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THE BIOCHEMICAL AND GENETIC METHODS OF ANALYSIS OF HISTORICAL OSSEOUS MATERIAL

ABSTRACT: A 15th century church cemetery was excavated during the renovation works near the Holy Mary church in Szczecin-Dabie (north-western Poland). A mummified left female forearm with a cat skeleton on it was found among other bones. This finding was then examined and the results are enclosed in this article. The aim of the study was to assess the sex, age, body height, type of body constitution and the social status of this individual. The use of modern radiological, biochemical and genetic techniques helped to enhance knowledge about the person whose mummified forearm was found. The analysis of the cuff material was performed in order to assess the century in which the mummified forearm was buried. Metallic elements like Ca, Mg, Zn, Mn, Pb and Fe were measured using atomic spectrometry. In order to assess possible diet of the individual, fluorides were measured using standard potentiometric method. DNA was isolated and then amplified using standard PCR technique.

KEY WORDS: Historical bone – Biochemical methods – DNA – PCR

INTRODUCTION

An old church cemetery was discovered during renovation works performed in 1972 in the Holy Mary Church in Dabie near Szczecin (north-western Poland). The church was erected in the second half of the 13th century by prince Barnim I after the city was founded. It became a Protestant church in the 16th century and after 1945 the designation was changed into Roman-catholic (Kalita-Skwirzynska 1980). The church cemetery, where the osseous remains were found, had the area of 0.7 ha and reached from the southern parts of the old city up to the church. It was founded in the beginning of the 15th century and officially closed in 1816 after the nearby Plonie cemetery was opened. After the WW I the Dabie cemetery area was levelled (Kroman, Wielkopolski 1960).

In order to further describe the site of the excavations it should be noted that there was a hunting chalet connected with the church cemetery and located at the south-eastern side of the church. It was raised around 1600 by Jan Fryderyk or Philip II who reigned afterwards on the remains of the building owned by the Kolbacz Monastery which was the supervising body of the Dabie church. The chalet was used as a retreat by prince Otto I and Boguslaw XIV during the hunting games in the Bukowa Forest and by guests of the prince during their visits to Szczecin.

After the expiry of the princely Gryfit's line in 1637 the chalet went under supervision of the Holy Mary parish and became living quarters for the pastors' and preachers' widows. It fulfilled this role until 1934 (Kroman 1980).

It is highly probable that the pastors' family members living in the chalet were buried at the church cemetery even after its official closure.

During many years bones, apart from skulls, were not given much attention. Examination of mummies excavated in large numbers at the end of the 19th century from burial sites in Nubia, Egypt and the New World have been for long years in the centre of attention of paleopathologists. These examinations have undoubtedly broadened the study material of anthropologists with extra-cranial bones and started paleopathology as a branch of science. It appears that even isolated bones can provide valuable information about the health structure and enrich knowledge about the studied group (Gladykowska-Rzeczycka 1982).

MATERIAL AND METHODS

Material consisted of mummified left forearm with preserved cuff fragment and mummified cat on top *(Figures 1, 2)*.

The overall preservation of bones was good. Because of mummification of the extremity there were problems with measuring the distal ends of ulna and radial bones. Therefore radiological methods, which are very useful in studies of excavated material, were used (Kroman 1980, Kroman, Wielkopolski1960). The measurements were taken using standard Martin's method (Martin, Saller 1921). The proximal ends of both bones and the corpuses' diameters were measured directly on the bones. Appropriate anthropometric indices were calculated in order to assess the type of bodily constitution (Malinowski, Strzalko 1985). L. Manourier's method was used to estimate the height of the individual (Piontek 1985).

FIGURE 1. Mummified forearm with the cuff fragment and with the mummified cat on top.

FIGURE 2. Mummified forearm with the cuff fragment – top view.

Sexual dimorphism was assessed using G. Olivier's method (Piontek 1985). The probable age of the individual whose bones were examined was estimated by examining the spongial structure of the distal end of ulna and radial bone as well as the marrow cavities size using the method recommended by Nemeskerie (Piontek 1985).

The following measurements were used in morphological examination of ulna and radial bones:

The analysis of the cloth found along with the bones was performed in the Prehistory Institute of Adam Mickiewicz University in Poznań. Type of raw materials, average thickness of elementary fibre forming the structure and plot thread, thickness and yarn kink, kind of weave, density of the cloth and its dating were examined.

Apart from standard anthropometric methods, macroscopic anatomical and radiological examination biochemical analysis was performed in order to establish the possible diet of the individual whose bones were studied. Phosphorus amount was assessed using colorimetric method. Metallic elements amounts, such as calcium, magnesium, mangane, lead and iron, were assessed using atomic spectrometry. Fluorides were examined using potentiometric standard method. The last stage of work was an attempt to identify bacterial DNA of *Mycobacterium tuberculosis* complex in mummified skin fragment. The PCR method was used to amplify the DNA. Since the first isolation of historical DNA from the bones, the oldest being dated to 5450 BC, this method has been more and more often applied to analyse historical DNA in this most commonly found material in excavations. Apart from population studies, evolutionary, migrations and sex related problems were also solved using this method. Another field of potentially fruitful application of PCR technique to historical genetic material is the area of hereditary and infectious diseases.

RESULTS

Examined bones were well preserved, delicately sculpted, had no visible muscle adhesion areas and the articular surface of the proximal end of ulna was undivided, which allows to ascertain that the examined bones belonged to a woman. We found no pathological features on the examined bones. Because the articular surfaces of proximal ends of both ulna and radial bone were not much used, we can say that the woman whose bones we examined died at young age.

There were serious problems to establish the exact age of the individual using only such a small part of her skeleton. Nevertheless we attempted to estimate her age based on the fact that up to maturity mineralization of the bone is a good determinant of the individual's age. We assessed the stage of accretion of proximal and distal ends of both bones with their respective corpuses. Macroscopic findings were complemented with radiological examination of our material. We found that the structure of spongial

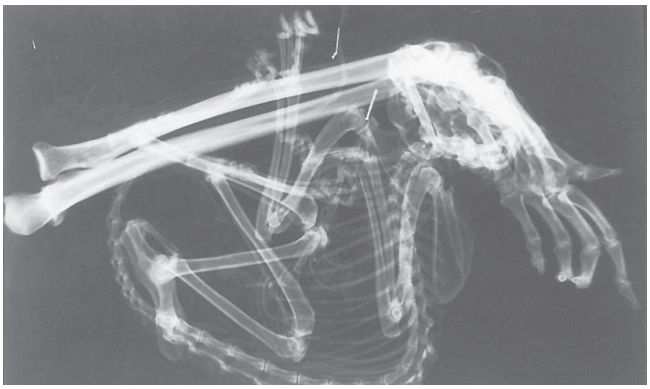

FIGURE 3. X-ray of the forearm with the cat on top – lateral view.

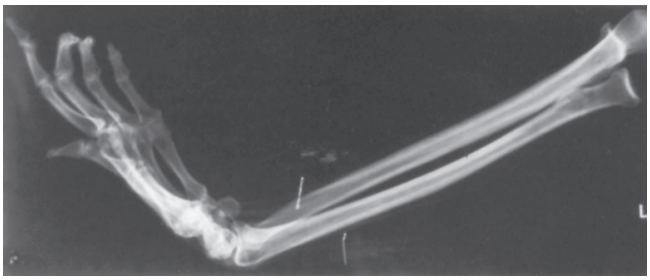

FIGURE 4. X-ray of the forearm – lateral view.

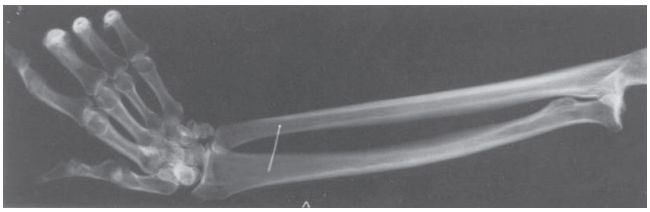

FIGURE 5. X-ray of the forearm with the cuff fragment – top view.

zone is dense, trabecules have a radial pattern and marrow cavities are elongated. Both ends of the ulna and radial bones were connected with their respective corpuses with no demarcation lines *(Figures 3, 4, 5)*.

All the above findings allow to estimate the age at death of this individual to be between 25–29 years. In order to improve morphological characteristic of the person whose bones we examined we attempted to estimate her probable height. We used proper indices based on the biggest length of radial bone and ulna recommended by L. Manouvier and M. Trotter and G. Gleiser. According to the results the woman whose bones we examined was 165.5 cm tall according to Manouvier's method, and 171.5 cm tall according to Trotter's and Gleiser's method.

Corpuses' massiveness and cross-section indices allow to assess the probable constitutional type of the individual. They show that the forearm bones belonging to the studied woman were slender with hardly visible muscle adhesion areas, therefore allowing to ascertain leptosomatic type of body constitution. Individuals with such type of body build tend to have height predominance over their shoulder and hip breadth.

Cuff cloth analysis performed in the Prehistory Institute of Poznań University allows to date the material as coming from the 18th century. The expertise revealed that the cuff was made of linen cloth with medium yarn breadth and with following features: structure – 0.190 mm, plot – 0.244 mm, yarn kink of ZZ type, cloth plot type 1/1. The cloth seems to be of a "stall" type produced in a workshop from second rate quality yarn (indicated by breadth of yarn used in structure and plot). We decided that the analysed fabric might come from uncoloured linen cloth – e. g. the element of underwear.

The results of elements amount assessment are shown in *Tables 1, 2*. We found no *Mycobacterium tuberculosis* complex DNA present in the examined mummified skin.

Historical findings concerning church cemetery, church and the hunting chalet exclude that the woman might have come from the princely family or court. We cannot however

TABLE 1. Dimensions and indices of the bones.

Dimension/Index	Radial bone (mm)	Ulna (mm)
Biggest length	245.0	267.0
Physiological length	237.0	245.0
Smallest perimeter	40.0	35.0
Diameter of the proximal end	24.0	27.0
Diameter of the distal end	32.0	–
Corpus' massiveness	16.8	15.9
Corpus' cross-section	55.5	28.4

TABLE 2. Elements of biochemical analysis.

Elements	Zn	Cu	Fe	Ca	Mg	P	Pb	Mn	F
mg/g	0.561	0.169	1.18	170.96	3.71	99.83	0.95	0.31	989.43 ppm F^-

exclude that she was a parson's widow or daughter living in the chalet. We found it very interesting that she was buried along with a cat.

DISCUSSION

We can grossly enhance our knowledge by applying modern scientific methods for evaluation of excavated material. Such methods broaden the information spectrum received as a result of their application. The biochemical assessment of excavated material can provide a lot of valuable data concerning populations often living in very distant past. Biochemical analysis of osseous material brings information about dietary habits and food preferences as well as about diseases, lifestyle and social hierarchy. However, mineral composition of bone does not depend solely on conditions directly influencing members of historical populations during their life. Diagenesis processes change a natural bone composition during the time they spend in the soil because of various chemical and physical effects. Those effects can cause enrichment, substitution or depletion of various mineral components of bone. For instance, it is a well known fact that high strontium concentration in bones, which suggests diet rich in meat, indicates high social status of an individual (Gonzales-Reimeres, Roman 1992). Calcium is a mobile element, therefore its concentration decreases along with the time a bone spends in soil. Concentration of such elements as lead, barium, copper and manganese increases with age and is higher among males (Molleson 1992). High lead concentration in bones can indicate high social status, since the primary source of lead was wine, fruit and juices kept in mortars and these were consumed almost exclusively by rich people (Molleson 1992). We could not compare the amount of elements in the examined mummified forearm with other findings from the same site. However, we compared our results with those available in literature and they may indicate high social background of the woman whose bones we examined.

CONCLUSIONS

Analysis of the results of anatomical, anthropological, biochemical and genetic evaluations of the found osseous remains and the cloth allows to formulate the following conclusions:

1. The examined bones belonged to a woman.
2. Radiological data such as high density of spongial zone, radial pattern of bone trabecules and elongated marrow cavity indicate that age at death of the individual whose bones were examined might have been between 25–29 years.
3. Measurements of ulna and radial bones allowed to estimate the height at death as 171.25 cm (Trotter's and Gleiser's method), respectively 165.5 cm (Manouvier's method).
4. Massiveness and cross-section indices of the bones' corpuses, their perimeters and cross-sections dimensions indicate the leptosomatic type of constitution.
5. Cuff cloth analysis suggests that the remains can be dated in the 18th century.
6. Biochemical analysis confirmed a diet rich in meat which can suggest high social status of the woman whose bones we examined.
7. Genetic analysis of the remains showed no *Mycobacterium tuberculosis* complex DNA presence in the examined mummified skin.

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