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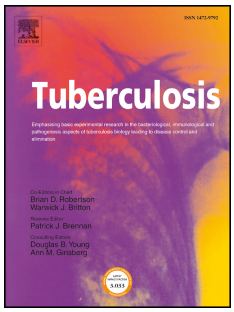

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Two positive tuberculosis cases in the late Nigrovits family, 18th century, Vác, Hungary

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Summary

Two mummies of the Hungarian mummy collection from Vác were the subjects of anthropological, paleopathological, radiological, paleomicrobiological, paleohistological and paleoproteomic studies. Both individuals belonged to the same family. The father, József Nigrovits (No 29), died at the age of 55 on the 11th of November 1793; his son, Antal Nigrovits (No 54), died on the 16th of July 1803, at the age of 22. They lived in the 18th century in Vác, a small town in northern Hungary.

The macroscopic examination of the son showed a severely deformed neck and back region; the father has no visible mark of any illnesses. As earlier researches showed that tuberculosis was widespread in the community, the etiology of these deformities were examined. The paleomicrobiological results found both individuals were infected with tuberculosis. Although they suffered from TB, the CT scan data of the bodies and their 3D reconstructions showed no skeletal evidence of tuberculosis. The deformity of the son turned to be a developmental abnormality of unknown origin, but no Pott's gibbus was present.

Keywords

Mycobacterium tuberculosis; Vác; Hungary; mummies; paleopathology; paleomicrobiology

1. The background of the mummies

The small Hungarian town of Vác lies to the north of Budapest on the bank of the Danube. During

the renovation of the Dominican Church in 1994-95, coffins were found that had remained untouched for 200 years and contained the remains of 265 deceased individuals[1]. Many of the individuals were naturally mummified and well preserved. The human remains from the crypt are housed in the Department of Anthropology of the Hungarian Natural History Museum, Budapest. Based on the descriptions on the coffins and in the parish registers, the crypt served as the burial site of the people who lived in Vác between 1674 and 1838.

The bodies were preserved through natural processes, with no human interference. Natural mummification was made possible by the unique microclimate of the crypt. The average temperature of the crypt was 8-11^{o}C (46.4 – 51.8^{o}F), independent of the outside temperature. The relative humidity was generally constant and the air pressure changed between 991 and 1009 hPa (0.99 and 1.01 bar). The weak but constant ventilation along the narrow tunnel connecting the undercroft to the outside world was a very important factor in the mummification process[2,3].

Inscriptions on the coffins and available contemporary archives enabled determination of the age at death and the identities of the buried persons – 166 individuals from the 265 are known by name. Based on the sources, most of the dead were citizens of the town. Clergymen were in the minority, and were buried in a separate part of the crypt. The long list of names and dates found in the registers and other documents gradually revealed the network of kinship relations, families, and fates. In some cases the cause of death and the profession of the deceased were also indicated. The kinship relations of some families can be traced back to several generations and whole family trees can be delineated.

2. Materials and Methods

The mummies of two members of the Nigrovits family, József Nigrovits (No 29), the father, and his son – Antal Nigrovits (No 54), are the subjects of this study. Based on the inscription on his coffin, József Nigrovits died on the 11th November 1793, at his age of 55 (“*Josephus Nigrovits anorum aetatis suae 55/obiit die 11 Novembris Ano Domini 1793”).* His son, Antal Nigrovits died on the 16th July

1803, at his age of 22, unmarried ("*P[erillustris] D[ominus] Antonius Nigrovits Caelebs Annorum 22 Obiit 16a Iulii An[n]o 1803").*

Philips Brilliance 16 CT equipment was used for radiological examination. The slices were 1mm thick, so between 1600-2500 slices were needed, depending on the range to be covered. Using the raw data, the slices were reconstructed in HRCT mode. During the post-processing, a narrower, so-called bone-window was used. For the 3D reconstruction, the inbuilt program of the Philips CT equipment was applied.

The skeletal and mummified tissues from the two Nigrovits' were examined for the presence of *Mycobacterium tuberculosis* complex as part of earlier researches on the Vác mummies[4,5]. The examination showed that 55% of the examined individuals were positive, and that the incidence varied according to age at death and sampling site in the body[4]. A later, more comprehensive study[5] gave a positive result in 67.7 % of individuals, ranging from 46.5 % in children, 89.7 % in middle-age and 69.6 % in individuals older than 65 years. Single samples proved a positive result in 55.8 %, multiple samples in 78.5 % of the cases. Recommended ancient DNA (aDNA) protocols[6] were followed throughout the DNA extraction, with separate rooms for different stages of the process. The procedures have been described previously[4,7]. In brief, small quantities of crushed or powdered sample were demineralised in Proteinase K/EDTA at 56^{o}C for 1-4 days. One aliquot was treated with 0.1M N-phenacylthiazolium bromide, a reagent that cleaves glucose-derived protein cross-links[8] and has been found to be useful in DNA extractions from some archaeological samples. Thereafter both aliquots were lysed in guanidium thiocyanate solution and DNA captured onto silica in suspension or by isopropanol precipitation of the residual supernatant, washed, and dried until use. Negative extraction controls were always included and extractions and analyses repeated.

The DNA amplification details have been described previously[4,5,7]. In brief, the *M. tuberculosis* complex (MTBC) was detected by targeting a specific region of the repetitive element IS*6110* using a two-tube nested PCR that yields an outer product of 123 bp and a nested PCR product of 92 bp (Table

1). Qiagen Hotstar® Taq polymerase and reagents (Qiagen, West Sussex, UK) were used. Negative controls were always routinely included. PCR products were electrophoresed on agarose gels, visualised by ethidium bromide staining exposed under ultraviolet light and recorded with a Polaroid camera. Later, the lung tissue of the body 54 was re-examined using real-time PCR[9] with specific primers and probe (Table 1) for the target IS*1081* (6 copies/cell).

Bone samples were taken from both individuals for histological and paleoproteomic investigations. A left rib fragment (7^{th}–10^{th}) and a vertebral body (5^{th}–7^{th}) of No 29, and a fragment from the shaft of the right fibula of No 54 were examined. All bone surfaces were investigated using a magnifying glass. Thin-ground sections were prepared as described by Schultz[10]. Additionally, all three samples were used to extract and detect extracellular bone matrix proteins[11]. The paleoproteomic analysis of these cases is still in progress.

3. Anthropological, paleopathological, radiological, paleomicrobiological, and paleoproteomic results of the two family members

Body No 29

Approximately 70% of József Nigrovits' body is mummified; his back is skeletonised. The individual is cachectic. The neck region is slightly curved. Slight irregularities can be seen on vertebrae, but there are no traces of skeletal tuberculosis (Fig. 1).

The early paleomicrobiological results obtained were as follows. The chest sample was positive by nested PCR (92 bp), but negative for single stage PCR (123 bp). The sample from the abdominal tissue proved to be negative.

The rib and the vertebral body of József Nigrovits showed no macroscopic sign of bone inflammation. Microscopically, the spongy bone of the vertebral body exhibited discrete vestiges of osteoclastic resorption. There are remnants of slightly developed and partly incompletely remodelled

Howship's lacunae in several trabeculae of the spongy bone substance (Fig. 2a-b). Furthermore, there are a few trabeculae that exhibit pronounced osteoclastic resorption (Fig. 2c). These findings do not correlate to the normal situation of an individual of his age. In an old-age osteoporosis, there are, as a rule, no Howship's lacunae observable. Thus, there is the probability for the existence of an initial inflammatory process that might be connected with early tuberculosis infection.

Due to the preservation and the lack of compact bone substance, no extracellular bone matrix proteins could be detected.

Body No 54

The body of Antal Nigrovits is partially mummified. It is markedly cachectic. His back shows extreme deformity and early stage vertebral lesions. The gross morphology suggests a possible tuberculosis infection. A virtual 3D model was reconstructed of his deformed back using the CT scan data of the vertebral column to investigate the morphology of each affected bone. His back displayed serious kyphosis, lordosis, and scoliosis, but there were no traces of skeletal tuberculosis. The severe deformation must have been caused by developmental abnormality.

The early paleomicrobiological results obtained were as follows. Both the lung tissue as well as the left abdomen samples demonstrated negative results. The abdominal tissue gave a strong positive result by nested PCR (92 bp), but it was negative for single stage PCR (123 bp). The result of the re-examined lung tissue using real-time PCR with specific primers and probe for the target IS*1081* showed that all fractions of this DNA extract were negative.

Antal Nigrovits' fibula initially showed no convincing vestiges of a pathological process. However, there is evidence of some signs of inactivity atrophy in the lamellar structure of the compact bone substance on the medial side of the shaft (Fig. 2d). This morphology might be induced by a longer period of being bedridden, provoked by chronic diseases such as pulmonary tuberculosis. Although

147 other causes are possible, the physical stress to the musculoskeletal system caused by a long period of
148 immobility should be taken into consideration.

149 In the fibular shaft fragment, extracellular bone matrix proteins, such as osteonectin and IgG, could
150 be detected. However, at present no specific proteins characteristic of tuberculosis disease have been
151 found (e.g. Ag 85).

152

153 **4. Relationship of bony lesions to MTBC aDNA**

154 Body No 29 was noticeably cachectic. The rib and the vertebral body showed no macroscopic sign
155 of bone inflammation. Although no abnormalities were detected in a chest radiograph, the chest sample
156 tested positive for tuberculosis by nested PCR (92 bp). The sample from the abdominal tissue was
157 negative for TB aDNA. The positive chest result suggests pulmonary TB. Paleohistological results
158 suggest the presence of an initial inflammatory process that might be connected with an early
159 tuberculosis infection.

160 Based on gross morphology, the severe deformities of the neck and back as well the noticeable
161 cachectic appearance suggest tuberculosis in the case of Body No 54. The radiological images gave
162 evidence of developmental abnormalities of unknown origin, but no Pott's gibbus was present. The
163 paleomicrobiological test yielded a positive nested PCR (92 bp) result but for only the abdominal
164 tissue. All the others samples and methods demonstrated negative results. These results suggest an
165 active tuberculosis infection. The positive abdominal sample may indicate secondary infection of the
166 abdomen caused by swallowing sputum. It is of course possible that the paleohistology result indicates
167 chronic diseases in general, pulmonary TB is thus one of the possible causes for this finding.

168 Body No 54 demonstrates similarities to another Vác mummy case, that of Antónia Tauber (No 97).
169 In this case, her back showed a prominent humpback, which indicated the possibility of Pott's disease.
170 The radiological images showed idiopathic scoliosis, a serious developmental abnormality of unknown
171 origin (Fig. 3). The molecular biological tests indicated that DNA residue of the MTBC was present.

The tuberculosis bacillus was present in her body although it had not caused lesions in the vertebral column[12].

It is estimated that around 40% of skeletal tuberculosis cases result in tuberculosis of the spine[13]. However, it is important to emphasize that spinal tuberculosis is comparatively rare, and possibly occurs in only 3-5% of all cases allowed to run their natural course. Therefore, the great majority of cases are unlikely to have skeletal lesions. Thus, the incidence of tuberculosis is undoubtedly far higher than can be suggested by the level of bony lesions observed by a paleopathologist[14].

The new cases from Vác (Bodies Nos 29 and 54) and the earlier one (Body No 97) support other reports that MTBC aDNA can be detected even in bones without morphological changes[15,16].

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Ethical approval

Not required

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Author contributions

ISz, IP and GyP conceived the study. ISz, IP, EM, GyP and MSp performed the macromorphological analysis. AK, KK, BKK and CsK performed the radiological analysis. HD performed the aDNA studies. MS and THSS performed the paleohistologic and paleoproteomic analysis. ISz and IP wrote the manuscript and all authors approved the final version.

194 **Competing interests**

195 None declared.

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199 **5. References**

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Legends

Fig. 1: Body No 54 (Antal Nigrovits). View of the extremely deformed and cachectic body (left); Deformed neck, a closer look (right above); Virtual 3D model of the neck and back region using the CT scan data (right below).

Fig. 2: Vertebral body of József Nigrovits (No 29). Thin-ground sections (thickness 70 µm); a and b viewed in plain, c viewed in polarized light using a hilfsobject red 1st order (quartz) as compensator. Magnification 200x. Arrows points to Howship's lacunae; d: Fibula of Antal Nigrovits (No 54). Inactivity atrophy in the lamellar structure of the compact bone substance on the medial side.

Fig. 3: Body No 97 (Antónia Tauber). Virtual 3D model of the extremely deformed body (left); Radiograph showing gross spinal deformity (right).

Table 1 *M. tuberculosis* complex-specific primers used in this study

Name	Target region	Target size	Primer
P1	IS*6110*	123 bp	5’CTCGTCCAGCGCCGCTTCGG 3’
P2	"		5’CCTGCGAGCGTAGGCGTCGG 3’
IS-3	"	92 bp	5'TTCGGACCACCAGCACCTAA 3'
IS-4	"		5'TCGGTGACAAAGGCCACGTA 3'
NF	IS*1081*	72 bp	5' TGATTGGACCGCTCATCG 3'
NR	"		5' CTTGATGGGGGCTGAAGC 3'
1081 Probe	"		5’-FAM-GGGCTACCGCGAACGCA-BHQ1-3’