 Ampli type PM + DAQ A1

Commonly known as poly marker with all advantages of PCR. This increases power of discrimination. Several markers at different loci were analyzed at the same time (procedure known as multiplexing).

Each of the fine additional markers contains less individual variation than DQ A1. power of discrimination increases from 1:200 to 1: 2000. Disadvantages of PM loci are that is often difficult to interpret from samples containing DNA from more than one contributor because of low power of discrimination per locus [51].

5.3 D1 S 80

Also known as Amplified Fragment Length Polymorphism (AMP-FLPs, AFLPs, AMFLPs). In D1 S 80 analysis, fragment in the range of 100 s of base pairs are amplified, about on order of magnitude smaller than fragments normally analyzed in RFLP typing.

In D1 S80, PCR amplified sections are efficiently purified before DNA analysis. In RFLP technique complete DNA is analyzed and then important sections of DNA molecule are distinguished with help of molecular probes.

D1 S80 loci are found as distinct alleles and easily compared with allelic ladder which is run on the same gel [52, 53].

5.4 Short tandem repeat (STRs)

This is similar to D1 S80, except that repeat units are shorter. For Forensic purpose loci selected usually have tandem repeat unit of 2–5 bp and it can be repeated up to dozens of times. The number of alleles varies from 5 to 20 bp depending on the locus. The size of DNA fragment produced by amplification of STR loci is in the range of 200–500 bp (base pair). Due to above specifications STRs is an ideal for degraded DNA. Also, PCR amplification of many different loci performed simultaneously in same test tube saves material, time and most important sample. STR loci also can be analyzed manually by silver stain by using fluorescence to detect the bands either during or after separation [54].

5.5 Gender identification

For tooth pulp tissue an Amelogenin locus is used which detects the variation of length in male and female. In female gene one of part contain a small detection (6 bp) in nonessential DNA and gives a shorter product by amplification with use of PCR. When this region analyze female with 2 X chromosomes will show one band and male with both X and Y chromosomes show two bands (one is same size as female the other one is slight larger) [55].

5.6 Y-STRs

STRs found on Y-chromosomes are amenable to typing small degraded samples of DNA and can be analyzed on the same instrumental platform. Male specific information thus obtained.

i. Can be helpful for non-sperm containing samples comprised of both male and female contributions such as mixtures of blood or male saliva deposited on female victim.

ii. Information from Y-STRs can also be successful where only incomplete separation has been achieved using a differential extraction produced, particularly where only a few sperm are present among many non-sperm cells.

iii. Sometimes detect male profile where only single female profile was evident using standard automated STR typing.

iv. Also helpful in determining number of male donors by eliminating any information contributed by female resources [56].

5.7 Mitochondrial DNA

Mitochondria in human cells contain an autonomous circle of DNA that codes for some protein that control function like cellular respiration. The mitochondrial genomes are about 16.5 kb and of interest to Forensic scientist and contain a non-coding hyper variable control region.

Mitochondrial DNA sequences are highly variable between unrelated individuals. Complete 16,569 nucleotide sequences of mitochondrial DNA have established for a reference individual [57].

Mitochondrial DNA circle is a genetic element that lacks a homogenous counterpart in the genome and it can be described as hemizygous. Mitochondrial DNA is haploid hence these genes survive for many generations and transfer to many generations intact, without change and retain its uniqueness. Up to thousands of copies of mitochondrial DNA genome present in small, old, badly degraded sample, and if no results obtained with any other systems. Mitochondrial DNA is commonly used to type the dead cells in hairs, shafts of bone and teeth.

5.8 Interpretation

Detection of allele specific sequences difference was in the form of Dot blot. This is constructed in such a way that a particular sequence was either present (signal on) or (signal of) each dot is represented for one allele.

In length-based system – PCR products are run on a gel through capillary and they are visualized as bands, similar to RFLP or as peaks on automated equipment [58].

5.9 Disadvantages of RFLP

1. The samples have to be in good condition to be analyzed.

2. Fragments isolated/identified by this method are in the ranges of 2 to 20 kbps.

5.10 Disadvantages of PCR

1. It is susceptible to contamination.

2. Most PCR loci have fewer alleges than the VNTR areas utilized in RFLP.

3. Some of PCR loci are functional genes.

5.11 Advantages of PCR over the RFLP

1. It is technically easy.

2. The reports can be given in short time.

3. It permits analysis of extremely tiny amounts of DNA.

6. Forensic application of DNA fingerprinting

1. Homicide: In homicidal cases, blood stains on clothes and weapon can be compared with the blood of victim. Also, hair roots present on weapon may be compared with the blood of suspected criminal and victim. In sexual assault cases, identification of accused by analysis of semen samples obtained from the vagina of the survivors of rape, blood stains or hair found at the scene of crime or on clothes.

2. Disputed paternity: The DNA samples of child are compared with that of alleged father and similarity is noted. With this technique paternity can be confirmed 100%.

3. Maternity testing: This technique is also used for maternity testing specially in cases where the child is exchanged, misplaced, stolen or kidnapped from the hospital.

4. Identification of mutilated remains: As in cases of accidents, mass disasters, bomb blasts, burnt bodies, putrefied bodies etc. The DNA fingerprint obtained from such remains can be compared with previous prints if available or with that of close blood relative of the disease which can establish link between family members.

5. Extortion cases: Saliva samples from envelope, face mask, nasal secretion, saliva from cigarettes butts etc.

6. To acquit a falsely implicated person of any crime.

7. Identification of bodies in exhumation cases.

8. For tracing pedigree and for establishing familiar relationship.

9. Diagnosis of inherited disorders: DNA fingerprinting is used in diagnosis in inherited disorders in prenatal and newborn babies.

10. Migration of population: DNA fingerprinting can be used in determining how the races migrated from one region to another by comparing the DNA fingerprint. Thus, it helps for study of history and confirmation of races.

7. Discussion

DNA fingerprinting can be proved for one's innocence as well as a guilty person. Most of the errors can be made during the samples collection. If the DNA samples have not been contaminated only then DNA evidence is completely conclusive. The advancement in molecular genetics avoids the types of contamination but sometimes lack of suitable tests might leads to the wrong perception. Allowing the trained person to educate about standardized tools and technologies of DNA fingerprinting [59]. Collection of profiles DNA of previous culprits' cases, victim, offenders and as well as the witness of the crime scene is known as DNA database. United State America (USA) has the major DNA databank known as Combined Index DNA System (COIDS). Combined Index DNA System (COIDS) is used for identification. Forensic Science DNA Database also contains evidence of persons DNA pieces who have been involved in a crime (victim, offender, crime affected, witness of the crime scene and crime suspect related). There is also human remains and missing person database. Databank of DNA is much useful in solving with its help many old cases are resolved. Country likes U.S.A and Great Britain which has DNA database, but they totally do not dependent on this technique to cracking out all the crime scene. Gattaca (1997) dystopian American science fiction film written and directed by Andrew Niccol in which Ethan Hawke (as Vincent Freeman) who becomes a cosmonaut. But due to his genetic problem, he would not get some essential benefits like insurance, etc. For becoming superhuman he found genius Genetic Engineer who can transform him completely. He knew that a hair like structure known as DNA creates overall personality of human being. He is aware that if his genetic defect is exposed, people will know about his true identity. Moral of this movie states that DNA databank is unsafe [60].

As most of the People are afraid by computer systems hackers who exploit the systems and easily gain someone's personal information and as a result get profit through black-mailing. Databank is the heart of whole this mechanism, any hacking or tempering of data by the corruption and dishonesty person such as DNA finger print experts can ruin an individual's life.

Sometimes wrong interpretations are identified in fake or synthetic DNA identification. The limitation to believe in DNA evidence as truthfulness in these fake DNA's and causes incorrect perceptions. In one of the, A Canadian physician (DNA fraud case), Dr. John Schneeberger alleged that he raped one of his patients in 1992 and at the crime scene semen left as a DNA evidence. Police investigated the case and matched the Schneeberger bloods with crime scene semen, never showing a match results drew totally different.

In another case, police identified the DNA samples from the same woman on different crime scenes in Austria, Germany, and France among them robberies, burglaries, and murders. Only after the DNA sampled from the burned body of a male asylum seeker in France exactly matched with that of the "woman". Investigators had serious query about DNA traces, then after careful investigation they found that these DNA traces was already present on cotton swab. These Cotton swab found at the scene of crime was manufactured by an Austrian Company. The Product description of cotton swab mentioned that swabs were sterilized but were not DNA free. The technique for differentiating false DNA and original DNA was afterwards developed by a company in Israel.

In India many cases were solved by DNA fingerprinting. Rajiv Gandhi former Prime Minister of India was killed in bomb blast. His body remnant and accused suicidal bomber remnant were also confirmed by DNA fingerprinting in 1991.

In 1995, Naina Sahani was murdered by husband Sushil Sharma, he chopped her body in to pieces and burnt in tandoor. Even from burnt body remnants, DNA fingerprinting was done and confirmed that it was of Naina Sahani. Naina Sahani case was solved with conviction to the accused.

Priyadarshini Mattoo was raped and murdered by IPS Officer's son in 1996. Trial court in 1999 acquitted him, but due to this it became sensitive case. In 2006, accused was convicted to death sentence due to DNA fingerprinting. The traces found on victim's undergarments matched with accused. This were possible due to DNA technology only.

United States Supreme court in its recent judgment clarified that in serious life-threatening offenses, investigating officer can take cheek swab for DNA analysis as routine procedures like taking photograph or simple fingerprint.

For Forensic analysis DNA database is very useful. Familial DNA Database Searching for matching of crime scene stain with near relative helps reaching up to accused. In 2004, US first familial DNA search was done in Craig Harman's case. He was convicted due to partial match with his brother's DNA.

In 2009, bollywood actor Shiney Ahuja raped the maid. Maid. In court Ahuja became hostile and witnessed that she was not raped but DNA traces from her private parts matched with Ahuja. Due to DNA evidence, Court convicted him.

The public outrage sensitive Nirbhaya case, six men raped college girl with brutal way, she died during treatment. All six men sentenced to death with DNA evidence.

In Hyderabad Blasts Case (2014), first intelligence agencies searched suspected house in Zephyr Heights in Mangaluru, but the team got no evidences. Afterwards Forensic team found DNA evidences collected DNA samples from the same house. Five accused was caught and DNA evidences collected from house were matched with all five accused.

Rohit Shekhar Tiwari alleged that Shri Narayan Dutt Tiwari is his father. Shri Narayan Dutt Tiwari was Chief Minister three times of Uttar Pradesh state, and a famous political leader. At first, he denied for DNA sampling but afterwards by compulsion of Delhi High Court, DNA mapping was done and subsequently confirmed his fatherhood.

8. Future

8.1 United States of America

The Federal Bureau of Investigation developed the Combined DNA index system (CODIS). DNA database in United States. Supreme court in Mayland v. King

Sentenced that if the officers made an arrest for serious offense, DNA samples like cheek swabs can be legally taken. The DNA samples can further be used in the Law of Court under the Fourth Constitutional Amendment and the Individual's privacy is valid.

Thus, in USA 28 states and federal government, swabs can be taken for DNA fingerprinting from any accused as a part of normal investigating procedure. These swabs can be compared to Combined Index DNA System database to identify the person and for creating links to unsolved cases. Due to technical advances DNA is one of the confirmatory and quickest methods of Identification [61].

8.2 United Kingdom of Great Britain and Northern Ireland

National DNA Database (NDNAD) in United Kingdom is based on The Criminal Justice and Public order Act. In case of certain offenses mentioned, Police are duty-bound to take the DNA samples of the arrested person. The samples are supposed to be taken before the Investigating process begins in order to make it faster [62].

8.3 China

A Law was passed by China which allowed Ministry of Justice and Ministry of Interior to setup DNA Banks.

The fundamental points integrated in this legislature are:

1. The Offenders – The accused and who are sex offenders have to provide DNA Samples willingly.

2. If the offender refuses to provide DNA samples, the Prosecutor can compel the person to do so.

3. The written and photographic samples of DNA can be preserved for 10 years.

4. If the accused is suspected of committing a crime for which, the punishment period is more than 5 years, are required to give non-intimate samples.

In India the DNA Technology (Uses and application) Bill is introduced some silent features are [63]:

1. The main goal is to establish identity in relation to many civil and criminal cases.

2. Establishment of supreme regulatory board of 12 members called as DNA Profiling Board.

3. Formation of National DNA Data Bank and various Regional DNA Data Banks.

4. Usually Consent of the person is required for collecting DNA samples, but if the person has done serious offenses where custody is more than seven years or death, then DNA samples can be taken without any consent. In this special circumstance the magistrate can order to take biological material for DNA analysis.

5. Labs are allowed to do DNA fingerprinting only after permission of DNA profiling board.

There is always conflict between the technology and ethical issues. In India article 21 gives Right of privacy in all aspects. To avoid conflicts, the court must use its powers only after balancing the interests of the parties and on due consideration whether for a just decision in the matter, DNA test is extremely needed.

Universally Right of privacy is accepted as one of the most important Basic Human Right. The Universal Declaration of Human Rights, 1948 states that 'no one shall be subjected to arbitrary interference with his privacy, family, home or correspondence, attacks upon his honor or reputation. Everyone has a right to protection by law against such interference or attacks. Also, shall not be forced to admit the culpability.

The International Society of Forensic Genetics has laid down guidelines which are to be followed by DNA laboratories while dealing with such cases in order to adhere to the moral obligations on them. The Establishment of DNA Database has many ethical and legal concerns which are to be handled properly in order to avoid possible violation of Fundamental Human Rights [64].

9. Conclusion

DNA evidence is a reliable and confirmatory tool for victim/criminal investigation, but many experts have warned because there are few instances of man-made mistakes. It has led to the wrong consequences as the DNA evidence can be tampered. As few studies have found that DNA analysis reports can have personal variation in opinion and is likely to make mistakes. Bias may arise due to presence of trace amount of DNA in biological evidences and also burden of conviction based on report. Although the margin to biological challenges is near to nothing, the room for human mishandling the sample always cannot be over ruled here. False results can be seen in poor laboratory practices. There is a possibility that DNA at crime scene could also be replaced by another person, who was not a criminal actually. Forensic DNA fingerprinting had a tremendous positive impact in the criminal judicial system but its reliability should not be taken for granted. DNA is a God's signatures in each and every person which discriminate every individual. DNA technology is now becoming an integral part of any investigation all across the world. It is now accepted universally in solving many mysterious cases with motto *Do Not Ask, it's DNA, stupid!*

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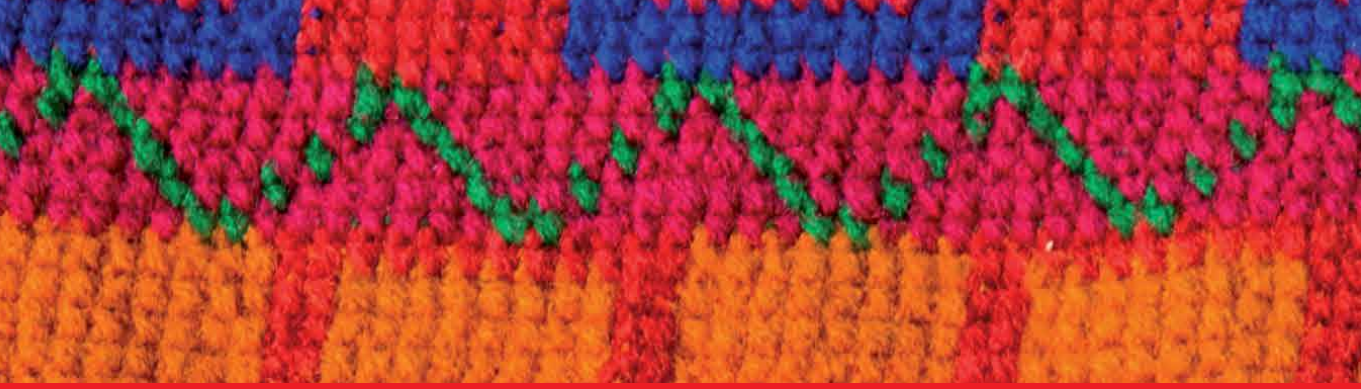

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