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Special Monographic Issue on the History of Human Anatomy and the Anatomical Bases of Palaeopathology

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It is our distinguished pleasure to introduce this special monographic issue of the *Italian Journal of Anatomy and Embryology* dedicated to the topics of the history of the anatomical sciences and the morphological bases of palaeopathological studies. These two branches of human anatomy, though deemed interesting and relevant to the field by a large number of scholars worldwide, are too often neglected or not developed into a self-standing, productive field of research and teaching, especially in Italy. This is particularly sad and detrimental if one considers that human anatomy was reborn in Italy after centuries of intellectual stagnation first at Bologna thanks to the teaching of the Mediaeval scholar Mondino de' Liuzzi and later, during the Renaissance, owing to the interest showed by artists in the correct representation of human morphology and bodily proportions.

This shows how rediscovering the discipline's history inevitably leads its students to the realisation of the existence of an interplay between the figurative arts and the study of the human body, a bond which until the late 19th century was perceived as evident, self-explanatory and indissoluble. Moreover, it was precisely in that century that, from the anatomical discipline and its sub-branch osteology, stemmed biological anthropology, a subject which, now greatly enriched by biomolecular studies and by the assessment of mummified human remains, can help us discover the antiquity of humankind, its evolution as much as that of the diseases that characterised its historical path, a field traditionally called "palaeopathology".

In this collection of contributions encompassing all of the aforementioned areas of research and anatomical knowledge, we aim to stimulate our colleagues and students to rediscover the importance of these topics and to develop them into a higher research platform capable of bridging the so-called hard biomedical sciences and the humanities.

Finally, we would like to express our deepest gratitude to Professor Domenico Ribatti, the journal's editorial board and the *Società Italiana di Anatomia e Istologia* for kindly accepting our proposal and for the invaluable support they gave us throughout the editorial process. In addition, we would like to thank all the contributors and anonymous reviewers who have made this issue possible.

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What lies behind the embalmed body of Rosalia Lombardo (1918-1920)?

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Abstract

Mummy studies help scientists to reconstruct both the evolution and manifestation of past diseases as well as the lifestyles and the habits of the ancient populations. They also help to gain insights into their funerary rites, which are a reflection of the community spiritual beliefs. Last but not least, the study of embalmed corpses reveals the evolution of the mortuary practices. After the 1840's, new embalming methods were developed; these coupled the use of chemical solution and arterial injection thus allowing the corpses to maintain their integrity (no external lacerations) together with a life-like appearance. An extremely interesting case of modern chemical embalming is that of Rosalia Lombardo (1918-1920), a two years old girl who died in Palermo. Her cadaver is housed in the Capuchin Catacombs of the Sicilian capital. Both her cause of death and the procedure used in her embalming are still enigmatic. Her embalming has been allegedly attributed to Alfredo Salafia, a renowned Palermitan embalmer. This paper addresses the most recent findings emerged from our re-analysis of Rosalia's case; furthermore, the cosmetic treatment of Rosalia's mummy is compared with those of Ernesto Salafia Maggio and Giovanni Paterniti, two individuals whose bodies were embalmed by Alfredo Salafia.

Keywords

modern embalming; anatomy; mummification; wax; restorative arts; history of medicine; mummy.

Introduction

Mummy studies are relevant both to clinical medicine, the history of medicine and the anatomical sciences (Brenner, 2014; Shin & Bianucci, 2021). Over the past three decades thanks to the application of modern scientific techniques, several pathological conditions of soft tissues and internal organs have been identified; this has allowed scholars to trace back the antiquity, evolution and manifestation of a multitude of diseases in historical settings (Shin & Bianucci, 2021). In parallel, the lifestyles and the habits of ancient populations, including their spiritual and religious beliefs, which are reflected into their mortuary rites, have been reconstructed (Shin and Bianucci, 2021). Through a careful analysis of the textual sources and the concomitant investigation of naturally mummified and embalmed bodies, scholars have

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progressively identified and characterised the compounds used to guarantee bodily preservation over time. Aside the anthropo-paleopathological approach, our research team- the Human Embalming Project© team- is devoted to the analysis of the chemical and surgical techniques applied to cadavers in Southern and Central Europe (with a special, yet not exclusive, focus on 16th to 20th century mummies). We also attempt to reproduce these methods experimentally. Particular attention is paid to the analysis of the documents, texts and scientific publications concerning each single examined individual/community (Bianucci & Nerlich, 2022).

During the reassessment of published literature on the topic of mummification and embalming, we recently focused on the embalmed body of a little girl named Rosalia Lombardo, who died shortly before turning two-years-old on the 6th of December 1920 (Galassi et al., 2021).

Materials and Methods

The embalmed body of Rosalia Lombardo has been on public display in the Capuchin Catacombs of Palermo for decades (Nerlich & Bianucci, 2021). However her cadaver still represents a scientific enigma: her real cause of death (flu vs diphtheria), the exact chemical formula used in her embalming, and the arterial site of injection of the embalming fluid (i.e. femoral artery) have not been so far scientifically assessed (Nerlich & Bianucci, 2021; Galassi et al., 2021).

In February 2009, a group of mummy researchers (Piombino-Mascali et al., 2009) claimed the discovery of the unpublished memoirs of the Palermitan embalmer Alfredo Salafia (1869-1933). The manuscript was reported to contain also the secret formula of the "Perfection fluid", an embalming fluid that allowed to maintain a cadaver in a "fresh state" (Piombino-Mascali, 2009; third edition 2012). These scientists also associated the embalming of the body of Rosalia Lombardo (1918-1920) with Alfredo Salafia and his work (Piombino-Mascali, 2009; third edition 2012; Piombino-Mascali et al., 2009; Panzer et al., 2013; Piombino-Mascali, 2020).

Rosalia's facial features were morphologically compared with those of Ernesto Salafia Maggio (died in 1914) and Giovanni Paterniti (died in 1911), two individuals whose bodies were reported to have been embalmed by Alfredo Salafia (Piombino-Mascali, 2009; third edition 2012).

Results

A reassessment performed by our team has shown that:

i. Salafia's handwritten memoirs were discovered before the 2009 publications (Piombino-Mascali, 2009; third edition 2012; Piombino-Mascali et al., 2009); a book by Di Cristina et al. dated February 2007 entitled "La Dimora delle Anime" describes the existence and content of Salafia's handwritten memoirs (Di Cristina et al., 2007; Galassi et al., 2021);
ii. Only "extracts" taken from the Salafia's memoirs have been published in their original Italian and English (Piombino-Mascali, 2009; third edition 2012; Piombino et al., 2009).

iii. Quite surprisingly, to date, no full-length independent review of the manuscript has been published (Nerlich & Bianucci, 2021; Galassi et al., 2021).
iv. No written statement by Alfredo Salafia that he embalmed the little cadaver of Rosalia has been published (Nerlich & Bianucci, 2021; Galassi et al. 2021).
v. No direct visual examination of her body occurred and no small biopsies nor swabs were taken from Rosalia's face, hair, body, dresses and coffin (Galassi et al., 2021). Therefore, no toxicological evidence that Rosalia was embalmed with the "Perfection fluid" exists (Nerlich & Bianucci; Galassi et al., 2021). Similarly, no molecular investigation were performed to ascertain whether the child had died of flu or diphtheria, as claimed by Rosalia Lombardo's relatives (Lanza, 2010).
vi. No experimental reproduction of Salafia's "Perfection Fluid" has been so far successful (Bianucci et al. 2022).

An additional element was overlooked. In their 2009 paper, Piombino-Mascali et al. (2009) wrote that Salafia's "*precious memoir discloses his occasional use of paraffin wax diluted in ether, hypodermically introduced into the deceased's face in order to keep the features life-like and plump, which reveals his great attention to the details of cadaver preparation such as the facial expression and the overall appearance of embalming the deceased*".

In 2013 the Multidetector CT investigation (performed in December 2010) of Rosalia's mummy was published (Panzer et al., 2013). With reference to the use of wax, the authors stated: *"…also we could not find clear evidence of the application of a facial paraffin treatment although it seems very likely that this method was used considering how perfectly preserved Rosalia's face appears to be"* (Galassi et al., 2021).

Interestingly, in 1981, Prof. Andrzej Niwiński published a paper in which the use of wax injections in Rosalia's embalming was described: "*In the Franciscan monastery in Palermo, among the famous mummies which can be seen in this rather gruesome cemetery, there is the mummy of a little girl, Rosalia Lombardo who died in 1920. The excellent preservation of the corpse (Pl. II) has been obtained through injection of liquid wax"* (Niwiński, 1981) (Figure 1a).

Professor Niwiński's publication represents his contribution to the conference proceedings of a congress which took place in 1979 [L'égyptologie en 1979: axes prioritaires de recherches (actes du colloque organisé par le CNRS dans le cadre du 2e Congrès international des égyptologues, Grenoble, 10-15 Septembre 1979)].

Discussion and Conclusions

Besides the important wax issue, Professor Niwiński's is the *de facto* the very first English-language anthropological publication that discusses the case of Rosalia Lombardo. Quite surprisingly, this paper has never been mentioned by scientists who have recently studied Rosalia's mummy (Piombino-Mascali, 2009; third edition 2012; Piombino-Mascali et al., 2009; Panzer et al., 2013; Piombino-Mascali, 2018; Piombino-Mascali, 2020).

As further clarified by Prof. Niwiński himself (private communications with Dr Raffaella Bianucci dated November 11th, 2020 and November 12th, 2020 reproduced with permission), in 1979, he read a leaflet printed most likely for the Franciscan Catacombs in Palermo, which was brought to Poland and given to him by an acquaint-

Figure 1. a: Plate II taken from Prof. Andrzej Niwiński's above mentioned paper. Available at: https://www.persee.fr/doc/bmsap_0037-8984_1981_num_8_3_3844, Accessed on June 13th, 2022; b: Close up of Rosalia Lombardo's face (scan copy of postcard from FMG's personal archive); c: A picture of Rosalia Lombardo dated the 23rd of February 2008 (Credits: Dr Giuseppe Carotenuto) shows the first signs of oxidation; d-Despite the mummy being introduced in a high-tech case, the oxidation proceeds rapidly (© Mark Benecke, Entomol heute 2019, SI; Reproduced with permission).

ance. According to Niwiński, the leaflet, which has been lost over the years, showed the picture of Rosalia (reproduced in Niwinski's paper, 1981) (Figure 1a) accompanied by a text stating that she had been embalmed with a liquid wax injection [*The excellent preservation of the corpse has been obtained through injection of liquid wax*].

Prof. Niwiński's eye witness testimony supports the notion that the use of wax in the embalming of Rosalia was already common knowledge, which predates by thirty years the 2007-2009 discoveries. Historical postcards show that Rosalia's face was originally plump and glowing with a greasy tuft of hair falling on her forehead, thus, suggesting a meticulous cosmetic treatment of her face (Figure 1b).

Although the knowledge that Rosalia's face underwent a cosmetic treatment dates back at least to the Seventies, the chemical composition and exact concentrations of the compounds used in her cosmetisation are unknown. This leaves the enigma of her remarkable preservation temporarily unsolved and calls for more in-depth investigations. Over the past decades, the face of Rosalia Lombardo has undergone a progressive process of degradation (Figure 1c); despite her introduction in a high technology passive display (Samadelli et al., 2019), the oxidation process progresses rapidly (Figure 1d).

Alfredo Salafia developed a method of preservation of the cadavers in a "fresh state"; this method was successfully demonstrated in the treatment and restorative arts applied to the corpse of Italian Prime Minister Francesco Crispi (died August 1901) (Johnson et al., 1993). Similar successful embalming procedures were performed with the bodies of other important Italian personages and commoners who could afford the treatment (Piombino-Mascali, 2009; third edition 2012). He also demonstrated the validity of his method in New York where the trademark of the "Perfection Fluid" was patented on the 16th of November 1911 without disclosing the embalming formula (Galassi et al., 2021).

Among the numerous bodies embalmed by Alfredo Salafia were those of his brother, the renowned fencer, Cesareo Ernesto Salafia Maggio, known as Ernesto (1854-1914)(Figure 2a), and of the Deputy and Vice Consul of the United States, Giovanni Paterniti (died in 1911) (Foreign Service List, US Department of State, 1901) (Figure 3). These embalmed bodies are of particular interest because both are housed in the Capuchin Catacombs of Palermo (Piombino-Mascali, 2009; third edition 2012; Piombino-Mascali, 2018).

Following Piombino-Mascali (Piombino-Mascali, 2009; third edition 2012), both were embalmed using the same embalming fluid ("The Perfection Fluid") that was allegedly used to embalm Rosalia Lombardo. Quite surprisingly, none of two mummies embalmed by Alfredo Salafia has ever undergone multidisciplinary scientific investigations.

The body of Ernesto Salafia Maggio has been left to decompose over the decades (Figure 2b). The glass top of his coffin broke away (it was already broken as early as February 23rd 2008) and was never replaced (Le catacombe di Palermo, uploaded on Jan 29th 2018, https://www.youtube.com/watch?v=yDpNv3DhQJ8, minutes 13:34 to 14:04).

As a consequence, a drastic modification of the microclimate inside the coffin occurred. Water infiltrated into the coffin. Humidity along with the action of insects, bacteria and fungi has resulted in a progressive decay of his corpse half skeletonising the lower portions of his body.

The cadaver of Ernesto Salafia Maggio is not on display; nevertheless, it was filmed and shown to the popular audience in the National Geographic docufilm "Le catacombe di Palermo" in 2008 (uploaded on Jan 29th 2018, https://www.youtube.com/watch?v=yDpNv3DhQJ8, minutes 13:34 to 14:04).

Figure 2. a: An image of Ernesto Salafia Maggio (1895) taken from the inside of his book (see ref. 19) (Salafia Maggio, 1895) (Credits: scan copy of the photograph taken from FMG's personal archive); b: This photograph dated the 9th of June 2008 (Credits: Prof. Luca Sineo) shows the embalmed body of Ernesto Salafia Maggio lying in his original coffin. The cadaver shows an extremely poor state of preservation and is surrounded by dust and mold which have grown on his face.

This action raises bioethical concerns since it breaches the dignity of the deceased whose body has been shown in an evident state of abandon (Bianucci et al., 2022). Furthermore, no action has taken place to restore his body and coffin over the years.

Similarly, the body of the Deputy and Vice Consul of the United States Giovanni Paterniti lays in his original casket (Foreign Service List, US Department of State, 1901).

A historical picture (see ref. 16, p.94) and a photograph taken in 2008 (Figure 3) show that both the the head and the shroud cloacking the body are covered by a whitish material which appears morphologically consistent with mold.

Only a small portion of the left forehead and left periorbital region is not covered by the whitish material and allows revealing the presence of an apparently preserved skin. The area where the skin can be directly observed does not show the same glowing and plumpy appearance displayed by Rosalia's face.

Previous studies have investigated the impact of microbial contamination on the indoor air quality at the Catacombs and on a limited number of mummies (Piñar et al., 2013; Piñar et al., 2014). As stated by Piñar et al. (2013) *in some areas of the crypts, the amount of fungal spores present in the air exceed 2.000 spores* m^{-3}. *Medically, this amount must be classified as posing potential health risk to the visitors. Indeed there are Italian Standards: UNI 10829 (1999) and UNI 10969 (2002) providing the guidelines to choose and control the indoor climate in order to keep correctly the artifacts. To date this guidelines have not been adopted to control the indoor air quality of the Catacombs of Palermo* (Piñar et al., 2013). Furthermore, *the analysis of samples taken from human mummies showed a contamination with halophilic microorganisms* and *the*

Figure 3. A picture of Giovanni Paterniti dated February 23rd 2008 (Credits: Prof. Luca Sineo) shows the presence of mold (?) on his face, on the shroud cloacking the body and the headrest.

surface of many mummies (heads, clothes and stuffing) *was heavily contaminated with mold. A superficial growth of fungi was identified as well as deep infection materials* (read organisms) (Piñar et al., 2013).

Similar studies have been carried out on insects and pests; these showed appalling conditions of degradation of the mummies and of the environment (Querner et al., 2018; Baumjohann & Benecke, 2019).

Since the two embalmed bodies prepared by Salafia in 1911 and 1914 represent the last examples of an early 21st century embalming technique, an immediate process of characterisation of the pests that colonise their bodies is ideally needed. This requires proper scientific multidisciplinary investigations and conservation procedures, which have not been carried out so far.

From a paleopathological and toxicological point of view, the embalmed body of Rosalia Lombardo has not revealed her secrets, as yet. Our findings suggest that her body should be re-investigated following strict scientific methods.

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History of Medicine as a bridge between Paleopathology and the Medical Humanities. New Technologies Applied to Bioarchaeology: reconstructing Lifestyles in Ancient Rome

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Abstract

The research project *Diseases, health and lifestyles in Rome: from the Empire to the Early Middle Age* (PRIN 2015), covered a significant area of research, ranging from the historical and historico-medical content that emerged during the multi-disciplinary investigation on the subject, to the intersection between different methods and approaches and the full enhancement of truly primary sources that included human skeletal remains, food residues, housing situations and burial contexts. From this perspective, the interest in the so-called "material culture" has taken on a more important meaning than the simple response to a conceptual and ideological historiography. Since the 1980s, there has been a sort of limitation regarding a more objective reconstruction of Antiquity. In general, written and iconographic sources convey information that is more or less intentionally mediated by the cultural and anthropological coordinates that produced it, requiring the historian to make a philological exegesis effort that even in the case of manuscripts and epigraphs needs to dialogue with tools of support and writing. On the other hand, the importance of molecular data would be partial and misleading if it were to be based on a more traditional documentary framework. Therefore, the history of medicine, always accustomed to operating in an area dedicated to a mixing of different areas of expertise and study, has once again proved capable of creating a dialogue among areas of study that are seemingly distant from one another. However, these areas of study are complementary, leading to a reliable historical reconstruction. In fact, the choice to focus the investigation on the human body has also inevitably highlighted the οἶκος in which it finds itself. This is part of a dynamic natural and social system, as was perfectly clear to the legislators of the late-Republican period and to doctors of imperial age, including Galen, who were well aware of the connection among health, productive and manufacturing activities, urban planning, pollution and food. Therefore, the anthropological, paleo-pathological and molecular investigation on skeletal remains, in dialogue with the historical-medical, literary and iconographic sources, has become the opportunity for a historical reconstruction. This reconstruction is all the more interesting as it encompasses a time frame that contains the transition from the Late Antiquity to the beginning of the Middle Ages, allowing for the identification of a series of indicators on continuity and discontinuity.

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Keywords
multi-disciplinary investigation; history of medicine; bioarchaeology; lifestyles in Rome; Early Middle Age.

Introduction

Our research focuses not only on the history of the concepts of lifestyle, health and disease and medical theories, but we also look at material history, made up of unwritten documents, architectural constructions, objects, artefacts and bones. For a long period of time, the historiography of medicine has left the material findings confined to a liminal dimension, in some way an accessory to a more intellectual sphere – that which was represented by the study and understanding of the written text. Certainly, historical research based only on unwritten testimonies will never be able to fully reconstruct the complex panorama of lifestyles and medicine in antiquity. Equally true, however, is the fact that the traditional historiography of medicine, limited to considering the "noble" voices written on the concepts of health and disease, on therapy and on the control of lifestyles, has ended up neglecting some fundamental aspects for the same internal understanding of texts. Moreover, the analysis of ancient texts does not ignore the fact that the written sources are also "material". Consequently, the examination of the epigraphic literature, together with the investigation of the funerary steles (halfway between texts and findings) allows, over a long period of time, to focus the Western medical tradition also through reflection on the history of certain supports (Cilione, 2016).

A good example of how a "collateral" and integrated approach to ancient scientific and medical knowledge is useful for the reconstruction of disease and pathocenosis in ancient times was already available for some time thanks to Mirko D. Grmek (Grmek, 1969).

Today, prospects for investigation have been enriched and enhanced. Data from material history, specifically those related to the studies of physical anthropology, paleopathology and molecular anthropology, have increased in number. Moreover, this type of data is more structured than in the past. Above all, they can be reinterpreted through the use of cutting-edge technology. The aim of this project was to increase, promote and spread the knowledge and protection of the culture of humanity, history and biology of a representative portion of the population in the European tradition.

The importance of the study lies in the intention of offering the possibility of presenting the results of an integrated approach applied to the reconstructive practice of the daily living conditions of the population of ancient, late ancient and early medieval Rome. In this interaction, the anthropological and molecular investigation and the study of the excavation come together in order to allow for a cross-referencing of the paleo-pathological and paleo-nutritional data with the ancient medical sources (clinical pictures, diseases, pharmacological preparations) in order to reconstruct around the finds a series of case studies that identify the physiognomy of an individual in Rome on the basis of biological specificity and lifestyle, as well as nutritional and socio-cultural habits.

Through the collaboration between Sapienza University of Rome and the Anthropology Service of the Special Superintendence for the Colosseum – the Roman

National Museum and the Archaeological Area of Rome, the University of Tor Vergata, the University of Roma Tre and the University of Pisa, this project aimed at reconstructing the lifestyle during the Empire and the Middle Ages in Rome through a cross-examination of material sources based on medical and literary texts.

Through a diachronic analysis, we focused on the understanding of the biological and social landscape of the population of ancient Rome, late ancient and early medieval Rome. This approach was carried out in order to also correlate – in comparative terms – the current re-emergence of related infectious, nutritional and socio-environmental risks to health determinants. Furthermore, these data were enhanced when the epidemiological factors concerning the displacement of populations were compared – a phenomenon that today as in antiquity has affected the European territory. By comparing the literary, epigraphic and archaeological data with the paleo-demographic, paleo-pathological, paleogenetic, paleoparasitological, paleo-nutritional data and from ergometric and morphometric analyses on the skeletal remains of numerous Roman individuals, the study aimed to contribute to the reconstruction of lifestyles, working and living conditions, as well as socio- environmental conditions. Consequently, the study attempted to reconstruct the Roman pathocenosis from the Empire to the early Middle Ages. The comparative study on molecular data provided by bioarchaeological finds, excavation materials, as well as historical-medical and literary sources is also aimed at obtaining information on the evolution and genetic variability of pathogens. This study also focuses on clarifying the role of encounters between populations, lifestyles and socio-environmental conditions on the etiopathogenesis of some diseases (i.e. infectious pathologies vs. trade and transport routes; infectious pathologies vs. frequency of spas; infectious pathologies vs. migration routes and flow; nutritional deficiency pathologies; pathologies due to poor food storage; pathologies and workloads; social care of sick individuals, etc). The relevance of this project was also determined by the large number of biological and archaeological remains available. This allowed for a singular and rather important statistical elaboration in the study of the populations of ancient Rome, considering that other European archaeological sites do not contain this wealth, despite being intensively studied.

Ancient human remains represent a biological archive of information that is fundamental for the study of humankind. These remains make it possible to obtain data on habits, quality of life, state of health and disease of the populations of the past, as well as on hereditary and evolutionary aspects in the strict sense (Ferrari 2010). In addition to the scientific results obtained from medical examinations, we should also underline the importance of ancient human remains and archaeological finds as a cultural heritage to be preserved, protected and valued. These cultural assets are the subject of multi-disciplinary study in which archaeology, history, biology, natural sciences and medical sciences collaborate to address very complex problems such as variability, microevolution, environmental adaptation mechanisms, and, in general, pathocenosis (Gourevitch, 2011).

Today, the progress of scientific knowledge allows for achieving a vast archive of anthropological data that allow us to accurately describe many aspects of the biology of the past populations and the environment where they lived, also providing information complementing the culturally-pertinent aspects. The molecular analyses to establish the genetic information and the dietary pattern, and the paleo-pathological investigations carried out on these assets not only increase the biological and histori-

cal archive, but also provide important information for the space-time location of the single individual. Isotopic analyses carried out on bone tissues allow for obtaining concrete elements for dating and, due to the recent bio-technological developments, for the identification of the geographical area in which the individuals lived, in order to implement the current information on preserved human artefacts. The investigations of paleo-immunology and molecular paleo-microbiology carried out on skeletal findings allow us to formulate a retrospective diagnosis of specific diseases, as well as to obtain epidemiological data to be used in the reconstruction of the history of the same diseases. The most recent developments in this area of study concern the application of immunology and molecular biology on ancient human remains. Paleo-immunology uses modern immunochromatographic tests and serological analyses (WB) in order reach a retrospective diagnosis of ancient pathogens in human remains. Thanks to this technique, important results have also been obtained for the study of diseases that were widespread in the past in Italy, such as the plague and malaria (Bianucci et al., 2015; Cesana et al., 2017).

The exploration of aspects that have long been neglected by traditional historiography sheds a new light on life in Rome between the imperial age and the High Middle Ages and imposes a rewriting of events and socio-political phenomena that consider nosological frameworks, dietetics, lifestyles and migration flows.

In the light of this multidisciplinary approach to the theme of research and the spirit of scientific collaboration that has animated the scholars involved in the project, the results of the work of the individual units can easily be disrupted and reorganized in thematic areas.

Nosological framework

The paleopathological study focused its work on the bioarchaeological and paleopathological studies of skeletal remains recovered in some Roman necropolises from the Imperial Age to the Early Medieval Age with the aim to obtain information about the social, demographic, and health patterns of ancient Rome.

Examination of hundreds of individuals from Roman Imperial Age necropolises allowed the identification of pathological conditions that resulted in bone alterations.

The most relevant diseases observed regards neoplasms. Nowadays cancer represents the second most common cause of death (Boyle, Levin, 2008) in developed countries. In particular, malignant tumors are considered the result of carcinogenic environmental factors in industrialised societies. (Siegel, 2020)

Tumours have been documented since the most ancient times, but the paleopathological evidence of these diseases among past populations are scarce. The main reason for the lower incidence of cancer in the past is represented by the lower life expectancy, considering that these diseases generally occur in the elderly. Furthermore, in paleopathology only cancer types affecting the bones can be detected, whereas malignancies often led to death before involving the skeletal apparatus. Finally, the poor preservation of ancient bones, especially those affected by destructive disorders, may prevent the observation of some evidence.

An important and rare case of metastatic carcinoma was brought to light from the Imperial Age necropolis of Casal Bertone, located near some residential structures

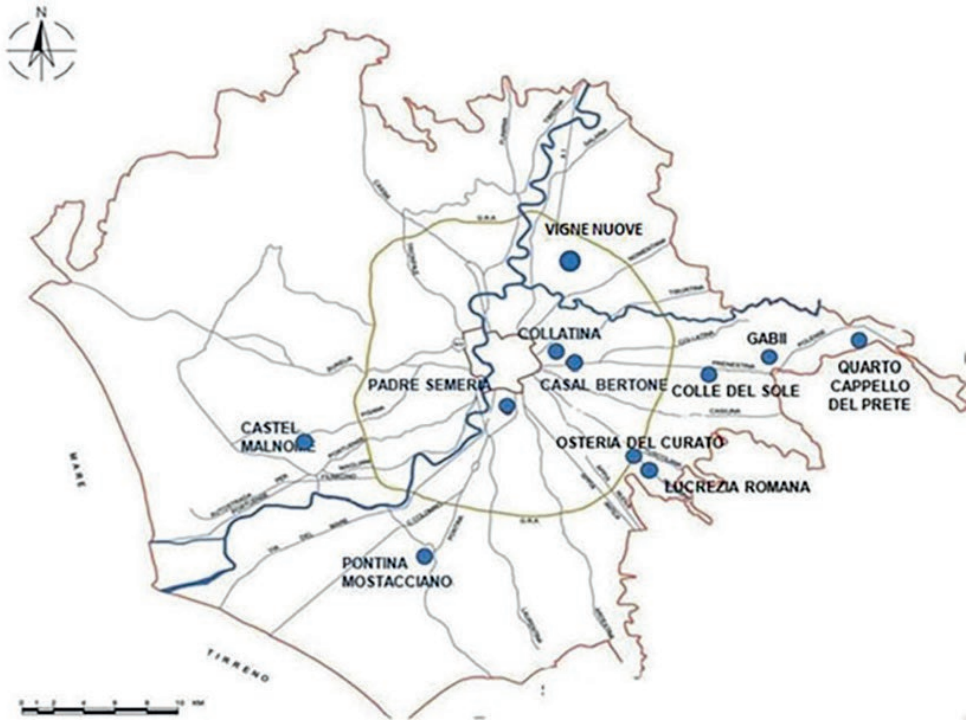

Figure 1. Location of the examined graveyards.

and a large place identified as a fullery (*fullonica*). The skeleton of an adult man displayed severe and widespread bone alteration consisting in mixed osteoclastic and osteoblastic lesions mainly involving the axial bones. The morphology of the lesions, which are both destructive and proliferative, and their anatomical distribution suggested a diagnosis of metastatic carcinoma arising from a soft-tissue primary cancer. The age and sex of the individual, together with the radiographic and histological aspect, permitted to hypothesize a prostate cancer as the site of origin. The archaeological evidence suggests that this individual probably worked in the *fullonica*. These workers were in daily contact with chemical agents such as soda, urine and sulphurous vapours used to scrub, wring and whiten the cloth. However, there is no evidence in the current clinical literature that these chemical substances can be related with the occurrence of malignancies. Therefore, it is not possible to verify the impact of environmental factors on the onset of tumour in this individual. The good state of preservation of the skeleton and the advanced stage of the prostate cancer represented a relevant opportunity to observe in dry bones the natural course of the disease in absence of treatment. (Minozzi et al., 2018).

Furthermore, two cases of benign neoplasm were found in skeletal remains from the Imperial Age necropolis of Collatina. The skull of two adult males displayed evidence of paranasal lesions. In both cases, the detection of the new bone formations

Figure 2. Left humerus osteochondroma.

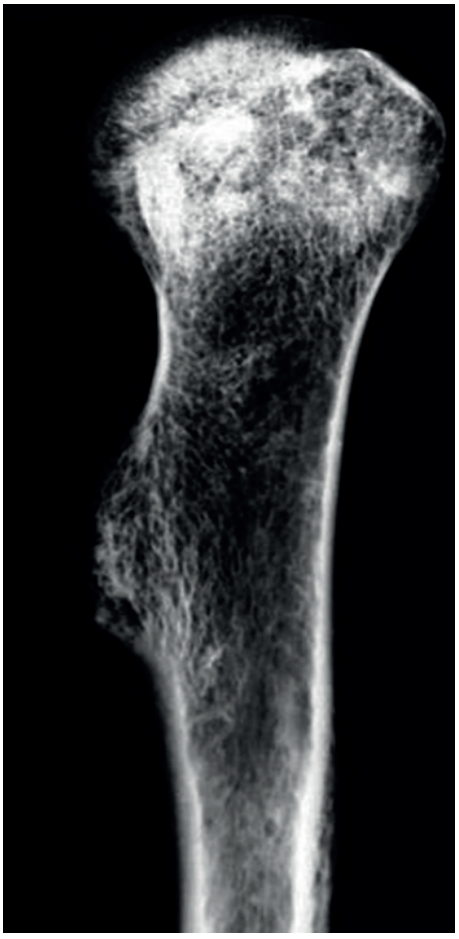

Figure 3: X-rays antero-posterior view of the proximal.

has been possible thanks to *postmortem* damage in the frontal bone. Radiological examination confirmed the presence of pediculated-based bone formations arising from the right frontal sinus, which can be interpreted as osteomata. In both cases the reduced dimensions, lower than 10 mm permitted to rule out mechanical complications and compression of the adjacent structures. The osteomata of paranasal sinuses are rarely documented in paleopathology, as they can be observed only incidentally after bone breakage or radiography (Riccomi et al., 2018).

Another case of benign tumour regards a new bone formation observed in the upper arm of an adult man from the Imperial Age necropolis of Castel Malnome. The lesion, localized in the distal portion of the left humerus, was observed by X-ray showing continuity between the cortical portion of the humerus and the neoforma-

tion. In addition, the marrow cavity of the neoformation is continuous with that of the underlying bone. These characteristics point towards a case of osteochondroma in the differential diagnosis.

Osteochondroma is one of the most common benign bone lesions; it arises in bones by endochondral ossification and the most common site of occurrence is the metaphyseal region of the long bones, like the distal femur, proximal humerus, distal tibia and fibula. The growth of the tumour stops with the end of the skeletal development (Alabdullrahman and Byerly, 2021). In the Castel Malnome skeleton, the neoplasm originated from cartilage tissue at the proximal metaphyseal plate of the humerus and during growth the tumour reached a point that also involved the diaphysis.

Every new case of neoplastic disease, both malignant and benign, is important. These cases add information on the presence of these pathologies in the past. The knowledge of past patterns of neoplastic diseases could contribute to the understanding of present trends of carcinogenesis.

Another interesting pathological case was observed in the necropolis of Casal Bertone. The skeleton of a young individual – 18-20 years in age – showed severe bone alteration affecting the right coxal bone, localized in and around the acetabulum, changing its margins and internal cavity. The lesions in the acetabulum, mostly consisting in new bone production, match those of the head and neck of the right femur that shows similar modifications. In addition, osteolytic lesions have been observed in two lumbar vertebrae resulting in a large erosion of the vertebral bodies of L3 and L4 producing cloacae and the almost complete destruction of the L4 body. This condition is accompanied by irregular new bone production with similar characteristics to those observed in the femur and pelvis.

Differential diagnosis takes some diseases that can stimulate new bone production accompanied by osteolysis, located in a few anatomical districts, into account. The location of lesions in two adjacent lumbar vertebrae and the presence of abscess cavities and fistulas in the vertebral bodies suggest that this individual was affected by tuberculosis (Pott's disease).

This infectious bacterial disease mainly involves the respiratory system, but in 3-4% of cases affects the skeleton (Buikstra, 2019).

Documented in Italy since the Neolithic, it is not surprising that it may have been present and widespread in a city with high density of population like Rome, as indeed testified by other cases from the Roman Imperial Age (Via Nomentana, Herculaneum) (Canci et al., 2005).

Traumas represent one of the most widespread and easily observable diseases in skeletal remains (Giuffra and Fornaciari, 2021) Hematomas, muscle tears, and bone fractures induce reparative bone processes and are well identifiable in the skeleton, providing information on the risks of work activities, interpersonal violence and socio-environmental vulnerabilities. Healing patterns can also clarify the degree of medical care and social cooperation within a community (Buikstra, 2019).

In the Imperial Age necropolis of Castel Malnome, 243 individuals were examined and over half of them showed evidence of traumas, mainly affecting males (Caldarini, Zavaroni, Benassi, 2015). Bone fractures were observed in 25% of individuals. The lower limbs were the most affected bones, followed by ribs, clavicles, and upper limbs. Among individuals with bone fractures, 19% show multiple traumatic evi-

Figure 4. Enamel hypoplasia in permanent teeth.

dence; although some injuries may be attributable to a single event, others occurred in different times suggesting heavy work activities and dangerous lifestyle pattern. The higher prevalence of affected males in comparison to females, and the low prevalence in subadults, combined with the framework provided by the skeletal indicators of biomechanical activity, suggest that the Castel Malnome population, mainly males, was employed in the difficult work in salt flats that were found close to the necropolis. Trauma healing outcomes suggest the application of effective medical treatments and a good level of social cooperation within this community.

Another important point developed during the project regards the analysis of dental enamel hypoplasia, an enamel defect that occurs in childhood and results from nutritional deficiencies/diseases (Hillson, 1996).

Examination of hypoplastic lesions in ancient populations provides relevant information about developmental stress-levels the Romans during the Imperial Age and can help investigating the relation between social status and health and nutritional patterns (Minozzi et al., 2020)

Enamel hypoplasia was scored in 177 individuals from the Casal Bertone and Collatina necropolises, grouped in two sub-samples on the basis of their social status, which was identified by the types of grave typologies and equipment used (i.e. mausoleums or simple tombs).

In all the parameters considered, statistically significant differences were observed. In particular, the lower classes were more affected by linear enamel hypoplasia (LEH) than upper classes both in term of frequencies of affected teeth and of affected individuals.

Differences between males and females, or adults and non-adults were not statistically significant. The mean number of hypoplastic lesions per individual was higher in the lower class as well. It has been observed that the defects started to occur since the first year of life, with higher frequency in the lower class than in the upper class; this trend could indicate a correlation between the chronological distribution of age at onset of the stressful events and the social status.

These differences could be attributed to the more accurate parental care to the children of the upper, with better or prolonged breast-feeding. This study evidenced the relationship between LEH and social status reflecting difference subsistence patterns in the two sub-samples of the same population and indicating that the group of high social status enjoyed better health in Imperial Rome (Minozzi et al., 2020).

Regarding the Medieval period, the research was focused on the Roman necropolis recovered in Piazza Celimontana al Celio (Rome).

Anthropological and paleopathological studies were performed in 37 individuals dated back to 6^{th}-12^{th} centuries CE.

The reconstruction of biological profile evidenced that subadults compose 40% of the sample with high infantile mortality. Adult individuals (11 males and 10 females) are characterized by robustness and, in the same cases, marked biomechanical stress indicators. Fractures affect 50% of individuals and are mainly located on the chest and small bones of the hands and feet, likely due to dangerous living and working environments.

The study of oral pathologies shows a high rate of tooth decay, abscesses and *ante-mortem* tooth loss, which can be associated in part to a cariogenic diet as confirmed by biomolecular analyses. Consequently, the results suggest rather poor living and health conditions related to subsistence pattern typical of modest social classes.

In conclusion, the paleopathological evidence from ancient Rome represents a valuable contribution to compare and interpret with the data obtained from written and literary medical sources in order to expand our knowledge on the health and living conditions of this important civilization of the past.

Dietary habits

In order to account for the nutritional significance of the Imperial-Middle Ages shift, an extensive multi-element isotopic survey was performed (De Angelis et al., 2020a; De Angelis et al., 2020b).

Collagen extraction from bone remains was carried out for every available skeleton in order to perform carbon and nitrogen stable isotope analyses (Brown et al., 1988). Carbon (C) and nitrogen (N) isotope measurements provided general information on subsistence strategies, which could differ based on sex, age, social status, and period. Selected faunal remains for several archaeological contexts were also evaluated for addressing the ecological background and establish the trophic baseline.

More than two hundred samples from the six above mentioned cemeteries (Castel Malnome, Casal Bertone Mausoleum, Casal Bertone Area Q, Casal Bertone Necropolis, Via Padre Semeria, and Quarto Cappello del Prete) represent the leading sample for Imperial Rome isotopic analysis so far. Their information has been joined to the few previously published results (De Angelis et al., 2020a, 2020b and references in it)

to obtain a broad diet reconstruction for people buried in archaeologically defined contexts in Rome (1st-3rd centuries CE), that was merged with archaeological and biological evidence for figuring out common patterns of food preferences. The dietary landscape we provide is heterogeneous, reflecting the multifaceted reality of the capital of one of the most influential empires in Antiquity.

Untangling the complexity of Roman Imperial society remains a challenge, even from a dietary point of view. However, some elements can be highlighted. One of these is the pivotal role of C3 plants, which seem to have been the staple foodstuff of the lower class. However, C4 plants also seem to have been consumed, albeit they were not as widespread and were not always used for human consumption. The environment played a critical role for Romans of lower social classes. Even though grain supplements granted by the central administration partially sustained them, the topographical location of the settlements pushed the preferential consumption of food that people could obtain from their neighbourhood to sustain active lifestyles defined by heavy labour. Nevertheless, the complexity of Roman society and trade that passed through Imperial Rome accounted for the broader range of foodstuffs available for people.

A crisis of the centralised economy marked the transition towards the Early Middle Ages (IV- V century CE), and the simultaneous closure of several trade routes shifted toward an autarchic subsistence economy (Pounds 2014). This trend was also highlighted by the carbon and nitrogen stable isotope analysis of more than one hundred skeletons (Varano et al., 2020). This resulting analysis revealed that the Medieval dietary behaviour was the direct result of the events that occurred with the collapse of the Roman Empire. The social, demographic, and economic crisis of the Roman world after the 4th century CE had a critical impact on the lifestyle.

The result was a change in the dietary pattern. A more autarchic economy was established with a significant decrease in the consumption of high protein resources. The values obtained in our survey are consistent with a diet mainly founded on the exploitation of C3 plant resources and terrestrial fauna. Grains and grain-derived products became the primary foodstuffs for disadvantaged Medieval communities that farmed a variety of grains.

Meats were the prominent component of the diet, and the data on faunal remains suggest that they were likely a food source for the people. At the same time, the results do not offer clear evidence for aquatic resource exploitation, both marine and freshwater.

However, San Pancrazio is atypical, and its high $\delta^{15}N$ values could be related to the harnessing of riverine resources. The demographic dissection of the samples does not reveal significant differences between males and females, likewise adults and children. This evidence is striking against historical sources suggesting religious rules as a leading modifier factor for women (Mion et al., 2019)The dietary evaluation clearly detected a diachronic nutritional transition in ancient Rome, demonstrating how the complex historical period following the downfall of the Empire might have influenced the lifestyle of the Roman society. The dietary transition in the hearth of the Empire also revealed to be different from that in other areas of Europe, which could be experienced different strategies to face the collapse of the Roman Empire (Varano et al., 2020).

Furthermore, tooth enamel was studied in order to analyse the oxygen and strontium composition in people presumed not to be local based on items found in their

graves and other particular features. This evaluation could shed light on mobility patterns in ancient Rome, clarifying questions of whether the movement of ancient people was essentially unidirectional or bidirectional, occurred over long or short distances, and followed a pattern of urban to rural migration or vice versa.

More than one-hundred-fifty samples were screened. Remarkably, the relative abundance of non-local people is quite low, and people moving Rome-ward are concentrated in funerary contexts related to productive activities such as Castel Malnome and Casal Bertone, as well as in ritual area such as Quarto Cappello del Prete (De Angelis et al., 2022) The extent of mobility patterns in Medieval times is currently under debate, and we are looking for the enlargement of the sample size for providing reliable mobility models in the Middle Ages at Rome.

Selected well-preserved samples were submitted to aDNA analysis. DNA extraction was performed with improved precautions as well as a series of recently introduced methods in order to avoid the contamination (De Angelis et al., 2021; 2022 and references herein included). The genome-wide typing of human aDNA was achieved using high throughput techniques via Illumina-indexed libraries that were submitted to shotgun sequencing (Meyer and Kircher, 2010).

Genomic regions were evaluated in order to elucidate the putative phylogeographic origins of people living in Rome at the edges of the Empire. This striking task was accomplished and represent the second batch of ancient Roman genomes to date. Our results show an ancestry shift toward the eastern Mediterranean area with only a few samples suggesting a central and western European origin. The results obtained are consistent with the only previously published data (Antonio et al., 2019), even accounting for a novel north African ancestry in selected individuals (De Angelis et al., 2021; 2022).

Whenever possible, human genomic regions associated with susceptibility and/or immunity to several diseases were analysed in order to evaluate the disease-driven variation of Roman populations over time.

Some interesting information was achieved to refine the differential diagnosis based only upon osteological evaluation. Indeed, the primary evidence for diagnosing disease in the past consisted of pathological lesions in skeletal remains, so the analysis of ancient DNA allows us to look for the genetic basis of some specific phenotypes that could be linked to metabolic, genetic, or infectious diseases. Dedicated genomic studies were accomplished in cemeteries (such as Quarto Cappello del Prete e Casal Bertone De Angelis et al., 2021; 2022) characterized by the unbalanced death ratio between children and adults for the detection of the causal factors for those phenomena.

The Quarto Cappello del Prete necropolis was chosen as a paradigm for the integrated genomic approach, and the data obtained shed light on the fact that this site was home to a number of people suffering from metabolic and genetically based osteo-dysmorphic disorders. Remarkably, the genomic and isotopic approaches are consistent for suggesting people buried in that burial ground came from different geographical areas, supporting the hypothesis concerning the specific social and religious feature for this site (Musco et al., 2010).

The drastic changes between the two time periods dramatically changed Rome's demographics (Séguy 2019). Accordingly, the biological challenges of new and worsened health conditions and lifestyles arose, requiring adaptation. These turns affected

the genetic structure of the *Urbs* and, consequently, the evolution of host/pathogen interactions, resulting in the blooming of several diseases. Ancient microbial DNA was sequenced using the same procedure used for human DNA (De Angelis et al., 2022) and the analysis of specific microbes have been prioritized according to osteological and archaeological suggestions. *Mycobacterium* sp. and *Treponema* sp. were the leading microbes for which we have searched, following pathological characterisation of skeletal alterations from several individuals from the Casal Bertone area, even though the environmental microbiota represented most of the findings (De Angelis et al., 2022). This approach enhanced our understanding of the prevalence of selected diseases whose etiology could be highlighted by microbial genetic fingerprinting (imagine DNA fingerpriting)

Lifestyles

A thorough study of the living conditions of ancient populations requires the approach of physical anthropology and paleopathology accompanied by the historical and archaeological studies. Quite certainly, this pairing is important even during the data collection phase. This approach allows, through stratigraphic analysis and typological study, for the definition of the chronology of the analysed burials and their cultural and social classification. Moreover, the study can then segment the results according to the differences of social status and follow its evolution and transformations through time.

The archaeological-historical investigation helps to clarify the framework of the analysed sample, with particular reference to nutrition, the living habitat, the likely physical activities carried out, the conditions of epidemiological risk (level of hygiene, level of interpersonal promiscuity and with pets, etc.).

The current studies on the style of life of people living during the Imperial Age precisely engaged the upper classes and their residences, with a structural architectural approach (Guidobaldi 1986; Priester 2002); only recently the attention was addressed to the social aspects of living (Allison 2004; Bowes 2010). Looking to the Late Antiquity and the early Middle Ages, conversely, the studies have been dedicated to the populace and their houses (most recently: Fronza, Santangeli Valenzani 2020 with former bibliography; see also: Galetti 2010; Santangeli Valenzani 2011), albeit here too with an interest focusing on typological and structural aspects.

Regarding the specific case of Rome, the archaeological data related to houses of the Early Medieval period (7th-11th centuries) have been treated in detail (Santangeli Valenzani 2004), with the attempt of associating the different types of buildings and the social classes of the Roman society of the time.

As part of the project, the research – focusing on Rome and the surroundings – also dealt with the building types related to the middle-low parts of the population (*insulae* for the Roman Imperial era, *domus terrineae* for the early Middle Ages) analysing a series of variables that could affect the living conditions and the state of health of the inhabitants: level of promiscuity and space availability *per capita* inside the housing unit, its health conditions (presence of isolation humidity, insolation, etc.), availability of toilets and sewage disposal systems, distance from water supply points, heating systems, presence of pets. The comparison with the

best studied cases of the upper classes residential system (*domus* with the *atrium* and similar types for the Roman era, *domus solarata* for the early Middle Ages) gave to the analysis the needed social depth, contributing to define, the articulation of the ancient societies.

Eating habits have been dealt with so far on the basis of written sources, here too with a focus on the high social classes for the Roman era, and on the peasant population for the Early Medieval era (Montanari 1979), while the archaeological approach to the topic was essentially based on the study of animal bones from excavations (De Grossi Mazzorin 2016, Salvadori 2015).

The research focussed thus on nutrition, using indicators such as the remains of meal (especially animal bones), and the cooking methods, basing the analysis on the containers used for cooking, also with the aid of molecular analysis of the incrustations preserved inside the objects. The data were provided by two ongoing excavations held in Rome by the University of Roma Tre, at the Colosseum and at the *Templum Pacis*, compared and integrated by others around Italy

Regarding the archaeo-zoological remains during the Imperial phase the pig was the most exploited animal: this species was prolific and characterized by high meat yield with cheap production costs. Between the 3rd and the 4th c. CE, the percentage of male individuals remains is high, between 73% and 88% of the entire mammal domestic population. There are rarer cases of slaughtering of old female individuals, due to their use for reproduction, and of suckling pigs, which were considered delicacy for the wealthy classes. Ovicaprines, poultry and cattle are less exploited.

This scenario starts changing during the 5th c. CE, when there is a progressive increase of the breeding of ovicaprines, which witnesses the search for different sources besides the pigs. A similar trend, together with a decrease in the slaughtering age, occurs among the cattle.

During the Middle Ages, from the 12th c. onwards, the evidence of ovicaprines progressively increases and surpasses that of pigs, to become predominant with the 13th c. The higher percentage of young individuals suggests an increase of the dairy production. At the same time also cattle grow, though with a discontinuous trend, and poultry too: the higher number of older female individuals is probably related to a larger use of eggs.

An interesting amount of remains of aquatic fauna was retrieved in the archaeological excavations too. They show that fishes in Antiquity were a sort of *status symbol*, part of the diet of wealthy people not by chance, indeed, the majority of finds, saltwater fishes and clams, comes from the sewers of the Colosseum, underneath the senatorial seats (De Grossi Mazzorin 2016).

Fishes continue to be well represented also during the Middle Ages, still with lower percentages compared to meat. The presence of fine marine species, during the 12th and 13th c., is particularly remarkable: while the consumption of freshwater fishes is better attested through the Middle Ages, still the marine life continues being fished along the coasts and being consumed in those cities tightly connected to the sea, like Rome. Several remains of turtles were found too: the animal was used during the Lent and on those other days when the meat was not allowed.

Regarding the pottery vessels, the cooking set remains the same until the Late Antiquity and includes a various number of shapes: casseroles, pans, pots and lids, which attest the coexistence of many different cooking methods. However, the cas-

serole is the predominating type, with a percentage around 80%. These are wide vessels, with diameters between 20 and 30 cm, meant for fast cooking, like frying big chunks of meat – like those provided by the *Annona* – on a layer of fat.

The set changes through the Middle Ages: the pot, previously only limitedly used, becomes almost the only type of cooking, with a percentage around 78%. The sizes also diminish, with the mouth from 8 to 12cm wide, never bigger than 14cm. This type of vessel, characterized by a rim narrower than the body, was essentially meant for long boiling cooking, like soups and broths, which witnesses a change in the diet and the cooking habits.

It is likely that other cooking methods were still used too, but the related vessels are lost because made by other materials, like metal: some sherds of soapstone cookware were found at the excavation of the *Templum Pacis*, confirming this hypothesis. The organic remains still preserved in there were analysed and showed that broths and stews were cooked inside the soapstone pots.

During the 12th c., the types of cooking ware increased again; the sizes though remained small in relation to the use of small cuts of meat. The improvement and increase of the shapes of ware show the return to a broader variety of cooking methods.The study of the living and working environment also focussed on the analysis of changes between Antiquity and the Middle Ages, investigating two important ancient monuments and shedding light on the ways in which their structures were reused in the post-ancient era for residential and working purposes.

The two sites of the Colosseum and the *Templum Pacis* were stratigraphically excavated. Concerning the Colosseum, the study focussed on reconstructing the phases of life of the site after the end of its use as an amphitheatre (Facchin, Rea, Santangeli Valenzani 2019).

The place, between the 11th and the 13th c., had many different uses and was under the property of many owners, who reused the vaulted rooms under the *cavea* as stables and warehouses. Written sources of the same period also witness the presence of higher-level dwellings (which, however, are still invisible to the archaeological evidence) and also of an aristocratic fortified residence, the fortress of the Frangipane, of which some elements started being recognized.

In the area of the *Templum Pacis*, the most meaningful results were provided by the excavation of some rooms flanking the *vicus ad Carinas*, a road which shows a rather long continuity of life, from Antiquity to the contemporary age. These spaces, most probably with a business or commercial function during the Imperial age, were transformed, during the high Middle Ages, in productive structures, linked to the use of fire for the ceramic and metal productions. This transformation is coherent with what the recent archaeological discoveries have suggested: the crisis of the urban town planning during the Late Antiquity in Rome produced a shift of the productive activities that from the suburbs entered the centre of the city (Molinari, Santangeli Valenzani, Spera 2016), with serious consequences on the health conditions of the most populated part of the city, also due to the sewage and water problems.

History of Medicine and Physical Anthropology: the role of transversal skills in PRIN goals

Studies carried out thus far at an international level have been defined by poor interactivity between scientific and humanistic skillsets. The design of this research was carried out with the fundamental conviction that cross-referencing and comparing the data already partially available in historical-medical, anthropological research, in the history of religions and burial rituals, could serve, in addition to interpreting specific pathophysiological conditions, to give contextualise scientific data within a historical-cultural background in an appropriate manner.

On the other hand, the epigraphic, literary and iconographic sources, a reflection of an aristocratic perception of the world, have always helped only tangentially to reconstruct contexts attributable to marginal population groups, while biological materials have made it possible to give a voice to a segment of the population that is by definition mute, also from a historiographical point of view. Consequently, these materials represent the only objective evidence at our disposal that can be used to reconstruct the life stories of the ancient population from the historical-medical point of view.

Medical and non-medical literary sources have allowed scholar to develop a connection between the analysis of social status and diseases, as well as a description of epidemiological trends in relation to environmental archaeology and population flows, including etiological interpretations in the therapeutic systems adopted (Fornaciari et al., 2019; Iorio, Marinozzi, Gazzaniga 2018; Piccioli et al., 2015) in ancient medicine.

The analysis of medical, pharmacological and literary texts was integrated with culinary recipes provided by some authors, including descriptions of banquets and ceremonies, the use of food in medicine as a preventive regimen and in the same way the documentary sources in relation to living conditions, work, nutrition, origin, hygienic conditions, habits and customs, parental relationships, traumas and diseases that have characterised the living conditions of the population. Regarding the aforementioned work, the differences between the actual pathological conditions that were frequent in most population groups and those described by doctors for their patients, who generally belonged to an elite class of the population, were also highlighted (Gourevitch 2001; Buzzi 2019)

We refused to rely solely or primarily on historical demographic data (Virlouvet 1997), believed to provide only partial and generally approximate data.

The focus of our research on nutritional history, which includes not only the study of food itself, also included the methods of procuring raw materials, conservation techniques, the history of communication routes and transport systems, reflection on routes geographical areas of a very extensive world (which was the Roman Empire at the time of the explosion of the "Antonine" Plague), in which the movements of the armies carry, among other things, diseases and contagion (Cilione-Gazzaniga 2020).

The character of the disease that repeatedly affected the Roman territory was that of a "new" disease, attacking populations that immunologically lack the means to combat it. Specifically, in the marginal areas of the empire, the people often experienced conditions of economic hardship and housing problems.

This part of history does not always adhere to the theories of medical explanation, but also relies in late stages on popular beliefs in "contagion" – with interesting

consequences, such as that of the precautions taken to avoid the risk of pollution during burials – to oracles, magic and sacred rites. Such an articulated and detailed path within Marco Aurelio's "unnameable disaster" makes the plague the most useful paradigm of reflection on the possibility that the history of medicine has to go beyond the narrow confines of its specific competence, becoming an undeniable part of social, economic and cultural history (Duncan-Jones 1996; Harper 2017).

First and foremost, the study of History of Medicine played a role in connecting all units by facilitating scientific comparison among the research groups.

Specifically, focus was placed on the conceptualization of the body in anatomical votives and in the tradition of vascular iconography as a vehicle for the anatomical and physio-pathological theories of the contemporary medical literature (Cilione, Marinozzi, Gazzaniga, 2018).

The molecular survey of the diet, which is the main therapeutic tool in ancient medicine, has partly confirmed, albeit also partly not, the historical-medical sources of imperial age – the relationship between gender and nutrition is in fact less binding than that found between nutrition and labour activities.

On the other hand, anthropological and paleo-pathological analysis confirmed the therapeutic approaches related to bone traumatology documented in the sources.

Lastly, the comparison between anthropological, molecular and paleo-pathological data on the contexts of the spread of infectious diseases, paired with the epigraphic, legal, literary and medical sources has revealed a consistent representation of the relationship between mankind, the environment, nutrition and disease that seems to testify to a strong awareness of public health problems already in the late-republican legal texts and in the medical treaties of the Imperial Age.

A sub-unit working group (coordinated by dr. P. Catalano) shared with the all the other research units the data of a multi-year work conducted by the Anthropological Unit of the Soprintendenza Speciale per i Beni Archeologici di Roma of which the PRIN has helped to enhance scientific results in a broader perspective of historical reconstruction.

Specifically, bio-archaeological studies and historical documents have proved crucial for the reconstruction of the lifestyle and health conditions of ancient populations and have also been a valuable tool for understanding human-environment interactions over time.

Participating in the activity of territorial protection conducted by the Soprintendenza Speciale per i Beni Archeologici di Roma, the Servizio Antropologico has recorded and preserved over the years an enormous amount of data, all potentially usable to reconstruct the biological history of Roman society, especially of Imperial Age (Catalano 2015).

The in-depth analysis of the skeletal remains in the laboratory allowed us to define the life and health conditions of the population, while also assessing the incidence of degenerative, traumatic, dental, metabolic and infectious diseases in the various human groups.

Ergonomic studies have made it possible to evaluate enthesopathic alterations and degenerative joint pathologies in order to reconstruct work and labour activities, through the study of the following aspects: paleo-demography, paleopathological analysis, anthropometry, detection of musculoskeletal markers, analysis of dentoalveolar diseases and stress indicators, macroscopic analysis: CT, SEM and histology (Mosticone, Pescucci, Porreca, 2015).

In recent decades, new excavation methodologies and approaches applied to human skeletal remains have yielded considerable information on Roman burials. The PRIN has allowed to expand and go more in-depth during the laboratory investigations. This work led us to a greater understanding of the complex biological landscape represented by the population of ancient Rome, in light of the biodemographic and social processes that concerned it. The sites were selected on the basis not only of the numerical consistency, but also of the peculiarities and unique aspects of the contexts. This choice allowed us to formulate reliable hypotheses on the different subsistence economies of the reference populations.

Specifically speaking, this included: on the far eastern outskirts, along the Via Prenestina Polense, of considerable interest what is found in the site of Quarto Cappello del Prete, located not far from the ancient city of Gabii. Proceeding towards the urban center, there are the large necropolis Collatina (which stretches between Via della Serenissima and Via Basiliano, near the ancient route of the Via Collatina) and, about 1 km from Porta Maggiore, the funeral complex investigated in the district of Casal Bertone, between the streets Tiburtina and Prenestina, adjacent to a production structure. Along the Via Tuscolana, in the immediate vicinity of the Villa of the Settebassi, is located the funeral set of Osteria del Curato. To the south is the burial ground of Via Padre Semeria, a crossroads of Via Cristoforo Colombo, near the southern section of the Aurelian Walls. Finally, in the south-west, not far from Ostia Antica, (Zona Ponte Galeria), on a sandy hilly area the necropolis of Castel Malnome has been unearthed

Conclusion

A great deal of time has passed since historians learnt how to make use of various different kinds of sources, apparently with nothing in common among one and another. This is especially true for the Empire and the Early Middle Ages, due to the vast array of archaeological materials found in the area of Rome over the last three decades and the new technological tools developed to analyse these findings. It is the notion of source itself that has likely changed. The study of everyday life allowed new evidence to enter the historian's laboratory, in which many humble objects, meaningless at first sight, started filling the shelves of the official history in order to draw a more complete image of man and woman.

The body itself has become a site to be excavated – certain lifestyles left important traces that need to be interpreted according to the traditional medical and literary written sources available.

Food, health and disease are to be examined first. Nutrition in relationship with the class or status that people belong to and the active life they lead, in terms of job practice and social roles, can provide plenty of information especially if connected to the pathological framework in which people should be placed.

Moreover, the natural and architectural environment in which humankind lived and worked literally shaped his body, turning into a treasure chest for investigation. The collaboration and teamwork of several specialists ensured a comprehensive analysis of the different factors involved in biological and cultural changes during the transition from the Empire to the early Medieval period. Research on daily life from

the beginning of the Roman Empire to the early Middle Ages in the Rome area has consequently provided new historical evidence to reconstruct a reliable image of society in those periods, enriching not only the scientific universe – through publications of papers and scientific volumes or through the organization and participation in scientific conferences – but also transforming the data emerging from the specialist's analysis of the various disciplinary groups into tools that could convey new knowledge outside pure academics. With the intent of deconstructing the more common approach of understanding ancient Rome mainly through archaeology and literary evidence, we focused more on how this culture was also made up of men and women, whose bodies can tell us a great deal about daily life, work, nutrition, healthcare and social customs of the most populous city in the ancient world.

Consequently, we decided to set up an exhibition inside the Sapienza University of Rome and then inside the Museo Nazionale Preistorico Etnografico "Luigi Pigorini" in Rome. The exhibition "Life Stories: Ancient Romans told by science" was made up of several exhibits of complete or partial skeletal reconstruction, developed as case studies that identify the physiognomy of an individual in Rome due to their biological specificity, eating and socio-cultural habits, able to tell the visitor in detail individual life stories.

They have given voice back to abused women, mothers and children, the elderly and workers who, through the signs of the body, tell us about living conditions, work, food, origin, hygienic conditions, habits and customs, parental relationships, trauma, diseases and medical treatments that defined their existence.

The narratives of everyday life were accompanied by the data and the related scientific techniques used to achieve them. This work was an attempt to give substance to the idea of a musealization as a dynamic centre that shows and tells the story of an ancient civilization, explaining with what techniques and what tools it was able to reconstruct it, which therefore plays an active role in the spread and awareness of cultural understanding, as well as scientific and didactic training within society.

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The controversial case of Cosimo I de' Medici (1519-1574): reflections on the interaction between anatomy and art (iconodiagnosis vs misdiagnosis)

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Abstract

A recent study maintains to have identified a case of severe Graves' disease in the bronze statue of Cosimo I de' Medici forged by Benevenuto Cellini (between 1545 and 1547). We carefully examined the artistic sources, the medical primary sources and the paleopathological findings with the aim of showing that Cosimo I de' Medici (1519-1574) was not affected by severe Graves' disease. The artistic analysis of different statues and portraits of the Grand-Duke of Tuscany confirms the medical interpretation. Cosimo I was thick-necked with a well-developed laryngeal prominence of the thyroid cartilage (Adam's Apple) and slightly bulging eyes. Plagued by obesity, Cosimo I was affected by DISH (diffuse idiopathic skeletal hyperostosis), chronic malaria, and severe osteoarthritis. The Grand Duke had a stroke on February 18th 1568, aged 49, and suffered from the sequelae for six years. Previous scholars proposed chronic cerebral vasculopathy as a possible diagnosis. We surmise that, as in modern day obese patients with DISH, he had increased risk for cardiovascular morbidity. The presence of a familiar thick-neck and a well-developed Adam's Apple can be observed in several other members of the Medici family such Giovanni dalle Bande Nere, Cosimo I de' Medici's father. The same features can also be found in several other predecessors of Cosimo I such as Piero the Gouty and his sons Lorenzo the Magnificent and Giuliano de' Medici, Pope Leo X, Giuliano de' Medici, Duke of Nemours and Lorenzo de' Medici, Duke of Urbino. This paper shows that the combination of literary, artistic, paleopathological sources is fundamental to correctly assess disease manifestation. A constant interaction between anatomy and art is recommended so to avoid over-interpretation of pathologic conditions in personages of the past.

Keywords

endocrinology; Graves' disease; art; history of medicine; iconodiagnosis; pitfalls.

Introduction

In a recent contribution (Pozzilli & Nicolai, 2021) it has been postulated that Cosimo I de' Medici (1519-1574), the second Duke of Florence, was affected by an endo-

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crinological condition (Pozzilli & Nicolai, 2021); the authors maintain to have identified a case of severe Graves' disease (untreated hyperthyroidism, atrial fibrillation and thyroid eye disease) in the bronze statue of Cosimo I de' Medici forged by Benvenuto Cellini in between 1545 and 1547. The statue is currently held by the *Museo Nazionale del Bargello* (Florence). Following the Authors' interpretation, the severe Graves' disease represented a considerable risk factor for the thromboembolic stroke which severely affected Cosimo I's last months of his life (Pozzilli & Nicolai, 2021).

Materials and Methods

A careful examination of the artistic sources, the medical primary sources and the paleopathological findings was performed with the aim of assessing the likelihood of Cosimo I de' Medici's diagnosis of severe Graves' disease, following recent guidelines on the integration of non-osteological sources in retrospective paleopathological analyses (Rühli et al., 2016; Mitchell, 2017; Nerlich et al., 2021; Varotto et al., 2022).

Results

By resorting to primary sources previous scholars have reconstructed the medical history of Cosimo I and the course of his 6 years progressive illness (Pieraccini, 1986; Arba et al., 2014). An obese man affected by DISH (diffuse idiopathic skeletal hyperostosis) and chronic malaria, the Grand Duke also suffered of diffuse and severe osteoarthritis affecting the lower portion of the dorsal and on lumbar spine; numerous Schmorl's nodes were identified on the vertebral plates of T8, L2 and L3 (the lumbar vertebrae L2-L3 were merged by a bony bridge on the left side) (Pieraccini, 1986; Fornaciari et al., 2007).

Studies on modern patients with DISH diagnosed at an age between 46 and 51 (Mader et al., 2021) have shown that these were significantly more affected by pain in the thoracic spine and in the lumbar spine (Mader et al., 2021) compared to patients with similar age and gender not affected by DISH. Patients with DISH also suffered bouts of pain around joints in the arms and legs due to the bony growths affecting those tendons and ligaments; this condition was also described in the case of Cosimo I who constantly lamented pain in the legs, knees and feet (Pieraccini, 1986). Patients with DISH also had a significant higher prevalence of obesity (Mader et al., 2021) and increased risk for cardiovascular morbidity (Bahn, 2010).

On February 18th 1568, aged 49, Cosimo I had a stroke. He died of bronco-pneumonia, six years later, at the age of 55 (April 21st 1574) (Pieraccini, 1986). His initial recovery from the 1568 cerebrovascular event was exceptionally good from a functional point of view (in October 1568 he could walk without a sticks, read, sign, play board games and hunt) (Pieraccini, 1986; Arba et al., 2014). However, from the autumn of 1572 onwards, the signs and symptoms of his slow but progressive illness (escalating loss of autonomy, hypophonia, dysarthria, pathological laughing and crying, gait impairment and dragging feet, urinary incontinence, mood swings and apathy) manifested. Chronic cerebral vasculopathy, also known as cerebral small vessel disease, was proposed as a possible diagnosis (Arba et al., 2014).

Figure 1. Artworks- statues and paintings- indicate that the members of the Medici family were thick-necks and had bulging eyes which, however, do not imply the presence of severe Greave's disease; **a.** Benvenuto Cellini, *Cosimo I de' Medici.* Bronze with partial silvering. 1545-47. Florence, Museo Nazionale del Bargello; **b**. Baccio Bandinelli, *Cosimo I de' Medici.* Marble. 1544. Florence, Museo Nazionale del Bargello; **c.** Giambologna, *Cosimo I de' Medici.* Bronze. 1565-74 c. Florence, Galleria degli Uffizi; **d**. Agnolo Bronzino, *Cosimo I de' Medici in armour.* Oil on panel. 1543. Florence, Galleria degli Uffizi; **e.** Giovan Pietro Naldini, *Cosimo I de'Medici.* Oil on panel. 1585. Florence, Galleria degli Uffizi; **f**. Agnolo Bronzino, *Cosimo I de' Medici.* Oil on panel. Before 1566. Turin, Galleria Sabauda ©MiC - Musei Reali, Galleria Sabauda; **g.** Mino da Fiesole, *Piero de' Medici.* Marble. 1453. Florence, Museo Nazionale del Bargello; **h.** Benozzo Gozzoli, Detail of *The Journey of the Magi* with the portrait of Piero de' Medici. Fresco. Since 1459. Florence, Palazzo Medici Riccardi, Chapel of the Magi; **i.** Florentine 16th century artist, after a model by Andrea del Verrocchio and Orsino Benintendi, *Lorenzo de' Medici.* Painted terracotta. 1513-20 c. Washington, D. C., National Gallery of Art, Samuel H. Kress Collection; **j.** Domenico Ghirlandaio, Detail of *The Confirmation of the Franciscan Rule* with the portrait of Lorenzo de' Medici. Fresco. 14. Florence, Santa Trinità, Sassetti Chapel 1483-1485; **k.** Workshop of Agnolo Bronzino, *Lorenzo de' Medici.* Oil on tin. 1565-69 c. Florence, Galleria degli Uffizi, Vasari Corridor; **l.** Sandro Botticelli, *Giuliano de' Medici.* Tempera on panel. 1478-80 c. Washington, D. C., National Gallery of Art, Samuel H. Kress Collection; **m.** Andrea del Verrocchio, *Giuliano de' Medici.* Terracotta. 1475-78 c. Washington, D. C., National Gallery of Art, Andrew W. Mellon Collection; **n.** Raphael or Giulio Romano, *Leo X as Clement I,* 1520 ca., black and white chalk drawing, Chatsworth, Devonshire Collection; **o.** Workshop of Raphael (?), *Giuliano de' Medici duke of Nemours.* Tempera and oil on canvas. 16th century. New York, The Metropolitan Museum of Art; **p.** Raphael, *Lorenzo de' Medici duke of Urbino.* Oil on canvas. 1518. Private collection. Reproduced with permission. For the Florentine Museums: Images reproduced with permission of the Italian Ministry of Culture.

Discussion

From a medical point of view, it shall be underlined that Cosimo I's adult life was plagued by obesity (Arba et al., 2014); this condition is incompatible with a diagnosis of Graves' disease since severe hyperthyroidism accelerates the body's metabolism and causes an unintentional weight loss independently from the food intake. The medical history of Cosimo I excludes both pathological slimming and Graves' ophthalmology (Pieraccini, 1986; Arba et al., 2014; Bahn, 2010). Cosimo I was thick-necked with a well-developed laryngeal prominence of the thyroid cartilage (Adam's Apple) (Fitzpatrick & Siccardi, 2021) and slightly bulging eyes (Figures 1a-f) (Fitzpatrick & Siccardi, 2021); he never suffered of inflammatory disorders of the orbit and periorbital tissues, as attested both by the primary sources (Pieraccini, 1986) and by several portraits of the Grand Duke over the decades (Figures 1d-f). In Giambologna's bust (Figure 1c), the signs of the stroke occurred in 1568 are clearly evident; Cosimo I's shows a facial asymmetry: the left nasolabial fold is deeper than the left one, the right upper and lower lids are sagging and the right corner of the mouth is turned down. The evidence of post-stroke facial features allows dating the bust between 1568 and 1574.

If Cosimo I's busts by Baccio Bandinelli (Figure 1a) and by Benvenuto Cellini are compared (Figure 1b) striking differences emerge. Both portraits are inspired by Roman imperial portraits dating to different centuries. Bandinelli's bust (circa 1544, Florence, Museo del Bargello) was based on a portrait of Hadrian (Uffizi, Florence). Bandinelli's shows the same accuracy observable in the portraits of Cosimo I over the years (Figure 1d-f). In these artworks, Cosimo I displays a quiet expression; his eyes are slightly bulging, but not protruding, and a thick-neck with an Adam's Apple can be appreciated.

Benvenuto Cellini's bust shows a personal artistic interpretation of the features of the duke: Cosimo I's countenance overflows with a vitality enhanced by the richly decorated cuirass and by the cloak falling over the Duke's left shoulder and caught up in his right arm. The tousled hair, knitted eyebrows, drilled pupils and tightened lips give an effect of intense concentration and military strength, together with the rapid turn of the head on the powerful neck. This effect was emphasized by the silvering of the eyes (retrieved through restoration) and the original gilding of the surface (Langedijk, 1981; Pope-Hennessy, 1985; Pope-Hennessy, 2002).

Cellini's bust may have been inspired to a cuirassed portrait of Julius Caesar (Musei Capitolini, Rome); according to one of the authors (EZ), the winged gorgon at the centre of the cuirass and the wide eyes both recall the portrait of Septimius Severus (Museo Nazionale Romano, Palazzo Massimo, Rome). The sharp, furious turn of the neck, the musculature of which is accentuated through the pose to suggest power, might have been modelled on Caracalla's portraits (Musei Capitolini, Rome, and Museo Pio - Clementino (Vatican Museums) (Gardner Coates, 2004).

The artistic analysis confirms the medical interpretation: Cosimo's eyes and neck are not consistent with severe hyperthyroidism; they rather represent a stylistic choice inspired by 3rd century CE Roman sculpture whose main characteristic was the unnatural enlargement of the eyes coupled with the indomitable vitality (Bianchi Bandinelli, 2002) [11].

The presence of the familiar thick-neck can be observed also in the statue of Ludovico di Giovanni de' Medici (1498-1526), also known as Giovanni dalle Bande

Nere, Cosimo I de' Medici's father. Cosimo I had slightly bulging eyes, a facial feature which can be found in the statues and paintings of several of his predecessor such as Piero the Gouty (1416-1469) (Figure 1g-h) and his sons Lorenzo the Magnificent (1449-1492) (Figure 1i-k) and Giuliano de' Medici (1453-1478) (Figure 1l-m).

Piero the Gouty, Lorenzo and his brother Giuliano all had thick-necks and an Adam's Apple; these can be appreciated both in Lorenzo the Magnificent's life-like terracotta by Verrocchio and Benintendi (ca. 1478, National Museum of Art, Washington, USA) (Figure 1i), in his portrait in *The Confirmation of the Franciscan Rule,* one of Domenico Ghirlandaio's frescoes in the Sassetti Chapel in S. Trìnita (Florence)) (Figure 1j) and by his posthumous portrait by Agnolo Bronzino (Uffizi Gallery, Florence) (Figure 1k). Similarly, Giovanni de' Medici, the son of Lorenzo the Magnificent and future Pope Leo X (1475-1521), had bulging eyes vividly depicted by Raphael (Figure 1n). The posthumous statue of Giuliano de' Medici, Duke of Nemours (1479-1516), carved by Michelangelo between 1519 and 1534, shows no evidence of goitre but the rapid turn of the head on the powerful neck. The copy of Raphael's portrait of Giuliano de' Medici, Duke of Nemours (1479-1516), originally painted around 1515, does not show any evidence of goitre (Figure 1o). The same is for the portrait of Lorenzo de' Medici (1492-1519) Duke of Urbino by Raphael (1518); the duke shows a short and thick-neck and slightly bulging eyes (Figure 1p).

Conclusions

Cosimo I de' Medici is highly unlikely to have suffered from Graves' disease. Making a more general point, as underlined in the context of studies of the auricular anatomy of members of the Medici family (Bianucci et al., 2021), it should be stressed how a virtuous combination of literary, artistic – and, whenever possible, mummy or osteological sources – should be implemented; this in order to correctly assess disease manifestation in the past, both at population and individual levels. Following the rheumatologist Jan Dequeker, paintings – and one may well add statuary – can teach medical scholars very much about disease presentation (Dequeker, 2006), with a particular focus on soft tissue analysis, but one should never forget that sometimes they can also lead to pitfalls.

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An anthropological and paleopathological analysis of a peculiar skeleton from the Necropolis of Zancle (1st century BCE – 1st century CE): a case report

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Abstract

The work presents the results of the anthropological and paleopathological analysis carried out on human skeletal remains of an individual (T-173) found in a burial from the necropolis "sector 96", Messina, Sicily, dating back to the Roman Empire (1st century BCE – 1st century CE). The study aimed to acquire the information necessary for the reconstruction of the biological profile. In fact, T173 is an adult male which is particularly interesting from a paleopathological point of view, showing skeletal anomalies from the cranial to the post-cranial skeleton probably caused by variations of genetic, neoplastic, articular and dental nature. Furthermore, the analyses have highlighted the possible cause of death, related to two *perimortem* traumatic lesions on parietal bones.

Keywords

physical anthropology; biological profile; paleopathology; Sicily.

Introduction

This work deals with the anthropological and paleopathological analysis of the bone remains belonging to an individual buried in Tomb 173, "sector 96" (Fig. 1), of the Necropolis of Zancle (Messina, Italy), found during the excavation campaign 1998-1999. The necropolis was used for an extended period, from the Hellenistic Period to Late Antiquity (4th century BCE – 5th century CE). Specifically, relining on the material culture recovered in the burial - a *Piriform Unguentarium* -, T173 was archaeologically dated between the 1st century BCE and the 1st century CE. The unguentaria are small ceramic bottles, representing the most common grave offerings of the Hellenistic and early Roman periods, especially in cemeteries of the Mediterranean area (Anderson-Stojanovic, 1987). The pear-shaped body of the *Piriform Unguentarium* is associated with graves of the 1th century BCE and it continues in use for a period of about a hundred years, until the latter part of the 1th century CE (Robinson, 1959; Anderson-Stojanovic, 1987).

The observation of the various skeletal areas highlighted numerous anomalies both in the cranial and post-cranial skeleton, attributable to a pathological spectrum and injuries caused by a violent episode (Messina et al., 2013) in an individual already characterized by a very intense occupational activity in life. Therefore,

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Figure 1. Sector 96 of the necropolis (Messina, Italy). (Tigano, 2017).

we proceeded with an in-depth anthropological analysis of the entire skeleton with the aim of reconstructing the biological profile and investigating the etiology of the numerous and different anomalies found, especially to understand better the activities carried out by the individual.

Historical background

The first consistent settlements in the Messina area date back to the Bronze Age, but it is only with the arrival of the Greeks - in 740 BCE - that the area assumes the maritime role for which it is known. In book IV of "*History of the Peloponnesian War*", Thucydides tells that the first founders of the city were Greek pirates from Cumae (Campania) to whom, later, the inhabitants of Chalcis would be added. Thucydides informs us that the first name of the city – Zancle - is of indigenous origin and it was so named for the sickle shape of the port (the indigenous named the sickle "*zanclon*").

Because of its strategic position in the Strait, the city acquires a new importance within the new communication routes, especially for what concerns shipments. The acquired wealth with the commerce stimulated competition and development and Zancle becomes a crucial Mediterranean hub - not always of peaceful nature - between Greeks, Carthaginians, Phoenicians, Etruscans and Sicilians. Messina became

the first Sicilian city in the hands of the Romans during the First Punic War and, at the end of the war, the city obtained the status of *civitas libera et foederata* (free and allied city), unique in Sicily together with *Tauromenium* (Taormina) and *Neaiton* (Noto).

Romans will rule in Sicily for other six centuries, as Sicily was very important for the Empire as a point of supply, for keeping a close watch on Carthage, for the production of grain that supported the Roman armies and – in general - as a central point to oversee the Western Mediterranean (Serrati, 2000; Serrati, 2020).

The necropolis of Zancle

The excavations of the Necropolis of Zancle (in the South/South-Western part of the town of Messina) were directed by *Sovrintendenza dei Beni Culturali di Messina* (Sicilian Heritage Office) from 1991 to 1997. In particular, the interest was placed along via Cesare Battisti, where the sections 83 (I-II sector) and 96 (V-VII compartment) (Fig. 2) brought to light about 400 tombs.

The stratigraphic investigation and the analysis of the material culture found made it possible to isolate three main chronological phases that covered a wide timespan ranging from the early Hellenistic age to the Late Ancient (last quarter IV BCE - V CE) and seem to coincide with the moments of change in the organization of the space intended for burial. From a stratigraphic point of view, these spaces are distinguished by alluvial deposits that seal the tombs of the lower level, thus creating the opportunity for new burials (Tigano, 2017).

Figure 2. Aerophotogrammetry of part of the necropolis, modified from Tigano (2017). The sector 96 is highlighted in grey.

Material and Methods

The anthropological study began with a preliminary analysis of the skeletal districts related to Tomb 173, proceeding with the basic anthropological characterization in order to acquire the necessary information through the use of current anthropological diagnostic methodologies and techniques (Buikstra and Ubelaker, 1994).

For the determination of sex, the dimorphic sexual characteristics of the pelvis and skull were primarily investigated according to the criteria of Acsádi and Nemeskéri (1970). Due to the partial fragmentation of these skeletal areas, it was not possible to observe all the characters reported by the standard, so other qualitative methodologies were applied such as the Phenice (1969) and Bruzek (2002) for the pelvis. As a quantitative method, the Probabilistic Sex Diagnosis (DSP) (Murail et al., 2005; Bruzek et al., 2017) was used. The measurements required by the standard were obtained both physically – by sliding jaw gauge and curved jaw gauge – and digitally through the development of 3D models of the coxal bones through Close Range Photogrammetry (Luhmann et al., 2019).

To estimate the biological age at death were applied methods based on the metamorphosis of certain skeletal areas during aging, such as the auricular surface of the ilium (Lovejoy et al., 1985), the pubic symphysis (Suchey and Brooks, 1990) and the sternal end of the fourth rib (Ïşcan and Loth, 1984).

The formulae of Trotter and Gleser (1952; 1958; 1977) were used to estimate stature.

When possible, the study proceeded with paleopathological assessments, which are fundamental for reconstructing the state of health and lifestyle. In investigating the onset of a disease, it is crucial to identify the nature and distribution of the "anomaly" on the skeleton. Thus, we performed with a preliminary analysis to differentiate the normal development in shape, size, and topography of a healthy skeleton from the ones we observed on T173. Once the "variation" was identified, its description and diagnosis continued with the aid of appropriate manuals (Capasso et al., 1999; Ortner, 2003; Roberts and Manchester, 2010; Grauer, 2011; Waldron, 2020) and from other sources in the scientific literature.

Results

T173 skeleton was found in an excellent state of preservation, despite some of its skeletal elements lacking. The missing skeletal elements are the sternal bones, the whole right upper limb, the left hand (except the first metacarpal), the left foot and phalanges of the right one, the left patella.

Regarding the determination of sex, the application of the Acsádi and Nemeskéri method (1970) gave mixed results. The qualitative analysis of the skull shows marked masculine morphological characters with a sexualization index 0.95. In detail, the characters with a greater degree of relevance – glabella, mastoid process and nuchal plane – have a strong male morphology, while the frontal bossing and the inclination of the frontal bone match with a female morphology. The pelvis' qualitative analysis results in contrasting morphological characters with a sexualization index 0.1. In fact, some of the characters have a male morphology such as the lack of compound arch,

Table 1. Results of the three methods applied for the estimation of biological age at death.

Method	Phase	Range
Pubic symphysis (Suchey & Brooks, 1990)	5	27-66
Auricular surface (Lovejoy *et al.*, 1985)	3	30-34
Sternal end of the fourth rib (Iscan & Loth, 1984)	4	26-32

the body of the ischium, the morphology of the iliac crest and fossa, while others express a female morphology, such as the presence of the preauricular sulcus – albeit minimal –, the shape of ischial incisura, the obturated foramen and the overall shape of the coxal bone.

Because of these conflicting results, the Acsádi and Nemeskéri method (1970) was integrated through the use of other methodologies. The application of Phenice (1969) and Bruzek (2002) methods provide a male assessment of T173, which was further confirmed by the outcome of DSP that gave a male probability of 0.988% (for physical measurements) and 0.96% (for digital measurements).

A preliminary observation of the skeleton has highlighted the belonging to an adult age class, since the epiphyses and the diaphysis of the long bones are welded. Multiple qualitative methodologies were applied as well to be able to obtain an age range of about 10 years (table 1).

The stature estimation was obtained by applying the Trotter and Gleser formulae (1952; 1958; 1977). Femurs and tibiae made it possible to apply the formula showing the lowest margin of error, returning a value of 169.5 +/- 2.99 cm.

Regarding the state of health, on the skeleton of T173 joint, dental, neoplastic and, presumably, congenital anomalies were found.

There are four osteomas on the skull, benign tumors deriving from excessive proliferation of the bone tissue. Furthermore, the skull is characterized by plagiocephaly, accompanied by what appears to be the premature synostosis of the left coronal suture, which could lead to craniosynostosis. A secondary joint of the right mandibular condyle is evident, a phenomenon that has been associated with skeletal dysplasia. Moreover, the skull of T173 manifests two traumatic events on the parietal bones as already described (Messina et al., 2013).

The skull exhibits a series of anatomical variants known as non-metric traits or discontinuous (Ossenberg, 1969) or discrete (Rightmire, 1972) or epigenetic (Berry and Searle, 1963). Usually, these present as a wide range of differences in the morphology and number of foramina, tubercles, ossicles, sutures, and grooves, representing deviations of the normal skeletal development.

The post-cranial skeleton appears afflicted by osteoarthritis, evident in most of the joints, which also show signs of eburnation caused by friction between the joint heads due to the thinning of the articular cartilage. There is evidence of osteophytosis in the vertebrae which also manifest the Schmorl's nodes on the vertebral bodies.

A probable intense physical activity is highlighted by the regions of origin and insertion of some muscles.

Discussion

This work presents the study of an adult skeleton from the necropolis of Zancle (Messina, Italy), that is characterized by a remarkable series of skeletal and pathological markers. In our opinion, it is a paradigmatic example of what could be the level of skeletal impairment in ancient periods.

The anthropological investigation conducted had the purpose of acquiring the information necessary to reconstruct of the biological profile of the individual buried in Tomb 173 of the Necropolis of Zancle – "sector 96" (Messina, Italy).

The first step was determining sex, which was evaluated on the skull and pelvis. The first method used was the combined one of Acsádi & Nemeskéri (1970), which simultaneously evaluates a series of morphological characters of the two skeletal areas mentioned. The results obtained are contrasting, as the skull shows marked masculine morphological characters – sexualization index 0.95 - while the pelvis shows some characters matching with a female morphology and others that are morphologically masculine – sexualization index 0.1 -. When the result of the sexualization index (**Σwx/Σ**) is between the values –0.5 and 0.5 the sex of the element cannot be determined (Acsádi and Nemeskéri, 1970). Therefore, there is a discrepancy using this method on T173 since the skull is morphologically male and the pelvis is undetermined. Due to this uncertainty, others methods have been applied, establishing that T173 was a male.

Similarly, multiple methodologies have been applied to evaluate the biological age at the death, providing further valuable data for an accurate estimation. The results gave a range between 30 and 40 years old.

The estimation of stature is essential to define the physical constitution and body size of skeletal remains. Having previously diagnosed the sex and the biological age and having both right and left femur and tibia available, we obtained an estimate of about 169.5 cm +/- 2.99. However, the Trotter and Gleser formulae (1952; 1958; 1977) are valid for subjects aged between 18 and 30 years, and for older ages it is suggested to subtract 0.06 cm for each year of age (Canci and Minozzi, 2015). As previously mentioned, the age of T173 is estimated in a range between 30-40 years, therefore, the actual estimated stature is about 168.4 cm +/- 2.99.

The paleopathological diagnosis completed the anthropological investigation as an essential step for reconstructing the lifestyle and activities carried out by T173. The beginning of any paleopathological investigation consists in the identification of qualitative and quantitative changes in the bones: the skeleton is a dynamic and flexible structure that adapts to internal and external stresses, therefore when a disease arises the bone tissue reacts and its response may involve in modifications that deviate from the normal skeletal morphology with consequent variations in size, bone strength and formation of accessory facets. So "consistent anatomical alterations" (Grauer, 2011) must be recognized and the single or multifactorial causes of such alterations must be investigated. The anomalies found on the skeleton of T173 are of joint, dental, neoplastic and, probably, congenital type. Furthermore, the individual died for a violent episode (Messina et al., 2013).

Observing the neurocranium a bone deformity was found. Its cause can be attributed to craniosynostosis (Fig. 3), a malformation of the skull's bone structure caused by the premature synostosis of one or more cranial sutures (Kabbani and Raghuveer,

Figure 3. T173 skull, anterior view. Asimmetry of the cranial vault is shown. Scale bar: 5 cm.

2004). This process results in an irregular growth of the neurocranium which expands to the side where it finds no resistance (Sgouros, 2005). The craniosynostosis can arise as a manifestation of a syndrome or as an isolated defect (non-syndromic). There are more than 150 syndromes that can involve craniosynostosis (Zoller et al., 2003). The etiology of the non-syndromic form is not known but is probably caused by various factors commonly of an environmental nature (e.g., the advanced age of the pregnant woman, permanence of the mother at high altitudes, exposure to chemical or physical agents), but presumably linked also to genetic factors such as *de novo* mutations or alterations in gene regulation. Mutations that arise in fibroblast growth factors and their receptors (FGFR) are among the main causes of craniosynostosis (Wilkie, 1997). On T173 skull, it is possible to notice premature synostosis of the left coronal suture, which could have involved a deforming plagiocephaly, modifying the shape of the skull (Fig. 3). This phenomenon could be an explanation of the frontal bone morphology which lacks useful characters for sexual diagnosis, for example the frontal bone inclination and the glabella. The involvement of a single cranial suture allows to hypothesize that the presumed craniosynostosis of T173 is of a non-syndromic form. However, it is not possible to define it precisely due to the still little known etiology and the difficulties in making the appropriate genetic investigations relating to possible factors on skeletal remains.

The craniosynostosis of T173 could also be the cause of the asymmetrical morphology of the mandible, which shows a secondary joint of condylar articulation that can be categorized as skeletal dysplasia (Ortner, 2003). Indeed, the right condyle manifests

Figure 4. Superior view of the mandible, on the right; inferior view of the maxillae, on the left. Scale bar 5 cm.

a double joint resulting in an oval shape with a slight depression in its epicenter and widens in the anterolateral norm, exceeding the size of the condyle (Fig. 4). Several factors may have contributed to the particular morphology of T173's mandible, including arthritis, tumor, trauma suffered during growth, and congenital anomaly (Williams and Polet, 2017). Both arthritis and tumors were excluded as possible causes because the mandible does not show signs of erosion or lipping and inflammatory signs of the periosteal tissue. Among the factors that could cause the formation of a second condylar joint are *postpartum* (Dennison et al., 2008) or adolescent trauma (De Luca et al., 2013). An antenatal obstruction or injury suffered during adolescence can cause a dislocation of the jaw, which, leads to the formation of a second condylar articulation if not treated. This hypothesis was also excluded as a unilateral traumatic phenomenon affecting the mandible is generally accompanied by a very pronounced musculature on the malformed side, affecting the masseter, mylohyoid and pterygoid muscles (Williams and Polet, 2017). The mandible of T173 does not show roughness, exostosis or anomalies in the region of insertion of the aforementioned musculature. The possible presence in T173 of craniosynostosis allows inferring that the malformation of the mandibular condyle is presumably to be traced back to a congenital anomaly, as a collateral results of prenatal craniofacial anomalies (Williams and Polet, 2017). The teeth - already affected by numerous degenerative diseases such as caries, calculus, periodontitis and abscess - do not seem to show an alteration due to the abnormal mandibular morphology, even if the latter could be one of the causes of the marked degree of dental wear, which manifests symmetrically between the hemi arches. Presumably, the dysplasia caused a phenomenon of malocclusion which resulted in constant friction between the teeth of the maxillary and mandibular arches. However, an opposite cause cannot be excluded, namely that it was the phenomenon of malocclusion that caused the formation of a second joint facet of the condyle.

On T173 skull, it is possible to observe four osteomas: two on the frontal bone, one on the occipital squama and one on the right parietal. Osteoma is a benign tumor

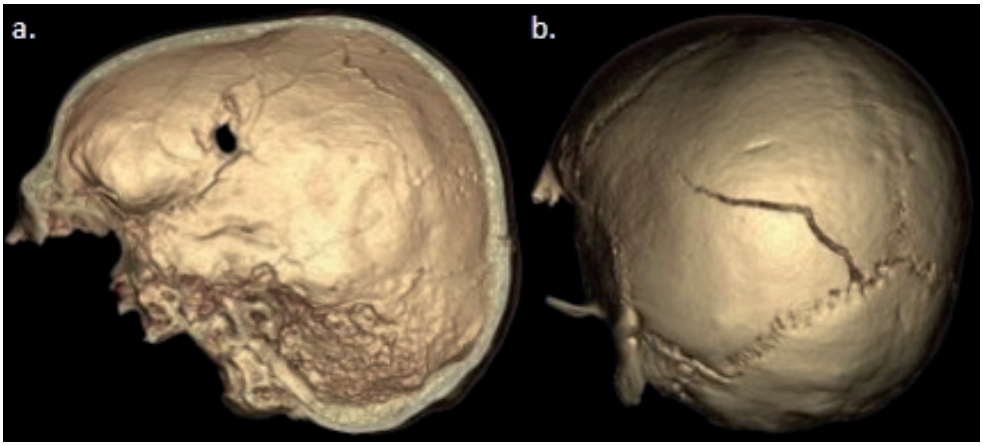

Figure 5. T173 skull computed tomography. a: endocranial, trauma on the right parietal. b: left posterolateral view, fracture lines on the left parietal.

that occurs mainly in the cranial vault and long bones (especially the femur and tibia). This neoplasm presents as a bone formation composed of osteoid and woven bone with a size that rarely exceeds 1.5 cm and is generally asymptomatic (Kransdorf et al., 1991).

The parietal bones of the skull of T173 exhibit two traumatic injuries. Trauma is defined as injury caused by forces or mechanisms extrinsic to the body (Lovell, 1997). Its study is significant since traumatic events testify the physical activities, their degree of intensity and the degree of interpersonal violence. The traumatic injuries found in T173 were investigated by Messina et al. (2013) that wanted to examine these cranial lesions with regard to their possible etiology and to formulate a hypotheses about the events, probably evidence of interpersonal violence. The authors established that the injuries are *perimortem*, i.e. they were inflicted shortly before or at the time of death. Two traumas were found: one involves the right parietal (Fig. 5a) and a secondary one involves the left parietal (Fig. 5b). The outer surface of the right parietal has a trapezoidal perforation located between the coronal suture and the temporal line. It was suggested that the injury was inflicted *perimortem* by a sharp object that hit the bone from left to right and from top to bottom, at an angle of 45°, causing a fracture and perforating the bone.

Through the CT scan analysis, it was found that the perforation also affected the *sulci* of the middle meningeal vessels causing an epidural hematoma, the probable cause of death. This methodology made it possible to detect the presence of a plastic response by the bone, a reaction typically associated with *perimortem* fractures. The hypothesis that the perforation had been inflicted shortly before the individual's death was further validated by the presence of bone splinters attached to the margins of the fracture, evidence that this was inflicted on 'fresh' bone. In addition, the left parietal bone has a lesion in a point near the lambda from which three fracture lines radiate. This injury appears to have been caused by blunt force trauma, possibly associated with a blow or fall. The hypothesis is that T173 was first hit by a sharp object that

injured the left parietal bone, creating a tear in the blood vessels resulting in an accumulation of blood between the intracranial surface and the *dura mater.* That caused an epidural hematoma. Due to this event, losing consciousness, the individual fell on a hard surface which caused the fracture on the left parietal bone (Messina et al., 2013).

Finally, the skull exhibits non-metric traits such as left supraorbital foramen, symmetrical parietal foramina and the lack of the jugular foramen septum.

The study of the skeletal material belonging to T173 also focused on dental analysis and the pathologies found. The teeth of the mandibular arch are all present, except the first and third molars of the left hemiarch, which were lost *postmortem* (Fig. 5). In contrast, the maxillary arch is almost devoid of teeth. Several and often interrelated factors can cause tooth loss in the lifetime. When a pathology affects one or more teeth, there is a reaction of the alveolar tissue that responds with remodeling, resorption and obliteration processes (Canci and Minozzi, 2015). The high number of *antemortem* tooth loss in T173 (Table 2) could find an explanation in the variety of dental pathologies that have been found. The presence of caries, abscess, periodontitis and *antemortem* loss of the teeth is indicative of a diet with high consumption of carbohydrates; however, the role played by poor oral hygiene should not be forgotten. Furthermore, the finding of a strong degree of dental wear on the occlusal plane could be related to the consumption of solid foods with a strong fibrous component that generally requires vigorous chewing. This factor could be one of the causes that led to such a degree of wear, which could also be caused by the friction deriving from the contact between the teeth of the upper arch and the teeth of the lower arch, from the use of the mouth as a third hand, from the friction by exogenous material on the tooth surface and the chemical dissolution resulting from the consumption of acidic food (Kaifu et al., 2003).

Regarding the post-cranial skeleton, T173 is affected by osteoarthritis, a degenerative change with a chronic course that affects the articular cartilage whose progressive thinning - being a non-vascularized tissue and therefore not subject to remodeling - involves a direct contact between the articular heads. Their friction causes the eburnation that represent the shiny appearance of the articular surfaces that's also furrowed by striae in the direction of movement and by porosity. Osteoarthritis is generally related to biomechanical stresses affecting the joints, genetic factors, health factors (e.g., obesity) and tends to increase with age (Gardner, 1983). The alterations caused by arthropathy are clearly visible in T173.

Arthrosis also affects the entire spine of T173. In fact, the vertebrae present tissue erosion, marginal lipping, osteophytosis and osteophytic spicules. It is hypothesized that the etiology of arthrosis in T173 is due to the intense activity practiced in life and it seems to be evidenced by other markers of occupational stress found, such as Schmorl's

Table 2. Teeth in situ; antemortem and postmortem tooth loss.

	In situ	Antemortem	Postmortem
Maxillary arch	L: I2 - C – P3 R: C - M2	L: I1 - P4 - M1 R: I1 - I2 - P3 - M3	--- R: P4 - M1
Mandibular arch	L: I1 - I2 - C – P3 - P4 - M2 R: I1 - I2 - C – P3 - P4 - M1 - M2 - M3	---	L: M1 - M3

Figure 6. Left upper limb: humerus, ulna, radius. Scale bar 5 cm. A: ulnar proximal end. B: radial proximal end.

nodes, accessory facets and squatting facets. Schmorl's nodes affect the spinal column and represent the degeneration of the intervertebral discs. The degeneration of the vertebral body bone tissue produces cavitation, and a bony "barrier" is created at the margin to prevent the progression of the herniation of the nucleus pulposus in the vertebral body (Schmorl and Junghanns, 1971). Schmorl's nodes can result from congenital defects of the vertebral column, traumatic events or senescent processes (Resnick and Niwayama, 1978; Capasso et al., 1999; Dar et al., 2009). In particular, on T173 the cause could be traced to a traumatic event affecting the thoracic and lumbar vertebrae, causing the deformation and rupture of the intervertebral disc. This hypothesis is supported by the presence of other skeletal alterations related to stress events found in T173.

The skeletal system of T173 is remarkably robust, therefore it is possible to hypothesize that the musculature was equally powerful. Most of the muscular insertions on the upper appendicular bones have a very rough and irregular surface (Fig 6). The clavicles show a region of depression at the insertion site of the conoid ligament and roughness in the sites of origin of the *M. pectoralis major* and *deltoideus*. The humerus shows a

depressed and osteophytic region at the insertion site of the *M. deltoideus,* osteophytosis and porosity in the insertion regions of the *M. latissimus dorsi* and *teres major*. The origin site of the *M. brachioradialis* (lateral supracondylar ridge of humerus) shows bone growth while the insertion site (proximal to the styloid process of radius) is eroded. The radial tuberosity is depressed with protruding margins. The ulna presents bone growth and roughness at the insertion sites of *M. brachialis*, *pronator* and *supinator.*

The stresses affecting these muscles - involved in the movements of adduction, abduction, flexion, external and internal rotation, extension of the arm and forearm - and osteoarthritis affecting the sternoclavicular, acromion-clavicular, glenohumeral and elbow suggest that the individual carried out activities requiring constant use of the upper limbs, with frequent movements of flexion, extension, rotation and elevation of the arms, requiring the effort of the entire shoulder girdle and arm and forearm. Furthermore, Schmorl's nodes in the thoracic and lumbar vertebrae, the stresses on the elbow joint and the alterations in insertion areas of *M. deltoideus*, *M. brachialis* and *M. pectoralis major* suggest the transport of heavy objects on the vertebral column and the arms.

Moreover, the left radial head and the distal end of the right first metacarpal show signs of eburnation. Radial eburnation can be related to alternation movements of supination/pronation, while the first metacarpal eburnation (Fig. 7) can be connected to rotational movements.

Agriculture and maritime activities such as fishing or working with fishing nets may be an explanation to the movements involving radium and thumb (Parks, 2002). However, this remains a hypothesis because the lack of the right upper limb prevents more accurate inferences.

Similarly, the bones of the lower limbs (Fig. 8) also show areas of osteophytosis, bone tissue apposition, porosity, bridges and marginal lipping.

The evidence of stress affecting the insertions of the main abductor, adductor, rotator and extensor muscles of the lower limbs are supplemented by other alterations of the skeleton, such as the enlargement of the lunate facies whose lower region extends into the region of the acetabulum margin, up to the region of insertion of the transverse ligament. There is a groove for *M. obturatorius externus* and a bridge in the acetabulum, the latter is formed due to the extension of the facies. The causes of the enlargement of the lunate facies are due to abduction and flexion of the hip joint and the stress on the margin and transverse ligament can cause osteoblastic activity (Capasso et al., 1999). The accessory facets of the sacrum appear at the level of the first and second posterior foramen of the sacrum, probably caused by stress on the vertebral column, which flexes and compresses during the transport of heavy objects. A depression is observed in the medial condyle of the femur, usually in the posterior side, which is generally caused by contact with the tibial condyle following repeated flexor movements; this phenomenon is called tibial imprint (Capasso et al., 1999). The patella has an incisura on the supero-lateral surface, probably for a presumed constant contraction of *M.vastus lateralis* following flexion and squatting movements. The tibia has a lesion on the tubercle presumably related to the partial avulsion of the insertion of the patellar ligament caused by the movements of the quadriceps (Capasso et al., 1999). Squatting facets in the tibia and talar are evident. This joint morphology occurs on the anterior surface of the distal tibia and on the lateral-superior surface of the talus, and it is caused by the habitual dorsiflexion of the foot in which

Figure 7. Left first metacarpal, scale bar 5 cm. Posterior view (left) and superiore view (right).

Figure 8. Anterior view of left femur (up, distal end) and left tibia (down, proximal end). Posterior view of the patella. Scale bar 5 cm.

the elongation of the ligaments creates further contact surfaces between the tibia and talus (Capasso et al., 1999).

The evidence of anomalies described is part of a pattern of movements attributable to squatting movements that represent a bending movement on the legs that is

Figure 9. Plantar view of left and right calcaneus. Calcanear spur is shown. A: lateral view of the right calcaneus. Scale bar 5 cm.

carried out with the simultaneous flexion of the hip, knee and ankle. The manifestation of sacral accessory facets, knee osteoarthritis and changes in the muscular insertions of the femur and tibia lead to the hypothesis of carrying heavy loads.

It's possible to observe osteophytosis on tibiae, especially on the posterior insertion of *M. soleus*. However, there are stress markers at the level of the tibial tuberosity and on the anterior intercondylar region (the insertion site of the cruciate ligament), with the diffuse presence of marginal lipping both in the proximal and distal epiphysis. The variations in the muscle insertion regions of the femur and tibia, and the arthritic degeneration of the knee are presumably caused by the transport of heavy loads on the vertebral column. Furthermore, the morphological changes observable in the talus and calcaneus seem to confirm this hypothesis. In fact, osteophytosis and osteoarthritis affecting the Achilles tendon in the calcaneus, the modification of the trochlear surface, and the presence of squatting facets in the talus suggest movements on rough terrain, probably during long walks. Both calcaneus present plantar spurs (Fig. 9), a bony outgrowth of the calcaneal tuberosity that occurs in higher proportions in the elderly, the overweight, plantar fasciitis, and arthritis, which is also connected to abnormal foot biomechanics (Kirkpatrick et al., 2017). Due to the pervasive presence of biomechanical stress markers recorded on T173, it can be assumed that the plantar spurs may be an adaptative response to repetitive, vertically orientated forces (Kumai and Benjamin, 2002; Li and Muehleman, 2007; Menz et al. 2008) which could arise from a transmission of weight on the toes to maintain a balanced position, for example on steep terrain or wet terrain.

Conclusion

The anthropological investigation aimed to acquire the information necessary for the reconstruction of the biological profile, the state of health and lifestyle of the indi-

vidual found in a burial of the necropolis of Messina (Sicily). The results obtained made it possible to establish that the skeleton buried in the Tomb 173 belongs to a male individual, whose biological age at death was probably within a range of 30-40 years and with an estimated stature of about 168.4 cm. The paleopathological investigation allowed the evaluation of skeletal alterations presumably of a genetic, neoplastic, dental, joint and violent nature. Finally, the study of arthropathies and alterations related to occupational stress made it possible to hypothesize the activities that the individual carried out in life. The alterations affecting the bones of the shoulder girdle, the pelvic girdle and the upper and lower appendicular skeleton highlight a constant use of these elements and intense stress on the mobile joints. Furthermore, the alterations found in the vertebral column and the femoral, tibial and tarsal bones lead to activities carried out on rough and/or unbalanced terrain with a hefty load on the vertebral column.

At the moment, the study is limited to T173, but certainly expanding the studies on coeval skeletal remains could provide interesting data about the lifestyle and state of health during Roman Imperial period.

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Ancient Egyptian paleopathology – The population of the tomb of IPI, necropolis of Dahshur-South

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Abstract

This study investigated the human remains of the tomb complex built by the ancient Egyptian high ranking official "Ipi" (4th Dynasty, ca. 2700-2600 BCE) in the Lower Egyptian necropolis of Dahshur-South. Beyond burials from the tomb owner and his family from the Old Kingdom, the complex was re-used until the Late Period. The excavated material presented the human remains of at least 73 individuals. Unfortunately, initial excavations had collected and mixed individual burials, so that individual identification in most skeletons was not possible. Later excavations presented complete individual burials. The custom typical of the New Kingdom until the Late Period of transnasal trephination offered, even with the mixed burials, a collection of 23 disturbed adults' skulls (New Kingdom until Late Period) and 33 skulls of undisturbed adults (Old Kingdom). In these two groups sex ratios were fairly balanced. Most adult individuals died between 20 to 30 years. There were relatively few immature burials and a single skeleton was that of a 7th lunar month fetus, an obvious stillbirth; total number of immature was 10 (17.8%). Numerous paleopathological observations indicated the considerable impact of living conditions and health. There were 12 cases with healed traumatic sequelae including one with a healed ankylosed fracture of the knee and another with a fracture of the humerus, three cases of nonspecific osteomyelitis, three cases highly suggestive of spinal tuberculosis, one with evidence of metabolic bone disease from vitamin deficiency, and two cases with malignant bone tumours; one with multiple metastases of the vertebrae, and the other chondrosarcoma of the pelvis. Also, typical pathological changes were seen in teeth and jaws. Finally, the rate of osteoarthrosis of large joints and that of spondylosis suggest significant work load. These findings indicate various ailments either due to heavy work load (arthrosis and spondylosis, traumatic sequelae), or to the living conditions (tooth abrasion by sand ingestion; tuberculosis due to foundation of settlements and townships), metabolic osteopathies possibly due to parasitic diseases and/ or malnutrition. These skeletal remains therefore contribute to our understanding of ancient Egyptian life and diseases.

Keywords

paleopathology; trauma; inflammation; tuberculosis; malignant bone tumour; vitamin deficiency.

Introduction

Our current knowledge on daily life and living conditions of past populations is mainly derived from archaeological findings and written sources. In most instances, however, it remains unclear to what extent the resulting information is correct and/ or complete. More and more information on life and disease in ancient cultures is

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obtained through the meticulous analysis of human remains, in most cases by anthropological and paleopathological investigations of skeletons and mummies.

This holds also true for the ancient Egyptian high culture. This flourished for more than 3,000 years in the Nile valley with significant variation of the population size in different time periods and regions. Beginning with the most extensive investigations by Smith and Jones (1908) in the early 20th century when emergency excavations in the Nile valley south of the first cataract (i.e. in the south of the Upper Egyptian city of Aswan) had to be performed due to the building of the first Aswan dam and the following flooding of the valley, several study groups have analysed specific populations in later periods. Since then, however, beyond differences in the populations also the techniques of analysis, the diagnostic certainty of diseases and their traces, and the extent of preserved material have been subjected to significant changes over time. It is therefore of considerable interest to collect as many data on as many available populations of the ancient Egyptian empire in order to refine our knowledge on their historic living conditions.

Besides Smith and Jones' (1908) studies, some analyses have been performed on the large necropolis of Qubbet-el-Hawa near Aswan, Upper Egypt by Rösing (1990) and Minshat-Abu-Omar in the Nile delta (Parsche 1991). These analyses provided considerable information on the population of townships at the periphery of the Egyptian empire. Significant data on distinct populations have previously been made available for the New Kingdom capital of ancient Egypt, Thebes, describing the anthropological and palaeopathological findings from various major tombs of the necropolis of Thebes-West which are part of the "Tombs of the Nobles" (Nerlich et al., 2000); these cover burials of mainly higher class individuals of the capital of the empire. Further, though limited, studies have been presented on other Theban tomb complexes (Dra-Abu-el-Naga) from Middle to New Kingdom period (Polz et al. 1999), the Theban tomb complex TT-196 (Asasif) (Zink and Nerlich, 2007) and from the Old Kingdom necropolis of Abydos (Zink and Nerlich, 2002) and Buto (Hartung et al. 2009).

The present study provides information on a specific population; the burials of the tomb complex of Ipi, an Old Kingdom royal court official during the reign of Pharaoh Sneferu (4th dynasty). This covers both Old Kingdom (OK) and Late Period (LP) burials in the large necropolis of Dahschur-South (Alexanian et al., 2006). The tomb complex comprises not only the original burial in a typical *Mastaba* complex, but also later intrusive burials. However, all material has been subjected to significant grave robbery. In addition, early modern period excavations did not take care of the human remains which were moved and intermingled. Although this setting renders the analysis much more difficult, a methodical investigation using anthropological and palaeopathological approaches can provide useful information, even in those instances where the material is mixed and/or partly destroyed.

Materials and Methods

The necropolis and the tomb complex of "Ipi"

The necropolis of Dahschur, some 30 km south of present-day Cairo and only few km in distance from the ancient Egyptian capital of Memphis, covers a large area

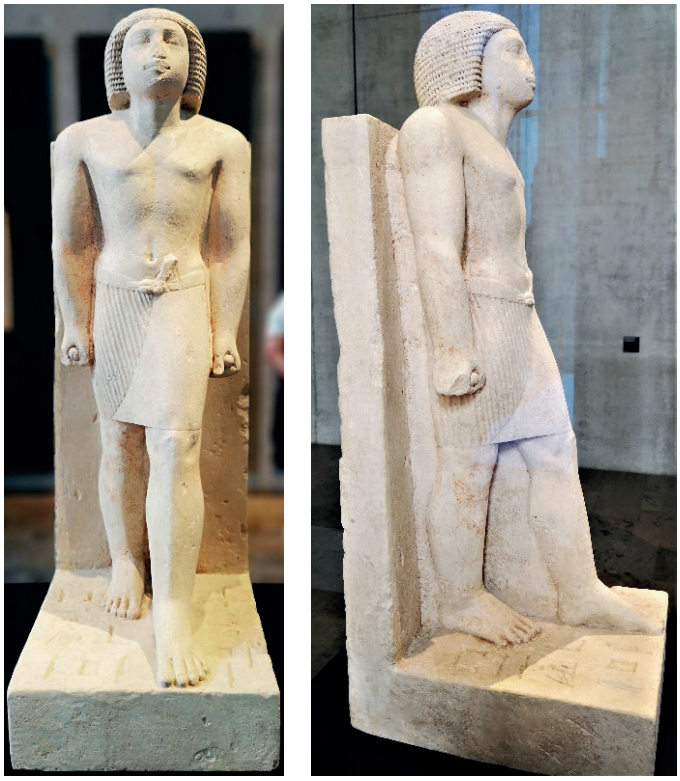

Figure 1. Standing-striding figure of the Court Musician Ipi (4th dynasty, limestone, Dahschur, inv. nr. 1600, Staatliches Museum Ägyptischer Kunst, München, Germany). Frontal (a) and lateral view (b) (Photos: A. Nerlich, with permission).

at the interface between the Great Western Desert and the irrigated Nile valley. The necropolis was used from the early period of the first pyramids that had been erected in Dahschur by Pharaoh Sneferu (4th dynasty, ca. 2700 – 2600 BCE). In close proximity to the cemeteries of the pyramids, high royal court officials were granted the right to build their own tombs in the necropolis area as a special favour of the Pharaoh.

The high court official "Ipi" (Fig. 1), musician at Pharaoh Sneferu´s court, built a *mastaba* for his family and household, several hundred metres south of the so-called "bent pyramid", one of the three pyramids that had been erected by the King. The tomb complex was first scientifically analysed by the American journalist and archaeologist Muses (Gabra, 1971, Muses, 2000) and later extensively re-analysed by the mission of the German Archaeological Institute Cairo by Alexanian (Alexanian and Seidlmayer, 2000 and 2002). These investigations proved the correct attribution of the tomb complex to Ipi, but also showed an extensive re-use of the burial complex in later periods into the Late Period (until 330 BC). The complete complex was found to contain seven burial sites, with burial DAS-9 being the by far largest tomb, and contained the original inhumation (Alexanian and Seidlmayer, 2002).

Unfortunately, during the first archaeological excavations (by Muses) the tomb complex DAS-9 had been opened and all the human remains were collected together and left mixed up, rather than separated as individuals. The subsequent excavations detected this pile of bones, removed all the human remains en-bloc, and provided this material as one sample for anthropological-paleopathological analysis. The subsequent excavation of the other tombs in the complex, DAS-8, DAS-11, DAS-12, G2, G4 and G16, were performed step by step; all human remains were collected separately and investigated as individual findings, although this material also showed significant previous grave robbery and destruction. In consequence, the present analyses comprised two sets of material; the mixed material from DAS-9, and the individual findings from the six other smaller burial sites.

Material

All material from the tomb complex comprised skeletonised human remains; there were no soft tissues. The isolated skeletal remains (except DAS-9) was evaluated for completeness of the skeletons (representability) and the condition of the bone material (state of preservation) using an established scoring system (Nerlich et al., 2015).

Methods

All skeletal material was subjected to an anthropological and paleopathological investigation as previously described (Nerlich et al., 2000). The mixed material was recorded and the numbers of skeletal elements were used to estimate the minimum number of individuals (MNI). In the isolated tombs, the number of individuals was determined by the (complete) skeletons detected. In order to estimate the number of individuals in the mixed material of DAS-9, all skulls were specially recorded for presence/ absence of a perforation of the lamina cribrosa of the nasal roof (the site for excerebration since the early New Kingdom, i.e. ca. 1500 BC) and/ or the presence of embalming substance residues within the skull (Ikram and Dodson, 1998). Thereby, a rough estimation of burials before and after 1500 BC could be performed.

All estimations for age and sex distribution were performed as previously described in detail (Nerlich et al., 2000) and in clear accord with suggested criteria (Buikstra and Ubelacker, 1996; Harbeck, 2014). For sub-adult individuals the criteria by Scheuer et al. (2010) were applied. Pathological changes in affected skeletal elements were recorded individually and differential diagnoses were established as previously described (Nerlich et al., 2000, Nerlich et al., 2015).

Results

Estimation of the number of individuals

In the tomb complex DAS-9 the mixed bones were first organised by anatomical site. There were at least 46 adult skulls present, 33 complete and 13 fragmented ones. There were 40 mandibles (either complete or fragmented), 43 right and 42 left femora, 37 right and 38 left tibiae, 43 right and 42 left humeri, 29 right and 34 left radii, and

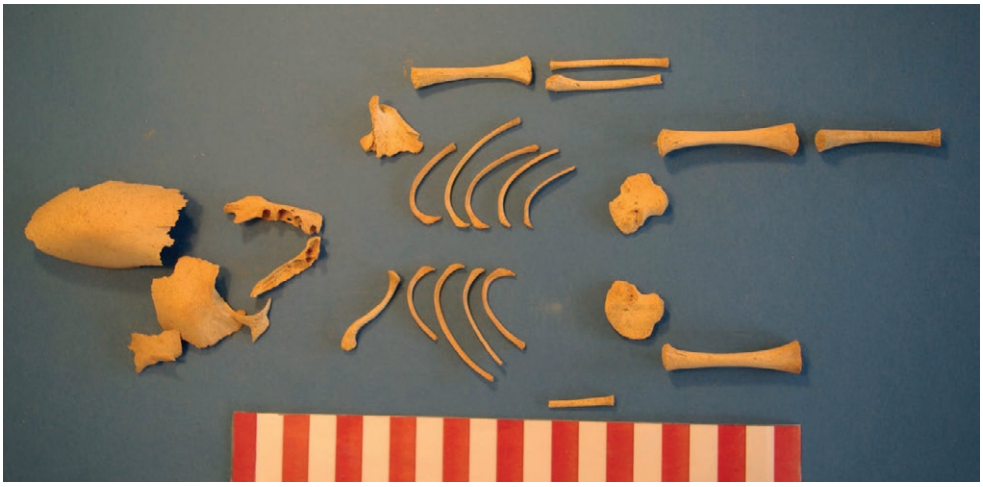

Figure 2. Fetal bones (7th gestational month) excavated from the tomb complex DAS-8. (Scale 1 cm).

41 right and 36 left ulnae. The number of complete or fragmented adult pelvic bones comprised 33 right and 29 left ones. In addition, at least four immature individuals could be identified; two *Infans I* (0-7 years), one *Infans II* (7-14 years), and one *Juvenis* (adolescent 14-18 years).

In total, there were at least (MNI) 46 adults and four sub-adults/ children in the mixed bone group. The macroscopic condition of the bone material suggests an overall good state of preservation, although for this material no further data on completeness or state of preservation could be evaluated.

For the other tombs the following observations were made. DAS-8 contained the burials of nine individuals, four adults and four sub-adults, three *Infans I* and *II* and one fetus (still-birth 7 months; Fig. 2). In DAS-11 only one adult burial was detected, while DAS-12 contained nine burials with five adults and four sub-adults *Infans I*. In the tombs G2, G4 and G16 each with one adult was found and adjacent to G16 one additional *Infans I* had been buried.

In summary, the complete tomb complex of the *mastaba* of "Ipi" contained the human remains of 73 burials, with 46 adults and four sub-adults in the main complex and 13 adults and 10 sub-adults in the adjacent parts of the tomb area. The rate of immature burials (fetus; Infans *I* and *II* and juveniles) was 10 out of 73 (17.8%).

Attribution to burial time periods

From time-specific embalming procedures 20 skulls of DAS-9 were attributed to the Old Kingdom and 23 to the New Kingdom until the Late Period; three skulls were not attributable. The four sub-adults could not be further classified due to major bone loss of the few residual and fragmented skull bones.

For the nine burials in DAS-8, the single burial in DAS-11, and the nine burials in DAS-12, there was no evidence of skull manipulations suggesting all may be of original

early burials dating to prior to 1500 BCE. At least one of the four burials in G2, G4 and G16 showed bitumen residue in the skull and seem to represent a later intrusive burial.

Representability and state of preservation

Representability could only be evaluated for the material in individual burials, but not for the material in DAS-9. In the 23 identifiable individuals only one was a fairly complete skeleton (30.8 points out of 42), in 10 cases moderately complete material (10 to 20 points out of 42 points), and in 12 samples little skeletal material (< 10 points out of 42 points). Therefore, most of those cases were only incompletely preserved.

The state of preservation in contrast was much better. Six individuals showed excellently preserved bone material (>70-100%), a further six good preservation (>50-70%), 10 samples were moderately well preserved (>30-50%) and only one case was poorly preserved (<30%).

Age and sex-distribution

In the mixed material of DAS-9, age estimation from skulls and pelvic bones in adults showed no significant differences between the Old Kingdom and the Late Periods with a mean age-at-death between 20 and 30 years (Fig. 3). However, the older material contained significantly more males than females when compared to the later material (p=0.02, Fisher Exact Probability Test) (Fig. 4).

The adjacent burials to Ipi´s tomb showed an age distribution that reflected the high rate of immatures which influenced the plot. The low number of adults has to be interpreted with great care (Fig. 5). Similarly, the sex distribution with mainly adult males (Fig. 6) obviously does not provide representative observations.

Paleopathological observations

The paleopathological findings of both study groups were combined since the material of the adjacent tombs to DAS-9 showed little evidence of pathology; two

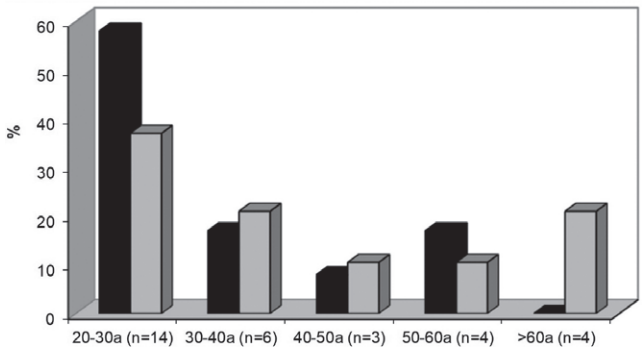

Figure 3. Age distribution of the skulls excavated in the tomb complex DAS-9, divided into material from the Old Kingdom and later material. (Black = Old Kingdom material; white = New Kingdom until Late Period).

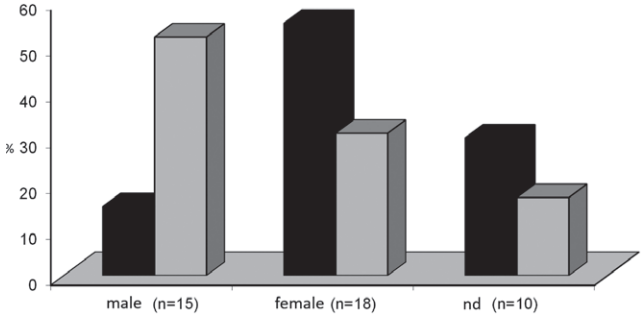

Figure 4. Sex distribution of the skulls excavated in the tomb complex DAS-9 (Black = Old Kingdom; White = New Kingdom until Late Period).

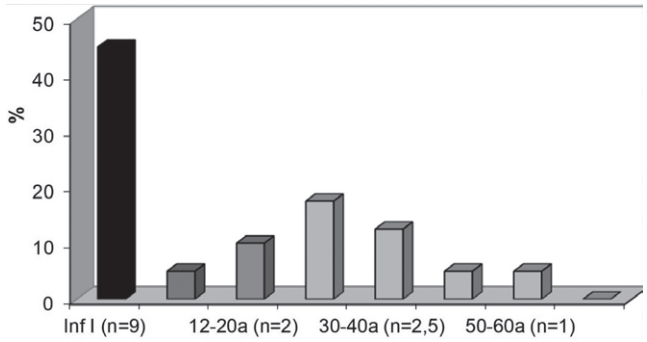

Figure 5. Age distribution of the human material from the tomb complexes except DAS-9(Black = *Infans I*; Dark grey = *Infans II*; Grey = Adults until mature age).

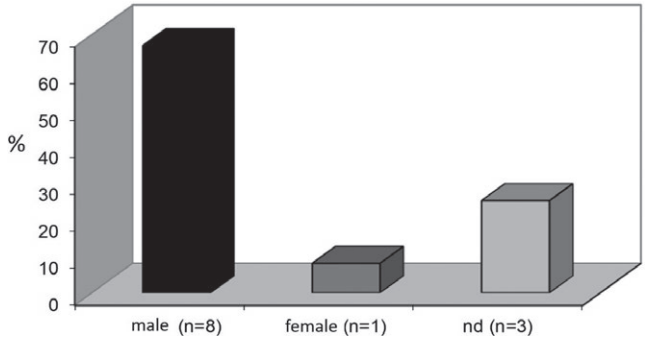

Figure 6. Sex distribution of the human remains from the tomb complexes except DAS-9 (Black = males; Dark grey = females; Grey = not determined).

Table 1. Summary of the pathological findings and anatomical variations.

Observation	DAS 9 N	Relative frequency %	Other tombs N	Total frequency %	Old Kingdom N	Later periods N
Spina bifida	3	6.5	0	5.1	0	0
Spondylolisthesis	2	4.3	0	3.4	0	0
Sutura metopica	3	6.5	0	5.2	2	1
Trauma	12+	27.9	2	24.1	0	0
Cribra orbitalia	5	10.9	0	7.4	2	3
Osteopenia	2+	4.3	3	7.3	0	0
Scurvy	2+	4.3	0	3.4	0	0
Arthrosis	36 out of 605	5.9	*	5.9	0	0
Spondylosis	85 out of 372	22.8	*	22.8	0	0
Nonspecific inflammation	3	6.5	0	5.2	0	0
Tuberculous Spondylitis	3	6.5	0	5.2	0	0
Benign Tumors	3	6.5	0	5.2	0	0
Malignant Tumors	2	4.3	0	3.4	0	0
Tumour-like lesions	2	4.3	0	3.4	0	0
Dental caries	5	10.0	0	8.6	0	2
Intra-vital dental loss	6	13.0	0	10.3	0	1
Dental abscess	11	23.9	1	20.7	2	3

NB:
Arthrosis = number of degenerated joints per the total number of joints evaluated
Spondylosis = number of degenerated vertebral bodies/ facet joints per total number of vertebral bodies investigated
*The adult joints and vertebrae in the adjacent tomb complexes were so poorly preserved that these were not included in this table.

traumatic injuries, three with osteopenia, and one with a dental abscess. A detailed summary of findings is reported in Table I. This contains not only the frequencies for the respective lesions, but also the burial period (OK vs. LP) where possible (i.e. in skull findings).

Out of these overall data, several specific observations have to be described more in detail. First, a high rate of traumatic sequelae was noted with 20 showing fracture healing in 14 skeletal sites either callus or bony ankylosis. In eight cases metatarsals and phalanges had posttraumatic reactions consistent with a stress (or march) fracture (Fig. 7), and a further march fracture in an individual from an adjacent tomb (DAS-12). Further fracture sites and types were; the rib, clavicle (three) (Fig. 8), and forearm (two ulnae, and one radius), and the fibula with a typical spiral fracture of the shaft. There was one bony ankylosis of the knee with bridging callus following fractures between the distal femur and the patella (Fig. 9). There was also a case of severe trauma to the shoulder with almost complete destruction of the head of the

Figure 7. Metatarsals with the 5th (on the left) showing a healed distal shaft transverse stress fracture (left, DAS-8).

Figure 8. Old healed short oblique fracture of the mid-shaft of the clavicle.

humerus and the glenoid with extensive new bone formation (Fig. 10). These last two injuries required considerable force, probably from an accident and survived for a long period afterwards despite significant disability in the right leg and right arm respectively.

Figure 9. Severe traumatic injury of the right knee joint with bridging ankylosis of the patella to the distal femur. Despite some *post-mortem* bone loss severe *in vivo* destruction of the joint is clearly evident. Note also the malunion of the lateral condyle of the distal femur indicating an old-healed fracture line. A: ventral view; B: dorso-lateral view.

Figure 10. Severe traumatic injury of the right shoulder joint. There is almost complete resorption of the humeral head presumably following avascular necrosis from a humeral neck fracture, plus severe deformity of the glenoid joint surface, consistent with a displaced fracture dislocation of the glenohumeral joint. A = anterior view; B = close-up posterior part.

Figure 11. Considerable osteopenia of the humeral head. Note the very thin metaphysel cortical bone and the loss of cancellous bone in the marrow cavity.

Evidence for metabolic disease was present as cribra orbitalia in five of the 46 skulls from DAS-9, mostly mild to moderate. Two of the affected individuals came from the Old Kingdom population, and the other three burials from the LP. Osteopenia, which in younger populations typically is due to vitamin D deficiency, was seen from DAS-9 in eight samples belonging to at least two individuals (typically multiple bones are affected) (Fig. 11), and in three individuals from adjacent tombs, included an *Infans I*. Since the latter also showed some bowing of the long bones, the diagnosis of rickets is most likely. Finally, at least two individuals showed subperiosteal new bone formation close to the joint which is typical of an ossifying haematoma from chronic vitamin C-deficiency (scurvy). Again several bone fragments were affected which must have belonged to at least two individuals.

A further group of paleopathological findings demonstrated degenerative lesions of the joints and the vertebral column. In the mixed bone material of DAS-9, 36 out of the 385 complete long bone joints mild to moderate arthrotic lesions were present. In seven joints severe osteoarthrosis was seen. The shoulder, hip and knee joints were the most affected. These data, however, have their limitations since considerable number of the joints were only poorly preserved and did not allow a detailed evaluation. This held particularly true for the material from the tombs outside DAS-9. Similarly, most vertebral bodies and facet joints did not show degeneration. In the spine, degeneration was mostly seen in 34% of lumbar, 24% of cervical, and 10% of thoracic vertebrae.

Inflammatory reaction in bones and joints were clearly seen in six samples from three individuals with typical non-specific inflammatory bone changes and three with probable tuberculosis. The latter all affected the spine with destruction of the anterior part of the vertebral body; two upper lumbar and one lower thoracic (Fig. 12). All came from DAS-9.

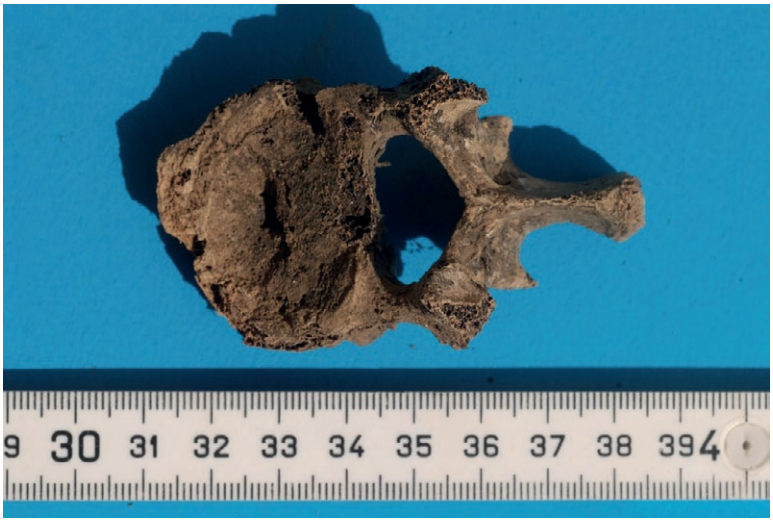

Figure 12. Lumbar vertebral body with major destruction mainly affecting the ventral part including the end (ground) plate. This type of lesion leads to a kyphotic spinal deformity and is very suggestive of spinal tuberculosis.

The final group of pathological lesions, which have a great clinical impact in modern medicine, were bone tumours, either primary or metastases, which have to be distinguished from tumour-like lesions. In the material from Ipi´s tomb complex, all three types of lesions were detected. First, there were two femora with metaphyseal osteochondromata with 19x11 mm and 15x4 mm bony spurs respectively. Then there was an Old Kingdom skull with an incomplete osteolytic lesion of the inner table and an adjacent large blood vessel bony impression consistent with the diagnosis of meningioma. The blood vessel impression was outside the area where normal blood vessel impressions (Pacchionian granulations) are typically seen. Although meningiomata are currently classified by the "Union International Contre le Cancer" (UICC) as semi- or low-grade malignant, most meningiomata have a benign clinical course. In the material from Ipi´s *mastaba* a typical tumour-like lesion close to the shoulder was a well-defined lobulated bone cyst with a smooth inner wall. An Old Kingdom male skull showed thickening of the inner table of the frontal bone (hyperostosis frontalis), another tumour-like lesion.

In addition, there were two lesions highly suggestive of malignant growth. The first was a large cauliflower-like lobulated tumour of the lower pelvic bone (os ischium), 39 x 24 x 11 cm in size, which extended beyond the outer cortex into the adjacent soft tissues (Fig. 13). The location and morphology strongly suggested the diagnosis of a well-differentiated chondrosarcoma; a large malignant tumour arising from cartilage with secondary calcification and reactive bone formation. These tumours are rare and become more frequent with increasing age. They most often affect the pelvic girdle and usually grow slowly without metastasising until they dedifferentiate when they spread haematogenically.

Figure 13. Tumour arising from the os ischium of the right pelvis with an irregular extension of the bone surface with protrusion into the soft tissue. This type of lesion is strongly suggestive of a chondrosarcoma of the pelvis. A = caudal view; B = lateral view.

Figure 14. Three lumbar vertebrae with the bodies showing irregular osteolytic defects in the central to posterior part, highly suggestive of spinal metastases. Type and location excludes degenerative lytic lesions as well as Schmorl´s nodes.

The second case of suspected malignancy affected several vertebral bodies with confluent small osteolytic lesions (Fig. 14). The most likely diagnosis is metastases from a malignant solid organ tumour. Lytic bony metastases have to be distinguished from osteoblastic (bone forming) ones. However, there no indicators for age, sex or any other possibly affected skeletal elements, so the underlying primary tumour is not certain.

Finally, teeth not only allow evaluation of nutrition, but may show distinct pathological lesion such as dental loss, abrasion and caries. The latter are frequent findings in most historic populations, mostly due to poor oral hygiene and the use of stone mills for bread making. In the DAS-9 material, dental caries was seen in five individuals of DAS-9 (11 %), while none of the other burials were affected (9%). This parallels the rate of *in vivo* dental loss, mostly from caries; in DAS-9, 13%, and other burials, 10%. Dentogenic abscesses were seen in 11 individuals from DAS-9 (24%) and the other burials (21%). Dental abrasion in these younger individuals was surprisingly more pronounced than in older ones, possibly as the result of differences in social ranking.

Discussion

The investigation of human remains using a combination of anthropological and paleopathological techniques offers a direct approach to understanding the life and living conditions of historic populations. Ancient Egyptian populations are ideal because their funerary arrangements provide, not only human remains, but pictorial and written material. A number have already been investigated (Smith and Jones, 1908; Rösing, 1990, Parsche, 1991, Nerlich et al. 2000). This paper presents a further collection of human skeletal material which, despite some being disrupted and mixed, still provides useful and important data. It also shows the advantages of modern day excavation techniques using careful planned recovery and storage of human remains for further analysis.

The tomb complex under investigation was of a high royal court officer named Ipi dating to the Old Kingdom (4th dynasty, c. 2700 – 2200 BC). Both the archaeological investigation (Alexanian, 2006), as well as the present anthropological-paleopathological study, support the notion that the *mastaba* was initially used for Old Kingdom burials, namely that of Ipi, his family, and possibly members of his household. However, as seen very frequently in ancient Egyptian necropoli (Nerlich et al., 2000), later intrusive burials destroyed part of the tomb complex and may also have disturbed the original ones.

The complete excavation of the tomb complex showed the human remains of at least 73 individuals, the number being determined by Harbeck's anthropological analysis (Harbeck, 2014). Since ancient Egyptian embalming techniques changed considerably at the end of the Middle Kingdom to the New Kingdom period, i.e. ca. 1500 BCE (Ikram and Dodsen, 1998), it was possible to divide most of the skulls into two separate sub-populations; one presumed from the OK period, and one of later burials which may be dated into the Third Intermediate to Late Period (LP) (Ikram and Dodsen, 1998). From this, 22 burials were assigned to the OK, and 24 burials to the LP periods.

The sex distribution in the OK group of DAS-9 significantly favoured females but was near equal when the other OK burials were included. It may well be that the initial incomplete excavation in 1957 led to an unequal retrieval of the material and that the combination of both groups presents a more realistic population distribution. This is supported by the addition of immature skeletons of both settings coming to a total of 14 individuals (ca. 18% of all individuals), covering a broad range between one fetus through to two juveniles.

Most interestingly, the estimated sex ratios and the age at death distribution were not significantly different between the "OK" and the "LP" (at least as far as this can be reconstructed) indicating that basic living conditions could have been comparable between both periods. This is similar to previous data which also showed that most adult individuals died between 20 and 40 years, an immature ratio of between 10 – 20% and a balanced sex ratio in various populations of the Nile valley (Smith and Jones, 1908; Rösing, 1990; Parsche, 1991; Nerlich et al. 2000).

A repeated, but nevertheless surprising observation, was the low rate of infants and sub-adults in the study population. A similarly low rate of immatures was seen in various previous studies of between 10 and 30% with minor differences between pre-dynastic to Christian period in Nubia (Smith and Jones, 1908). The analysis of a large necropolis in Lower Egypt (Minshat Abu Omar in the Nile Delta region, Parsche, 1981) revealed an infant rate of 32%. Several Theban necropoli closely resembles the present data with an immature rate of 19% which is also significantly lower than supposed from Egyptian data of Khedive Period around 1900 CE where the immature death rate ranged at about 70% (Smith and Jones, 1908). The low amount of immature mummy residues may be due either to a rather selective loss of the much more fragile skeletal elements of the infants to adults, or a truly lower rate of immature deaths than expected, or that not all immature individuals were buried in the tombs. The latter has been observed since in-house burials of infants have been detected in the ancient Egyptian city of Elephantine, Aswan (Schulz and Schmidt-Schulz, 1993) or as separate cemeteries for infants as seen in Deir-el-Medineh (Bonnet and Valbelle, 1976). However, since the conditions of tissue preservation in general are excellent with storage in a dry, arid climate, the amount of lost infant skeletons can assumed to be limited. This may be supported by the presence of fetal and neonatal bones despite being the most fragile ones that should perish quickly. The answer to this open question awaits further archaeological findings.

The pathological findings in the individuals of the Ipi tomb complex need to be compared with the findings in other populations. The rate of dental pathology seems to be slightly lower in the Ipi population of Dahschur when compared to the Aswan and Theban material. Nevertheless, the severe abrasion of teeth, in combination with the low age at death, indicates the effects of stone mill debris and sand inclusion in the daily diet. The rates of dental caries, dental apical abscesses and *in vivo* tooth loss are comparable to those of other ancient Egyptian populations.

In contrast, the population buried in Ipi´s *mastaba* contained a high number of traumatic sequelae (24%) which clearly exceeds those of previous ancient Egyptian studies e.g. 3% in the Nubian material of Smith and Jones, 1908, 9 % in the necropolis of the Qubbet-el-Hawa(Rösing, 1990), and 16% in the Theban necropolis(Nerlich et al., 2000), but which is comparable to a study on Nubian material, south of Aswan, dating in the Late Period until Christian time with 21% trauma cases (Alvrus, 1999). The reason for the increased trauma rate in the Dahschur material may relate to the type of fractures; most occurred in the small bones of feet and hands and may be the result of working injuries during the building and/or maintaining of the necropolis. The two cases with severe trauma injuries of the shoulder and knee joint however were due to high energy mechanisms, such as falls from a height, or a blow by a heavy or speeding vehicle and would have led to severe, debilitating loss of the func-

tion of the affected limb. To have continued in the community would have required the individuals to have a supportive social network.

As seen in previous studies on ancient Egyptian human remains, significant bone changes consisted with chronic metabolic disease were found; typically, this is due to chronic anaemia or vitamin deficiencies. Cribra orbitalia which is assumed to occur as a result of chronic anaemia, were noted in around 7%, which is lower than in other populations at 15-20% (Parsche, 1981; Nerlich et al., 2000). The most likely cause for chronic anaemia is chronic infection, in particular parasitic infections such as schistosomiasis (Ruffer, 1910), pulmonary parasitosis (Nerlich et al., 1995), leishmaniasis (Zink et al., 2006) and chronic malaria (Nerlich et al., 2008).

Similarly, the rates for osteopenia and periosteal new bone formation at the metaphyses of long bones were also considerably high. Periosteal new bone formation is characteristically seen in scurvy Around 4% of individuals were found to have this abnormality, which is less than found, for instance, in the Theban necropolis at 8 - 11.5%. In a country with a wealth of vitamin C-containing fruit, nutritional deficiency is unlikely; thus it is more likely to be due to disorders that "consume Vitamin C" such as in chronic infectious diseases. Interestingly, in previous cases with molecularly proven tuberculosis (Nerlich et al., 1997), periosteal new bone formation was seen e.g. in the distal femora. Osteopenia is a feature of several pathological conditions, in particular pre-osteoporosis (mainly as senile or hormonally induced), but can also occur in chronic vitamin D-deficiency (osteomalacia) or overactivity of the parathyroid glands (hyperparathyroidism). Since this study population were mostly young when they died (see above) then the osteopenia is much more likely to reflect chronic vitamin D-deficiency and osteomalacia. Since vitamin D-deficiency may also result from chronic intestinal infection, such as with parasites, then the deficiency is more likely to be from intestinal worms than lack of sunlight.

In this study the rate of inflammatory osseous lesions was low at around 5% with half of these of tuberculosis. This is still significantly higher than that seen elsewhere. Smith and Jones' (1908) in their series of 6000 ancient Egyptian mummies/skeletons specimens showed no inflammation at all or any osteomyelitis. This has already been extensively discussed in previous papers on various ancient Egyptian human remains (Nerlich and Lösch, 2009; Zink et al., 2005). However molecular testing (Zink et al., 2005) supports the view that the high incidence of tuberculosis in ancient Egypt fits in with the present study's findings.

The osteoarthrotic and spondylotic bone changes found in this study in a relatively young population implies extreme mechanical loads on the large joints especially the hip and knee, and the spine. The rate of affected joints, and the degree of degeneration, is in keeping with previous study populations. Likewise, populations of several Theban tomb complexes showed a joint osteoarthrosis rate of 4 – 7% (Nerlich et al., 2000; Polz et al., 1999; Zink and Nerlich 2002) which is comparable to the Dahschur material from Ipis tomb complex at 5%. Degenerative lesions of the vertebral column (spondylosis) were 23% in the Dahschur material and 12 – 36% in the Theban material (Nerlich et al., 2000, Polz et al., 1999; Zink and Nerlich 2002). The Ipi's *mastaba* complex rates suggest comparable mechanical load and stress on joints and the vertebral column to those of the New Kingdom until Late Period tomb material from Thebes. Furthermore, the fairly constant figures suggest only little changes in the work load of populations of comparable social status and possible function over many centuries of ancient Egyptian history.

Finally finding a number of benign and malignant tumours affecting the skeleton in this study was unsurprising since all other previous studies on larger series of human remains were able to detect tumours. Although repeatedly discussed, tumours, in particular malignant ones, have been seen in the majority of ancient populations (Nerlich, 2018), but the relatively high frequency should be noted (and is at variance to the interpretations by David and Zimmerman, 2010). While there do not exist comparable data on benign tumours, the malignant tumour rates have extensively been documented and discussed (Strouhal, 1976; Waldron, 1996; Nerlich et al., 2006; Nerlich, 2018). The identification of two cases of malignant tumours, one a genuine osseous tumour (the pelvic chondrosarcoma and the other metastatic infiltration of the spine, add to the previous lists of malignant tumours found in ancient Egypt and strongly support the notion that malignant tumours were not rare at that time especially as the age at death was much younger than today (Waldron, 1996; Nerlich, 2018).

In summary, the analysis of all the available human material from a large tomb complex of the necropolis of Dahschur, erected by Ipi in the Old Kingdom, presents novel data on the structure and the occurrence of distinct diseases in that population. Beyond some basic data on the structure of the population and further observations of an unexpectedly low rate of immature burials, this study adds information to the spectrum and frequency of diseases in ancient Egyptian populations, with the surprisingly comparable types and rates of diseases with other populations of the Egyptian high civilisation over almost 3,000 years. Therefore, this study concludes that life and living conditions were very comparable over a long period of time.

This study shows that analysis of poorly curated material with intermingling of skeletal remains at a burial site can still provide useful data that enhances the knowledge of the lifestyle and diseases of an ancient population.

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The limping nuns. Two cases of hip dislocation in a medieval female monastery

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Abstract

We observed the upward displacement of the femoral heads in the skeletal remains of two females unearthed from the medieval cemetery of the monastery of Santa Maria in Valle, in Cividale del Friuli (North-Eastern Italy). Examination of bone vestiges suggests the diagnosis of hip dislocation as the consequence of developmental dysplasia of the hip. In addition, in the youngest subject the first sacral vertebra appears lumbarized and shows the unilateral defect of the right pars interarticularis. Developmental dysplasia of the hip is one of the most common congenital diseases of the musculoskeletal system in newborns. Findings suggest that the skeletons belong to two nuns, who perhaps enter monastic life precisely because of their pathology.

Keywords

hip joint; developmental dysplasia; lumbosacral transitional vertebra; spondylolysis.

Introduction

Cividale del Friuli is a small and ancient town located near Udine, in the North-Eastern Italy, close to Slovenian border. According to tradition it was founded by Julio César and called *Forum Julii*, but Cividale dates back to before the Roman times, as documented by archaeological findings remounting to Palaeolithic and Neolithic times. Occupied in 568 by the Lombards led by King Alboin, *Forum Julii* became the capital of the first Lombard Duchy. In the 8th century, after the victory of the Franks, the city changed its name to *Civitas Austriae*, which today has become Cividale. From 737 to 1238 it was the residence of the patriarchs of Aquileia. It fell under Venice domain along with the rest of Friuli in 1419-20. In 2011, Cividale was declared UNESCO World Heritage Site as part of the "*Italia Langobardorum*" project.

Probably in the middle of the 7th century, the Lombards founded the Benedictine monastery called Santa Maria in Valle which, in the medieval period, became one of the two most important female monasteries in the region, achieving significant prestige and power thanks to numerous donations. Female monasteries often provided shelter and food for orphans, old people, poor and beggars as well as medical care. Santa Maria in Valle always remained a women's convent, in which lived women of high social status (Tilatti, 2002). The number of the nuns was not particularly high,

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Figure 1. The skeletons of MSM5 (left) and MSM12 (right). In both images the anomalous position of femora is well appreciable, particularly on the right side.

probably around twenty; the nuns lived in the monastery until the end of the 20th century (Quendolo and Villa, 2009).

In 2008, an archaeological excavation revealed the presence of tombs. Only part of the cemeterial area of the monastery was investigated. Few good graves were found, mostly parts of clothing (buttons, buckles), and a coin dating from the second half of the 10th century. The purpose of this article is to depict two cases of hip dislocation in developmental dysplasia in the skeletal remains of two females (Fig. 1).

Materials and methods

Overall, skeletal remains belonging to 34 individuals, 11 subadults and 23 adults, have been examined.

The skeletons object of the present study are named respectively MSM5 and MSM12. Sex estimations has been conducted using morphological characteristics of the pelvis as well as morphological and metric characteristics of the skull and the post-cranial skeleton. The age-at-death was estimated with standard physical anthropologi-

cal methods (dentition, long bone length, epiphyseal closure, pubic symphyseal face and sacroiliac joint morphology, root dentine transparency, cranial suture obliteration) (Lamedin, 1992; Buikstra and Ubelaker, 1994; Scheuer and Black, 2000). Calculations for stature were performed using long bone measurements. The analysis of metric and non-metric morphological characters was also carried out, as well as the study of the activity markers. All bones were analysed for the presence of pathologies and traumas.

In MSM12, the femoral neck anteversion, which indicates the degree of torsion of the bone, has been assessed by determining the angle between the projection of the line going through the proximal femoral neck region and the surface of the lab bench representing the transepicondylar axis. The angle has been measured on digitally photography in cephalocaudal view (Unnanuntana et al, 2010; Scorcelletti et al., 2020)

Results

MSM5. The skeletal remains are poorly preserved, and many bones are damaged or absent. The remains of MSM5 are those of an adult female (>50 years old); her stature, calculated with the formula by Trotter and Gleser (1958) from the ulna, was 158,5 cm. Muscle attachments are well developed. On the right humerus and on both ulnae the attachments are pronounced (the left humerus was not preserved); the left femur exhibits a prominent *linea aspera*.

The left hip, fragmented and damaged, displays a smooth, shallow depression located posterosuperiorly to the acetabulum. The preserved upper edge of the acetabulum results irregular and flat (Fig. 2). Although in a fragmented state, the head

Figure 2. MSM5, comparative view of the lateral surfaces of the left hip bone of MSM5 (right) and a normal hip bone (left). A shallow depression is appreciable on the posterior superior aspect of the left wing of MSM5 (black arrow). The acetabular roof slopes upwards (white asterisk).

Figure 3. MSM5, left femur. Sagittal view. The head, largely fragmented, appears flat.

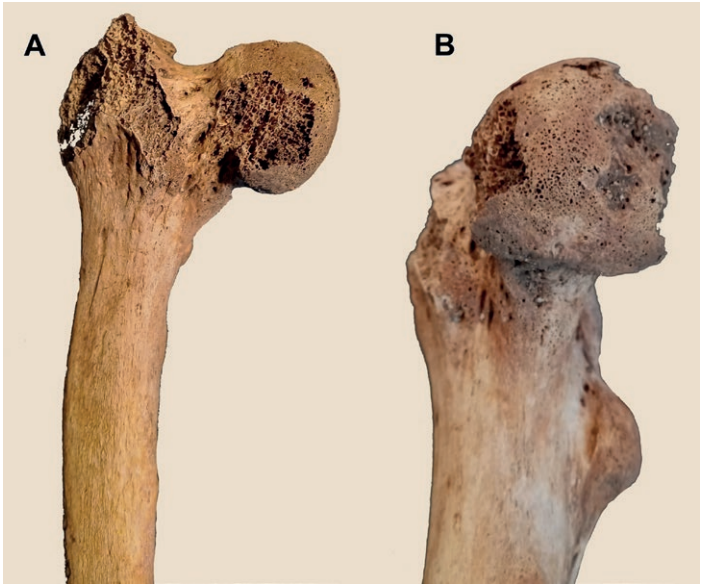

Figure 4. MSM5, right femur. A) Coronal view; B) Sagittal view. In A), ovalization of the femoral head and short neck are well appreciable. In B) Porosities and degenerative changes of the head.

of the left femur appears deformed, with a markedly flattened area (Fig. 3); the less trochanter is very irregular, with macroporosities and spicules (Fig. 3). The great trochanter is too damaged to be evaluated.

The right hip lacks. The right femoral head is small and oval, with porosities and eburnation areas indicative of osteoarthritic changes (Fig. 4). The neck is short.

The few vertebrae that could be examined show large, horizontal osteophytes originating from the margin of the bodies.

MSM12. The skeletal remains are well enough preserved. The remains are those of a young female, 15-16 years-at-death. The muscular attachments are regular, indicating a normal muscle development.

Some malformations can be identified. In the spine, the failure of the first sacral vertebra (S1) that fuses with the sacrum, leaved it with only four sacral segments, being present an extra lumbar vertebra (lumbarization of S1) (Fig. 5). Additionally, the lumbarized vertebra displays the unilateral defect in the right *pars interarticularis* (spondylolysis) and a posterior arch schisis (Fig. 6); the fourth sacral vertebra shows a posterior schisis.

Hip bones are incomplete. The right iliac wing exhibits a deep, rounded depression with degenerative changes and areas of pitting (Fig. 7). The right femoral head is oval and shows marginal osteophyte development; the neck is short (Fig. 8). The femoral neck anteversion is increased (anteversion angle 54.4°) (Fig. 9). The right tibia and fibula do not present alterations.

Figure 5. MSM12, lumbar spine. Sagittal view. Lumbar spine is formed by six vertebrae.

Figure 6. MSM12, L6. Posterior anterior view. Schisis of the spinous apophysis and defect of the right pars interarticularis (spondylolysis) (black arrow).

Figure 7. MSM12, right hip bone, lateral surface. The secondary acetabulum is well visible on the iliac wing (black asterisk). The true acetabulum, incomplete, looks flat (white asterisk).

Figure 8. MSM12, femora, coronal view. Comparative view of the proximal epiphysis. The black arrow indicates osteophyte developing of the inferior margin of the right head.

The left iliac wing displays a thin, irregular layer of bone on the cortex (Fig. 10).

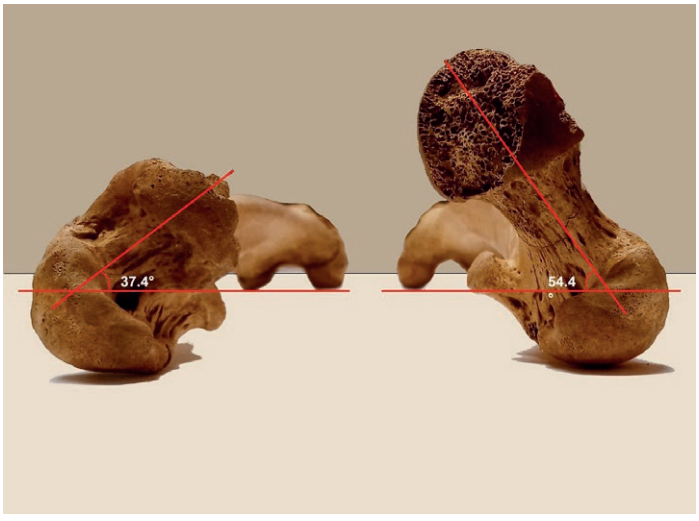

Figure 9. MSM12, femora, cephalocaudal view. Anteversion angles.

The left femoral head is small and deformed; the neck is short (Fig. 8). The femoral neck anteversion is increased (anteversion angle 37.4°) (Fig. 9). The left tibia and fibula do not present alterations. The lesser trochanters are clearly different in shape.

Examination of the rest of the skeleton was unremarkable.

Discussion

The concomitant presence of a false acetabulum in MSM5 left hip (the right hip is missing) and in both hips of MSM12, the aspect of the preserved portion of the true acetabula and the alterations of the proximal femora are consistent with the diagnosis of hip dislocation in developmental dysplasia of the hip (DDH). In DDH, the acetabular cavity is too shallow and the acetabular roof undeveloped, leading to superior dislocation of the femoral head in some instances. In time, the contact between the head and the ilium wing results in formation of a false acetabulum (Fig. 11) The femoral head becomes deformed and the whole geometry of the proximal epiphysis changes, leading in many cases to an increase of anteversion and neck-shaft angles (Clohisy et al., 2009). Even if the incidence varies largely between populations, DDH is considered one of the most common congenital diseases of the musculoskeletal system in newborns. In Italy, the incidence is around 1% and the disease is bilateral in 30-40% of the cases (Farsetti, 2021). Female gender (male to female ratio approximately 1:6), family history, limited foetal mobility, breech position in the last trimester are risk factors for DDH, which shows different severity ranging from a complete dislocation at birth to asymptomatic acetabular dysplasia in adult. (Yang et al., 2019; Rubini et al., 2021). In some cases, DDH occurs with neuromuscular diseases and

Figure 10. MSM12, comparative view of the lateral surfaces of the left hip bone of MSM12 (right) and a normal hip bone (left). The black circle indicates a patchy layer of bone on the posterior superior aspect of the left wing. The acetabular roof seems to slope upwards.

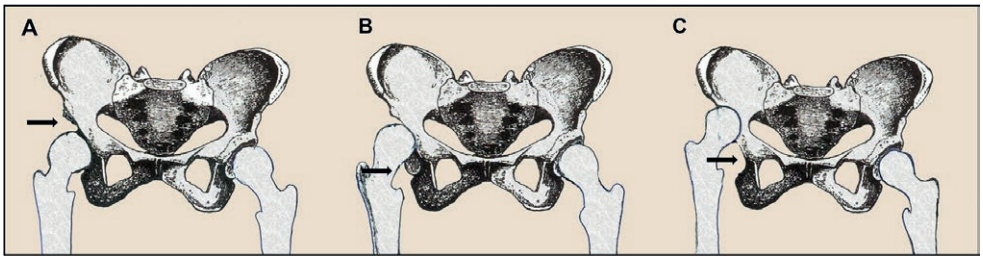

Figure 11. Natural history of DDH. A) The left hip joint illustrates the normal relationship between the femoral head and the acetabular cavity. The right hip shows a flattening of the roof of the acetabular cavity (black arrow), but the femoral head is in the normal position (dysplasia). B) The left hip joint illustrates the normal relationship between the femoral head and the acetabular cavity. The right joint shows the displacement of the femoral head, with some contact between the articular surfaces (subluxation); the true acetabulum is partially empty (black arrow). C) The left hip joint illustrates the normal relationship between the femoral head and the acetabular cavity. The right femoral head is completely displaced, and a false acetabulum is present on the iliac wing (dislocation). The true acetabulum is undeveloped and shallowed (black arrow).

syndromic and nonsyndromic disorders, especially those characterized by joint laxity (i.e. Marfan syndrome, Ehlers- Danlos syndrome) or, conversely, by joint contractures (Castriota-Scanderbeg and Dallapiccola, 2005). Another causal or contributory factor of DDH is the traditional practice of the tight swaddling of infants until walking age, widespread in Middle Ages. Swaddling infants with the lower legs in adducted and extended position results in an altered contact between the acetabulum and femur and may increase the risk of hip dysplasia and dislocation (Clarke, 2014).

The most severe form – that is congenital dislocation – consists of a condition known since ancient times, already described by Hippocrates (400 - 300 BC) (Musielak et al., 2015). Between 1935 and 1936, Marino Ortolani, an Italian paediatrician, first evaluated, diagnosed, and began treating hip dysplasia. Even today, Ortolani's manoeuvre is utilized to assess hip instability in newborns (Spina, 2009).

Symptoms of dislocation depend on age. They may include limping, different lower leg length, limited flexibility in the hip joint. It may cause little or no pain for years, but eventually leads to osteoarthritis.

Diagnostic criteria for developmental dislocation of the hip on skeletal remains are described by Ortner (2003) and, more recently, developed by Mitchell and Redfern (2008). Specifically, Mitchell and Redfern (2008) described the characteristics of the true acetabulum (small, oval or triangular, shallow and with little roof), the false acetabulum and the proximal femur. Located superiorly or posterosuperiorly to the true acetabulum, the false acetabulum can have four different morphologies: smooth and shallow depression (type 1), fine layer of bone without a depression (type 2), elevated bony plaque (type 3), deep and round cavity (type 4). The femoral head can conserve good proportions but results oval in anteroposterior plane in type 4 acetabulum, or be flat or mushroom-shaped in type 1, 2, 3.

The present case MSM 5 exhibits a shallow depressed area on the lateral aspect of the left iliac wing (type 1); the true acetabulum is quite incomplete; the only visible part of the roof is deformed and receding. Regrettably, the right hip is missing. Both femoral heads, even if incomplete, are deformed, with flat aspects (the left one) and

asphericity (the right one). Degenerative changes are appreciable, indicating the use of the joints.

Both hips of MSM 12 are present. On the right iliac wing, a deep, rounded depression is evident, indicatives of a type 4 acetabulum; the left one exhibits a fine layer of new bone (type 2 acetabulum). Howsoever, different types of false acetabula have been documented in the same individual (Mitchell and Redfern, 2008). The preserved parts of the true acetabula appear flat and irregular. Accordingly with the different morphology of the false acetabula, also the proximal femoral epiphysis shows different aspects: the right femoral head, incomplete, seems nearly normal in size but ovalized and, despite the young age, initial osteoarthrosis changes are present along the inferior border. These aspects are congruent with the type 4 of acetabulum. The neck is short and the anteversion angle (value 54.4°) largely exceeded the normal value of about 15°. The left head is severely deformed, the neck is short and the anteversion angle measures 37.4°. The right lesser trochanter is more elongated, while the left one seems to be in a higher position. Remarkably, MSM12 also displays a lumbosacral transitional vertebra. A lumbosacral transitional vertebra occurs as either sacralization of the lowest lumbar segment or lumbarization of the most superior sacral segment of the spine. It arises because of mutations in the Hox genes (Gopalan et al., 2018). In the present case, the first sacral vertebra is lumbarized and presents a unilateral defect in the right pars interarticularis (spondylolysis) and a posterior arch schisis. A recent study has demonstrated the association of the increased frequency of spinal anomalies, seen on standard hip radiographs, and DDH (Sun et al., 2021). Specifically, acetabular deficiency characterized by anterior acetabular undercoverage can result in anterior pelvic tilt and compensatory hyperlordosis which, in turn, enhance axial load on the pars interarticularis (Sun et al., 2021). Moreover, hip osteoarthritis, even at a young age, appears to be more frequently associated with this type of acetabular deficiency (Jessel et al., 2009). A similar case has been described by Wakely (1993) in a medieval female.

In the present cases, the post-mortem damage of the bones does not certainly allow to rule out a traumatic cause of the dislocations. However, it seems very unlikely: bilateral traumatic dislocations are very rare with few cases reported in literature. A high energy trauma is needed and nowadays it occurs more commonly among young male patients, due to vehicle accidents (Buckwalter et al., 2015).

Mostly sporadic cases of dislocations caused by DDH have been described in paleopathological literature, but in two large skeletal series excavated from a London cemetery and from Digne (France), the prevalence of dislocation in developmental dysplasia was found to be 2.7‰ and 13.1‰ respectively (Mitchell and Redfern, 2007; Mafart et al., 2007). To explain the occurrence of two cases in 34 individuals (prevalence 5.8%) we should remember that, from the 6th century, the oblation, that is the offering to God and therefore to the monasteries, of boys and girls was widely practiced, especially in Benedictine monasteries. Carried out by the parents, until the Council of Trent (1563) it had an irrevocable character. At first, only the children member of upper class or noble families were oblates, and a large gift (money or lands) was expected to accompany the child. Later, the oblation was extended and the poor were allowed to donate their children. In many cases, the oblation was a method to safeguard the well-being of other siblings or the family. Daughters were more often oblates, even by wealthy family. Frequently, the oblates children were

physically deformed or mentally defective, and it was believed that children born deformed were the result of incorrect behaviours (i.e., conception occurred during the menstrual cycle) (Boswell, 1984; McCracken, 2003). However, not all the nuns were child oblates: in many cases they were adult converts, often widows (Osheim, 1983).

Hence, we assume that the two cases here presented concern two women that during childhood, or later, became nuns, probably due to the pathology they suffered. Since in DDH genetic factors play an important role, a consanguinity cannot be excluded.

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Anatomy and Ceroplastic School in Bologna: a heritage with unexpected perspective

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Abstract

Ceroplastic, the art of wax modelling, is a depiction mode that has been used widely throughout time for artistic, religious and social purposes. During the 16th Century, ceroplastic became also an essential tool for medical education thanks to the creation of the first anatomical wax models. For centuries, anatomists have worked with artists with the aim of producing faithful and long-lasting wax artefacts that reproduce physiological and/or pathological conditions. Important figures such as Ercole Lelli and Anna Morandi Manzolini refined the techniques of the anatomical wax modelling and created works that are to date preciously conserved. The scientific and didactic value of these works was indisputable, for wax anatomical models have deeply facilitated the education of medical students in that time. Nowadays, a similar impact on medical education is given by new technologies such as augmented reality, that allows the addition of virtual contents to tangible anatomical 3D models. Under this light, anatomical wax models and augmented reality can be compared as two innovative tools that have changed the history of anatomical teaching.

Keywords

anatomy; augmented reality; ceroplastic; history of medicine.

A brief history of ceroplastic art

Ceroplastic is the ancient art of wax modelling. Ceroplastic has been used for a long time in the funerary, devotional and portraiture fields but, in the 18th and 19th centuries, it became also a useful and frequently used tool for the reproduction of anatomical, zoological and botanical models for didactic purposes. Wax readily availability as well as its properties of malleability and ductility have always favoured its use for various purposes. Indeed, wax has always been considered a precious material: for thousands of years it has been used as a functional tool to protect the wood of ships and to prevent weapons from oxidation. Additionally, its properties have also been applied in the dyeing process, in agriculture and in writing. Ancient populations such as the Greek, the Egyptians, the Phoenicians and the Romans modelled wax to make amulets, bases for metal casting of jewels and coins and small toys for children. The Greeks used wax to build dolls (*pupae*) used to decorate the graves of young women. *Pupae* were playful symbols, but were also considered as a company during the journey to Hades. In the courtyards of the patrician Roman residences, within individual

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niches on the walls, it was customary to place portraits depicting the ancestors of the family, created thanks to wax-based masks glued on the faces of the dead; this tradition permitted in time to build sorts of family trees which allowed the deceased's family members continuous, even though illusory, presence in the house and also their participation to domestic life and to the most important family events. During the Middle Ages, wax gained a particular relevance in the context of witchcraft, since life-size portraits of demons were used in rituals that ended with their liquefaction to symbolize the cast out of the demon depicted (Simonetti, 2012). Given its various applications, in time wax modelling became a real sculptural art that, although considered minor and being practically ignored by critics until 1911, for centuries has produced works decorated and carefully defined in the smallest detail.

The wax modelling technique applied to human anatomy

At the beginning of the 16th century the numerous opportunities offered by the material allowed its use not only in the visual arts, but also in the scientific field. Indeed, Leonardo Da Vinci was among the firsts to inject melted wax through a syringe into the ventricular cavities of the brain and heart to investigate their internal shapes (Bramley, 2005) . The techniques used for anatomical wax modelling varied according to the periods and the different workers (Maraldi, Mazzotti, Cocco, & Manzoli, 2000). Wax was initially used in substitution of or associated with other fluid substances to inject the vascular system. Obviously, this technique is particularly suitable for showing the vascular tree of parenchymal organs, such as kidney or lung, but does not allow the reproduction of the whole organ structure. However, the technical basis for the wax anatomical modelling is surely derived from vascular injection. Two main techniques were developed in time: the superimposition of wax handmade models of organs on the original skeletal bones and the fusion of the wax into a plaster mould of the organs. The first technique was used by Ercole Lelli's School in Bologna, the second by Clemente Susini's School in Florence. In the first case, the specimens are the only copy extant; in the other, several copies could be obtained. While the use of skeletal bones, reinforced by a metal scaffold, allowed to arrange the statues in anatomical or artistic positions and to ensure an absolute balance of the relative proportions of the different parts of the body, the use of moulds allowed the artists to reproduce variants of organs, with changes in surface details or colours. The Bologna School technique firstly required the coating of the bone skeleton with tow or cloth to prevent the detachment of the wax from the bone surface and to reduce the amount of the wax needed. Then, the wax was poured on the bone and the anatomical structures were modelled (Ballestriero, 2006). On the other hand, the Florentine school technique required four phases: coating the anatomical specimen with clay, making the plaster mould, casting the melted wax in the mould utilizing superimposed layers of differently coloured waxes, and manually finishing the surface. Small structures, such as surface vessels and nerves, were obtained by coating iron or cotton threads with the wax. Lymphatic vessels having a knobby appearance were obtained by extracting in an intermitted way a silk thread from the melted wax. The metal brightness of the tendons was obtained by gold powder entrapped in a transparent paint coat (Dal Forno, 2009).

The wax used to make anatomical models was the one secreted by the honey bee *Apis mellifica* to construct the honeycomb. After the removal of the honey, the honeycombs were melted in boiling water and the filtered product constituted the wax, whose colour depended on the age of the honeycomb and the flower species of the territory. However, the waxes could be of different origin, each characterized by their melting point and colour: the Italian wax was an intense yellow, the German one a light yellow, and the waxes coming from Eastern countries were characterized by a dark red colour. The most-used waxes were the so-called *Levante* and *Sottana,* diluted in turpentine or melted with tallow and putty. Different colours were obtained either by melting or painting the wax with silver, gold, carmine red, lapis-blue, cinnabar, ceruse, minium, and China ink (von Schlosser, 2011).

Anatomical Waxes modelling in Bologna

The study of anatomy has been widely practiced in Bologna since 1595, when the Anatomical Theatre was established inside the *Archigymnasium*, which is the historical seat of the University of Bologna. The Anatomical Theatre was utilized mainly during the Carnival feast to perform public demonstrations on the cadavers of executed criminals. The cold weather at that time of the year was suitable for dissection and cadaver preservation. Later, the Anatomical School of Bologna was founded. The Bologna Anatomical School was active for over a century and a half and gave much prestige to important anatomical artists such as Ercole Lelli (1702-1766), Anna Morandi Manzolini (1714-1774), her husband Giovanni Manzolini (1700-1755), Domenico Piò (1715-1801), Francesco Mondini (1729-1803), Giovan Battista Manfredini (1742-1829), Giuseppe Astorri (1785-1852), Cesare Bettini (1801-1855) and his successor Luigi Calori (1807-1896) (Riva, Conti, Solinas, & Loy, 2010). However, at the end of the 17th century, the University of Bologna underwent a cultural crisis due to the dogmatism of the public teaching. To compete with the University, several private academies were founded in Bologna in the fields of Arts, Philosophy, and Sciences. Among them, the most famous was the Academy of Sciences, established in 1711 by Luigi Ferdinando Marsili.

Ercole Lelli, who had already made two statues of skinned humans in wood to support the canopy in the Anatomical Theatre of the *Archigymnasium*, designed the Anatomy Room at the Academy of Sciences in 1742. There, he placed life-size statues made up of real skeletons covered with superficial and deep muscle bundles in wax, coloured with natural substances; he also created two healthy and two abnormal kidneys which were some of the earliest representations of malformations and pathologies. Nowadays the wax specimens are collected in two separate locations both belonging to the University Museum Network (Sistema Museale di Ateneo – SMA). The Lelli and Morandi Manzolini preparations are collected in the main hearth of the Network in Palazzo Poggi, the site of the ancient Academy of Sciences of the Institute of Bologna and Chancellor residence, whereas the preparations of their scientific descendants are collected in the Anatomical Waxes Museum located in the Centre of Clinical Surgical Experimental and Molecular Anatomy of the University (Fig. 1).

Figure 1. The corridor of the Anatomical Waxes Museum at the Centre of Clinical Surgical Experimental and Molecular Anatomy in Bologna. At the top, the plaque commemorating Ercole Lelli and Pope Benedict XIV.

Two great figures of anatomical waxwork in Bologna

Ercole Lelli (1702-1766) was the son of a gunsmith who, as a boy, worked in his father's workshop and distinguished himself for the construction of a very refined arquebus. His inclination for painting directed him to the study of the figurative arts; he studied under the supervision of Francesco Merighi, Ferdinando Galli Bibiena and above all Giovan Pietro Zanotti, who were all important artists of the period. Zanotti directed Lelli to anatomical studies and facilitated him in winning the Marsili Prize for the first class of figure with black pencil drawing in 1727. From that moment on, Lelli's name began to acquire credit from private individuals as a painter of histori-

cal and religious subjects. However, Lelli began to be interested in anatomical illustrations; in 1732 he created an anatomical statuette which achieved extraordinary success: it was replicated in numerous plaster versions, while the original bronze remained at the Clementine Academy of Bologna which in 1772 gave it to the Academy of Saint Petersburg, Russia. After this successful debut Lelli devoted himself more and more to anatomical sculpture. In 1733-34 he created two wooden male statues (called "the skinned"), the result of a study for the Bologna anatomical Theatre of the *Archigymnasium* that had required the dissection of approximately fifty cadavers (Medici, 1856). The public consent for Lelli made him obtain the "Master of coinage" qualification at the Mint facility of Bologna. Lelli was then contacted by Cardinal Prospero Lambertini, that in 1740 became Pope Benedict XIV, who in 1742 welcomed Lelli to the Accademia Clementina and, in the occasion of the Museum of human anotomy of the Institute of Sciences establishment, commissioned him to make eight anatomical wax statues of life-size, including two naked and six flayed models, and over forty plates depicting various muscles and bones of the skeleton. Indeed, the fame of Lelli is mainly due to his work on muscle apparatus. His preparations, with the representation of the whole-body motor apparatus, are collected in Palazzo Poggi in eight cabinets made from painted wood and glass of the 18th century (Fig. 2). The first two cabinets, one in front of the other, contain the nude statues of a young man and a blond woman, referred to as Adam and Eve, whose surfaces are carefully modelled to underline the muscular and vascular structures. Then, four cabinets contain statues dedicated to the representation of the myological system from the more superficial muscle layers to the deeper ones. The last two cabinets contain two skeletons, one of a woman with a sickle in her hand, the other of a man with a scythe, which recall the usual representation of Death. In the female skeleton, the joint-capsules and ligaments are modelled by wax, while in the male skeleton, the bones are assembled with artificial joints in iron, which allow the bones to be moved according to the functional requirement of the joints. All these statues were made according to the classical Bolognese school technique by assembling the waxworks of the muscles onto the bones of human skeletons.

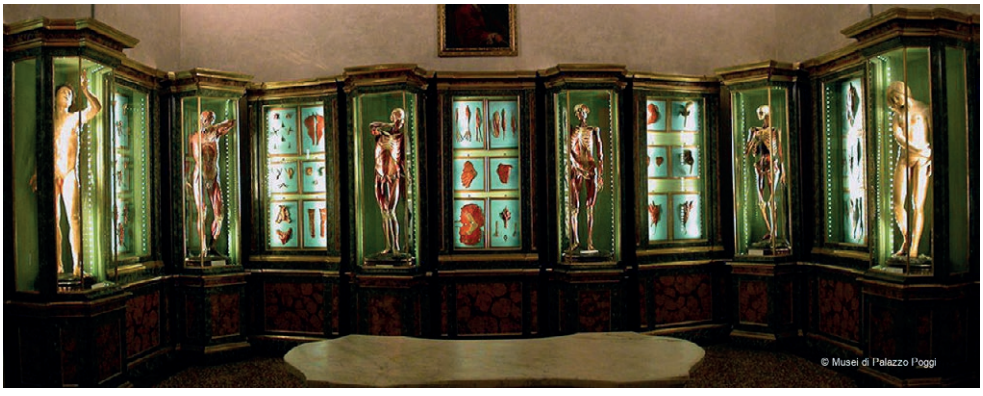

Figure 2. The eight wax statues by Ercole Lelli conserved in Palazzo Poggi, Bologna.

Anna Morandi Manzolini (1714-1774) was the daughter of Carlo Morandi and Rosa Giovannini and studied drawing and sculpture in Bologna in the schools of Giuseppe Pedretti and Francesco Monti. Here she met Giovanni Manzolini, professor of anatomy and her future husband. Anna Morandi soon had the opportunity to demonstrate her abilities as an anatomist when her husband fell ill with depression, in fact she helped him concretely in the dissection of corpses; in doing so she became an expert in the reproduction in wax of anatomical parts. Later, when her husband fell ill with tuberculosis, Morandi receives the special permission to teach on his behalf. After her husband's death in 1755, she was appointed by the Bolognese Senate as a wax modeller at the University's chair of anatomy. Thanks to her wax works she became famous throughout Europe: The Royal Society of London, Catherine II of Russia and other European courts have invited Anna Morandi several times with very attractive economic offers, but she never wanted to leave Bologna. Just a piece of curiosity: on the tombstone it is defined first of all as "loving wife and mother" and only in the third line does it read "Educated artist researcher brilliant teacher". The specimens by Anna Morandi Manzoli are collected in the wing of Palazzo Poggi which resembles the ancient Anatomy Room designed by Lelli. A large room contains all the two vertical cabinets which exhibit the wax self-portrait of Anna Morandi Manzolini (Fig. 3) and the portrait of her husband Giovanni Manzolini. Both are

Figure 3. Wax self-portrait of Anna Morandi Manzolini conserved in Palazzo Poggi, Bologna.

represented in the attitude of dissecting, in one case a skull to show the meninx, in the other, the liver to show the gall bladder. Manzolini's other works, which reveal her interest in detecting the functional connections among the organs, are shown in a large vertical cabinet and in a showcase table. Among them, eight wood panels, delimited by gauze materials as in an actual dissection field, show the muscles of the limbs, exhibiting the tendon insertions and the nerves. In addition, the 28 models describing in very realistic detail the senses of sight, hearing, taste, smell, and touch, and the nine tables dedicated to the organs of the pelvis and of the chest are modelled as dissection preparations to underline functional details of the vascularization and innervation of the organs.

From wax modelling to augmented reality (AR)

The scientific and didactic value of wax anatomical models was indisputable, since they have deeply facilitated the education of medical students in that time. Nowadays, on the other hand, technology is undoubtably the most promising tool to implement in teaching human anatomy. In particular, augmented reality (AR) is the form of technology that holds the higher potential in this field. AR represents an innovative educational tool applied to human anatomy in terms of adding virtual contents to tangible anatomical models (Ma et al., 2016) (Fig. 4). The possibility of having a three-dimensional

Figure 4. Medical students using augmented reality to explore the structures of the skull: Virtual exploration of the bone structures. Images taken from AEducAR study by Cercenelli et al., 2022.

and topographical learning approach has been widely tested obtaining a very positive outcome. Indeed, numerous recent studies have reported the positive effects of implementing AR in medical teaching, with increased learner immersion, a higher engagement and a better perception of study time being just some of them (Cercenelli et al., 2022; Duncan-Vaidya & Stevenson, 2021; Moro, Štromberga, Raikos, & Stirling, 2017).

Conclusion

For thousands of years, anatomical models have served as essential tools in medical instruction. Thanks to the work of important anatomical artists such as Ercole Lelli and Anna Morandi Manzolini, medical students have been able to deeply study and understand human anatomy. Nowadays, augmented reality constitutes a promising tool that will likely have the same positive impact on medical education that the creation of anatomical wax models has had. Therefore, both wax anatomical models and AR represent strong innovations in medical teaching for the respective historical period thanks to the vast applicability that they share: wax anatomical models could be easily produced and, in time, extensive collections have been created, while AR, thanks to its characteristic of expanding the real physical world by using a location-aware system and interface with added and layered networked information, can be used to explore and study basically every medical condition at 360 degrees. Under this light, the possibility of combining these two tools for medical education purposes is an intriguing idea that looks at the future while keeping its roots to our solid past.

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"De oculis", a chapter on ocular anatomy by Constantinus Africanus

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Abstract

Purpose: The publication of the compendium *Pantegni* by Constantinus Africanus in the monastery of Monte Cassino in the eleventh century CE was a pivotal moment in the history of medicine in Western Europe. The work is predominantly based on the *Liber regalis* by Haly Abbas (Baghdad, tenth century CE). The earliest available manuscript of the *Pantegni* (KB 73 J6) is handwritten and has been supervised by Constantinus himself. It includes a chapter on ocular anatomy. In this paper we try to distill the anatomic description by Constantinus.
Methods: The chapter "*de oculis*" on ocular anatomy as found in chapter 13 of book 3 in KB 73 J6 is interpreted in the historical context and translated into Modern English.
Results: We present an English translation of the concerning chapter.
Conclusion: Constantinus bequeathed a compact but comprehensive account of the anatomy of the eye, which can be rendered into a contemporary representation with use of the historical context.

Keywords

Pantegni; Constantine the African; anatomy, history; ophthalmology, history.

Introduction

The history of European medical knowledge has a turning point in the middle of the eleventh century CE. A significant role at this moment has been played by Constantinus Africanus, the first translator of medicine from Arabic to Latin. He produced more than 30 different texts, whereas in the three ages before in Western Europe in total only about 70 different medical texts circulated with only minor impact (Glaze, 2019). One of the texts by Constantinus Africanus is the *Pantegni*, which reached an impressive circulation and remained influential for ages (Green, 2018). In the Royal Library of The Hague lies the oldest available version of the *Pantegni* (KB 73 J6).

Recently, more and more about this specific parchment manuscript has been elucidated (Green, forthcoming; Hogewind and Coebergh, 2021; Kwakkel and Newton, 2019). KB 73 J6 is written by known Cassinese scribes, supervised by Constantinus himself. He based this work predominantly on the Arabic medical compendium *Al-Kitāb al-Malakī* (Latinized name *Liber regalis*; Figure 1) by 'Ali ibn al-'Abbas al-Majusi (Latinized name Haly Abbas, Baghdad, tenth century CE), although he did not refer to Haly

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Figure 1. Digitalised scan of chapter 13 from book 3 of the *Al-Kitāb al-Malakī* by ʻAli ibn al-ʻAbbas al-Majusi, Leiden Universtity Library, Or. 94 a, 176-180. The scanned manuscript is an undated paper copy, written in the 13th century (Voorhoeve, 1980; Witkam, 2007).

Abbas (Zargaran et al., 2013). In order to evaluate the extend of the impact of the *Liber regalis*, Russell (1994) extensively studied how Haly Abbas addressed the ocular anatomy. She thereto analyzed part 1, discourse 3, chapter 13 in the Arabic text as edited by de Koning (Majūsī, 1903) and exposed the underlying distinguishing concepts of ʾAbū Zayd Ḥunayn ibn ʾIsḥāq al-ʿIbādī (Latinized name Johannitius, Baghdad, ninth century CE [Dalfardi et al., 2016]) and Galen (Rome, second century CE [Laios et al., 2016]). The purpose of this paper is to elucidate what Constantinus wrote on ocular anatomy.

Materials and methods

We accessed the *Pantegni*, manuscript KB 73 J6, via the website of the Royal Library of the Netherlands and we transcripted book 3, chapter 13 (Africanus, circa 1080c). In cases where legibility was impaired because of the handwriting, abbreviations or wormholes, we used as cross-reference the transcription of another manuscript of the *Pantegni*, which has been dated back to the same era (Kaltio, 2011 and 2020). Hereafter we distilled the content and translated the text into Modern English.

Results

Figure 2 shows a scan of the relevant chapter. Below we respectively depict the transcription in Latin and the translation of the text.

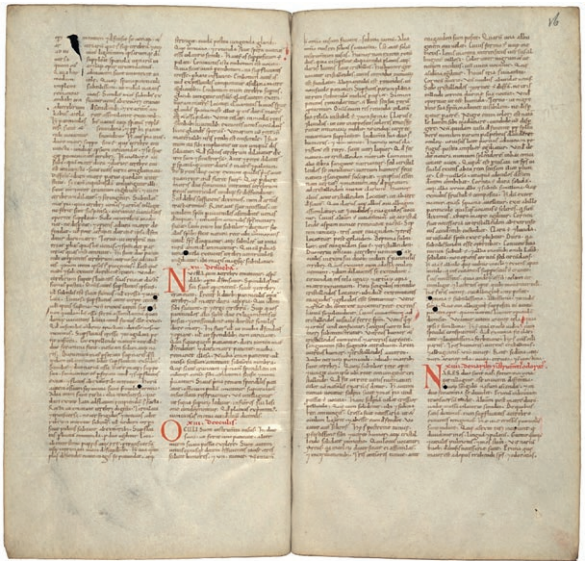

Figure 2. Digitalised scan of chapter 13 "de oculis" from book 3 of the medieval parchment manuscript *Pantegni*, Dutch National Library, KB 73 J6, folio 15^{v} and 16^{r}. Written around 1080 AD under supervision of Constantinus Africanus in the abbey of Monte Cassino, Italy (Kwakkel and Newton, 2019).

xiii. deoculis.
OCULI sunt instrumenta uisus, hi duo sunt, ut forte uno patiente, alter uicem suam possit explere. Sunt autem uniuscuiusque decem efficientes cause, tres scilicet humores, et vii, tunice. Nontamen hęc omnia uisum faciunt, sed una tantum. Alia omnia eius rei solius sunt iuuamenta.

Chapter 13, about the eyes
The eyes are the instruments of vision. There are two of them, so that if one were to suffer, the other can take over. Each of the two consists of ten components: three humours and seven tunics. Yet not all realize vision, only one does; all other parts are supportive elements.

Est autem solum instrumentum uisus, humor non ex toto rotundus, quia insuperficie aliquantulum planus atque clarus, lucens sicut cristallus. Hic humor graece uocatur cristalleidos, cuius inmedio tunicarum est fundatio. Aliquantulum est rotundus, ut nonfacile patiatur. Superficies parum plana, ut rem sentiendam melius percipiat. Si enim penitus rotundaretur, non bene sensui rerum aptaretur. Omnis enim res, rotunda inlatera sua redisa instabilis est et non firma. Clarus est et lucidus, ut cito inoppositos colores transferatur in tunicarum medio, ne undique careret iuuantium supplemento.

The only instrument we use to see is a humour, not quite spherical since its surface is somewhat flattened, and which is clear and shining as crystal. This fluid is called *cristalleidos*, by its Greek name. It is situated in the middle of tunics. It is somewhat spherical, making it less susceptible to damage. Its surface is a bit flattened, so as to better encompass all that is to be perceived. Moreover, if it was spherical allaround, it would be unstable and not firmly fixed in place. The *cristalleidos* is clear and bright, enabling it to quickly change into the different colours confronting it; it is centred between tunics so as not to lack the support from the surrounding structures.

Indumenta sua duo sunt humores, et vii. tunice. Humorum unus clarissimus est retro, sicut uitri liquor. Quod fecit natura, ut cristalleidon nutriat. Cum enim alia membra sanguine nutriantur, sed acristalleidos se moueatur, uitreum humorem fecit natura quis sanguinem suscipiat, et inipsius essentiam uertat, et mutatum atque depuratum ad cristalleidon mittat clarum.

The *cristalleidos* itself is covered by two humours and seven tunics. Of these humours one, situated behind the *cristalleidos*, is very clear, translucent as molten glass is; it was made like this by nature to nourish the *cristalleidos*. Whereas other parts of the body are nourished by the blood, the *cristalleidos* is an exception, nature has made the *humor vitreus* to receive blood, change it into the essence of the *cristalleidos* and so, altered and purified, to deliver it to the *cristalleidos* as a clear fluid.

Humor alius ante cristalleidon locatur, ut abaere desiccetur. Qui clarus atque albus oui albugini assimilatur, ut et euagaidos uocatur. Cuius alterum est iuuamentum, ut acristalleide asperum tunice remoueat tactum. Septem tunicarum, tres ante euagaidon, et tres locantur post gelaidon. Septima subtilior, inter euagaidon sita est, et cristalleidon.

The other humour is situated in front of the *cristalleidos*, to prevent it becoming desiccated by the air. This fluid is clear and whitish and is being compared to *albugo ovi* [egg white]. It is called *euagaidos*. Its second function is to prevent a rough contact of the *cristalleidos* with the tunic. Of the seven tunics three find themselves in front of the

euagaidos and three behind the *gelaidos*. The seventh, a delicate tissue, is positioned in between the *euagaidos* and the *cristalleidos*.

Duo nerui concaui acerebro uenientes ad oculos in exitu sui duobus induti sunt panniculis cerebri. Quos exuunt cum adossa oculorum ueniunt, et dum dilatantes se extendunt, circundat eos tela uenarum et arteriarum apia matre exeuntium. Horum singulus inmedio cristalleidos locatur, ubi due extremitates euagaidos et gelaidos esse sentiuntur. Vene et arteriae ibi contexte uocantur rete, ex retis tantum similitudinem. Cuius iuuamentum ad cristalleidos uisualem ferre spiritum.

Two hollow nerves approach the eyes where they exit from the brain, they are covered by two meninges, that enter the ocular orbits with them, where they separate, expand and stretch out over the length of the nerves, covering them with a network of veins and arteries which emerges from the *pia mater*. Each of these [nerves] is found halfway [at the equator] of the *cristalleidos*, where the two edges of the *euagaidos* and the *gelaidos* can be seen. This is called *rete* since the web of veins and arteries resembles a *rete* [network]. The *rete* serves to convey the *spiritus visualis* to the *cristalleidos*.

Venis quoque et arteriis unde conficitur, sanguis uitreo humori subministratur. Vitreus humor et cristalleidos cum uenis et arteriis, careant, sanguinem sibi sugendo attrahunt. A reti uitreus humor, et cristalleidos auitreo. Ambo neruorum panniculi, aduabus matribus sunt cerebri. Quorum subtilior rete operit et iungit cumipso ubi rete coniungitur cristalleido. Quod fit ut rete uenis nutriatur, calor uero naturalis ex arteriis detur. Hęc autem tunica uocatur secundina sicut et mater pia unde processit est uocata. Hanc secundinam uelat crossior pellicula. Quę cum solidetur, ubi et secundina reti coniungitur. Crossa fuit necessaria, ut et oculum ligaret, et ab osse eum defenderet. Vocatur autem scliros.

Blood coming from veins and arteries out of the retina, is provided to the *humor vitreus*. Since the *humor vitreus* and the *cristalleidos* are devoid of veins and arteries, they attract blood by suction. The *humor vitreus* from the *rete*, and the *cristalleidos* from the *humor vitreus*. Both the meninges of the nerves are from the two meninges of the brain. Of these the thinner covers the *rete* and is attached to it where the *rete* is attached around the *cristalleidos*. This layer serves to nourish the *rete* by the veins, and to supply natural warmth by the arteries. This tunic is also called the *secundina*, just as the *pia mater* from which it originates, is sometimes called *secundina* [second brain covering [Gerard Baader 1967]]. This *secundina* is covered by a fairly thick coat, which is tightened where it joins with the *secundina* and the *rete*. This layer was needed to keep the eyes located as well as to protect them from the hardness of the bone. It is called *scliros*.

Hę sunt postremę tunicę, perfectissime sibi et uitreo humorus atque cristalleido solidate inmedio. Qui locus uocatur yreus, quia in colorum diuersitate ei assimilatur, et incircuitionibus.

These are the posterior tunics, they are firmly connected with one another and with the *humor vitreus* and the *cristalleidos*, exactly halfway [at the equator]. This spot is called *yreus*, because in its variety of col-

ours and in its circumference it resembles an *yreus* [rainbow].

Tres anteriores tunice, ante euagaidon sunt posite. Quarum una albuginem oui uelat. Cuius forma est uuę medietas. Lenis inantea intrinsecus uersus albuginem uillosa. Color inter nigrum et uenetum medius, unde uuea uocatur. Quę asecundina egreditur. Huius tria sunt iuuamenta. Corneam nutrit. Unde multis abundat uenis. Secundo cristalleidos et cornee est differentia, ne cristalleidi cornea duritie sua noceat. Vnde oportuit ut esset humida. Tertio, ut nigredine sua spiritum coadunet uisibilem, ne dispergatur per aerem. Nigro enim colorus est naturale lumen sibi coadunare, candido uero dispergere.

Three more tunics are positioned in front of the *euagaidos*. One of these veils the *albugineus* and its form is that of the inside of a grape. It is smooth anteriorly and it's furrowed inside lies against the *albugineus*. The colour is in between black and blue. It is called *uvea* and originates from the *secundina*. This structure has three supportive functions. One is to nourish the *cornea*, to which end the *uvea* contains multiple veins. The second function is to keep the *cornea* separated from the *cristalleidos*, so as to prevent the *cornea* being injured by the hardness of the *cristalleidos*. For that reason the area needs to be moist. The third function is to ensure, by its blackness, to keep the *spiritus visibilis* (on the use of spiritus visualis and spiritus visibilis see Russell [1994]) collected so that it does not get scattered by the air. After all, black is the colour in which by nature all light comes together, whereas it is scattered by white.

Vnde quidam uisu deficiente propter splendorem nimium parum palpebras claudunt oculorum, ut uisus licet paruus aduueam refugus, postea amplior egrediatur. Vnde dedit natura nimium splendorem in hac concauitate uuea. Quę etiam est pertusata ut spiritus uisualis exiens abea rem sensam ilico percutiat. Est et uillosa, ut aquam adoculos descendentem combibat.

Therefore, if too much light hampers vision, one closes the eyelids a bit, so only a little vision effuses from the uvea, after which the *spiritus visibilis* emerges amplified. For that reason nature has provided an abundance of light in the cavity that is formed by the *uvea*. Since it is perforated, the *spiritus visualis* can directly exit to reach the perceived object. The *uvea* is furrowed, so that if water descends to the eyes, it swallows it.

Cornea est dura solida, atque alba cornu albo et subtili simillima. Quę exmultis frustulis est composita. Hęc dum excorticatur, quasi squama auellitur. Exit abillo panniculo qui supra uocatus est scliros, qui sicut diximus, adura matre egreditur. Cornea fuit necessaria ut cristalleidon ab extrinsecus accidentibus custodiat. Clara est et lucida, ut uisualem spiritum exire prohibe-

The *cornea* is hard, firm and colourless, very similar to the fine and white structure of horn, and consists of numerous small parts. When one removes the outer layer one can, as it were, peel of lamellae. The *cornea* is a continuation of the coat that we have previously called *scliros* which, as we have already mentioned, is itself a continuation of the *dura mater*. The *cornea* is need-

at. Dura, atque subtilissimam esse oportebat.

ed to protect the *cristalleidos* from external injury. It is clear and transparent, and to avoid any loss of the *spiritus visualis* to the outside, it needs at the same time to be hard and very delicate.

Coniuntiua tunica subtilis est, et alba inomnibus oculi lateralibus solidata, non operiens corneam sed circumdans. Hęc illa est albedo quę uidetur in oculo et exiens apanniculo, qui inter cutaneus suppositus est craneo. Eius necessitas, quia undique ossibus oculum circumligat, et lacertos operit quibus mouentur oculi.

The *coniunctiva* has a fine structure and is white, everywhere firmly attached to the sides of the eyes, including around the *cornea*, but not covering it. It is the white of the eyes that one sees, which derives from the membrane underneath the skull. It's function is that it binds with the bone all around the eye, additionally it covers the muscles by which the eyes are moved.

Hae sunt tres tunice, oui albugini ante posite. Septima est subtilissima, albissima, et nitidissima. Quę oui albugini supposita eam tantum partem operit, quam uitreus humor operiendam dimisit. Vocatur autem araneę tela, quia ipsi est simillima. Haec est quam in oculo uidemus, cum speculum conspicimus. Quod exnimia fit claritate, et lucentissima sorbitione.

These are the three tunics positioned in front of the *albugineus ovi*. The seventh tunic is very delicate and lustrous, and of a bright white colour. It is placed underneath the *albugineus ovi*. It covers only that part [of the *cristalleidos*] that ought not to be touched by the *humor vitreus*. It is called *tela arachnoidea* [spider's web] since it very much resembles it. This [the *tela arachnoidea*] is what we see in the eye, every time we watch in a mirror. It is of the most transparentness and is dissolved even by the brightest light.

Hę sunt omnis oculi partes. Tres humores, vitreus, cristallinus, et albugineus. vii. tunicę Rete, secundina atque dura araneę tela, uuea, cornea, et coniunctiua.

These are all parts of the eye. Three humours: *humor vitreus*, *cristalleidos* and *albugineus*. Seven tunics: *rete*, *secundina*, *dura*, *tela arachnoidea*, *uvea*, *cornea* and *coniunctiva*.

Discussion

First of all, the interpretation of ancient texts is a hazardous challenge, requiring a thorough historical comprehension (Hogewind et al., 2013). However in the recent years ample insights have been gathered on the setting and the period that Constantinus supervised the writing of this specific manuscript KB 73 J6 (Hogewind and Coebergh, 2021; Kwakkel and Newton, 2019). Nonetheless, although we attempted to choose the most accurate Modern English words for anatomical terms in the translation in order to obtain a contemporary rendering, the right historical interpretation of words like blood, nerve, meninx, orbit, artery, vein, eyelid, skull and muscle remains of course an important caveat.

As to the form, Constantinus identifies two cerebral meninges around the optic nerve and describes the eyeball as an elongation of these membranes which envelope three so called humours *(humor vitreus*, *cristalleidos* and *euagaidos)*. The outer meninx or *dura mater* continues as the *scliros,* which on its turn continues as the *cornea*. The inner meninx or *pia mater* perpetuates as the *secundina* till the equator of the eye and is perpetuated as the *uvea* anteriorly of the equator. The optic nerve itself prolongates within the eyeball as the *rete* encompassing only one humour: the *humor vitreus*. *Rete, secundina, scliros* and *humor vitreus* are intertwined with each other at the circumference of the *cristalleidos*. This junction lies at the equator of the eyeball and is called *yreus* (De Koning, 1903). (Note that Constantinus positions the *cristalleidos* in the centre of the eye, whereas the crystalline lens is located more anteriorly [Leffler et al., 2016]). Anteriorly of the *cristalleidos* is the *euagaidos* and the two are separated by a thin membrane called *tela arachnoidea*. Anteriorly the *euagaidos* is covered by the *uvea*. The frontal outside of the eye is formed by the *cornea* (which is the continuation of the *scliros* as described above). The sides of the eye are wrapped together with the extraocular muscles by the white *coniunctiva*, which tightens the eye to the orbital bones.

To understand what Constantinus writes on the function of the eye and its parts, it helps to read discourse 4, chapter 11 "de virtute visus" (Africanus, circa 1080d). Here Constantinus describes the concept that eyesight is realized by the *spiritus visibilis* which arises in the ventricles of the brain and travels through the hollow optic nerves to the *cristalleidos*. By effusing this *spiritus visibilis,* the *cristalleidos* makes contact with the object that is seen and subsequently changes itself in the objects colour, after which the conceptualized consciousness in the brains ventricles perceives this colour and intrinsically the form of the object (Ierodiakonou, 2014; Russell, 1994).

With this background information we can distill that Constantinus explains that the *spiritus visibilis* travels from the ventricles of the brain through the hollow optic nerves via the *rete* (which is a prolongation of the optic nerve) to the *cristalleidos* and then through the *euagaidos* and the perforation (or pupil) in the *uvea* through the *cornea* to make contact with the visualized object (Jordan, 1990; Reeves and Taylor, 2004; Salmón, 1996). Subsequently the clear *cristalleidos* adapts to the colour which is perceived by the effused *spiritus visibilis*.

Constantinus meticulously explicates the efficiency of the eye handling the *spiritus*: the spherical lens is somewhat flattened to maximize the passage of *spiritus visibilis*, the *cornea* prevents leakage of *spiritus visibilis*, the dark colour of the *uvea* facilitates storage of the *spiritus visibilis* within the eyeball and in case of bright light the palpebral aperture is decreased (de Koning, 1903).

Table. Etymological origins for ocular anatomical names as used by Constantinus Africanus in the *Pantegni*, book 3 chapter 13 (Africanus, circa 1080c).

Name by Constantinus Africanus	Constantinus' etymological explanation	Greek word as used by Galen in *De usu partium*	Rendering in the contemporary anatomical equivalent
Gelaidon		τὸ ὑαλοειδὲς ὑγρόν[@]	vitreous body
Humor vitreus (synonym for gelaidon)	sicut uitri liquor	τὸ ὑαλοειδὲς ὑγρόν[$]	vitreous body
Cristalleidos	lucens sicut cristallus hic humor graece uocatur cristalleidos	τὸ κρυσταλλοειδὲς ὑγρόν[@]	crystalline lens
Euagaidos		τὸ ὠοειδὲς ὑγρόν[@]	aqueous humour
Albugineus (synonym for euagaidos)	albus oui albugini assimilator	τὸ ὠοειδὲς ὑγρόν[$]	aqueous humour
Rete	ex retis tantum similitudinem[#]	ὁ ἀμφιβληστροειδὴς χιτών[$]	retina
Secundina	sicut et mater pia unde processit est uocata[*]	ὁ χοριοειδὴς χιτών[$]	choroid
Scliros		ὁ σκληρὸς χιτών[@]	sclera
Dura (synonym for scliros)		ὁ σκληρὸς χιτών[$]	sclera
Tela arachnoidea	quia ipsi est simillima	ὁ ἀραχνοειδὴς χιτών[@,%]	anterior lens capsule
Uvea	cuius forma est uuę medietas	ὁ ῥαγοειδὴς χιτών[$]	iris
Cornea	cornu albo et subtili simillima	ὁ κερατοειδὴς χιτών[$]	cornea
Coniunctiva		ὁ περιόστιος ὑμήν[$]	conjunctiva
Yreus[+]	quia in colorum diuersitate ei assimilatur, et incircuitionibus	ἡ ἶρις	conceptualized region near the ciliary body

[@] The term used by Constantinus is derived from the Greek word used by Galen (Baader, 1967).
[$] The term used by Constantinus is not derived from the Greek word used by Galen (Baader, 1967).
[#] For a comprehensive study on the etymology of the word *rete* see de Jong (2014).
[*] Secundina is another name for pia mater (Baader, 1967).
[+] Yreus is not so much an ocular component but a location (*locus*).
[%] Tela arachnoidea is derived from ὁ ἀραχνοειδὴς χιτών, which is used by Galen in his *Methodus medendi* (Baader, 1967).

Constantinus also addresses the blood supply. The ocular blood vessels stem from the cerebral *pia mater's* vessels, enter the eye in the *secundina* and continue in the *uvea* and in the *rete*. Constantinus distinguishes arteries and veins, which are required for

supplying the ocular parts with respectively natural warmth and nutrition. The clear cornea is lacking blood vessels but is nourished by the *uvea* veins. The *humor vitreus* transforms the blood from the *rete* vessels in a clear fluid to supply the transparent non-vascularized *cristalleidos*.

A lot of attention goes to the vulnerability of the most precious part of the eye: the *cristalleidos*. It is not spherical all-around preventing it to be unstably attached and anteriorly it is covered by the *euagaidos* which prevents its dehydration (and protects the *cornea* against the hard *cristalleidos*). Finally, the *cristalleidos* is protected by the *cornea* from trauma.

Introducing the anatomical names of the ocular components, Constantinus also supplies us with the etymological information wherever it is applicable. In this context the analysis by Baader (1967) is of much interest: he discusses elaborately which of the names are derived from the Greek terms that are already used by Galen. In the table we depict the etymological explanations by Constantinus, add the Greek terms as used by Galen and render the anatomical names into a modern anatomical equivalent.

One sentence about the tunic which has been called *uvea* forms an enigma; what was meant by "Est et uillosa, ut aquam adoculos descendentem combibat."? Haly Abbas mentions couching/cataract operation in this context (Majūsī, 1903; Russell, 1994), but the couching is not mentioned by Constantinus in this chapter. Haly Abbas' text is later also translated from Arabic to Latin by Stephanus Antiochenus (Majūsī, 1492). Stephanus writes here: "Facti que sunt in ea ab interioribus villuli: quibus aqua que ad oculos veit suspendit." It appears that Constantinus (and Stephanus) describe(s) a physiological phenomenon, which might be hampered in the pathological condition of *cataracta* (Africanus, circa 1080a and circa 1080b; Fischer, 2000; McVaugh, 2001).

We compared our translation with the interpretation of Haly Abbas' corresponding text by de Koning (Majūsī, 1903) and by Russell (1994). With respect to the content and the outlay, the description of Constantinus is almost exactly the same as what de Koning (Majūsī, 1903) and Russell (1994) distilled from the text by Haly Abbas. Constantinus specifically did not add any content or add new insights.

In conclusion, Constantinus bequeathed a compact but comprehensive account of the anatomy of the eye, which can be rendered into a contemporary representation with use of the historical context.

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The "prince of anatomists" Paolo Mascagni and the modernity of his approach to teaching through the anatomical tables of his *Anatomia universa*. A pioneer and innovator in medical education at the end of the 18th century and the creator of unique anatomical collections

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Abstract

In the wake of the great anatomists, Paolo Mascagni introduced important innovations into the methods of studying anatomy, as revealed both by his writings and, especially, by his approach to the analysis of anatomical sciences. He devoted his whole life to the study of anatomy, which he considered a mainstay of medical education.
Mascagni's great originality in this field lay in the fact that he designed new, modern tools for the teaching of anatomy – tools that enabled students to gain greater understanding of the human body even in the absence of dissection, which was regarded as indispensable in those days.
His two most important results were the first description of the lymphatic system and the clear and precise anatomical tables of the Anatomia Universa. This "modern and evocative anatomical atlas" was the first to present precise, life-size drawings of the human body.
The present overview aims to illustrate the modernity of this great scientist, his approach to the study of anatomy, his meticulous attention to topographical anatomy, and the utility of his innovative methods, which were to mark the evolution of medical education for many years.

Keywords

Paolo Mascagni, dissection, medical education, teaching aids, collections of anatomical tables.

Introduction

Paolo Mascagni (1755-1815) graduated in Medicine in 1778 at Siena University[1]. In the same year, he became the assistant of Professor Pietro Tabarrani, who had been a pupil of Giovanni Battista Morgagni "the father of modern anatomical pathology".

On the invitation of Tabarrani, Mascagni had already begun studying the lymphatic vessels before graduating, succeeding in highlighting their evolution, organization, functions and alterations through mercury (Hydrargyrum) injections. Published

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in 1787, this study was entitled *Vasorum lymphaticorum historia et ichnographia*[2,3,4]. This work was praised by anatomists for its description and systematic identification of the lymphatic system.

Subsequently, Mascagni devoted all his activity to studying and teaching the anatomy of the human body.

At the beginning of the 16th century, Berengario da Carpi (1466-1530), lector at the University of Bologna[5], defined anatomy as the "alphabetum medicorum"[6]. Likewise, Mascagni regarded the teaching of anatomy as a fundamental part of medical education.

Mascagni's *Vasorum lymphaticorum historia et ichnographia* provided the first description of the pathways of the lymphatic system, accompanied by 27 tables drawn by the artist Ciro Santi. Immediately after the publication of this work, Mascagni began to conceive his project to "create a much more extensive work that would reveal the whole of the human body without neglecting anything"[7]. Thus, he started work on a new anatomical atlas that would "by means of new Tables, display the Parts of the Human Body and depict them precisely as they are in nature, allowing each one of them to conserve its order and respective position"[8]. Mascagni's objective was to create an innovative teaching aid that medical students could use when they did not have the opportunity to study directly on cadavers. He therefore drew up his *Anatomia Universa,* which was published in nine fascicles between 1823 and 1832 and which reproduced the dissection of a cadaver in a virtual manner.

In the preface to this work, the great anatomist asserted that, in the study of the human body, two methods of investigation were possible. The first was to utilise verbal descriptions of the individual parts of the body; the second utilised images in order to place the complete and organic form of the human body in front of the eyes, which would therefore not be deceived[9].

In Mascagni's view, this latter approach was the better. Indeed, his anatomical tables resulted from the observations that he himself made during the course of the dissections that he carried out on a large number of cadavers both for his own research and for teaching purposes.

The study of anatomy based on dissection

Dissection can be regarded as the anatomy lesson par excellence, at least since the beginning of the 14th century, when it became a fundamental element of the new system of education and the transmission of knowledge that gained sway following the foundation of universities.

Once common in the third century BC in the flourishing cultural life of the Alessandria of Ptolemy II[10], the dissection of human bodies was soon abandoned and, up to the end of the 13th century, medical knowledge was based exclusively on the study of the ancient texts.

In 1316, Mondino de' Liuzzi (1275-1326), a teacher at the University of Bologna, published his *Anothomia*, a manual on the technique of dissection, which testifies to the re-emergence of interest in this practice. Dissection did not only have an educational purpose, however; it was also – in the case of public dissections – a kind of spectacle with a scientific value. Indeed, the manual was greatly appreciated by a vast readership.

That these "public displays" took place gives the lie to the belief that the Catholic Church had placed a ban on dissection. Indeed, the papal bull *De sepulturis* (also known as *Detestandae feritatis*) issued by Boniface VIII in 1299, which many cite as the basis of this ban, in reality targeted the custom of boiling the corpses of important persons who had died far from home – a practice that enabled the soft parts of the body to be separated from the skeleton, which could then be more conveniently repatriated.

According to this bull, anyone who boiled a corpse was liable to be excommunicated; it made absolutely no mention, either directly or indirectly, of the dissection of corpses for educational purposes. To settle any possible ambiguity regarding the interpretation of Boniface VIII's document, Pope Sixtus IV unequivocally stated in his *De cadaverum sectione* in 1472 that the study of anatomy was useful in medical and artistic practice.

As a result of Mondino's work, dissection was set to become a cornerstone of the teaching of anatomy. Indeed, it became not only a fundamental visual aid to understanding the ancient texts, but also, as stated by Berengario da Carpi in his *Commentaria,* it constituted an ongoing "open-body" verification of the assertions of the ancient anatomists: an *anatomia sensibilis* aimed at the senses through which the anatomist could and should explore the human body[11].

In this regard, Berengario wrote in his commentary on Mondino's *Anothomia*: *"Haec omnia ego vidi ad sensum in anatomiis"*[12, 13], concluding that *"Experientia* (in the sense of 'observation') *tamen in hoc est magistra cui credendum est"*[14].

Thus, anatomy is a form of knowledge that can be learnt and communicated above all through images[15]. This claim finds ample corroboration in Andreas Vesalius' (1514-1564) work *De humani corporis fabrica libri septem* (1543), which constitutes the basis of the scientific study of human anatomy by means of *manuum munus*[16] and defines the methodology and epistemology of the new medical education[17]. Indeed, this publication, which was magnificently illustrated by Johannes Stephan van Calcar, a pupil of Titian, constitutes a completely different approach from that of the previous anatomical treatises and from Galen's anatomical paradigm, in that it inverted the order of priority between text and dissection, between reading and observation.

In the preface, Vesalius scoffed at his predecessors and contemporaries who taught by repeating from memory what they had read in books written by others, without troubling to verify it or to draw upon new knowledge derived from the direct observation of human anatomy. At the same time, he assigned to dissection an investigative role as well as a didactic function.

Indeed, by opening, handling and observing the corpse, it was possible to acquire new information on the human body.

This enabled him not only to write a new chapter in the history of anatomy, but also to correct the erroneous descriptions of the ancients, who had not been able to study the body by means of the direct experience that only dissection could provide.

Vesalius explicitly stated these convictions in his book, beginning from the frontispiece, which depicted a dissection. In this dissection, the great anatomist was not preaching from a treatise on anatomy, as was customary at that time; rather, he was dissecting the corpse with his own hands, showing and explaining to the spectator the various anatomical parts. In this way, he brought together – as Mondino had done previously and Mascagni would do later – the three traditional figures of dissection: the *Lector*, the *Sector* and the *Demonstrator*.

In the centre of the illustration, a woman's body, in perspective, lies on the autopsy table, which is surrounded by a number of people: on one side stand the contemporary anatomists, who oppose Galen's theories; on the other, his supporters. All are gathered around Vesalius in a sort of ideal conciliation. The fact that Vesalius is depicted in the act of dissecting the corpse with his own hands, without the customary aid of the *Sector* and the *Demonstrator*, reveals his view that anatomical knowledge is gleaned from dissection performed personally by the anatomist. Moreover, the writing instruments and the sheet of paper seen on the side-table next to the body underline the idea of an anatomical text that is the result of the dissection and observation of the corpse, of direct perception through the senses. Thus, Vesalius fits into a school of thought that, while not repudiating the link with the traditional system of official teaching, began to emphasize the manual component of medical practice.

That Vesalius distanced himself from the traditional anatomy lesson is testified, *in primis*, precisely by the difference between the frontispiece of his publication and those that appear in volumes published only a few decades earlier, such as that of the German Johannes de Ketham's *Fasciculus medicinae* (1491), which shows a *Lector* reciting *ex cathedra* from a text of Galenic tradition and a Demonstrator, who is pointing with a stick to where the Sector has to cut the corpse.

Mascagni's anatomical tables: perfect teaching aids

It was precisely this renewed importance of dissection that prompted the creation of teaching aids that could be used when no corpses were available. In particular, anatomical tables constructed on the basis of direct observation of the human body were deemed to be more efficacious than any written text or speech, as they enabled numerous people in different places to observe what could otherwise be seen only on the autopsy table[18]. This conviction was also held by Paolo Mascagni, as is testified by his publications, in which images were given the greatest priority. Indeed, his *Anatomia Universa,* an extraordinary work in terms of both conception and commitment, immediately became the foremost anatomical atlas of the time. Mascagni wanted to provide medical students with anatomical tables that could reproduce the human body in extremely fine detail: a galaxy of vessels, bones and organs, both whole and sectioned.

Mascagni's tables depict the human body seen from the front *(facie adversa)* and from the back (facie aversa), as on the dissecting table. Moreover, 24 tables (102 cm long, 75 cm wide) are designed in such a way that, when arranged 3 to 3 vertically, they form life-size pictures of human bodies.

The body is represented stratigraphically, *"per diversa strata a cute ad sceletum"* ("in different layers, from skin to skeleton")[19]: the first layer *("stratum primum")* illustrates the skinned human body (i.e. deprived of the skin) and shows superficial muscles, nerves and vessels; the second layer *("stratum secundum")* depicts deeper muscles, nerves and vessels; the third *("stratum tertium")* shows the muscles, arteries and veins of the deepest layer, while the fourth layer *("stratum quartum")* shows the skeleton. These representations of the human body are followed by 20 tables illustrating the viscera and various other organs, again in life size and with extreme precision. This ground-breaking project, on which Mascagni worked for about 30 years, constitutes the very first depiction of the lymphatic system in tables of natural size ("3 braccia tos-

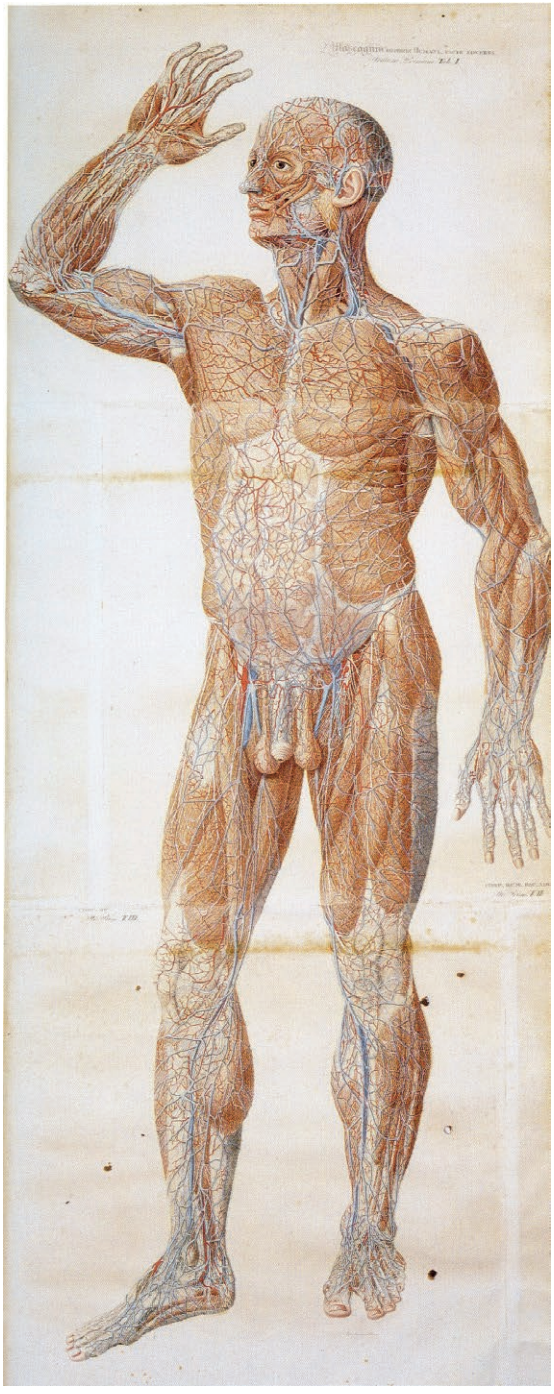

Figure 1. Paolo Mascagni, *Anatomia Universa*, stratum primum, facie adversa.

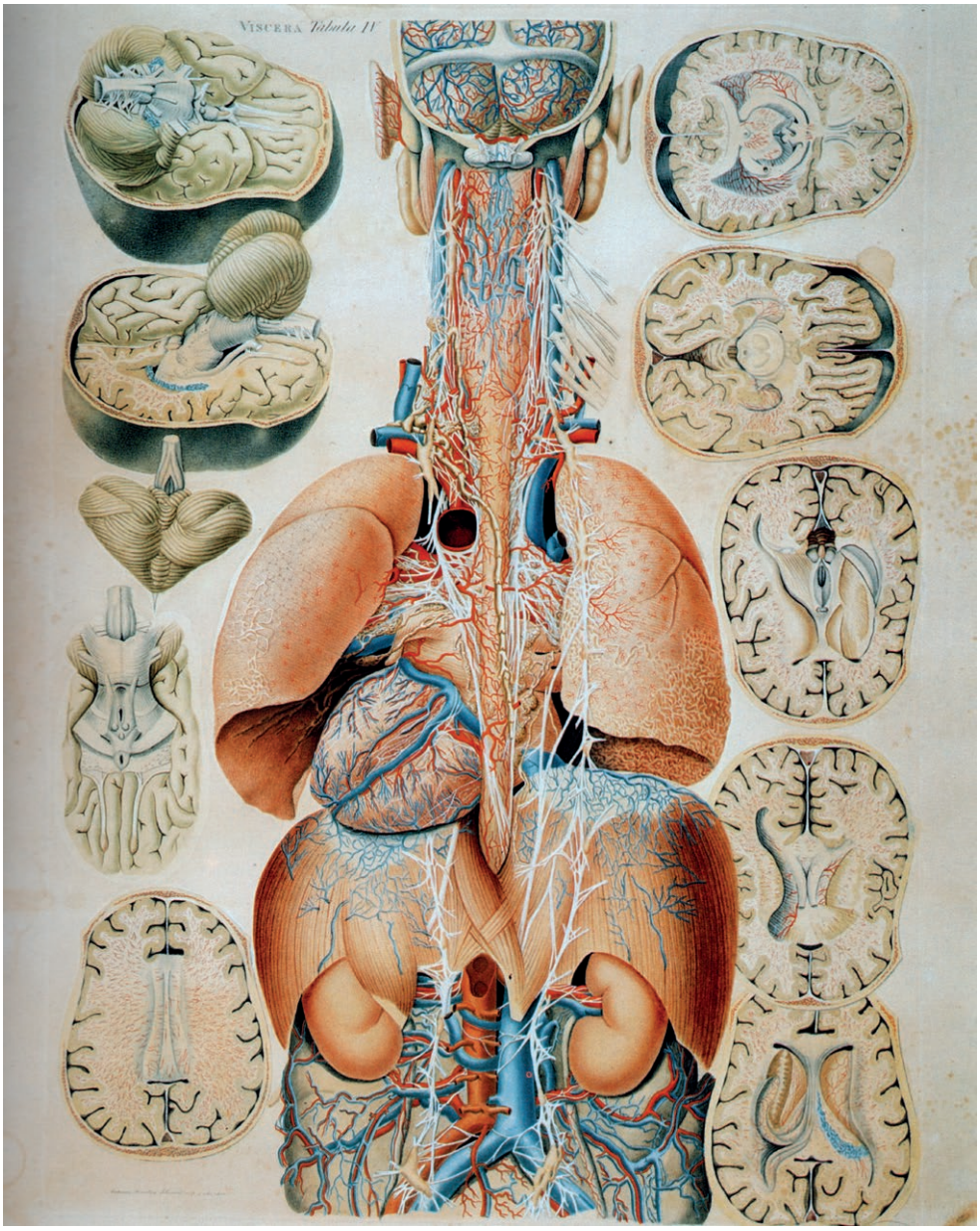

Figure 2. Paolo Mascagni, *Anatomia Universa*, Viscera, tavola IV.

cane", corresponding to 175 cm). Consequently, Mascagni is remembered not only for his fundamental work in the anatomical field, but also for his teaching methods and the high-quality materials that he produced in order to facilitate the study of anatomy.

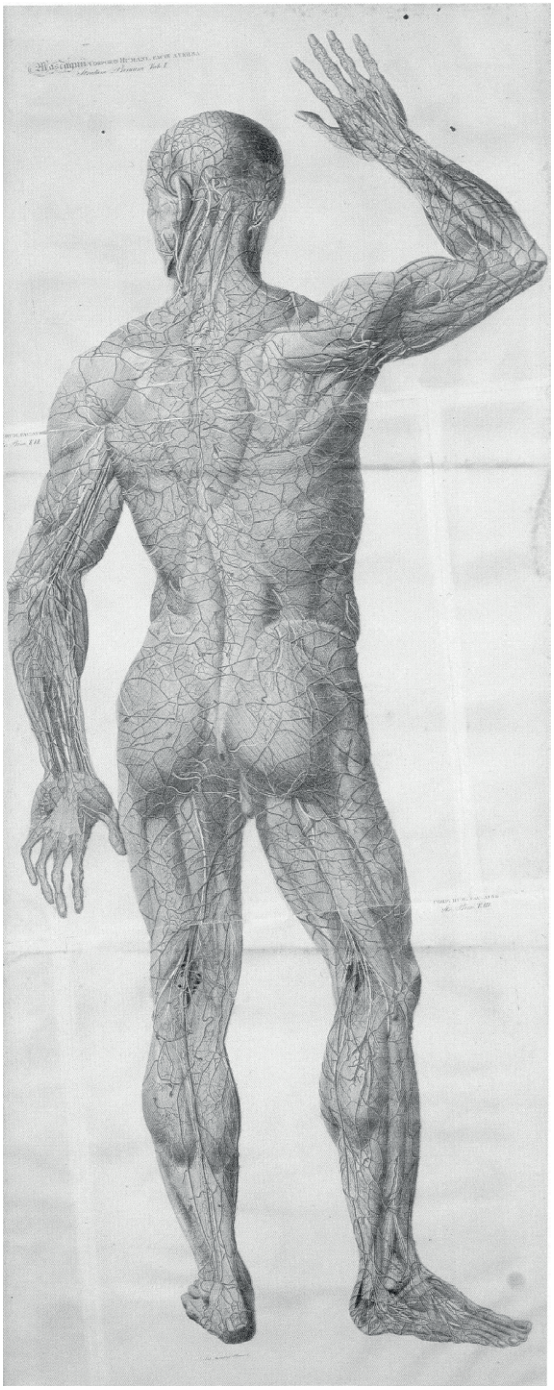

Figure 3. Paolo Mascagni, *Anatomia Universa*, stratum primum, facie aversa.

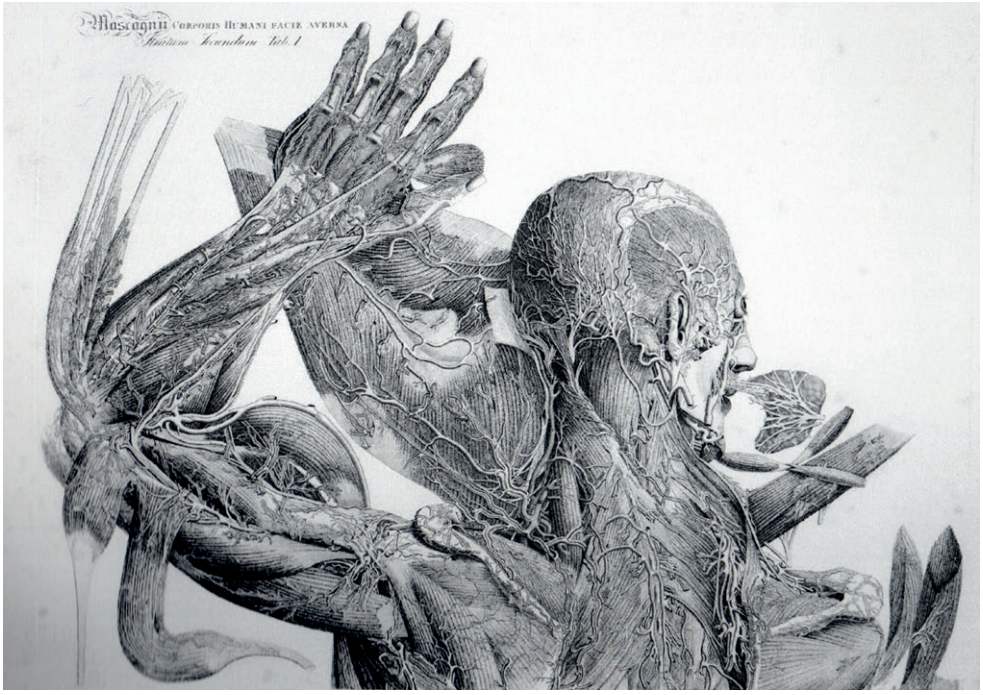

Figure 4. Paolo Mascagni, *Anatomia Universa*, stratum secondum, tav.

Mascagni's modernity and innovation in the teaching of anatomy

In their precise attention to proportions, movement and expressions, Mascagni's drawings fit into the academic tradition, indicating the extent to which he took into account the knowledge of those who had preceded him. At the same time, however, they distance themselves from this tradition.

Mascagni's vast culture was also based on the ancient texts, which abounded in his library. From the volumes of Andreas Vesalius to those of Gaspard Bauhin, Pietro da Cortona, Frederik Ruysc, Bernhard Siegfried Weiss – better known as Albinus – and Albrect van Haller, down to the most up-to-date Venetian edition of Antonio Scarpa, all the most important publications on anatomy were owned, read and annotated by him[20].

Likewise, he owned and studied texts on physiology. However, his tables clearly reveal the extremely detailed study that he conducted directly on cadavers through his continual dissections and observations. Thus, while maintaining a direct link with the classic iconographic tradition, Mascagni introduced innovations that are indicative of a new approach to research and teaching in the field of anatomy.

The tables of the *Vasorum lymphaticorum historia et ichnographia,* and especially those of the *Anatomia Universa,* display a further innovative feature; in keeping with their didactic purpose, the illustrations are not burdened by any extraneous finery, and the human body appears in its true dimensions, as it is seen on the autopsy table.

The above exposition clearly indicates the importance of Paolo Mascagni's contribution to medical education and, in particular, to the teaching of anatomy, a contribution that stemmed in no small part from his propensity to consider and to synthesise both past and contemporary knowledge, art and science.

Over the centuries, methods and technologies have evolved, but the understanding of anatomy is still based on the direct observation of the human body. Paolo Mascagni's *Anatomia Universa* is a perennial testimony to his ability as a scholar and a teacher and to his desire to contribute to the formation of a new medical class.

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Sarcoglycans and integrins in human thyrocytes: an immunofluorescence study

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Abstract

The sarcoglycan sub-complex is a protein system which plays a key role in sarcolemma stabilization during muscle activity consisting of six glycosylated transmembrane proteins. Integrins play a key role in the process of cell adhesion, linking the extracellular matrix to the actin cytoskeleton. Here we have analysed the receptor for thyroid hormone, identified on αvβ3-integrin that has an important role in the activation of non-genomic actions of the hormone. Many non-genomic actions of the thyroid hormone appear to contribute to basal levels of activity of a variety of proteins, including ion pumps, intracellular protein trafficking and protein turnover. The purpose of our research was to study the presence and behaviour of sarcoglycans and integrins on the thyroid gland, in both normal and pathological conditions, for the first time. Our results show a normal fluorescence pattern in patients without pathology, and a reduced fluorescence pattern in patients with thyroid disease. Moreover, colocalization in healthy patients was found in double localization reactions, whereas in patients with Hashimoto's thyroiditis sarcoglycans did not colocalize with tested integrin. These data could confirm the hypothesis of a close association between sarcoglycans and integrins, which, in pathological condition, are not found contemporarily hypothesizing that each single protein system could have a role in maintaining cell vitality.

Keywords

sarcoglycans; thyrocytes; integrin; immunofluorescence; thyroid.

Introduction

The sarcoglycan sub-complex (SGC) is a multi-member transmembrane complex which provides a connection between extracellular matrix components and the cytoskeleton. The SGC is made up of six glycoproteins, α-, β-, γ-, δ-, ε- and ζ-sarcoglycan (SG), linked by lateral binding to β- dystroglycan. β-, γ-, δ-, and ζ-SGs are similar to type II transmembrane proteins with the NH-terminal on the intracellular side; α- and e-SG is a type I transmembrane proteins with the NH-terminal on the extracellular side (1-3). Furthermore, ζ-SG, similar to δ- and γ-SGs has been identified and has also been found as a component of the vascular smooth muscle SGC (4).

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In recent years, SGs have been the most studied proteins, together with dystrophin, which plays an important role in the pathogenesis of many muscular dystrophies (e.g. Duchenne and Becker muscular dystrophies). Molecular genetic studies have allowed to demonstrate that a mutation in each single SG gene causes a series of recessive autosomal dystrophin-positive muscular dystrophies, known as sarcoglycanopathies or limb girdle muscular dystrophies (LGMD type 2D, 2E, 2C and 2F) (5-9).

Our previous studies on skeletal muscle fibers have demonstrated that SGs are organized in costameres representing a protein machinery made up of a dystrophin-glycoprotein complex and a vinculin-talin-integrin system, proving the colocalization of SGs and integrins in adult human skeletal muscle and supporting the hypothesis of the existence of bidirectional signalling between SGs and integrins (10-12).

Integrins, part of the vinculin-talin-integrin system, are transmembrane heterodimeric receptors, that play an important role in the process of cell adhesion, linking the extracellular matrix to the actin cytoskeleton (13).

These proteins would appear to be the main mediators of cellular processes such as adhesion, migration, survival, apoptosis, and cell differentiation (14) and they are primarily expressed in skeletal muscle fibers and in cardiac and smooth muscle fibers (15, 16).

Our previous studies, carried out on human biopsies obtained from airway, gastrointestinal, and urinary tracts, demonstrated the presence of all SGs in smooth muscle tissue (17, 18). In particular, all six sarcoglycans are organized in different tetramers, or even in a hexameric arrangement (18) Nevertheless, it is not clear yet what are the tetramers in these tissues. Also in glandular epithelium from the mammary and prostatic gland it was possible to demonstrate a normal presence of all SGs in healthy tissues and a reduced fluorescence in samples with mammary or prostatic pathology (19, 20). In particular, the study on mammary gland showed that in biopsies of normal breast tissue, immunofluorescence was detectable for all the tested SGs, the staining pattern for all SGs was distributed in all cells and immunofluorescence for all SGs was detectable also in myoepithelial cells. In samples of pathological breast tissue, we observed that staining for all the tested SGs appeared to be severely reduced or absent, both in epithelial and myoepithelial cells (19); these studies have demonstrated that the lack of SGs, observed in the biopsies of breast tissue obtained from patients with fibroadenoma and fibrocystic mastopathy, may provoke a loss of strong adhesion between epithelial cells, facilitating degeneration and progression from benign tumours in malignant tumours.

Similar results we have obtained in glandular epithelium of prostatic gland, analysing samples of patients affected by benign prostatic hyperplasia and by prostatic adenocarcinoma (20).

Based on our previous results in epithelial and glandular tissues, in the present report we performed a study on SGs and integrins in thyrocytes. In particular, we studied all SGs and the $\alpha v\beta 3$- integrin, a cell surface receptor for thyroid hormone, which is the initiation site for T4-induced activation of intracellular signalling cascades (21) by activation of the sodium pump (14, 22). As this integrin is also widely present on cancer cells, it was clear that the thyroid hormone has also been found to cause proliferation of a variety of human cancer lines via the cell surface receptor. The thyroid hormone analogue L-thyroxine (T4), widely studied in genomic mechanisms of hormone action as a prohormone antecedent to T3 through tissue deiodinas-

es, is biologically active at the integrin receptor (23). This integrin is considered the main exponent of that initiation/progression process that leads to many human diseases, both inflammatory and neoplastic (24-26).

Since the presence of SGs and their interactions with integrin, in thyroid gland epithelium, has not previously described, here we have analysed the behaviour of these transmembrane proteins, in healthy and pathological subjects, by immunofluorescence techniques. Relatively to pathological conditions, we have analysed samples of thyroid obtained by patients affected by Hashimoto's thyroiditis, since it is considered a risk factor for thyroid cancer (27).

Materials and methods

Patients

For the data obtained at the level of the thyroid gland, thyroid needle aspiration was used for the this study, a minimally invasive method that allows, in most cases, a precise diagnosis on the nature of thyroid nodules. Thyroid needle aspiration was performed according to the American Association of Clinical Endocrinologists (AACE), American College of Endocrinology (ACE) and Associazione Medici Endocrinologi (AME) 2016 guidelines. Cytological sampling was performed using a thin needle, 20-24 G, attached to a syringe by suction technique. Given the minimum duration and minimally invasive blood sampling, the 12 patients were not subjected to local anaesthesia. The needle aspiration was also performed under continuous ultrasound guidance. The ultrasound guide allowed us to pick up cells also from non-palpable nodules, to choose which nodule or part of the nodule to subject to needle aspiration and to increase the diagnostic power of the needle aspirator (28, 29). In detail, needle aspiration was performed on 12 patients: 2 control patients (female), affected by colloidal cysts, and 10 patients with Hashimoto's thyroiditis (8 female and 2 male), aged between 26 and 77 years. Once the sampling was carried out, we performed the preparation of the material by direct smear on slide. All patients gave their informed consent for the treatment of the cytology obtained by needle aspiration, for scientific purposes.

Immunofluorescence

The biopsies were fixed in 3% paraformaldehyde in 0.2 M phosphate buffer, ph. 7.4, for 2 hours at room temperature. They were washed extensively with 0.2 M phosphate buffer ph. 7.4, and then with phosphate buffer saline (PBS), containing 12 and 18% sucrose. The samples were snap-frozen in liquid nitrogen, and 20 μm sections were prepared in a cryostat for use in a protocol to perform immunofluorescence. The sections were placed on glass slides that were coated with 0.5% gelatine and 0.005% chromium potassium sulphate.

To block nonspecific binding sites and to permeabilize the membranes, the sections were pre-incubated with 1% bovine serum albumin (BSA), 0.3% Triton X-100 in PBS for 15 minutes at room temperature. Finally, the sections were incubated with primary antibodies. The following primary polyclonal anti-goat antibodies were used: anti-

α-SG diluted 1:100, anti-β-SG diluted 1:200, anti-γ- SG diluted 1:100, anti-δ-SG diluted 1:10, anti-ε-SG diluted 1: 100, anti-ζ-SG diluted 1: 100, anti-αvβ3-integrins diluted 1:100 (all from Santa Cruz Biotechnology Inc., Santa Cruz, Ca., USA). About SGs, primary antibodies were detected using Texas Red-conjugated IgG anti-goat diluted at 1:250 (Jackson ImmunoResearch Laboratories, Inc., West Grove, Pa., USA), about αvβ3-integrin, primary antibody was detected using fluorescein isothiocyanate (FITC) IgG-conjugated anti-mouse, (1:250 dilution; Sigma Chemicals, St. Louis, Mo., USA)

The sections were then analysed and images acquired using a Zeiss LSM 5 DUO (Carl Zeiss, Jena, Germany) confocal laser scanning microscope. All images were digitized at a resolution of 8 bits into an array of 2048 x 2048 pixels. Optical sections of fluorescent specimens were obtained using a HeNe laser (wave-length 543 nm) and an Ar laser (wavelength 458 nm) at a 62 second scanning speed with up to 8 averages; 1.50μm thick sections of fluorescent specimens were obtained using a pinhole of 250. Contrast and brightness were established by examining the most brightly labelled pixels and choosing the settings that allowed clear visualization of the structural details while keeping the pixel intensity at its highest (~200). Each image was acquired within 62s, in order to minimize photodegradation.

For image analysis, we used splitting, and the function called Histo, both belonging to software ZEN 2009 of the ZEISS LSM DUO. In particular, the splitting shows individual channels and relative mergers; the Histo allows to display a histogram of entire image calculating the distribution of pixel intensities and showing the fluorescent values in a table form wherein the mean intensity, standard deviation and number of pixel are indicated. The mean intensity and standard deviation for each images were reported in Tab. 1. Number of pixels was always the same because the images were all the same size and therefore not shown in the table.

Digital images were cropped and figure montages were prepared using Adope Photoshop 7.0 (Adobe system, Palo Alto, Calif. , USA).

Results

In this study, we performed immunofluorescence analysis of the thyroid tissue samples using antibodies against all SGs and against αvβ3-integrin. The 20-μm-thick cryosections were analysed by acquiring with a scan step size of 0.8μm.

Immunofluorescence investigations were performed in single-localization reactions analysing singularly SGs and αvβ3-integrin, and in double-localization reactions combining single SGs with αvβ3-integrin. The immunofluorescence reactions were carried out both in control thyrocytes and in thyrocytes during pathological conditions.

In particular, single-localization reactions (Fig. 1) using antibodies against α-SG (A), β-SG (B), γ-SG (C), δ-SG (D), ε-SG (E), and ζ-SG (F) in thyrocytes obtained from healthy subject samples show a normal fluorescence pattern for all tested proteins.

We observed the same result using antibodies against αvβ3-integrin in thyrocytes taken from healthy subject samples. Also in this case the fluorescence pattern for tested protein shows a normal staining (Fig. 2).

Instead, performing a single-localization reaction with antibodies against α-SG (Fig. 3A), β-SG (Fig. 3B), γ-SG (Fig. 3C), δ-SG (Fig. 3D), ε-SG (Fig. 3E), and ζ-SG (Fig.

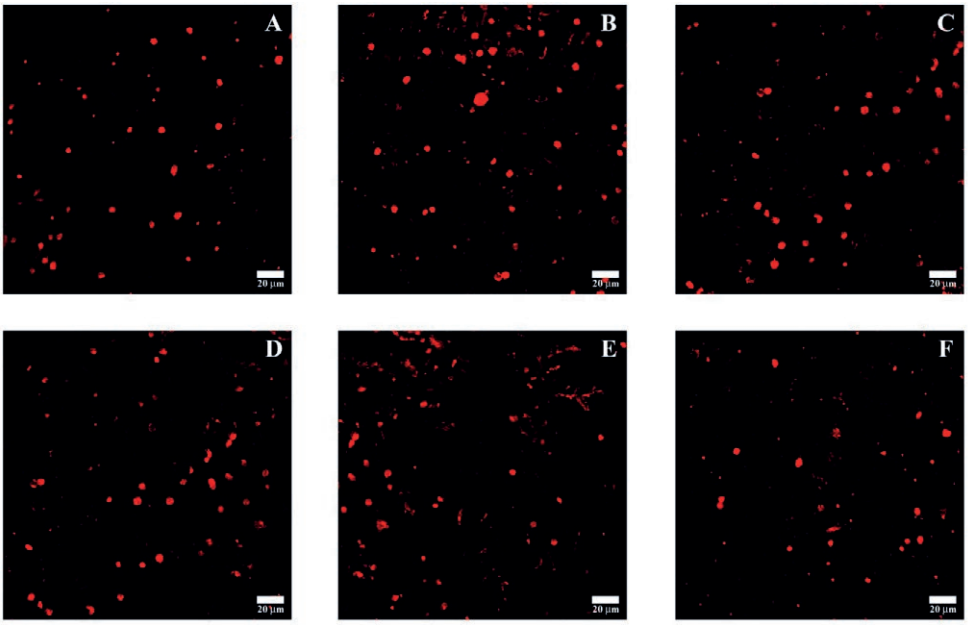

Figure 1. Compound panel showing immunofluorescence findings in thyrocytes obtained from healthy subjects. The samples were immunolabelled with single-localization reaction using antibodies against α-sarcoglycan (A), β-sarcoglycan (B), δ-sarcoglycan (C), γ-sarcoglycan (D), ε-sarcoglycan (E), ζ-sarcoglycan (F). All tested proteins show a normal staining pattern.

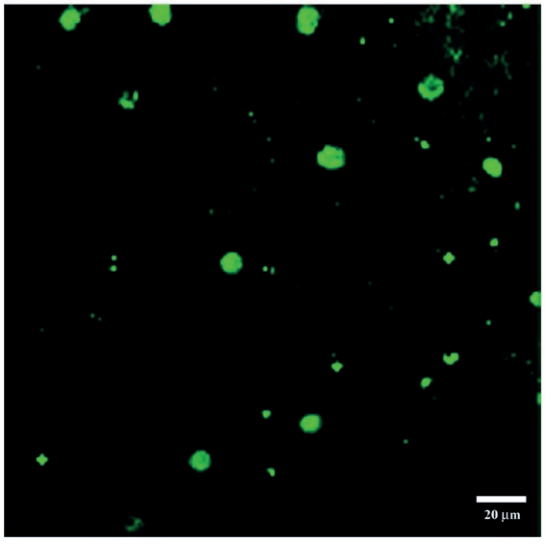

Figure 2. Single-localization reaction performed using antibodies against integrin αvβ3 showing a normal staining pattern for this protein in thyrocytes obtained from healthy subject.

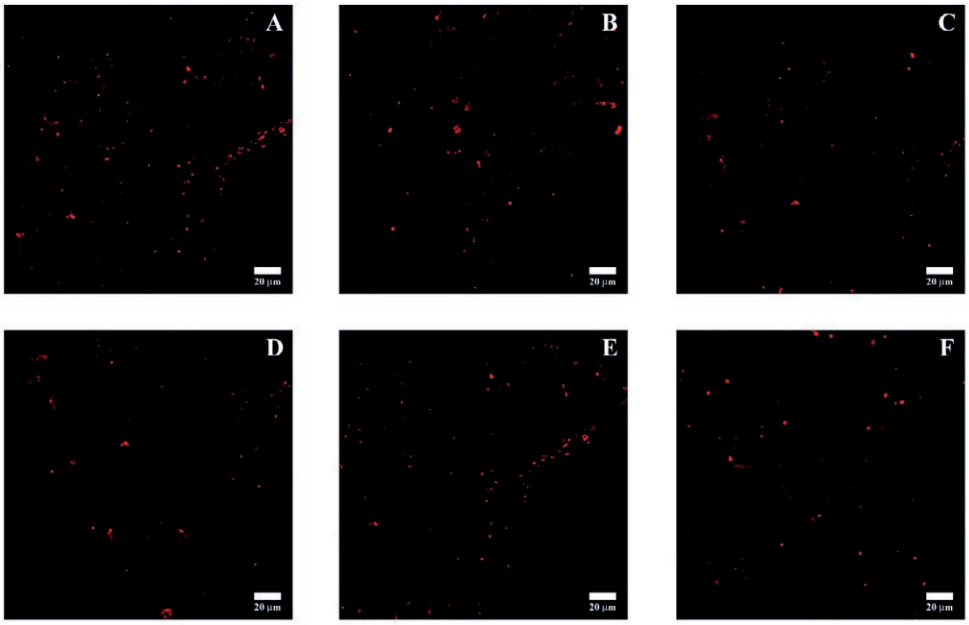

Figure 3. Compound panel showing immunofluorescence findings in thyrocytes obtained from subjects affected by Hashimoto's thyroiditis. The samples were immunolabelled with single-localization reaction using antibodies against α-sarcoglycan (A), β-sarcoglycan (B), δ-sarcoglycan (C), γ- sarcoglycan (D), ε-sarcoglycan (E), ζ-sarcoglycan (F). All tested proteins show a decreased staining pattern.

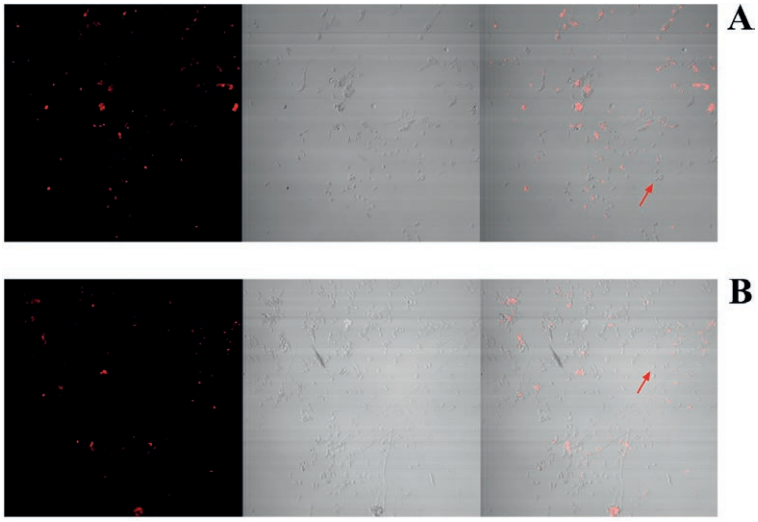

Figure 4. Single-localization reactions performed with antibodies against β- (A) and δ-sarcoglycan (B) respectively showed in Figure 5B and 5D. Merging the fluorescence channel (red channel) with transmitted light (gray channel) in the images to the right, it is possible to denote the absence of staining pattern in some cells. (red arrows).

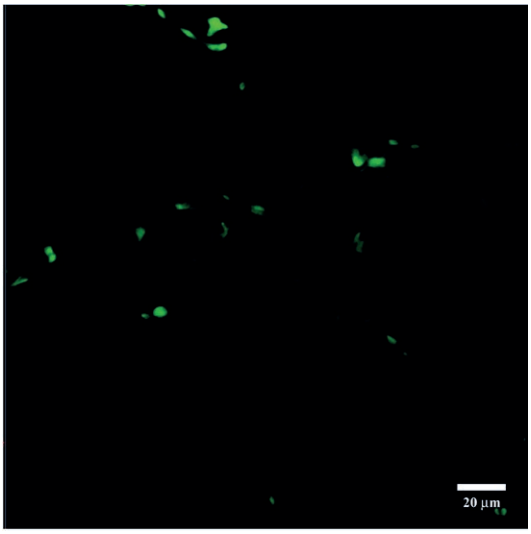

Figure 5. Single-localization reaction performed using antibodies against αvβ3-integrin showing a decreased staining pattern for this protein in thyrocytes obtained from subjects affected by Hashimoto's thyroiditis.

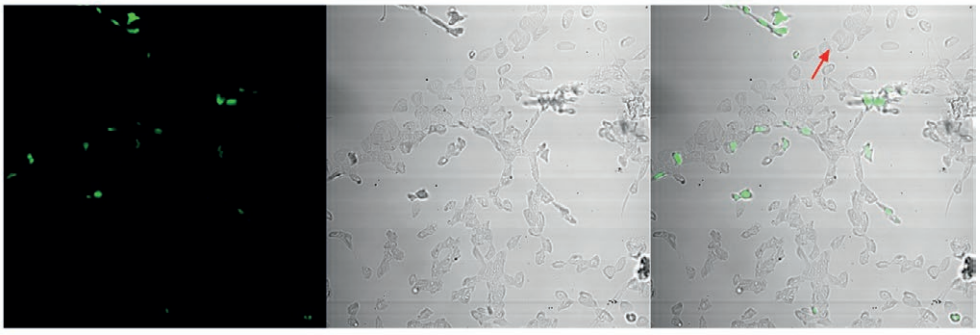

Figure 6. Single-localization reactions performed with antibody against αvβ3-integrin shown in the previous figure. Merging the fluorescence channel (green channel) with transmitted light (gray channel) in the images to the right, it is possible to denote the absence of staining pattern in some cells (red arrow).

3F) in thyrocytes obtained from subjects with Hashimoto's thyroiditis, a decreased pattern of all SGs in these cells can be observed, compared to thyrocytes obtained from healthy subjects.

Merging the fluorescence channel (red channel) with transmitted light (grey channel) using antibodies against β-SG (Fig. 4A) and δ-SG (Fig. 4B), respectively, shown in previous Figure 3B and 3D, it is possible to show the absence of staining pattern for sarcoglycans in some cells (red arrows). The same condition was observed for other SGs (data not shown).

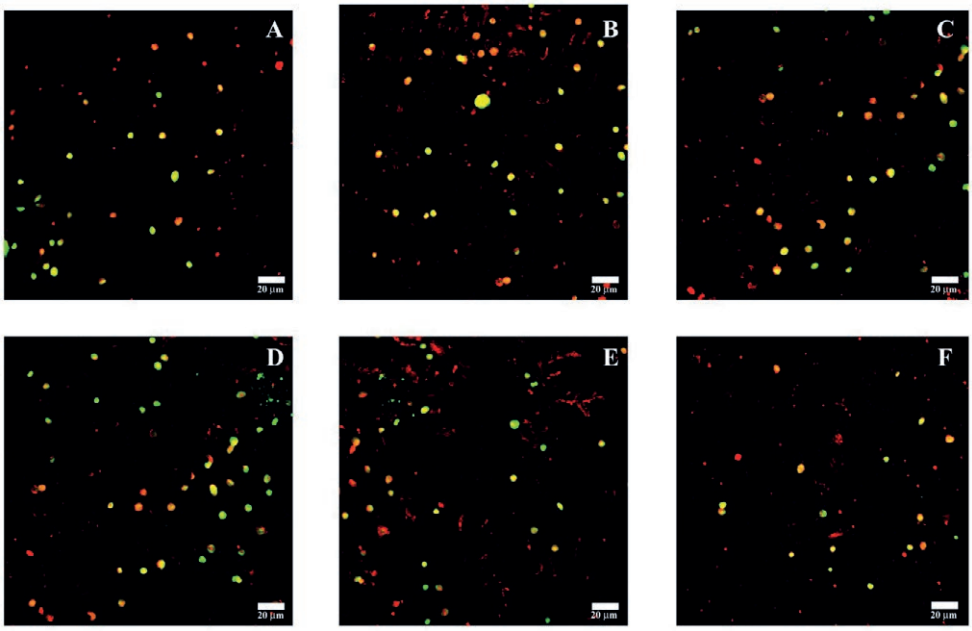

Figure 7. Compound panel showing immunofluorescence findings in thyrocytes obtained from healthy subjects. The samples were immunolabelled with double-localization reaction analysing antibodies against α-sarcoglycan (A), β-sarcoglycan (B), δ-sarcoglycan (C), γ-sarcoglycan (D), ε-sarcoglycan (E), ζ-sarcoglycan (F) (all in red channel) and αvβ3-integrin (green channel). It is possible to denote a yellow fluorescence due to an overlapping of red fluorescence (sarcoglycans) on green fluorescence (integrin) demonstrating a co-localization between these proteins.

Single-localization reaction carried out using antibody against αvβ3-integrin in thyrocytes obtained from subjects affected by Hashimoto's thyroiditis shows a decreased staining pattern for this protein compared to results obtained in thyrocytes taken from healthy subject samples (Fig. 5).

Merging the fluorescence channel (green channel) with transmitted light (grey channel) in single-localization reaction using antibody against αvβ3-integrin (Fig. 6A), it is possible to denote the absence of tested protein in some cells (red arrow), as seen for SGs.

Subsequently, we carried out double-localizations, analysing each single SG with αvβ3-integrin in thyrocytes obtained from healthy subject samples. In particular, superimposing α-SG (Fig. 7A), β- SG (Fig. 7B), γ-SG (Fig. 7C), δ-SG (Fig. 7D), ε-SG (Fig. 7E), and ζ-SG (Fig. 7F), shown with red channel, and αvβ3-integrin, shown with green channel, we observed a yellow fluorescence due to an overlapping of red fluorescence (SGs) on green fluorescence (integrin) demonstrating a co- localization between these proteins.

Double-localization reaction performed combining α-SG (Fig. 8A), β-SG (Fig. 8B), γ-SG (Fig. 8C), δ-SG (Fig. 8D), ε-SG (Fig. 8E), and ζ-SG (Fig. 8F), with αvβ3-integrin in thyrocytes obtained from patients affected by Hashimoto's thyroiditis show the

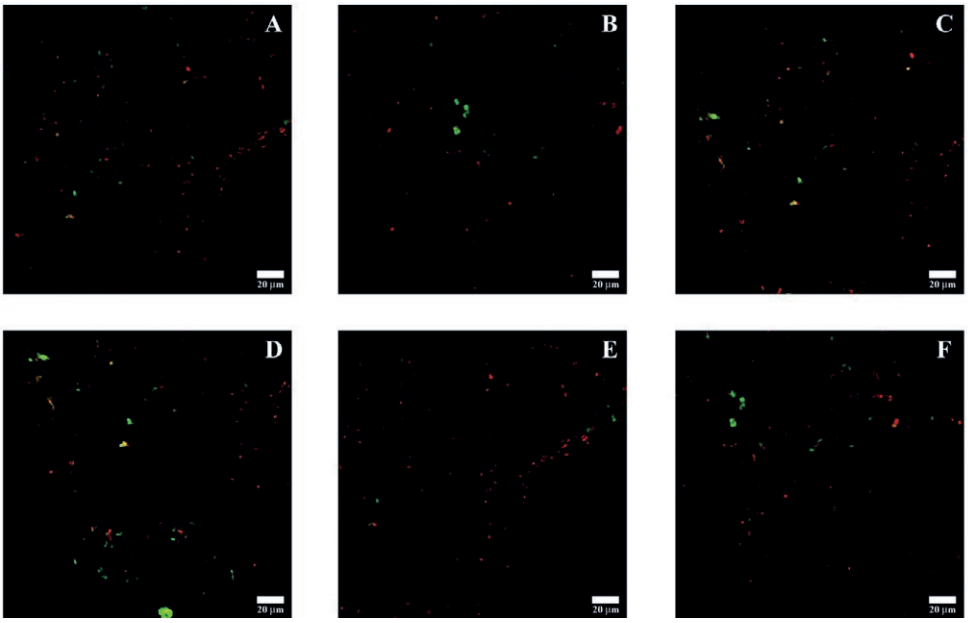

Figure 8. Compound panel showing immunofluorescence findings in thyrocytes obtained from subjects affected by Hashimoto's thyroiditis. The samples were immunolabelled with double-localization reaction analysing antibodies against α-sarcoglycan (A), β-sarcoglycan (B), δ-sarcoglycan (C), γ- sarcoglycan (D), ε-sarcoglycan (E), ζ-sarcoglycan (F) (all in red channel) and αvβ3-integrin (green channel). It is possible to denote the presence of the cells expressing either only red fluorescence or only green fluorescence, but always in a reduced amount compared to thyrocytes obtained from healthy subjects.

Table 1. Histo function applied in each immunofluorescent image in single-localization showing the mean intensity and standard deviation of real pixel intensity in entire image (M.I.= Mean Intensity; S.D.= Standard Deviation).

Protein	Control		Hashimoto's thyroiditis	
	M.I.	S.D.	M.I.	S.D.
α-SG	5.97	28.485	1.94	16.818
β-SG	6.79	35.041	1.48	14.651
γ-SG	5.21	30.267	0.96	11.063
δ-SG	4.98	28.152	1.02	11.146
ε-SG	6.04	31.012	1.91	16.823
ζ-SG	4.87	24.716	1.44	14.745
αvβ3-integrin	5.87	26.823	1.63	19.375

presence of cells expressing either only red fluorescence or only green fluorescence, but always in a reduced amount compared to thyrocytes obtained from healthy sub-

jects, indicating a no-colocalization between SGs and tested integrin.

In order to display the real values of the pixel intensity corresponding to tested proteins, for each previous images in single-localization, the function called Histo was applied and the mean intensity and standard deviation were reported in Tab. 1. In this table, really it was possible to denote how the pathological thyrocytes show low values compared to those of the control thyrocytes.

Discussion

Sarcoglycans are a subcomplex of glycoproteins mediating interaction between the extracellular matrix and sarcolemma of the myofibers, protecting also the muscle fibers from damage provoked by contraction and relaxation (30-33). In our previous studies on SGC, we examined smooth muscular districts, both in normal and pathological conditions, finding that in smooth muscle the SGC presented a hexameric structure (formed by all SGCs) which has a higher or lower expression of a single SG in conformity with the function of smooth muscle in the gastrointestinal, urogenital, vascular or respiratory tract (17, 18, 34).

However, it has been shown that only α- and γ-SG constantly are present in striated and smooth muscle, whereas other SGs are identified also in other tissues (2). Indeed, it has been hypothesized the presence of a bidirectional signaling between SGs and integrin adhesion system in cultured L6 myocytes (10).

Extracellular domains of the integrins, a known family of transmembrane glycoproteins, interact with a variety of ligands (35), including extracellular matrix glycoproteins, and the intracellular domain linked to the cytoskeleton (36). Moreover, it has been demonstrated that the thyroid hormone could influence the interaction of integrins with the extracellular matrix protein (22). At this purpose, $\alpha v\beta 3$-integrin has a large number of extracellular protein ligands, including growth factors and extracellular matrix proteins. In particular, the role of $\alpha v\beta 3$-integrin as a cell surface receptor for thyroid hormone at which non-genomic actions are initiated and as a mediator of thyroid hormone effects on plasma membrane ion transporters and on intracellular protein trafficking has been analyzed (37).

Then, in this work we carried out primarily a semiquantitative study on thyrocytes obtained from unaffected subjects, in order to analyze and to understand the behavior of SGs and $\alpha v\beta 3$-integrin in non-muscle cells.

In the present study, immunofluorescence results demonstrated, for the first time, that in thyrocytes of healthy patients: (i) all tested SGs were detectable with normal staining pattern in all analysed cells; (ii) $\alpha v\beta 3$-integrin was detectable in all analysed thyrocytes; (iii) each SGs co-localize with $\alpha v\beta 3$-integrin.

Our previous results on normal breast and prostatic epithelium have shown a clearly detectable immunofluorescence for all SGs, normally distributed in all cells, demonstrating a hexameric structure for this subcomplex (19, 20). Based on our present results, we can assert that also in thyrocytes exists a SGC structured with hexameric organization. Moreover, the present data, evidencing a constant colocalization between SGs and integrin, confirm that also in thyrocytes exists a bidirectional signalling between these proteins, as previously demonstrated in skeletal and smooth muscle (11, 12, 34) and that also in thyrocytes a protein machinery could be present.

Furthermore, as it has been demonstrated that the mutations in any SG cause limb-girdle muscular dystrophy (38, 39) provoking dysphagia and acute digestive dilatations (40), it was possible to assert that SGs play an important role in ethiopathogenesis of muscular pathologies and they seem to be functionally and pathologically as important as dystrophin (10). Moreover, our previous reports on breast and prostatic epithelium, have demonstrated a clear reduction or absence of SGs in samples obtained from pathological subjects (19, 20). On this basis, in order to understand the real role of SGs and αvβ3-integrin, it was necessary to analyze these proteins also in pathological conditions studying SGs and αvβ3-integrin in thyrocytes obtained from patients affected by Hashimoto's thyroiditis, as well known, risk factor for thyroid cancer (27). Our data on pathological thyrocytes revealed: (i) a severely reduced or almost absent staining pattern for all SGs and αvβ3-integrin; (ii) cells expressing exclusively SGs or exclusively αvβ3- integrins.

These data are similar to our previous results obtained in breast and prostatic epithelium in pathological conditions (19, 20) showing, for the first time, that SGs in normal epithelial cells display a wider distribution compared to pathological tissues in which all SGs are reduced; then, it can possible to hypothesize that SGs and integrins play a key role in regulation of vital functions of the cells also in thyrocytes. Moreover, on the basis of our present data showing the presence of the cells expressing exclusively SGs or exclusively αvβ3-integrins, we can also assert that in pathological conditions the structural and functional roles of these proteins can be reduced. In our opinion, it's possible to hypothesize that a self-defence system is activated to maintain cellular vitality. This system could be activated by SGs when there are no αvβ3-integrins and could be activated by integrins when there are no SGs.

Furthermore, while in normal conditions in the presence of both SGs and αvβ3-integrins, the opening of the membrane ion pumps and the leakage of T3 and T4 from the cells with normal thyrocyte function exists (14), in pathological conditions the reduction of thyrocytic functions could provoke a lack of opening of membrane ion pumps. This confirms that the tested proteins in the present study can play a key role in mediating signalling between the intracellular environment and the extracellular matrix, as evidenced by the reduction of these proteins in pathological conditions, and by the constant colocation between SGs and the αvβ3-integrin. This hypothesis could be confirmed by evidence that the inoculation of integrins in rats affected by autoimmune pathology reduces or abolish infiltration of lymphocytes (41) and so this finding could lead to future therapies.

Our present data, confirming the importance of SGs and αvβ3-integrin also in nonmuscle cells and showing a possible role of these proteins in pathogenesis of thyroiditis, reveal a new avenue of research. In this way, it will possible to understand the variations of SGC in many other tissues and their function in other pathological conditions also integrating these studies with molecular biology techniques.

Authors' contribution

AFa, GC and GR analyzed the data, and wrote the manuscript; GR, AFu and AC designed the experiments, performed the experiments; AC and AF conceived the study and revised the manuscript; GS and SP provided the patient samples.

Ethics approval and consent to participate

The study was performed in accordance with the Declaration of Helsinki, and was approved by the Ethics Committee of Istituto Dermopatico dell'Immacolata IRCCS (IDI-IRCSS) of Rome (Sezione Comitato Etico IRCCS Lazio (no. 523-1). The participants provided written informed consent to participate in this study.

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From morphological basic research to proposals for regenerative medicine through a translational perspective

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Abstract

Basic research, especially morphological research, often fails to get off the ground due to scarcity of opportunities and funding. There is a need to exploit ideas, while starting from a morphological basis, to channel them into pathways with translational value: patents, trademarks, alternative experimental models, etc., aimed at formulating new proposals for applied research.
Among the many sprouts emerging in laboratories where basic research is carried out throughout the country, one reality from Emilia Romagna region is represented by some insights from human anatomy teachers and researchers at the University of Modena and Reggio Emilia. They have developed an original idea whereby they propose to use very small bone segments (the scleral ossicles –SO) taken from the sclero-corneal boundary of lower vertebrates with protruding eyes (particularly from avian species) to supplement 3D scaffolds to be used in regenerative medicine (by triggering/improving angiogenesis and osteogenesis) for the recovery of severe bone injuries, defined as "critical size", i.e., unable to recover autonomously. The idea was followed by the patent application and, subsequently, the filing of a trademark (Pal-OS®) concerning to SO-derivatives (powders, sticks, caps, etc.).
In times when respect for the natural environment and attention to animal health are among the relevant aspects for an ecosystem's welfare, along with these patent and trademark a focus was developed on experimental methods alternative to animal testing, with which to be able to test the efficacy of the proposed products while respecting the 3 R's rules, using a model already known and exploited in the past, the chorio-allantoic membrane – CAM, revisited today from an ethical perspective.
The article traces the observations that led to the idea of patenting scleral ossicles, of filing the Pal-OS® trademark and of using the CAM model to test their validity for regenerative purposes, with the ultimate goal of underlining how morphological observations, interpreted from a translational perspective, can provide interesting insights for clinical applications.

Keywords

Scleral Ossicles (SO); Pal-OS®; chorioallantoic membrane (CAM) model; angiogenesis; osteogenesis; critical-sized bone lesion recovery.

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A local example at UNIMORE: scleral ossicles, Pal-OS®, CAM and their combination

Scleral ossicles – description and development possibilities

In recent years, with the rapid development of sophisticated bio-molecular techniques and the sharpening of the biotechnology mindset, morphology has undergone slowdown that results in term of scarcity of funding and human resources. To emphasize the importance of morphological studies, our research group, starting from mere morphological observations, proposed the use, in a translational key, of a peculiar natural scaffold, the Scleral Ossicles (SO), for bone regeneration.

SO (Fig. 1) are small bony plates, organized to form a ring of 13-14 elements articulated to each other with sutures, located at the sclero-corneal boundary of the eyeball of lower vertebrates with protruding eyes such as teleosts (Franz-Odendaal and Hall, 2006; Franz-Odendaal, 2008a; Lyon et al., 2017), amphibians, reptiles (Franz-Odendaal, 2006; Presch, 1970; Vieira et al., 2007), and birds (Franz-Odendaal, 2008b; Lima et al., 2009; Zhang et al., 2012), whose aim, at the end of their development, is solely to protect the eye from deformation during flight or swimming (therefore submitted lifelong to stereotyped loading), reason why they should not be subject to bone variations dependent on the body's mineral/metabolic requirements (as is the case for all other skeletal segments). Thus, in order not to undergo bone remodeling, once SO reach the final size, all their osteocytes massively go into apoptosis (Palumbo et al., 2012). Therefore, SO are "naturally decellularized" materials, and it has been shown that they do not induce adverse immune reaction (when transplanted into non-immune-depressed animals) and promote both angiogenesis and osteogenesis (Checchi et al., 2018, 2020). On this basis, and after developing and standardizing an appropriate extraction and preparation method, they were thought to be housed in special slots within 3D scaffolds (Fig. 2), to be inserted into critical bone lesions, to

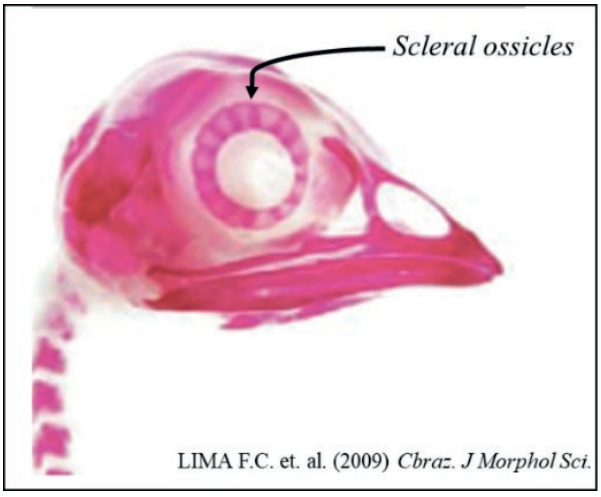

Figure 1. Photograph of the scleral ring in *Gallus gallus domesticus*. The arrow highlights the scleral ossicles.

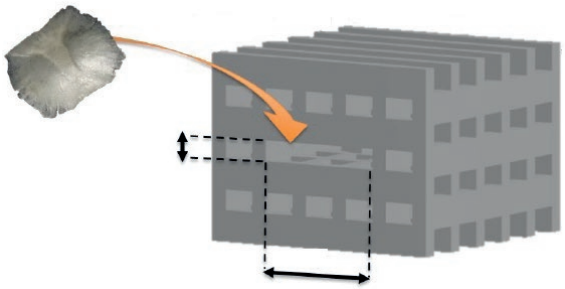

Figure 2. The picture represents a hypothetical 3D scaffold architecture that could host the scleral ossicles in a peculiar slot designed with appropriate dimensions.

trigger an angiogenic and osteogenic response in the host, in order to improve the recovery of critical-sized bone lesions.

In November 2022 (https://www.magazine.unimore.it/site/home/notizie/articolo820058150.html) the University of Modena and Reggio Emilia started the patenting process for the use of Scleral Ossicles (SO).

Pal-OS® – description and development possibilities

Preliminary observations on the realization of prototypes of complex constructs that could accommodate SO suggested, later, that it would be more effective to combine some derivatives of patent-SO to be processed in parallel with the printing of biomaterials that must include them. Thus, a trademark, Pal-OS®, was filed (January 2022)

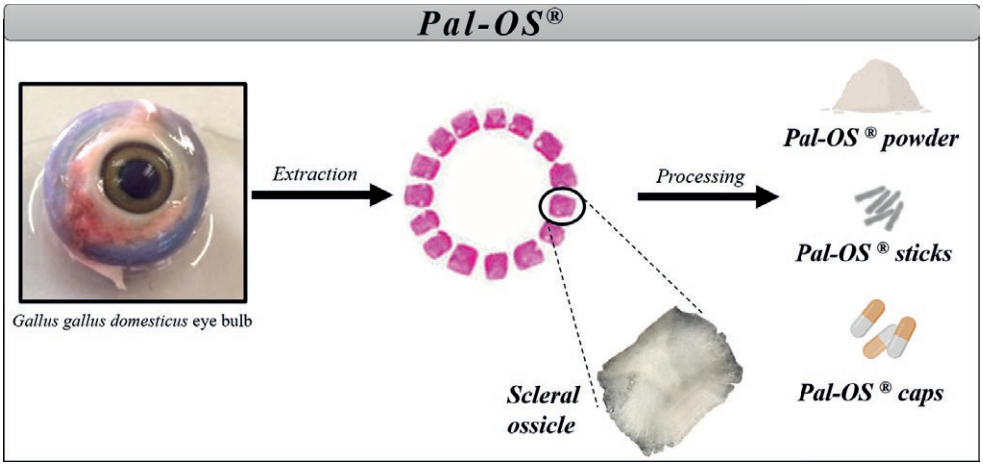

Figure 3. The picture schematically represents the extraction of the scleral ring from the chicken eye bulb. Each scleral ossicle is disjoined and it could be processed in different derivates: Pal-OS® powder, Pal-OS® sticks, or Pal-OS® caps.

denoting any product from comminution of scleral ossicles (powder, filaments, chips, splinters, caps, etc.). The idea is to obtain 3D printed gelatinous biomaterials, such as hydrogels, that include SO derivatives (Pal-OS®), for example Pal-OS® powder sticks (Fig. 3). This will optimize the angio-/osteo-inductive influence of SO on the host bone.

Tests on the application potential of Pal-OS® derivatives are currently in progress and propose the embedding of Pal-OS® within matrices of different nature, that can be customized by the manufacturer in order to adapt perfectly to the size of the bone defect to be to deal. "Critical size" bone defects are fractures which, due to their excessive extension, do not undergo self-regeneration and therefore require a method or therapy that allow complete healing. Essential requirements for the healing of a critical fracture are the restoration of an efficient vascular system and the reduction of the gap between the two bone stumps. Vascularization is an essential preliminary element without which ossification cannot proceed. As mentioned above, SO have been tested for biocompatibility and angiogenic potential *in vitro* and *in vivo* resulting biocompatible and able to induce angiogenesis; therefore, the use of these ossicles would ensure the angiogenic potential necessary for the recruitment of all the elements (cells and cytokines) to initiate the process of bone regeneration (Ferretti and Palumbo, 2021).

Our final goal is to use customized constructs containing Pal-OS® derivatives as triggers for bone regeneration. To do this, 3D printed constructs containing Pal-OS® derivates were tested onto the Chorioallantoic Membrane (CAM) in order to select the best combination between bio-ink and Pal-OS® derivatives in terms of angiogenetic response.

Description and ethical value of CAM

An important aspect of our research activity which deserves attention is performing (after the preliminary *in vitro* tests) *in ovo* experiments by means of Chorioallantoic Membrane (CAM) assay (Fig. 4A). The CAM is a highly vascularized membrane that performs the functions of the primitive respiratory organ of the chick embryo and contains high amount of both oxygen and growth factors (Ribatti, 2010; Schneider-Stock and Ribatti, 2020).

CAM forms on days 3–4 of embryo development by the fusion of the chorion and the allantois. It acts as natural bioreactor and largely vicariates the use of animal experimentation (in fact, if used within day 17 of development, it does not require ethics committee approval). In the past, it has been used mostly in cancer research to study the molecular mechanisms of anticancer drugs and the correlated angiogenic response (Ribatti, 2014; Ribatti et al., 2001, 2003; Ribatti and Tamma, 2019), as well as for various types of tests (Kundeková et al., 2021): angiogenic potential assay (in both physiological and pathological purview), experimental embryology, tumor growth, experimental metastasis (Ribatti, 2021), teratogenesis and toxicology, drug delivery assay (Fonseca et al., 2021). Recently, instead, CAM has been re-discovered as an alternative to the *in vivo* experimentation, which underlines its ethical value. In fact, according to the 3R's principles of the animal experimentation (Reduction, Refined, Replaced), CAM assay has been exploding in recent years, where animal *welfare* is absolutely to be pursued and developed to make research sustainable, in relation to the "health" of both the ecosystem in general and the animal world in particular (Fig. 4B). The authors were recently funded by the Emilia Romagna Region, which has

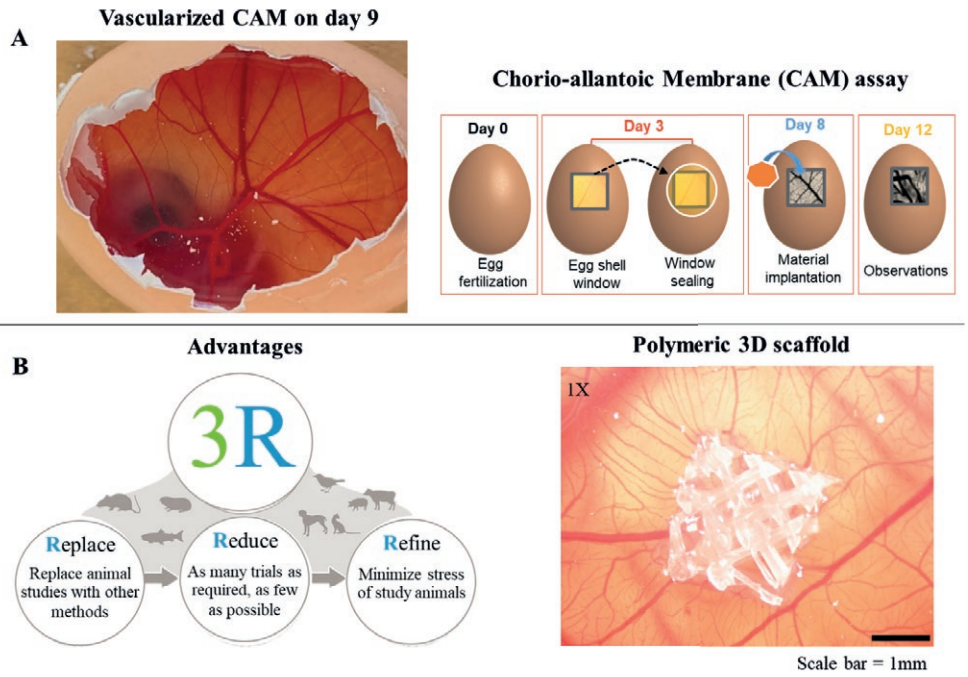

Figure 4. A. On the left a photograph of Chorio-allantoic Membrane (CAM) on day 9 of incubation. On the right, a diagram representing the CAM assay during 12 days of incubation: on day 3 eggs are opened in order to detect that fertilization has occurred. On day 8 the CAM is ready to host the material and the implantation is performed. Finally, on day 12 all the observations take place. B. All the advantages deriving from CAM assay are represented on the left of the picture: the 3R's principles are respected. On the right, a picture of a polymeric 3D scaffold on chorio-allantoic membrane that shows a large number of vessels around it.

been funding precisely alternative research models to animal testing for several years. The use of CAM assay cuts across several topics: from tests of biocompatibility, cytotoxicity and mechanisms of action of bioactive molecules, to validation of materials for tissue engineering; just to this last topic, our interest is particularly focused and declined in various projects.

Conclusions – Proposed development of eco-sustainable strategies for skeletal tissue regeneration

Studies on scleral ossicles and their derivatives (Pal-OS®) in combination with the use of ethical CAM assay are a clear demonstration of how morphology can overcome the risk of being confined and considered obsolete following the exuberant development of other disciplines. Morphology, instead, represents the indispensable basis of innovative proposals with translational significance and applicative possibility.

Moreover, our group's recent studies are absolutely based on environmentally sustainable research as well as respect of animal welfare: scleral ossicles are obtained

from poultry waste (whose extraction and processing procedure was patented by UNIMORE-Palumbo), thus no animal sacrifice is required for their procurement. CAM is a simple, quick, low-cost and ethically sustainable model, that replaces the use of animals in the primary stages of experimentation and does not require administrative ethics committee approval (unlike animal experimentation), because the chick embryo is not considered as living animal until 17th day of embryo development in most countries (moreover, the chick embryo younger than 10th day are assumed to be unable to experience pain (Institutional Animal Care and Use Committee (IACUC) - Brown University, 2019)).

In conclusion, the purpose of this prospective article is to emphasize how the "serendipity" (Dong et al., 2021; Hartl et al., 2021; Thomson, 2021) of morphological evaluations like the observation at the onset of this "story" concerning the generalized osteocyte apoptosis in some types of ossicles (Palumbo et al., 2012), can provide new application insights in the regenerative field, when combined with the scientific creativity, declined from a translational perspective, allowing the complementation or improvement of approaches that are already exploited by regenerative medicine.

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Histomorphometric changes in the ovaries of thymectomized guinea pigs

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Abstract

Hypothalamic-pituitary-gonadal axis function is necessary for maintaining proper female reproductive cycle. This study aimed to evaluate the ovarian histomorphometric and histoarchitectural changes in neonatal, prepubertal and pubertal thymectomized female guinea pigs. A total of 30 female guinea pigs, sham-operated (n-5) and thymectomized (n-5) were studied in each group. The diameter and number of ovarian follicles among the thymectomized and sham operated female guinea pigs during estrus phase of estrous cycle was compared. Gonadal and accessory reproductive organs weights and microscopic features were studied in the sham operated guinea pigs and thymectomized. There were statistically significant changes in the number and diameter of follicles in the ovary in neonatal thymectomized female guinea pigs, but no significant changes were observed in prepubertal and pubertal female guinea pigs. Neonatal thymectomized female guinea pigs showed significant changes in their weight as well as changes in the microscopic features including reduced thickness of myometrium of uterus and less mucosal folding in the fallopian tube compared to the sham-operated group. But prepubertal and pubertal thymectomy did not affect the weight and microscopic features of gonads and accessory reproductive organs. Depending on the time of thymectomy, these results indicate morphological changes in the ovaries after thymectomy in females.

Keywords

thymus; ovary; estrous cycle; morphology; histoarchitecture; thymectomy.

Introduction

Thymectomy performed in Tx3 inbred mice showed increased frequency of independent autoimmune diseases that target the ovaries, stomach, thyroid, lacrimal gland, prostate, and testis, and in the production of the respective organ-specific auto antibodies and pathogenic T cell responses (Kojima et al., 1976; Taguchi et al., 1981; Nishizuka et al., 1980; A. Kojima et al., 1985; Tung et al., 1987; Kosiewicz MM et al., 1990). The production of circulating auto antibodies against the ooplasm of oocytesin in Tx3 mice was an important autoimmune etiology for ovarian failure. The antioocyte antibodies, lymphocytes infiltration was detectable until 25 days of age. The disruption of the hypothalamic-pituitary–ovarian–thymic axis is caused by thymectomy, which resulted in autoimmune ovarian dysgenesis (Plant TM, 2015). The role of thymus during early life is essential for the normal develop-

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ment of immune system and as well as maturation of the hypothalamic – pituitary-gonadal axis. The Hypothalamic- Pituitary- Gonadal axis begins in the hypothalamus which secretes gonadotropin releasing hormone (GRH) that acts on the anterior pituitary and stimulates the synthesis and secretion of follicular stimulating hormone (FSH) and luteinizing hormone (LH). Follicular maturation and estrogen synthesis are promoted by FSH and LH which act on the ovarian follicle to promote ovulation and corpus luteum development (Calzolari. A, 1898). There has been a heightened enthusiasm in the previous decades on the connection between thymic and reproductive gonads. Studies show thymic enlargement after gonadectomy on rabbits and guinea pigs in both sexes (Dougherty SM, 2006). Androgen and estrogen induce the destruction of the thymocytes when administered to thymic bearing animals (Nishizuka Y, 1969). Nishizuka and Sakakura, 1969, reported a correlation between thymus and reproduction after they found that neonatal thymectomy in mice 72 hours after birth caused ovarian dysgenesis and created wasting sickness that occurred in 2- to 3-month-old female mice. These defects in female guinea pigs were due to immune suppression affecting the reproductive system. Its effects on the ovaries of thymectomized mice were extremely small, absence of follicle and corpus lutea, but no significant effect was seen in male mice. Besedovsky and Sorkin, 1974, and Listern – Moore and Norbaek Sorensen, 1976, noticed the development of ovarian formative disturbance after neonatal thymectomy in mice, Flanagan, 1966, was the first to observe decreased fertility, delayed vaginal opening and follicular atresia in athymic female nude mice. Reber et al., 1981a, reported congenitally athymic mice showed decreased level of pituitary gonadotrophins as well as circulating gonadotrophins in both sexes (i.e., puberty) and decreased level of estrogen in adult athymic mice showed. Previous results that showed enlargement of thymus while other studies showed atrophy of the thymus upon hypophysectomy gland were contradictory (Farookhie R, 1988). So far functional correlation between the thymic hormones and gonadal hormones has been sufficiently studied. However, histomorphometric and histoarchitecture changes of the gonads in female after thymectomy have not been explored.

Materials and Methods

Experimental Animal

Experiments were conducted in the neonatal (1st week animals, Average weight 90 to 100gm), pre-pubertal (5th week animals, Average weight 200 to 250gm) and pubertal (7th week animals, Average weight 280 to 300gm) female guinea pigs. A total of 30 female guinea pigs were studied. The animals were procured from the Institute of Experimental Animals in Karnataka. All guinea pigs were housed at the SRM Central Animal House; room temperature was maintained at 25±2^{o}C and adequate dark and light cycle for 12 h/ day. All the experimental protocols and procedure were approved by the Institutional Animal Ethical Committee of SRM Institute of Science and Technology Tamilnadu, in accordance with the guidelines of CPCSEA (16111/835re-S-04/IAEC2016).

Experimental design

The present study used 30 female guinea pigs divided into 2 main groups. In Group I (n-15), female guinea pigs underwent a surgical procedure without extirpation of thymus gland which was considered as sham operated group. In Group II (n-15), female guinea pigs with surgically removed thymus gland was considered as thymectomy group. In both groups 5 guinea pigs were each assigned to neonatal, prepubertal, and pubertal subgroups.

Transcervical Thymectomy

Thymectomy was performed according to the procedure described by Adams, 1977, as shown on Figure 1. In the Sham-operated group of guinea pigs (Group I), all the steps in the surgical procedures were followed except for the removal of the thymus gland. Post-surgical procedures included recovery under heat lamp, a special cage with soft corn cob bedding material with adherence to standardized aseptic methods.

Assessment of estrous cycle

The estrous cycle of guinea pigs consists of 4 stages: Proestrus, Estrus, Metestrus and Diestrus with a mean length of ovarian cycle of 16-18 days. Vaginal smear findings were used to assess the stages of estrous cycle. A moist cotton bud with normal saline was slowly inserted into the vagina at a depth of approximately 8-10 mm. Swab was gently turned (clock and counter-clockwise) against the vaginal wall and then removed. Immediately after withdrawal, the tip of the cotton bud was rolled along the whole length of a glass microscopic slide and immediately fixed in absolute alcohol for staining purpose (Jadarmkunti UC, 1999). The slide was then air

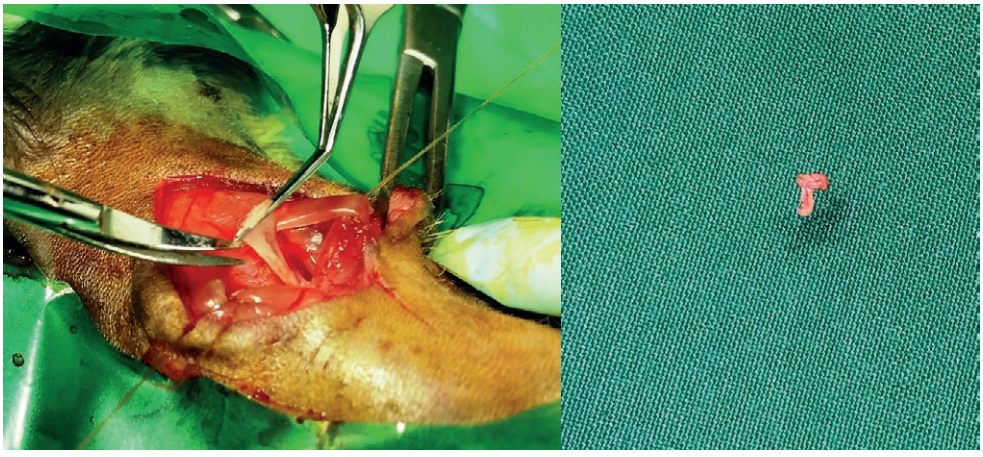

Figure 1. Surgical removal of the thymus gland in guinea pigs by transcervical approach.

dried and stained with Papinocolaou staining (PAP). In the proestrous phase, smears-stained nucleated cells with an intense pink colored cytoplasm; aggregated nucleated cells stained blue to purple with granulated nuclei. In the estrus phase –squamous epithelial cells were cornified. These cells were arranged in sheets, clustered and were uniformly stained orange and pink without nucleus. In the metestrus phase, the nucleated cells-stained pale blue and dark blue with polymorphic nuclei. In the diestrus phase, densely packed leukocytes in groups with presence of nucleated cells (Lilley KG, 1997).

Morphometric assessment of ovarian follicles

Both sham operated and thymectomized guinea pigs in each group were sacrificed in the estrus phase of the first estrous cycle after the procedure. The guinea pigs were euthanized using carbon dioxide for the removal and examination of their ovaries and accessory reproductive organs. Ovaries and accessory reproductive organs were fixed in Bouin's aqueous fixative. In the case of the ovaries every 20^{th} section was mounted and stained with hematoxylin-eosin and evaluated by confocal fluorescence microscopy at a 20X magnification. In the present study, follicles were categorized according to the layers of the granulosa cells (GC) surrounding oocyte; primary follicle (oocyte surrounded by a single layer of cuboidal GC), small secondary follicle (if at least oocyte surrounded by a two layers of GC), medium secondary follicle (oocyte surrounded by a three layers of GC), large secondary follicle (oocyte surrounded by four layers of GC without antrum) and antral follicle (follicle with the presence of an antrum) Karakaş, 2010. The mean diameter of the follicles in the ovary was measured under 40X magnification (Figure 2). The accurate calculation of diameter was taken by using the integrated measuring tools in the ZEN 2010 software Germany after calibration with a stage micrometer [LSM 700 Laser Scanning Confocal Microscope] Griffin J, 2006.

Statistical analysis

Two individual groups were compared and assessed by Student's t-test. Data values were expressed as mean and standard error of means (SEM). Values were considered statistically significant when $p< 0.05$.

Results

Effect of thymectomy on histomorphometry and histoarchitecture of ovarian follicles of thymectomized guinea pigs were compared with sham-operated in neonatal, prepubertal and pubertal female guinea pigs.

In Group I (Sham-operated), the mean values of follicle diameter expressed in mean ± SEM (μm) in the ovary were determined. Table 1 shows the statistical analysis of diameters of primordial follicles in neonatal, prepubertal and pubertal were 80.2±1.80; 87.5±0.97 and 86.8±0.82, respectively. The small secondary follicle diameter in neonatal, prepubertal and pubertal were 181.8±1.42, 189.3±1.52 and 191.8±1.76, respectively. The medium secondary follicle diameter in neonatal, prepubertal and

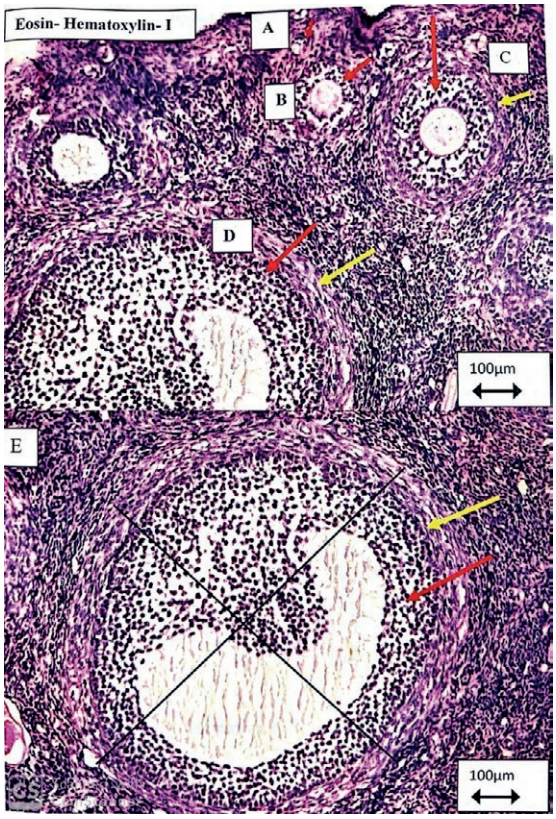

Figure 2. Hematoxylin-eosin staining of guinea pig ovary shows ovarian follicles at different stages. Yellow arrow – theca layer Red Arrow - Granular cells A. primary follicle with single layer of granular cells B. Small secondary follicle with two layers of granular cells C. Medium secondary follicle more than four layers of granular cells with formation of theca layer. D. Antral follicle E. Oocyte is surrounded by granular cells with theca layer and continuous line for measurement of diameter.

pubertal were 202±2.14, 213±1.16 and 223.5±2.4, respectively. The large secondary follicle diameter in neonatal, prepubertal and pubertal were 552±2.69; 621±1.74 and 628±1.25, respectively and the antral follicle diameter in neonatal, prepubertal and pubertal were 915±1.20, 934±2.60, 941±1.94, respectively.

In Group II (thymectomized), the same measurements were done as in Group I. The values are summarized in Table 1 that showed primordial follicle diameter in neonatal, prepubertal and pubertal were 62.5±0.8, 82.5±1.0 and 86.5±1.1, respectively. Small secondary follicle diameter in neonatal, prepubertal and pubertal were 141.7±1.0, 185±1.38 and 195±1.5, respectively. Medium secondary follicle diameter in neonatal, prepubertal and pubertal were 168±1.83, 195.3±1.53, 204.3±1.39, respectively. Large secondary follicle diameter in neonatal, prepubertal and pubertal were 364±1.67, 595±1.42 and 612±1.36, respectively and antral follicle diameter in neonatal, prepubertal and pubertal were 655±1.37, 893±1.16 and 930±1.71, respectively.

Table 1. Mean ± SEM for Different Follicle Diameter of Thymectomized and Sham-operated Group (*p < 0.05 significant).

Age	Primordial (μm)		Small secondary(μm)		Medium secondary(μm)		Large secondary(μm)		Antral(μm)	
	Shax	Tx	Shax	Tx	Shax	Tx	Shax	Tx	Shax	Tx
1st week	80.2±1.80	62.5±0.8	181.8±1.42	141.7±1.0	202±2.14	168±1.83	552±2.69	364±1.67	915±1.20	655±1.37
5th week	87.5±0.97	82.5±1.0	189.3±1.52	185±1.38	213±1.16	195.3±1.53	621±1.74	595±1.42	934±2.60	893±1.16
7th week	86.8±0.82	86.5±1.1	191.8±1.76	195±1.51	223.5±2.41	204.3±1.39	628±1.25	612±1.36	941±1.94	930±1.71
P value	1st week-0.001*		1st week-0.044*		1st week-0.003*		1st week-0.001*		1st week-0.041*	
	5th week -0.091		5th week –0.131		5th week -0.376		5th week –0.466		5th week –0.437	
	7th week-0.346		7th week-0.177		7th week-0.406		7th week-0.532		7th week-0.210	

Table 2 Number of Ovarian Follicles in Neonatal, Pre-pubertal and Pubertal in Thymectomized Guinea Pigs When Compared with Sham-operated Guinea Pigs. (*p< 0.05 significant).

Group	1st week		5th week		7th week	
	Shax	Tx	Shax	Tx	Shax	Tx
Primordial	605	395*	554	520	420	412
Small secondary	354	250*	329	330	295	296
Medium secondary	613	400*	552	510	432	421
Large secondary	400	258*	382	372	350	348
Antral follicle	432	291*	392	388	366	358

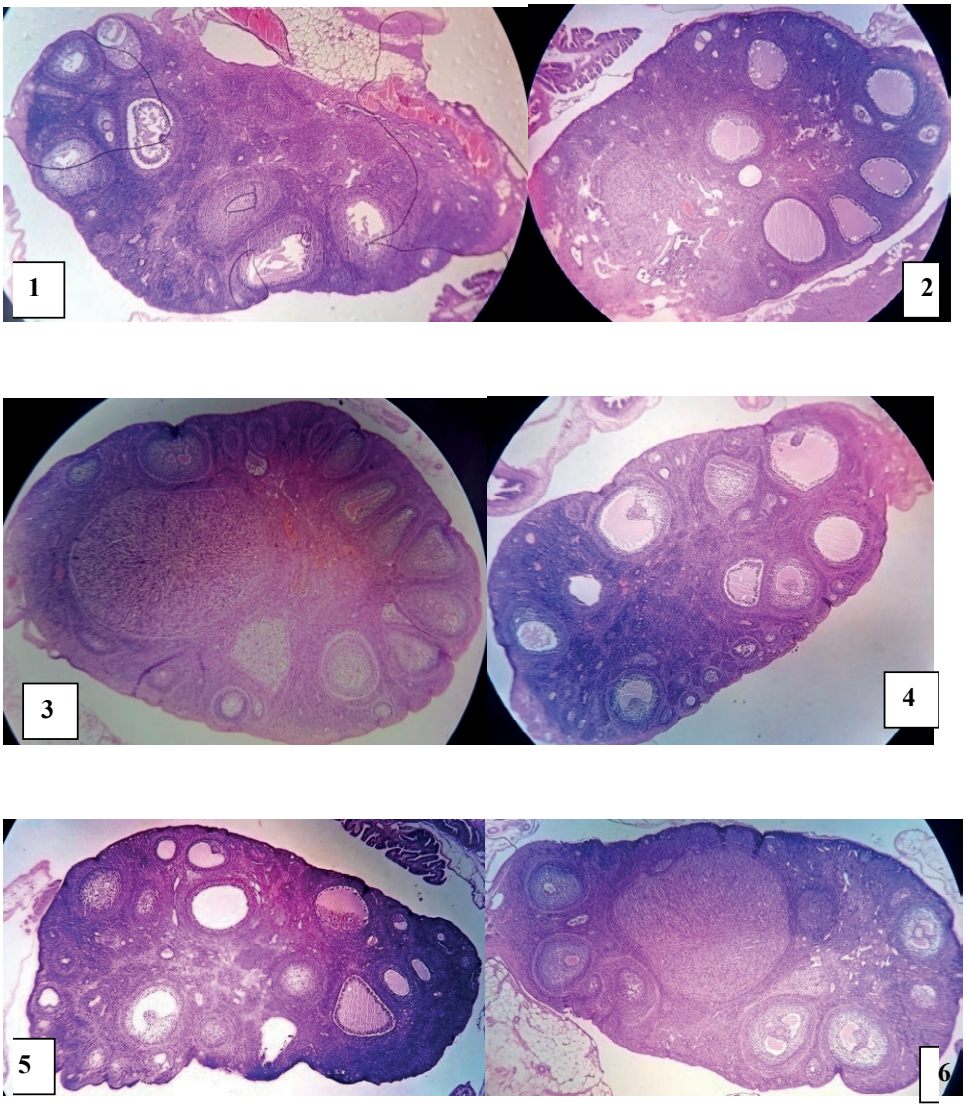

Figure 3. Female guinea pigs ovary Comparison between sham-operated and thymectamized.1. Neonatal Sham-operated. 2. Neonatal Thymectomized 3. Pre-pubertal Sham operated 4.pre-pubertal Thymectomized 5. Pubertal sham-operated 6. Pubertal Thymectomized.

The morphometric analysis showed a significantly higher average range of mean value of diameter of all types of follicles in the ovary in neonatal thymectomized animals when compared to the sham operated animals. In contrast, there was no significant difference in the mean diameter of different stages of ovarian follicles in the ovary in pre-pubertal and pubertal age thymectomized animals when compared to the sham-operated animals.

Statistically there was a significant difference in all kinds of follicles in the ovary between sham-operated and experimental animals in neonatal group animals with a $p < 0.001$, but there were no significant changes of different ovarian follicles ovary in pre-pubertal and pubertal age thymectomized animals when compared to the sham-operated animals (Table 2, Figure 3). Female neonatal thymectomized guinea pig results showed significant changes in their weight as well as changes in the microscopic features including reduced thickness of myometrium of the uterus and less mucosal folding in the fallopian tube compared to the sham-operated group. There were no significant changes in the accessory reproductive organs in pre-pubertal and pubertal thymectomized guinea pigs compared with the sham operated guinea pigs.

Discussion

To our knowledge, this is the first study to examine the effect of thymectomy on morphometric and histoarchitectural changes of the gonads during the estrus phase of the estrous cycle in neonatal, prepubertal and pubertal age female guinea pigs. The study proved that complete thymectomy in five to seven-day old female guinea pigs resulted in changes in the follicular morphology and a reduced number of follicles. Morphological changes in the follicles included the absence or advanced atresia of large size follicle, medium size follicle, primordial follicle, degenerated corpora lutea and proliferation of hypertrophied interstitial cell elements.

Earlier studies by Deschaux et al, 1979, reported that thymectomized guinea pigs showed development of a wasting disease with a loss of body weight and dysfunction of hormonal balance. In mammals, the immune system is essential for the optimal function of the reproductive system. This clearly shows that the immune system affects reproductive function linked to the hypothalamus Garcia L et al, 2000, pituitary gland (S. S. Walusimbi et al,2013, Meinhardt et al,2011 and gonads Greenstein BD et al, 1986, Walsh S et al, 2005. Thymic epithelial cells secrete hormones such as thymulin and thymosin alpha-1. The production of thymic hormones is inhibited by conditions like sex hormone treatment and ovariectomy. This in turn increases the weight of thymus in the mice Greenstein BD et al, 1987, Heng TS et al, 2005, Windmill KF et al, 1993,1998, Miller, E.M et al, 1967, Hardy, B, 1974. The factors which regulate the primordial follicle in the mammalian ovary are poorly understood.

The surgical removal of the thymus gland and ovaries during non-breeding and breeding phase of the animals, respectively results in marked changes in the ovaries of thymectomized and the thymus of ovariectomized animals. The role of thymic hormones in the development of the ovaries is further highlighted in a study that showed retardation of ovarian follicle development in a girl with congenital absence of the thymus Miller ME et al, 1967. Nishizuka and Sakakura 1969, 1971 have revealed that there was a functional connection between the thymus and the ovaries and noted that after neonatal thymectomy, ovarian dysgenesis is replaced by the invasion of lymphocytes into the ovary, a sudden loss of oocytes, an expansion in follicular atresia, a reduced or absence of corpora lutea, a lower weight of the ovaries and tumors developing in the grown-up stage. Besedovsky et al., 1979, have shown that neonatal thymectomized female mice demonstrated delayed primary ovulation and the presence of contracted ovarian follicles with the delay occurring in the

beginning of pubescence in thymectomized mice as well as in athymic nude mice. In the two cases, follicular morphology might be restored by exogenous gonadotropin Jones, E.C et al.,1961a.

A significant reduction of growing follicles has been reported at 10 days in congenitally athymic nude mice but restoration of multi laminar growth stages upon treatment with gonadotropin Jones, E.C et al., 1961b. The neonatal thymectomy at 2 to 4-day-old female mice results in reduced weight of the ovary, composed of mostly of interstitial- like cells, devoid of follicle and replaced by lymphocyte infiltration in and around the medium and large sized follicles that accompany the dysgenesis. The ovarian dysgenesis occurs between the age of 90'th to 120'th day and further reduction of weight of ovary when compared to the intact animals H.O. Besedovsky, 1979. Neonatal thymectomized female mice at the age of 10, 21 and 30 days did not show statically significant changes in the number of ovarian follicles in different stages and follicular atresia which begins after the 50th day. Complete destruction of follicles occurred at the age of 130th day Eshkol A et al.,1967, These findings were consistent with our present finding of ovarian changes after thymectomy in neonatal female guinea pigs.

Further studies have reported that nude female mice have shown that ovarian dysgenesis begins at approximately 24 days of age and is usually complete by 60 days of age Jones, E.C, et al.,1961. Around the 30'th day of life, the presence of heteropyknotic cells with lymphocytes infiltration and abnormal-looking cells have been reported Pedersen T, 1969a. Michael, 1983, has stated that third day thymectomized mice produce circling autoantibodies against the ooplasma of oocytes, showing an immune system etiology for the ovarian atrophic changes or failure. However, the invasion of lymphocytes and the manifestation of antioocyte antibodies were observable until 25 days of age Tung et al.,2005. Kosiewicz and Michael, 1990, suggested that the thymectomy impact on the ovary may include the interruption of the hypothalamic-pituitary– ovarian– thymic axis, which does not seem to include the immunological factors associated with ovarian dysgenesis. In addition, similar changes indicative of ovarian follicle destruction was recorded in hypophysectomized and mice treated with an antiserum injection to gonadotropins during neonatal period (2-7days) mice and rat Weisz J, 1970, Bagavant H1 et al., 1999, Kleinewietfeld M, 2014.

Neonatal thymectomy on Day 3 after birth showed organ-explicit auto immune system disease influencing various organs including the ovary Fitzpatrick, F. Q et al.,1985. Day three thymectomy after birth instigated oophoritis and ovarian atrophy because of aggravation and raised cytokine expression in the female ovaries, interceded by the proinflammatory Th1 T cells. Th1 cells prevent immunosuppressant and are less in number in female animals when compared to males. Th1cells are capable to maintaining the normal estrous cyclicity, ovarian follicular development, ovulation, and fertility Greenstein, B. D et al., 1986. Above outcomes suggest that loss of various functions of ovaries in autoimmune ovarian disease based upon mechanisms in Th1 cell-intervened oophoritis, and anomalous cytokine creation may produce untimely ovarian failure. Neonatal thymectomized mice initiated immunological disorder results from absence of a one-of-a-kind thymus-determined administrative CD4+T cell subset that constitutively communicates the IL-2 receptor αchain (CD25) Lintern-Moore et al., 1977.

Interestingly Sue Lintern-Moore et al., 1976 examination upon careful evacuation of thymus after 2 days in female Bagg rodent showed no statistical significance on the

number of small, medium and antral ovarian follicles during the initial 12 weeks of life. In this examination, results demonstrated 20% of thymectomized mice over 12 weeks of age created ovarian failure which incorporated the loss of corpora lutea and a general decrease in all parts of the ovarian follicle population. These findings were not consistent with our present findings in neonatal thymectomized female guinea pigs ovaries.

Conclusion

Reduction in the number of ovarian follicles and their decrease in diameter in neonatal thymectomized female guinea pigs strongly indicate that thymus plays a major role in the proper development and function of the hypothalamic –pituitary – gonadal axis in the early age of a guinea pig. But no significant difference was noted in the thymectomized prepubertal and pubertal female guinea pigs when compared with the sham operated group which indicates an age-dependent role of thymus in reproductive development.

List of abbreviations used

LH- luteinizing Hormone
FSH- Follicular Stimulating Hormone
Tx – Thymectomy
Shax – Sham-operated
GC – granulosa cells
ns – Not Significant
s- Significant.

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Morphological variations of human pulmonary fissures: an anatomical cadaveric study in Sri Lanka

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Abstract

The aim of this study was to identify morphological and morphometric variations of pulmonary fissures. A sample of 50 adult formalin fixed Sri Lankan cadaveric lungs (24 left and 26 right lungs) were observed with the help of magnifying glass and length measurements of the lung fissures were taken using a measuring tape. Complete oblique fissure was seen in 16 (66.67%) left lungs and 11 (42.3%) right lungs. Incomplete oblique fissure was seen in 8 (33.33%) left lungs and 15 (57.69%) right lungs. There was complete absent of horizontal fissure in 4 (15.38%) right lungs whereas rest of the 22 right lungs indicated incomplete horizontal fissure (84.61%). The mean lengths of the left oblique fissure, right oblique fissure and horizontal fissure were 26.88±5.88cm, 27.31±6.04 cm and 8.31±3.61 cm, respectively. Incomplete fissure was the most common variant of the fissures in the analyzed sample. There was a high prevalence of incomplete horizontal fissure of right lung followed by incomplete right and left oblique fissures. Absence of oblique fissure was not found in either left or right lungs. The mean length of right oblique fissure was slightly greater than the mean length of left oblique fissure. The knowledge lung fissures, indeed help clinicians and radiologists to identify alterations of the disease distribution and to reduce the misinterpretation of radiological modalities as well as to arrive at an accurate diagnosis with plan of management of a patient.

Keyword

lung; variant; fissure; morphology; Sri Lanka.

Introduction

Lungs are the vital organs of the body which are responsible for the process of gas exchange called respiration. They are paired organs situated on either side of the heart in the thoracic cavity. Lungs are separated into lobes by the presence of fissures. Presence of fissures are essential for the greater distension of the lungs during the respiration. The fissures allow greater degree of movement of the lobes over one another. Thus, they allow uniform expansion of the whole lung for more volume of air during inspiration (Rosse & Gaddum-Rosse, 1997). The left lung is composed of superior and inferior lobes separated by the oblique fissure whereas right lung has oblique and horizontal fissures separating it into superior, middle, and inferior lobes. The oblique fissure being less vertical on right lung than left lung separates inferior lobe from middle and upper lobe (Sudikshya et al., 2018). Normally fissures cut the

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whole thickness of the lung except at the hilum. The oblique fissure runs obliquely downward and forward. In the right lung, horizontal fissure runs horizontally to the anterior border and separates a middle lobe which is wedge shaped (Sudikshya et al., 2018). Knowledge about the fissures of lungs are clinically important for distribution of bronchopulmonary segments and lobar anatomy (Meenakshi, 2004).

The deviations from normal anatomy of lungs are somewhat common. Main anatomical variations found in the lung fissures are categories as the complete, incomplete, absent and accessory. When there is a complete fissure, the lobes are held together only at the hilum by the bronchi and pulmonary vessels. The fissures are said to be incomplete when there are areas of parenchyma is left in between lobes and the clefts do not reach the hilum. Accessory fissures are the clefts of varying depths lined by visceral pleura. Accessory fissures usually surround the boundaries of bronchopulmonary segments. Accessory fissure, common congenital variant which commonly occurs at the borders of bronchopulmonary segments, as a with clefts of varying depth lined by visceral pleura. The fissures are said to be incomplete when parenchyma is left in between lobes and the clefts do not reach the hilum. Accessory fissure, common congenital variant which commonly occurs at the borders of bronchopulmonary segments with clefts of varying depth. Commonly occurring accessory fissures are the superior accessory fissure (SAF), Left minor accessory fissure (LMF) and the inferior accessory fissure (IAF). SAF separates the basal segment of the lower lobe from the superior segment, which can either be complete or partial. This causes the superior segment to be called as posterior or dorsal lobe. The IAF associate with medial basal segment of the lower lobe. In the left upper lobe, the lingula is separated from the rest of the lobe by the LMF (Sudikshya et al., 2018). Some accessory fissures are not detected on computed tomography because of incompleteness, thick section and orientation related differences (Zareena, 2014).

Normal anatomy is vital for surgical procedures. Variations in anatomy can lead to interpretation difficulties in radiological investigations. In patients with endotracheal lesions, presence of an accessary fissure might alter the usual pattern of lung collapse and leads to difficulty in diagnosing the extent of the lesion (George *et al*., 2014). Usually, pneumonia is confined to the lobes affected by it, but in contrast, when there are incomplete fissures, pneumonia would spread to adjacent lobes due to incomplete parenchymal separation. (Aldur, 1997). There are many researches have been done related to variations in the anatomy of human lung cadavers all over the world (Sudikshya et al., 2018.; Meenakshi, 2004; Zareena, 2014; Aldur, 1997). In another research "Fissural balance" is used to describe the relationship between the pulmonary artery to oblique fissure (Craig & walker, 1997). In a "normally balanced" fissure, the pulmonary artery lies centrally to the oblique fissure. In an "anterior or posterior imbalance fissure", anterior or posterior displacement of the artery occurs. A cadaveric study in Nepal, revealed complete oblique and horizontal fissures in right lung and incomplete oblique fissure in left lung as their common findings (Sudikshya et al., 2018). Another study by indicated that the incomplete fissure predominates in right lungs whereas in few cases the horizontal fissure is classically absent (Radha and Durai, 2015). In an Indian study reported by Meenakshi, 2004 had found out 16.6% absence of horizontal fissure whereas absence of oblique fissure was not reported (Meenakshi, 2004). In a study conducted in Ethiopia had unfolded higher prevalence of 68.42% of incomplete horizontal fissure of the right lung (Gebregziab-

her *et al*., 2015). Although the anatomy of lung fissures and lobar pattern is studied in many populations, there is only one study been reported in Sri Lankan context where authors revealed 66 % of the right lungs had either an incomplete or absent horizontal fissure and the typical fissure pattern was seen less (Ekanayaka *et al*., 2019). In order to widen the Sri Lankan data base related to lung variations, the present study is aimed to analyze the morphological and morphometric variations of human pulmonary fissures and lobes related to local population.

Material and method

The 50 specimens of adult lungs of unknown gender, fixed with formalin and preserved in the dissection hall at the Faculty of Medicine, University of Kelaniya were analyzed in the present study. Overall the specimens were in good condition and their pleura are intact except at the hilum. Lung specimens that were damaged during dissection, undergone surgical procedures, and those that had been identified to be having pathological changes were excluded from the study. Each specimen was given an identification number for convenience in collecting data by observation and assessed under four key parameters; number of fissures, completeness of each fissure, presence or the absence of an accessory fissure and length of each fissure.

Length measurements of the lung fissures were taken using a measuring tape. In addition, magnifying glasses and dividers were used on requirement during the study. Three length measurements on the same fissure were taken from each specimen by the same observer. On data collection, same observer took 3 length measurements on same fissure of each lung. Calculated average length of the 3 recorded measurements was used for statistical inference. The Craig and Walker classification was used to determine the presence and the completeness of fissures; Grade I: Complete fissure with entirely separate lobes, Grade II: Complete visceral cleft but parenchymal fusion at the base of the fissure, Grade III: Visceral cleft evident for a part of the fissure, Grade IV: Complete fusion of lobes with no evident fissure line (Craig & walker, 1997). The collected data was analyzed using the software SPSS 25.0 whereas the significance level was set to $p< 0.05$.

Results

Out of the 50 lungs analyzed in the current study, 24 (48%) lungs were left lungs and 26 (52%) of the lungs were right lungs. Oblique fissure was present in all the left lungs and among those 16 lungs (66.67%) had complete oblique fissures (Figure 1A) whereas 8 (33.33%) had incomplete oblique fissures (Figure 1B). Oblique fissure was present in all the right lungs and it was complete in 11 (42.3%) lungs whereas incomplete oblique fissure was seen in 15 (57.69%) lungs.

Regarding the horizontal fissure of the right lungs, it was present in 22 (84.61%) lungs and absent in 4 (15.38%) lungs (Figure 1C). Engrossing fact is that oblique fissure is present in all the lungs unlike the horizontal fissure (Table 1).

A significant presence of accessory fissure was observed in both lungs (Figure 1D and Figure 1E). Out of the samples analyzed we observed accessory fissures in

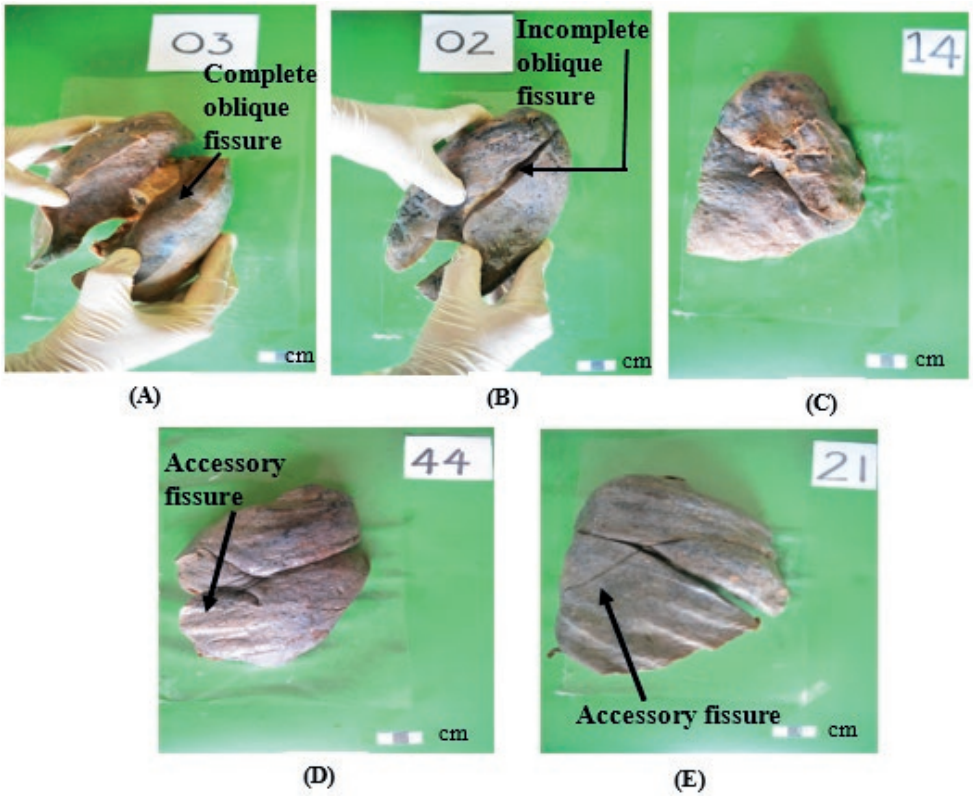

Figure 1. (A)-Left lung with complete oblique fissure, (B)-Left lung with incomplete oblique fissure, (C)-Right lung with absent horizontal fissure, (D)- Left lung with an accessory fissure, (E)- Right lung with an accessory fissure.

Table 1. The fissural pattern and measurements of the Sri Lankan cadaveric lungs.

	Complete (%)	Incomplete (%)	Absent (%)	Length(cm) Mean±SD	Range (cm)
Left oblique fissure	16(66.67)	8(33.33)	00	26.88+/-5.88	8.2-35.5
Right oblique fissure	11(42.33)	15(57.69)	00	27.31+/-6.04	13.2-35.8
Horizontal fissure	00	22(84.61)	4(15.38)	8.31+/-3.61	2.7-16.5

4 left lungs and 3 right lungs and the data pertaining to this variation was given in Figure 2.

The obtained fissural parameters related to oblique fissure of both left and right lungs, and horizontal fissure of right lungs were given in Table 1. Incidence of oblique and horizontal fissures found in the lungs according to Craig and Walker criteria is shown in the Table 2.

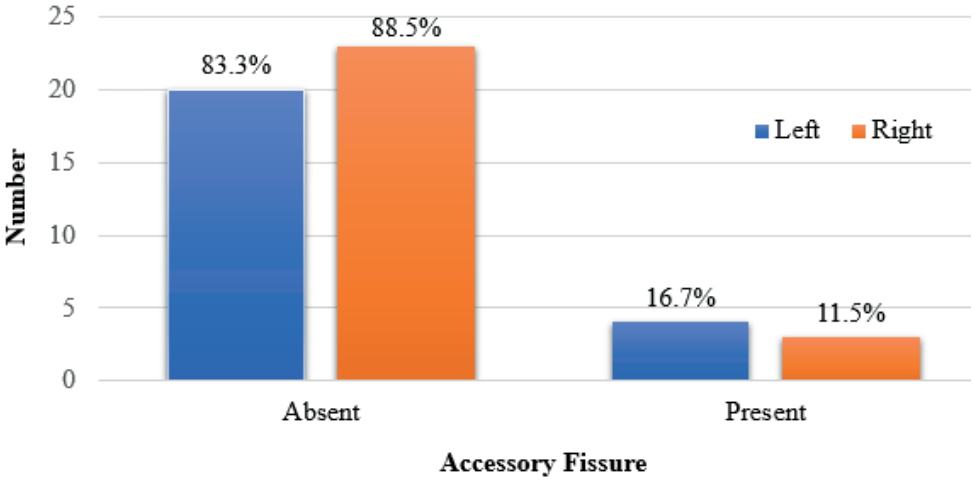

Figure 2. Presence of accessary fissures in left and right lungs.

Table 2. Craig and Walker classification of fissures of the lungs in the present study.

Side of Lung	Fissure	Grade I (%)	Grade II (%)	Grade III (%)	Grade IV (%)
Right	Oblique	11(42.3%)	4(15.4%)	11(42.3%)	0(0%)
Right	Horizontal	0(0%)	3(11.5%)	19(73.1%)	4(15.4%)
Left	Oblique	8(33.3%)	4(16.7%)	12(50%)	0(0%)

Discussion

The importance of understanding the anatomical variation of lobar anatomy in relation to fissures has been overlooked in the Sri Lankan context. Many studies have been conducted in the subcontinent, mainly in India with the objective of enhancing the understanding about this subject area, often widening the study on this anatomical diversity (Meenakshi, 2004; Zareena, 2014; George et al., 2014; Varalakshmi et al., 2014). The lack of similar studies in Sri Lankan population can hinder the future improvements in the field of lung surgery and this study will shed lights to fulfil this lacunae.

The variations in the fissures and lobes of the lungs were due to the defective pulmonary embryological fissures that initially separates individual bronchopulmonary buds. Incomplete or absence of lung fissures could be due to a defect in the obliteration of the prenatal fissures either completely or incompletely. A good understanding about the pulmonary fissural anatomy is helpful not only in surgically but also in radiological and pathological perspectives. Radiological signs can be vary depending on variation on fisural anatomy such as fluid collecting areas (Damor et al., 2012). It has also been identified to be playing a key role in the process of disease spread and progression. The most significant impact lies in the field of pulmonary surgery, where

the fissures demarcate the boundary of lung lobes acting as the landmarks based on, where the surgeons make their incisions. A wide knowledge on possible variations of fissures, both location wise and size wise can improve the outcome of oncological and benign surgical procedures (Standring et al., 2008). Clinically, in cases of endobronchial lesions, diagnosis of type of pneumonia may be altered by presence of an accessary fissure. Pneumonia may spread to adjacent lobes by parenchymal fusion.

Study conducted in India found that 16.7% right oblique fissure was incomplete (Varalakshmi et al., 2014) whereas another study revealed that it was 10.7% (Divya et al., 2015). In the studies conducted by Nishan et al., 2014, Abhilasha & Charulata, 2013, Thapa & Desai, 2016 have found out that incomplete right oblique fissure was present as 24%, 17.25% and 30%, respectively. However, in contrast to that, our study reported higher incidence (57.69%) of incomplete right oblique fissure. Similar results were found by Shivaleela et al., 2018 and they reported 63% of right oblique fissures. Lower incidence of absent oblique fissures of right lung was reported in some studies (Nishan et al., 2014, Shivaleela et al., 2018) but most of studies including our present study does not report, absence in oblique fissure (Meenakshi, 2004; George et al., 2014; Varalakshmi et al., 2014; Divya et al., 2015; Abhilasha & Charulata, 2013; Thapa & Desai, 2016; Mamatha et al., 2016).

Regarding the horizontal fissure of right lungs, the studies conducted by Varalakshmi et al., 2014, Abhilasha & Charulata, 2013, Divya et al., 2015 and Nisha et al. found 30%, 31.03%, 50% and 32% of incomplete horizontal fissures, respectively. Contrasting to these results, Shivaleela et al., 2018 had found higher incidence rate (63%) of incomplete horizontal fissures. However, present study reported remarkably higher incidence rate of 84.61% of incomplete horizontal fissures.

The results of absent horizontal fissures (15.38%) of the current study were comparable to published data by Thapa & Desai, 2016 (20%), Shivleela et al. (26%), Divya et al., 2015 (21.4%) and Varalakshmi et al., 2014 (10%). Considering the incomplete horizontal fissure left lung, very high incidence rate (70%) of incomplete horizontal fissure was reported by the, Shivaleela et al., 2018 and low incidence rate such as 14.8% recorded by Divya et al., 2015. Interestingly, current study revealed a 33.3% Varalakshmi et al., 2014 (29.4%), Nishan et al., 2014 (40%), Abhilasha & Charulata, 2013 (28.8%) and Thapa & Desai, 2016 (25%). Absent of left oblique fissure is the commonest variation observed by many published researches. Its rate varies between 3-15% (Varalakshmi et al., 2014; Divya et al., 2015; Abhilasha & Charulata, 2013; Thapa & Desai, 2016; Shivaleela et al., 2018). However, in the current study, we did not observe any absence in left oblique fissure. Also similar result was reported by Nishan et al., 2014. Variations in formation of lobes and fissures occur due to certain factors affecting fusion of lobes (Brahmbhatt et al., 2013). Accessory lobes developed by non-fusion of the spaces between the bronchopulmonary buds. Some studies had identified variations in the presence of lobes and fissures of the lungs (Sudikshya et al., 2018; Meenakshi, 2004; Zareena, 2014; Brahmbhatt et al., 2013). Accessory fissure may confuse the interpretation of X-rays and CT scans. Clinical conditions such as linear atelectasis, pleural scars or walls of bullae may be misinterpreted due to presence of accessory lobes and fissures (Brahmbhatt et al., 2013; Mayuri et al., 2013).

According to literature review several studies claimed the presence of accessory fissures of the lungs (Esomonu et al., 2013.; Mayuri et al., 2013). In George et al., 2014 reported 3 (4.61%) of right lungs and 2 (2.73%) of left lungs with accessory fissures.

In a study done in South Indian cadavers has found out presence of 4 (13.3%) accessory fissures in right sided lungs and 5 (27.7%) in left sided lungs (Jacob & Pillay, 2013). In our study, we found 4 (16.67%) of left lungs having accessory fissures while 3 (11.53%) of right lungs having accessory fissures.

Craig & walker, 1997 had proposed a classification based on degree of completeness of the fissures. To prevent post-operative hemorrhage and complications in the surgical procedures, it's important to grade the fissures. According to current study, similar percentages in grading of the above classification found as in previous studies, except that we found right horizontal grade 1 percentage is less than usual and grade 3 is more than usual pattern of distribution.

Some research had focused over the length of lung fissures (Gopalakrishna et al., 2017, Dutta et al., 2013). Gopalakrishna et al., 2017 states that the mean length of right oblique fissure is 29.36 ±5.61cm (29) whereas in the study conducted by Dutta *et al.* had found out that the mean length of right oblique fissure is 30.15±6.26 cm. Our results of 27.31±6.04 cm mean length of right oblique fissure was not much differ from the previous studies. The mean length of the left oblique fissure was measured as 26.81 ±8.18 cm by Gopalakrishna et al., 2017 while it was measured as 27. 32 ±7.29 cm by Dutta et al., 2013 In the current study the mean length of the left oblique fissure was 26.88 ±5.88 cm which is in the similar range as previous studies.

Conclusion

In the present study, the commonest variation was the presence of incomplete fissures on both right and left lungs. The prevalence of incomplete horizontal fissure of right lung reported from the Sri Lankan cadavers, probably is the highest reported from such researches all over the world. Incidence of incomplete oblique fissure was found to be greater in the right lung than the left lung. Absence of oblique fissure was not found in both left and right lungs. The similar prevalence of accessory fissure was observed in both right and left lungs. The mean length of right oblique fissure was slightly greater than the mean length of left oblique fissure. The knowledge on morphological and morphometric variations of lungs, indeed help clinicians and radiologists to identify alterations of the disease distribution and to reduce the misinterpretation of radiological modalities as well as to arrive at an accurate diagnosis with plan of management of a patient.

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