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Unusual spinal tuberculosis in an Avar Age skeleton (Csongrád-Felgyő, Ürmös-tanya, Hungary): A morphological and biomolecular study

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SUMMARY

The paleopathological analysis of a well-preserved young adult female skeleton from the AD 7–8th century (Avar Age) in Hungary revealed multiple lytic lesions in all of the thoracic and lumbar vertebral bodies. The lesions were characterized by smooth marginal zones and space-occupying mass appearance. The considerable loss of spongy bone in the thoracolumbar vertebrae resulted in angular deformity and fusion, characteristic of the healing stage of TB. Osteolytic lesions were also observed on the vertebral processes, ribs and sternum. On the endocranial surface, abnormal blood vessel impressions were revealed, indicating some kind of meningitis.

The X-ray and CT analysis of the affected bones detected abnormal structures and cystic zones of destruction. The lesions were however not always bordered by areas of increased density, which is typical in cystic TB. Vertebral remains were also subjected to biomolecular analysis in two different laboratories, which attested the presence of *Mycobacterium tuberculosis* complex (MTBC) DNA and supported the paleopathological diagnosis of TB. Spoligotyping analysis confirmed the presence of MTBC DNA and more specifically an infection caused by bacteria belonging to the *M. tuberculosis* lineage.

This case study provides new data for the paleoepidemiology of TB in this geographical area and historical period, and draws attention to the great variability of TB lesions in the human skeleton.

1. Introduction

In paleopathology, tuberculosis is one of the few infectious diseases that usually leave quite pronounced and rather specific bone lesions. The classic diagnostic criteria of skeletal tuberculosis are characteristic lytic lesions with little reactive bone formation and a predilection for areas of hemopoetic (red) marrow (mostly in the vertebrae, ribs, as well as metaphysis and epiphysis of long bones), although any bone can be affected. Vertebral tuberculosis (spondylitis tuberculosa/tuberculous spondylitis, also known as Pott's disease) is the most typical representation of skeletal tuberculosis, known since antiquity. The spine is involved in 25–60% of skeletal TB cases, particularly in the lower thoracic and lumbar regions. The Batson's venous complex has been suggested to play a significant role in this predilection. It has also been noted that two thirds of the vertebral changes are associated with other TB alterations [1,2].

Beside the classic bone alterations, there are also some early-stage or minor osseous lesions that can be indicative of tuberculosis, such as vertebral hypervascularization, endocranial alterations, proliferative rib changes and diffuse periosteal new bone formation [3–5].

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In the differential diagnosis of TB changes, numerous diseases causing similar bone alterations have to be taken into account, although quite a few skeletal changes due to TB can also contradict its classic criteria [2]. In the differential diagnosis of such cases, complementary analyses, especially biomolecular techniques, might provide invaluable help since they can detect ancient pathogen remains and therefore support the osteological diagnosis [6–11].

The case study presented here came from AD 7–8th century (Avar Age) Hungary and presented very severe pathological alterations on the vertebral column of a young adult female, although the unusual features of the alterations required complementary analyses.

2. Material

The examined 7–8th century skeleton originated from the area of Felgyő (in the proximity of the city of Csongrád, Southern Hungary). A total of 312 graves (240 inhumations and 72 inurned burials) were uncovered at the Ürmös-tanya site at Felgyő between 1960 and 1977 in the course of the excavations directed by Gyula László [12]. A small part of the site had been in use during the Bronze Age, while the largest part of the site, including 216 graves, was identified as an Avar cemetery. The Avar period (AD 7–8th century) in Hungary was characterized by the presence of Avar people, who led a sedentary life and were mainly involved with agriculture and animal breading. Some of the burials were very rich in grave goods (such as bone bows, quivers, mounted belts, jewellery items, horse harnesses) and were of great importance from an archaeological point of view. Various types of animal bones (e.g. cattle, sheep, hen, domestic goose) were also recovered during the excavations [13].

Although the skeletal remains of 219 individuals had been recovered from the 216 excavated graves of the Avar Age, only the remains of 160 individuals were preserved and added to the osteological collection housed at the University of Szeged. A general anthropological study was carried out most recently on this series [14]. Although the bone surfaces were fairly well-preserved in the whole series, approximately one half (48%) of the osteoarchaeological material was incomplete. The remains of the subject under study, individual no. 205, consisted of an almost complete adult skeleton with relatively well-preserved bone surfaces.

Although the newest archaeological studies have been using the name of “Felgyő, Ürmös-tanya” (“Ürmös-Farm”) exclusively as the name of the site, earlier paleopathological publications followed the works of Gyula László, who called the site “Csongrád-Felgyő” in his studies [15]. This was the name used in publications presenting the occurrence of skeletal TB from different parts of this site [4,16,17]. The abbreviation “CsoF 205”, used in these preliminary studies to identify individual no. 205 referred to this ancient site name “Csongrád-Felgyő”. In order to avoid any confusion, both site names have been used together for this study as follows: “Csongrád-Felgyő, Ürmös-tanya”.

3. Methods

3.1. Morphological studies

By utilizing standard sexing [18] and aging [19,20] methods, individual no. 205 was identified as a female adult, approximately 20–30 years old.

The skeleton was subjected to a detailed paleopathological analysis at the Department of Biological Anthropology, University of Szeged, where it is still currently stored. The detection of pathological changes was principally based on standard gross morphological analysis [1,2,21,22], taking into account normal anatomical variability and pseudopathology. In the inventory sheet, the alterations' location, laterality, type (destructive, proliferative or both), description (e.g. size, shape) and developmental stage (e.g. active, healed) were recorded and classified into nosological groups.

Complementary radiological examination was carried out at the Department of Radiology, University of Szeged. This complementary analysis consisted of plain film radiography (X-ray) and computer-aided tomography (CAT).

The presumptive diagnosis was principally based on current anatomo-clinical and radiological criteria.

3.2. Biomolecular studies

Biomolecular analysis was also carried out on the affected skeletal parts in two separate institutes (Munich and Bolzano) as an important additional source of information in the paleopathological diagnosis [6-11]. A first aDNA analysis was undertaken at the Institute of Pathology, Munich, Germany, which was later completed by a second biomolecular study in the aDNA laboratory of EURAC, Bolzano, Italy.

1) The first paleomicrobial analysis carried out at the Institute of Pathology, Munich used the following protocol:

- DNA extraction

To eliminate contamination, the vertebral bone samples were first cleaned with a 0.5% sodium hypochlorite solution and then the outer surface was removed mechanically by sterile instruments. Samples were taken from the inner part of the bones. These were then used to produce a homogeneous bone powder using a mixer mill (Retsch MM200, Haan, Germany). One gram of the pulverised material was then incubated with 2 ml of 0.5 M EDTA-solution containing proteinase K (0.25 mg/ml) at room temperature for two days on a rotatory mixer [23]. Following centrifugation for 15 min at 3000 g, 0.5 ml of the supernatant was removed and 1 ml guanidine isothiocyanate solution and diatomaceous earth were added [24]. After incubation on a rotatory mixer for another 2 h, the diatomaceous earth was pelleted by centrifugation and washed twice with 70% ethanol and once with acetone. The DNA was eluted with 80 μl sterile water. Finally, another washing and concentration step was performed with Microcon-30 filters (Millipore, Bedford, MA). The resulting DNA (c. 1 μl fluid) was then diluted with sterile water to a volume of 20 μl. This was done in order to have enough template for repeated PCRs (including subsequent restriction enzyme digestion or sequencing). The final DNA concentration ranged between 20 and 40 ng/μl.

- Precautions to avoid contamination

Precautions were taken to avoid contamination during the extraction procedure and in the PCR reactions. The extraction, PCR and post-PCR analyses were all conducted in separate rooms where no studies of modern mycobacterial or human DNA have been performed. All reagents were purchased as DNAse and RNAse-free molecular biology grade chemicals and autoclaved when appropriate. No positive PCR controls were used. Disposable gloves were worn during all procedures and changed frequently. Sterile aerosol protection tips (Safeseal tips, Biozym, Hess. Oldendorf, Germany) were used to avoid cross-contamination. Two extraction blanks were always performed in the same procedure and additionally a PCR blank was included in each PCR reaction.

- Amplification of mycobacterial DNA