Symposium 30: MISCELLANEOUS

Paleopathology
Moderators: Gino Fornaciari, Italy / Dina G. Tiniakos, Greece

Contents

| Presentation 1 | Paleopathology in the inner Abruzzo region (central Italy). A ten-year experience. | Luca Ventura, Italy |
| Presentation 2 | The Egyptian mummies of the Museum of Anthropology, University of Athens, Greece | Dina G. Tiniakos, Theodore Pitsios, Greece |
| Presentation 3 | Paleopathology studies in Spanish royal mummies: technical approaches and findings | Pedro L. Fernández, Jordi Esteban, Montserrat Tortosa, Spain |
| Presentation 4 | Paleoandrology in Italian mummies (15th -19th century) | Gino Fornaciari, Italy |
The inner Abruzzo region in central Italy, roughly corresponding to the province of L’Aquila, is a land of mountains and highlands with a cold, dry climate. These environmental conditions play an important role in preserving human remains by allowing tissue dehydration, a fundamental mechanism for the creation of natural mummies. Since 1997 a systematic study of human mummified and osteoarchaeological remains was undertaken, in order to create a map of the paleopathological material available in this area [1,2]. The main paleopathological results obtained in the last ten years in this particular region are presented.

The natural mummies of 5 anonymous individuals, dating back to XIX century, were found in the friary of San Giorgio degli Osservanti in the village of Goriano Valli (2). The 3 male and 2 female bodies, ranging 43-55 years of age at death, showed moderately high stature and good nutritional status; they underwent CT and autopsy study with histologic examination. In this series we observed frequent pathologic conditions of the respiratory system (silico-anthracosis, tuberculosis, pneumonia and pleuritis), possibly related to hard environmental conditions. Degenerative joint disease and arteriosclerosis were widely distributed. Occasional findings were represented by goiter, prostatic hyperplasia, DISH, femur osteochondroma and a possible ovarian neoplasm. Dental pathology showed high rates of diffuse periodontitis and marked attrition.

The church of San Sebastiano in Navelli contains hundreds of mummified or skeletonized bodies, presumably ranging XVI-XIX century [3]. During the first excavations the recovery yielded the remains of 206 individuals in different conditions of preservation. No macroscopic evidence of internal organs was observed in mummified subjects, whereas skin, muscles and other superficial tissues (eyes, ears, hair) appeared well preserved, suggesting the preminent role of desiccation in
the mummification process. The initial paleopathologic analysis allowed to identify degenerative joint disease, periodontal disease, healed bone fractures, occasional tumours, and a hydrocephalus. Traces of post-mortem examination in an adult male and a subdural chronic hematoma in an infant were also recognised.

The series from the church of the Santissima Trinità in Popoli (Pescara province) includes at least 8 mummified or skeletonized bodies dating back to XVIII-XIX centuries, buried in a crypt [4]. A preliminary investigation of a middle-aged male individual showed he had significant dental, pulmonary and renal pathological findings, without major arthritic changes suggesting a life free from extensive labor. The death of the subject could be related to infectious complications of renal urolithiasis and hydronephrosis.

The paleopathologic findings of the three series helped to understand the pathocenosis of these samples of rural populations from the same geographical area.

Paleopathologic investigations were also addressed to osteoarchaeological remains [1]. The necropolis of Fossa Casale yielded 560 individuals of the first millenium BC, while the medieval burials in Luco dei Marsi and San Potito di Ovindoli belonged to a rural population with high rates of osteoarthritis and periodontal disease.

Along with these collections of individuals, the remains of remarkable figures resting in L’Aquila should be investigated in the near future. The body of Saint Bernardino da Siena represents an interesting example of the embalming techniques during the Renaissance period. Among the historical figures of the XV century, the mummified bodies of the Blessed Jean Bassand, Antonia da Firenze, and Vincenzo dell’Aquila also repose in our town. In the basilica of Santa Maria di Collemaggio, the relics of Saint Pietro da Morrone (Pope Celestino V) represent the basis for an historical case. The hole in his skull has raised many questions about the death of this saint and should be investigated completely in the future.

External collaborations also led to interesting results in the field of paleoimmunology [5], as well as in the application of histological methods for the examination of egyptian canopic jars content [6], and tissue samples from natural mummies of south-western Lybia, dating back 6000 years ago [7]. All the research activities involved colleagues of different specialties and produced 20 journal articles and 28 congress presentations.

Among the educational events organised in the region and dealing with local paleopathologic studies, the National Conference “Eminent Relics of Saints: Crossroad of History, Science, and Devotion”, held in L’Aquila in 2000, should be remembered, as well as two television documentaries realized by the National Geographic and History Channel [1,2].
The inner Abruzzo region represents a new area of growing paleopathological interest. Further investigations of these and other series of ancient human remains will give the opportunity to trace a whole anthropological profile of our ancestors in this region.

References


The ethnologic collections of the Museum of Anthropology, University of Athens, Greece, include four Egyptian mummies of the Hellenistic/Roman period (332 B.C.-A.D. 395), with a series of glass containers, vials and fabric bands which accompanied the embalmment of dead bodies according to ancient Egyptian customs. In the inventory of the Museum of Anthropology, which was partially destroyed during the 2nd World War, only a single, laconic reference to these mummies exists, according which they belong to members of the same family, two adults - a man and a woman- and two young male children.

Information about the mummies is scarce. However, we know that after their discovery in Egypt by a German archeologic mission, they were, probably, transferred to Bremen, Germany. Before 1860, they were donated, along with other ethnologic collections (Spleiter donation?), to the University of Athens and were initially incorporated to the Museum of Zoology and subsequently to the Museum of Anthropology. The mummies were exposed to the public in the old exhibition area of the Museum of Anthropology until the 2nd World War and the requisition of the Museum building, first by the German and then, during the civil war, by the Greek army. In the decades following the war, after the re-opening of the Museum, they were re-exposed in the same area.

Anatomical and imaging (x-Ray, computed tomography scan) studies combined with 3-D virtual reconstruction of scull and bones were undertaken at the Museum of Anthropology, University of Athens, Greece, while studies of the linen strips covering the mummies have been performed by experts from the Louvre Museum, Paris, France.
Using the imaging techniques it was possible to identify foreign material in the body cavities of all mummies, while soft tissues still covered by linen strips and resin could be examined. A family relationship between the mummies was hypothesized based on biological age (as calculated by anthropometric and dental features) and on similarities of the skull and morphological features of the face (mainly between the woman and the children). The face of the woman and the two children showed a characteristic fronto-lateral projection of the zygomatic bone indicative of subsaharan anthropological influence.

The adult male mummy belonged to a man of 25-35 years. The body of the mummy was destroyed while on storage in suboptimal conditions during the 2nd World War and it was subsequently buried. The head had remained intact. Dental examination revealed 32 teeth with mostly normal morphology with the exception of a fracture of the right lower canine. The adult female mummy belonged to woman of 40-50 years with both head and body intact. The woman was 158 cm tall and had elaborately breaded hair. Dental examination revealed 26 teeth with signs of erosion. Whole body x-Rays and CT-scan did not reveal any significant bone abnormality. Her skin was intact. The viscera had been extracted and embalmed separately as was the costum in Egyptian mummification. The eldest child mummy belonged to a boy of 7-8 years (Demirijan method-diagram Ubelaker), 106 cm tall with intact skin and nails. Whole body x-Rays and CT-scan did not reveal any significant skeletal abnormality. The viscera had been extracted and embalmed separately as in the previous case. Dental examination revealed mostly deciduous teeth. The younger child mummy belonged to a boy of 2.5-3 years and was accompanied by a painted funeral mask. The boy was 83 cm in height with an estimated weight during life of 7kg. His skin, nails and hair were intact. The latter had evidence of breads. The face of the boy had traces of gold paint possibly indicating a noble origin. Dental examination revealed deciduous incisors, canines and molars. A very interesting finding was the radiological (X-ray) detection of a fracture in the middle of the left femoral bone most likely caused by pressure on the back side of the femur. There was no evidence of healing at the fracture site indicating occurrence close to or at the time of death. This finding raises suspicion of a violent death of the young child.

We undertook, for the first time in Greece, histological studies in samples of skin tissue from all four mummies [1]. Four different methods of tissue rehydration were applied for specimens from each mummy using a) 10% sodium bicarbonate in distilled water (dH2O), b) fabric softener, c) 5% glucose in dH2O and d) 3% dimethylsulfoxide in dH2O, followed by fixation in 10% neutral formalin and embedding in paraffin. Immersion of the specimens in 10% sodium bicarbonate solution for 8 hours was considered as the optimal (most rapid and with best tissue preservation) rehydration method for the desiccated mummy skin. Hematoxylin-eosin and Masson trichrome histochemical stains were performed on serial 5µm-thick tissue sections cut from each paraffin
Histological analysis using light microscopy showed absence of the epidermis in all tissue sections examined, while the dermis appeared well preserved with identifiable collagen fibers in the dense, irregular fibrous tissue which stained lightly with Masson trichrome, and blood vessels. No visible cells or cell remnants were present. Immunohistochemistry was performed using a standard biotin-streptavidin immunoperoxidase technique and antibodies to epithelial (pan-keratin) and endothelial markers (CD31, FVIII), glycoforin and α-smooth muscle actin (αSMA). In the dermis, there was no evidence of endothelial marker immunopositivity or traces of erythrocytes in the vascular lumens positive for glycoforin. Immunoreaction for pan-keratin was negative. However, possible immunopositivity for αSMA was focally identified in the dermis of all four mummies, indicating conservation of α-smooth muscle actin in 1700-2300-year-old human skin.

Future studies of our group will include histological examination and immunohistochemical analysis of tissues from organs (liver, heart) kept in canopic jars and molecular biological studies of preserved genetic material.

Reference

Paleopathology is a medical science whose main goal is to shed light on how diseases affected ancient subjects and the consequences which they brought to their lives. In some cases, the importance of the subjects is such that those consequences go beyond personal life and spread through History.

We present the paleopathological findings on two such individuals, both belonging to the Spanish Royal House, with different ages and historical importance but whose medical conditions could have historical relevance.

Case 1:
In September 2006, a mummified body was discovered under a small shrine of the Monastery of Santo Domingo el Mayor of Toledo, belonging to a sisterhood of Dominican nuns. Historical records were reviewed which confirmed that the subject corresponded to Sancho, a bastard son of Pedro I The Cruel, King of Castilla. The body had come to the monastery in the XV century from the fortress of Toro, where the boy had been kept in captivity and where he died in 1370 at the age of seven. Some historians supported the theory that the subject could have died by poisoning to avoid future claims of royal rights.

External and endoscopic examination were performed in the monastery and samples were taken from the internal organs. External examination showed that the head was almost detached from the spine and it preserved soft tissue from the left side of the face. The left arm and hand were well preserved and the latter showed a contracture similar to an ulnar claw. Endoscopic examination with a high-resolution digital flexible endoscope revealed an empty skull, presence of preserved ligaments in the vertebral channel and turgent lungs with abundant adherences to parietal pleura. The abdominal cavity showed a distorted and compact content in the lower abdomen, which could
not be identified and was not biopsied, and a recognizable liver of normal external features but too hard for biopsy.

Rehydration and optical examination of the lungs demonstrated abundant anthracotic pigment and the presence of abundant intraalveolar elements consistent with hemosiderophages and inflammatory cells which, coupled with the macroscopically evident pleural *sinequiae* and the absence of classical poisons such as arsenic, cyanide and mercury, suggested a natural cause of death related to a pulmonary infectious process in a subject chronically exposed to smoke, probably a fireplace in his reclusion chamber.

Case 2:
Holy Roman Emperor Charles V (also King Charles I of Spain) (Ghent 1500, Yuste 1558) was probably, in relative terms, the most powerful ruler of all times. He was the first global leader governing a conglomeration of territories in Europe, Africa, Asia and America, more extensive than those previously held by any ruler in European history. Charles V, afflicted by diverse pathologies, abdicated in 1556 and retired to the Monastery of Yuste in Cáceres

For many years, medical historians had speculated that the well documented medical condition of the Emperor during his last illness was compatible with *Plasmodium falciparum* malaria, and that this was the most likely cause of death. Charles V was buried in Yuste but his body was transferred to the Monastery of El Escorial in 1574. Historical reports of this transfer state that the body had spontaneously undergone mummification and that the Emperor was completely recognisable. The recent finding that the phalanx of the Emperor was kept outside the coffin allowed the investigation of the cause of his death.

The specimen consisted of part of the final phalanx of the fifth finger of one of the hands (laterality unknown). Externally, the finger fragment had a dark-brown colour and leathery consistence. The proximal part of the nail was attached to the ungual bed, whereas the outer half was completely detached. An X-ray study was performed.

A 3-mm-thick section of the proximal end was rehydrated by immersion in Sandison’s solution. After rehydratation, the specimen was embedded in paraffin following standard protocols used for fresh tissues. Four μm sections were deparaffinized, hydrated through graded alcohols and water and routinely stained with Hematoxylin & eosin, Masson trichrome and Giemsa’s stain.

The X-rays showed extensive erosion of the proximal epiphysis of the phalanx which had irregular borders and soft tissue calcifications. Macroscopic examination disclosed a yellowish deposit with chalky appearance that completely occupied the proximal edge of the specimen. At low magnification, no bone could be identified in the proximal margin of the specimen. In this location a massive deposit of weakly basophilic masses composed of fine acicular crystals eroding the bone
and extending into the adjacent soft tissues was observed. Polarization and Energy Dispersive Analysis by X-rays (EDAX) demonstrated that the material corresponded to urate crystals caused by a severe gout which had been historically suspected and which had impaired the emperor’s capacity as a ruler in critical moments [1].

Further histological analysis revealed a well preserved dermal collagen and bone, but no preserved cellular elements were detected except for isolated red blood cells. Giemsa’s stain showed what appeared to be young ring-form trophozoites of *Plasmodium* in some erythrocytes. The size and shape of those parasites correspond to the morphology of *P. falciparum*. No mature forms with hemozoin or free malarial pigment were identified. Electron microscopy showed erythrocytes containing irregular, ill-defined structures with membrane material, consistent with intraerythrocytic parasites. Molecular analysis with PCR and sequencing confirmed the presence of this parasite in DNA extracted from the sample previously to rehydration. These results definitively confirmed the historical suspicion of malaria as the cause of death of Emperor Charles V.

Reference
Prostatic hyperplasia, a very common condition today, was well known in the past as cause for bladder distension. At autopsy of natural or artificial mummies, the difficulty in identifying even a normal-volume prostate is likely to be due to putrefaction processes as well as dramatic reduction in size [1]. We report some ancient cases of prostatic hyperplasia observed recently in natural mummies from Italy.

The first case regards Pandolfo III Malatesta (1370-1427), a leading figure of the Italian Renaissance. He was a valiant soldier and horseman with a very active life style. The tomb of Pandolfo, containing his naturally mummified body, was explored in Fano (Marche, Central Italy). After careful X-ray and videographic examination, autopsy showed good preservation of the skeletal muscles, cartilage, internal and external organs, including prostate gland and penis. Macroscopic examination revealed a staghorn calculus (calcium urate) of the left kidney and severe enlargement of the prostate, with calcifications detected by X-ray and large nodules protruding in the lumen of an ectatic urethra. Histology showed fibrous bands of connective and muscular tissue surrounding circular and oblong lacunae, with no preservation of epithelial structures. The macroscopic and histological picture allowed us to diagnose prostatic nodular hyperplasia [2].

The second case, regarding the artificial mummy of Salimbene Capacci (+ 1497), rector of the Medieval hospital of S. Maria della Scala in Siena (Tuscany, central Italy), revealed well preserved pelvic organs at X-ray and CT scan. At autopsy, the cavity appeared filled with vegetable material, but some visceral organs were still in situ, in particular, the remains of the bladder, the prostate and the terminal segment of the intestinal tract. The prostate consisted of a central fibrous structure, surrounded by perineal tissues. Histology revealed dense fibrous tissue containing
muscular fibers and roundish cavities of variable size. Histological findings, distended urinary bladder, and age of the subject support the diagnosis of prostatic hyperplasia [3].

The third case (XIX century) concerns the natural mummy of an anonymous 50-60 year-old-man, recovered in an ancient friary near L’Aquila (central Italy), which underwent computed tomography and complete autopsy. Pelvic CT scans showed distended urinary bladder and a ring of fibrous dense tissue at the site of the prostate. At autopsy the bladder measured 7 x 6 x 5 cm and the prostate was 4 x 5 x 3 cm; the prostatic urethra had a 2-cm diameter. Histology revealed dense fibrous tissue containing muscular fibers and roundish cavities of variable size, filled with eosinophilic, PAS-positive material. Concretions were also present in some of these spaces. Strong immunohistochemical reactivity for PSA was observed in this material. The existence of glandular structures containing PAS-positive material, immunoreactive for PSA, confirmed the prostatic nature of the specimen, already suspected after CT scan and gross examination. The presence of the prostate, its histological appearances, the preserved and distended urinary bladder and the age of the subject support the diagnosis of prostatic hyperplasia [4].

To our knowledge, so far neither benign nor malignant forms of prostatic enlargements have appeared in paleopathology literature [1]. Therefore, the Italian cases of the Renaissance and Modern ages represent the only known reports of prostatic hyperplasia in mummies and clearly demonstrate that paleoandrological studies using diverse and modern technologies are possible. The good preservation of the external and internal genitalia of these three individuals may be related to the supine position of the bodies after death, which allowed rapid dehydration of these structures.

This type of approach, currently limited to the prostate but easily extensible to other organs on a larger number of mummies, could solve some important medical problems, as for example the origin and diffusion of some sexually transmitted diseases and genital tumors, whose natural history studied by the andrologists is still unclear.

References