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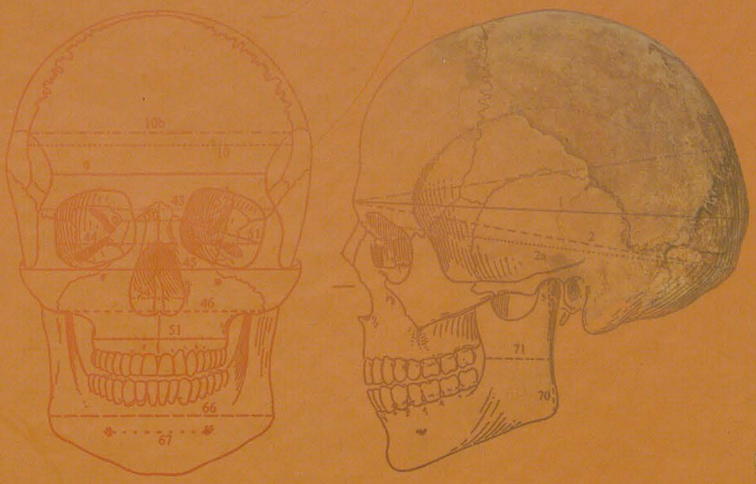

THE TALKING DEAD

NEW RESULTS FROM CENTRAL AND EASTERN EUROPEAN OSTEOARCHAEOLOGY

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New results from Central- and Eastern European Osteoarchaeology

Proceedings of the First International Conference
of the Török Aurél Anthropological Association
from Târgu Mureş

13–15 November 2015

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EDITOR
SZILÁRD SÁNDOR GÁL

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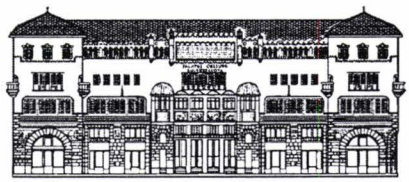

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In memoriam Török Aurél
(1842–1912)

FOREWORD

The International Conference organized in 13th–15th November 2015 by the 'Török Aurél' Anthropological Association was the first step of a long nurtured dream. The aim of the association awaiting registration is to provide a panel for bioarcheologists and archeologists as well as to create a scientific framework for the specialized study and analysis of historical single-graves and cemeteries from Romania and the Carpathian Basin. Through the organization of the above mentioned conference series we wish to honor the heritage of anthropologist Aurél Török, who inspired by Paul Broca established the Anthropological Institute in Budapest, in 1881, as the fifth anthropological organization in the world. We believe that a joint cross-border collaboration will greatly contribute to the unveiling of new information and data concerning communities from the past.

Szilárd Sándor Gál

VIOLENCE AND DEATH: BIOARCHAEOLOGICAL ANALYSIS OF TRAUMA IN A SKELETON SAMPLE FROM A MEDIEVAL OSSUARY DISCOVERED IN TAUT (FELTÓT), ARAD COUNTY, ROMANIA

Luminița Andreica-Szilagyi[1]

[1] Muzeul Județean Arad

Keywords: Tauț, ossuary, medieval period, *peri-mortem* injury, deliberate violence.

Abstract

The aim of this paper is to prove the existence of the interpersonal violence in Tauț (Arad County) during the medieval period (XIV–XVI centuries). Osteological material comes from an ossuary found in the northern area of Gothic churches. The skull was the most frequently traumatized element; peri-mortem trauma was observed in 4 male skulls while ante-mortem healed sharp force lesions were registered in 9 skulls (8 males and 1 female). The ante-mortem lesions indicated that these individuals had probably experienced other combats before the battle in which they died. The predominance of frontal injuries, as well as the presence of peri-mortem trauma and sharp force lesions, may suggest the presence of deliberate violence in this community. All the indicators of deliberate violence were recorded predominantly in males, suggesting that intentional violence in Tauț was exclusively the males' prerogative.

Introduction

The village Tauț is situated at the feet of Zărand Mountains (North face), on the plain of Cigher, in the eastern half of the Zarand County (Plate I.2.). The earliest reference of this place can be traced in a Papal document dated 1187, where a house belonging to the Order of the Hospitalers is mentioned in Tauț. Still, their presence in this area is not yet sustained by archeological evidences[1].

The archaeological campaigns were allowed to unveil an ensemble of complicated buildings that had belonged to a medieval church built in successive stages. The building of the first stage belonged to a Romanesque church from the 13th century. This community decided at a given moment to enlarge their religious settlement, its result being a Gothic church whose construction lasted until approximately the beginning of the 16th century[2].

Somewhere at the end of the 14th century, but certainly in the 15th century, in the Northern part of the Gothic church there was an osuary where the access was from the church nave (Plate I.1.). Settling an ossuary became mandatory when the cemetery became overcrowded, thus, the bones of those buried before were recovered and kept in such a place, organized in a compartment of the northern area of the Gothic church nave under the level of the wooden floor. In the middle of the space between the ossuary and the sacristy there was also a crypt.

[1] Kovács 1980, 198.

[2] Mărginean & Rusu 2010.

The aim of this paper is to prove the existence of the interpersonal violence in Tauț (Arad County) during the medieval period (XIV–XVI centuries) based of the bioarchaeological analysis.

Material and methods

The osteological material was recovered from an ossuary built in the North area of the Gothic church discovered in 2006 campaign[3] and is composed of 28 skulls, 10 mandibles, 32 femurs, 29 tibiae and 20 humeri. During the excavation, it was obvious that the osteological pieces were reburried carefully; those participating to the innitial exhumation knew or had received clear instructions on selecting only the lond bones and skulls and handling them with great care; this was possible due to the christian belief about the resurrection of the body[4].

Sex assessment from the skull is based on cranial features and age-at-death was estimated using cranial suture closure[5].

For femurs the maximum length and the transversal diameter of the head were indicators of the sex[6], while for the tibia, the maximum length was used as indicator of the sex[7]. Skeletal lesions were designated as *ante-* or *peri-mortem* according to the description from the literature[8].

Results

Ante-mortem injuries

Lesions caused antemortem were identified on nine skulls, out of which eight belonged to men and one was established to be female. The maximum number of strikes were recorded among the men (nine out of 10 strikes); six are localised on the frontal and three on the parietal (two on the left side, one on the right) (Table 1) (Plate II. 1.); the most affected age category is of 40–49 years of age, with five strikes, followed by 30–39 years of age, with two strikes and only one strike for the decade between 50–59 years of age. In the case of skulls established as female was noticed one single strike, caused during lifetime, on the occipital, on a woman of 30–39 years of age (Plate II. 2.). The long bones lack such trauma.

Peri-mortem injuries

On the skull, perimortem strikes were identified for four individuals, all of them males; five of these injuries are on the frontal and one strike is visible on the right parietal (Table 1) (Plate II. 1.). The maximum number of injuries (three strikes) was noticed on individuals in the age category between 20–29 years, followed by two strikes for the superior age category and one strike in the case of a male aged between 50–59 years.

Table 1. Distribution of cranial lesions by cranial element

Antemortem	FR	LP	RP	OC	N (%)
	n (%)	n (%)	n (%)	n (%)	
M	6 (66.7)	2 (22.2)	1 (11.1)	0 (0.0)	9 (100.0)
F	0 (0.0)	0 (0.0)	0 (0.0)	1 (100.0)	1 (100.0)
Perimortem					

[3] The research team included: G. P. Hurezan†, F. Mărginean, Z. Kopeczny and L. Andreica.

[4] Gilchrist & Sloane 2005, 41.

[5] Buikstra & Ubelaker 1994.

[6] Bass 1987.

[7] Olivier 1960.

[8] Merbs 1989; Sauer 1998; Botella et al. 2000.

Antemortem	FR	LP	RP	OC	N (%)
M	5 (83.3)	0 (0.0)	1 (16.7)	0 (0.0)	6 (100.0)
F	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

M: males, F: females, FR: frontal, LP: left parietal, RP: right parietal, OCC: occipital, n: number of lesions, N: total number of lesions,%: n/N.

The long bone peri-mortem trauma frequency is 18,8% (6/32 femurs) for femurs and 24.1% (7/29 tibiae) for tibiae. Regarding the number of injuries, for the femurs established as male, there was identified a number of 25 injuries vs. one injury on the femur that had belonged to a woman. For the tibia, the maximum number of injuries (11 lesions) was visible on those whose sex could not be identified. From the point of view of the distribution of the lesions depending on the side, for the femur, for both sexes, there can be noticed a higher incidence of the strikes on the left side (23 vs. 3 on the right side); on the contrary, on the tibia there were identified ten lesions on the right side vs. 3 on the left (Table 2).

Tabel 2. Long bone lesions (femur and tibia) frequencies by bone element

	Male n (%); N=27		Female n (%); N=1		Unidentified n (%); N=12	
	L	R	L	R	L	R
Femur	22 (81.4)	3 (11.1)	1 (100.0)	0 (0.0)	0 (0.0)	1 (8.33)
Tibia	0 (0.0)	2 (7.4)	0 (0.0)	0 (0.0)	3 (25.0)	8 (66.7)

L: left, R: right, n: number of lesions, N: total number of lesions,%: n/N.

Discussion

The percentage of peri-mortem lesions are compatible with certain studies that refer to the skeletons of individuals who had died on the battlefield; the percentage of 14.2% on the skull skeleton (4/28 skulls) recorded in the case of individuals from Tauț is very close to that obtained on the collection of skeletons in Čepin (Croația)[9], namely 15% and that of 21.3% in the case of long bones (13/61 femures + tibiae) is very close to the percentage recordedat Towton[10], of 33%. Similarly to other research[11], the demographic profile of the victims in the Tauț ossuary suggests acts of violence towards the age category of young adults and adults (20–29 and 30–39 years of age).

Fighting techniques

Poor protection of individuals' heads from Tauț is indicated by the large number of cranial injuries, some of which involve extremely deep cuts. The side distribution and location of skull traumas provide further indications on fighting modalities. At Tauț many cranial injuries were found on the left side of the parietal or other anterior elements (Plate II. 3.), indicating that a right-handed attacker successfully engaged their weapon while facing their victim[12]. The posterior lesion found on female skull may suggest that this victim was struck from behind while fleeing her attacker[13].

The traumas on the femurs (Plate II. 5.) and tibiae (Plate II. 6.) were interpreted as the result of a fighting technique where one tried to incapacitate the opponent by first striking at the legs and then attacking the head when the individual lay helpless on the ground[14].

[9] Šlaus et al. 2010, 361.

[10] Novak 2007.

[11] Novak 2007; Šlaus et al. 2010.

[12] Ingelmark 1939, 167; Boylston 2000, 361; Djurič et al. 2006, 174.

[13] Larsen 1997.

[14] Holck&Kvaal, 2000.

Can we talk about unjustified violence?

Porter at al. (2003) defined gratuitous violence as "...excessive, or unnecessary violence that goes beyond the level that would be necessary to accomplish homicide, and causes the victim unnecessary pain and suffering". Such lesions have been identified on the left femurs of two male individuals: 10 injuries in one case and 11 in the other case; they were produced with sharp blade and they were not healed.

Conclusions

The indicators of deliberate violence were recorded predominantly in males, suggesting that intentional violence in Tauț was exclusively the males' prerogative.

The majority of injuries, both those on the skull and those on the long bones consist of sharp force wounds. One exception is represented by the skull number 23 which shows a lesion produced by a blunt object on the frontal bone (Plate II. 4.). The great number of individuals with healed traumas may suggest that hostilities often emphasized injury over death. Similarly, the lesions healed without severe complications may indicate that these individuals had high immune system and also that they benefited from medical and social care.

Simultaneous appearance of both ante-mortem and peri-mortem lesions in the case of two male individuals indicate that these individuals had probably experienced other combats before the battle in which they died. Moreover, the reduced number of such cases may mean that these victims were rather simple locals than people trained for war. It is important to remember that the injuries observed in the bones today are likely to represent a fraction of the injuries that were sustained, since many injuries only affected the soft tissues of the body.

The documents that could offer some information about the conflictive events are missing, while an attempt of contextualization with the major events regarding our micro region are/ could be hazardous. Historical data reveal about Tatar invasion in the years 1241–1242 in the area of Zarand and fighting back the opposition of the local population[15]. At the same time, one of the obligations of the nobles towards the kingdom was sending people to fight in the cases when the king was in danger[16].

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[16] Engel 2006, 212.

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Figure legends

Plate no. I. 1. Attempt to reconstruct the earth fortification after Karczag & Szabó (2010). with framing layout of churches from Tauț after Mărginean & Rusu (2010).

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Plate no. II. 1. Distribution of perimortem lesions (black) and antemortem (blue) on the cranium in males.

Plate no. II. 2. Location of antemortem lesion on a female cranium.

Plate no. II. 3. The skull of amale individual (C 28) which presents on the frontal bone two completely healed injuries.

Plate no. II. 4. Blunt-force depressed fracture of frontal bone in mature male skeleton (C 23).

Plate no. II. 5. Sharp force trauma to the femur (F 02).

Plate no. II. 6. Sharp force trauma to the tibia (T 12).

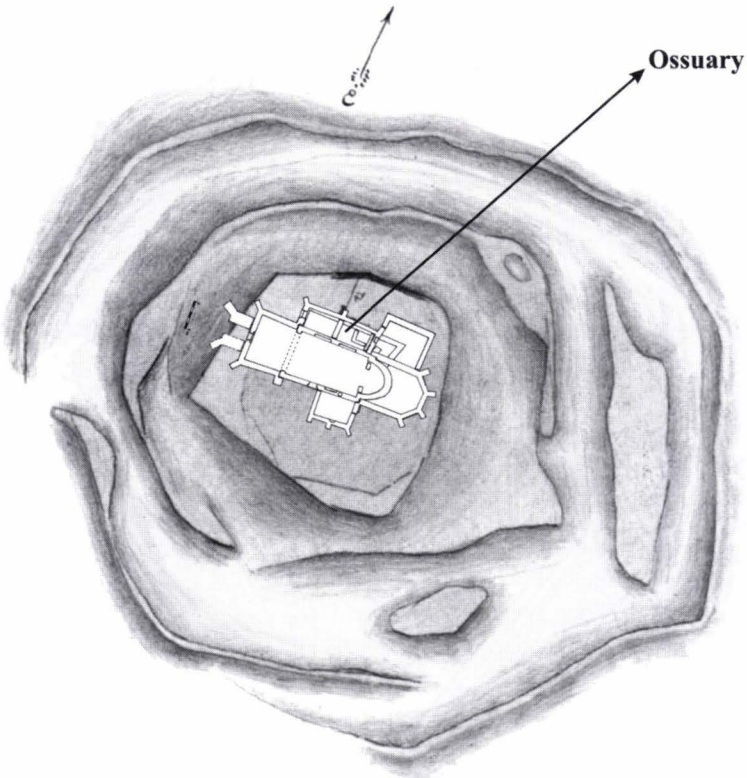

Figure 1

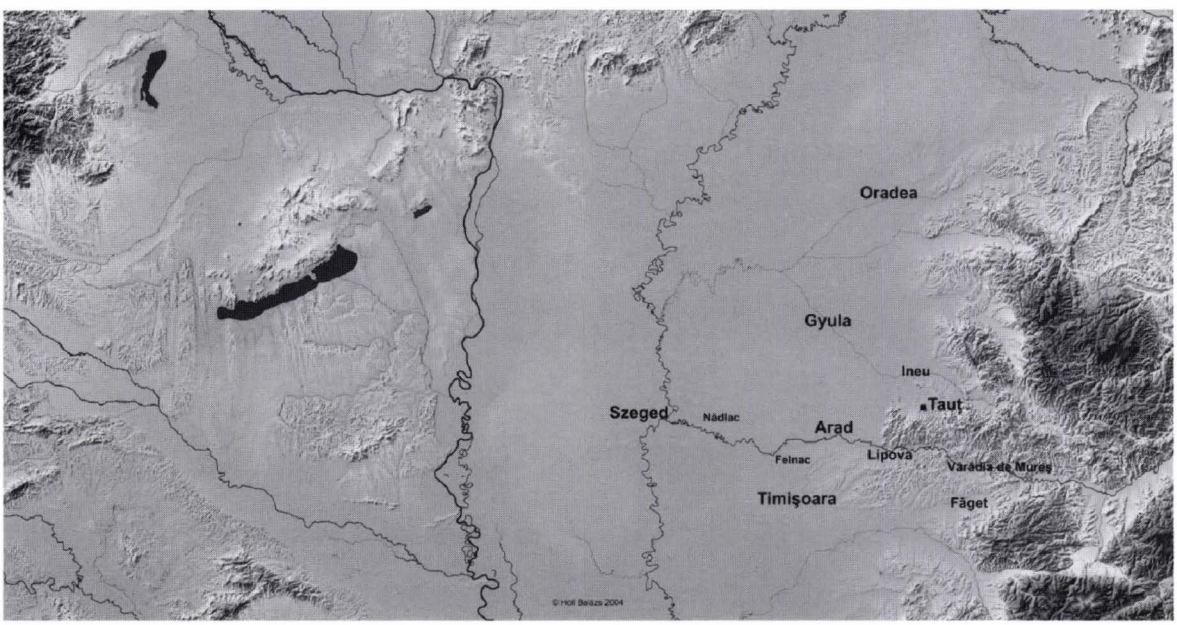

Figure 2

Plate no. I.

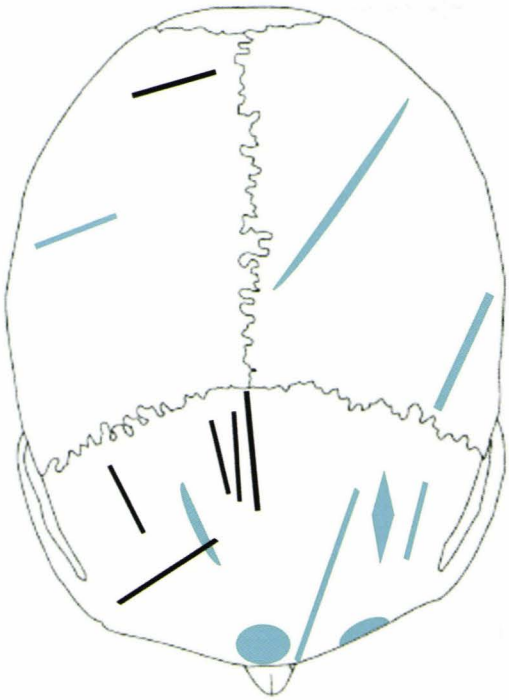

Figure 1

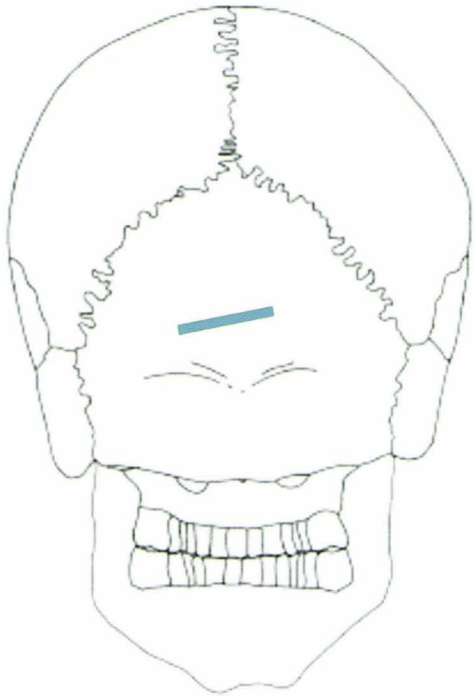

Figure 2

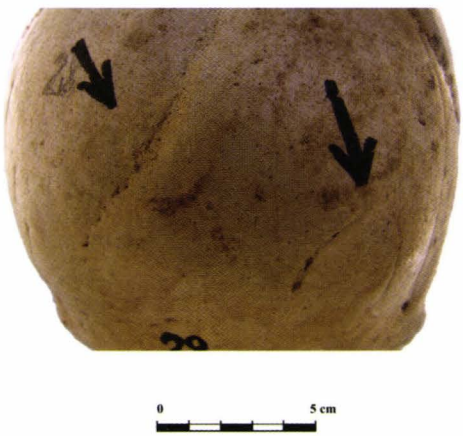

Figure 3

Figure 4

Figure 5

Figure 6

Plate no. II.

POSSIBLE SIGNS OF RITUAL HEALING OBSERVED IN THE 7–8TH C. AD AVAR AGE SITE OF CSÁSZÁRSZÁLLÁS-HANZÉLY TANYA (MRT 10. 385. 4/21. LH.)

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Abstract

During a rescue excavation in 1998, Pál Medgyesi unearthed a burial site consisting of 19 graves near Csárdaszállás (Békés County, southeast Hungary). 15 graves were dated to the turn of the 7–8th century AD, 3 to the 10–11th century, and 1 remained undated. In the following study, we report data of the remains of 17 individuals that were investigated (13 from the Avar period, 3 from the 10–11th century, 1 undated). During the collection and analyses of the data, standard macromorphological methods of historical anthropology were used. The data of the 7–8th century remains are well in accordance with the formerly collected knowledge on morphology, metrical characteristics, and paleopathological features in the era. Among the pathological cases, only a few stand out. The remains of several individuals (gr. no. 10 infant, gr. no. 12 adult male, gr. no. 14 adult female) show signs of probable infectious and other pathological conditions. The remains of an adult female from grave no. 15 exhibit infectious symptoms and symbolic trephination of the cranial vault, an intentional cranial modification that was probably performed as an attempt of ritual healing of the individual. In this study we shortly report the results of the osteological analysis and discuss the use of cranial intervention in the particular case.

I. Introduction

Pál Medgyesi led a rescue excavation in 1998 near Csárdaszállás (Békés County, southeast Hungary) where Júlia Kovalovszki in 1958 and Júlia Szénászky in 1988 had already excavated several graves from different archaeological periods.[1] During his excavation in 1998, Medgyesi unearthed a burial site consisting of 19 graves. 15 graves were dated to the turn of the 7–8th century AD, three to the 10–11th century, and one remained undated. The remains of 17 individuals were investigated (13 from the Avar period, 3 from the 10–11th century, 1 undated). During the collection and analyses of the data, standard macromorphological methods of historical anthropology were used.[2] In this study we shortly report the results of the osteological analysis, and discuss the findings.

[1] Kovalovszki 1959; Medgyesi oral communication.

[2] Vallois 1937; Schour–Massler 1941; Schinz et al. 1952; Martin–Saller 1957; Nemeskéri et al. 1960B; Olivier 1960; Éry et al. 1963; Alekszejev–Debec 1964; Rösing 1977; Szilvássy 1977; Stloukal–Hanáková 1978; Iscan et al. 1984; Lovejoy et al. 1985; Knussmann 1988, Ubelaker 1989, Ortner 2003; Aufderheide–Rodríguez-Martín 1998.

II. Results

The examined 7–8th c. AD graves contained 5 males, 5 females and 3 infants, while the 10–11th c. AD burials contained the remains of 1 male and 2 females. Preservation of the individual remains varied greatly, only a few cases were available for thorough morphological and metrical analysis. Because of the small sample size statistical analysis was not performed. The metrical data of crania can be found in Table 1, the metrical data of postcranial skeletons is summarized in Table 2.

Grave no. 1: 10–11th century, adult female. Both the skull and the postcranial elements are poorly preserved, damaged, fragmented and incomplete. The bones are small in size, the skeleton is gracile. The surface of the midshafts of tibias shows striation.

Grave no. 3: 10–11th century, adult female. The gracile skeleton is badly preserved, the bones are small. The skull was not present at the examination. Among the remains of the adult, a fragment of a subadult right femur was also found.

Grave no. 4: 7–8th century, male over 70 years of age. Poorly preserved, fragmented, eroded remains of a skull and a postcranial skeleton. The skull is *post mortem* deformed, also showing typical senile alterations (Pacchionian impressions, blood vessel impressions). Most of the dentition was lost *ante mortem*, the alveoli are remodelled, and the remaining teeth show carious lesions and heavy abrasion. A 15 × 8 × 4 mm depression of unknown (supposedly traumatic) origin is seen in the frontal bone along the lateral rim of the orbit.

Grave no. 6: 7–8th century, 4.5–5.5 years old child. Moderately preserved skull and postcranial elements. No considerable alteration was observed.

Grave no. 7: 7–8th century, 25–30 years old female. Both the skull and the postcranial elements are moderately preserved. An *os apicis* and several Wormian bones are observed in the lambdoid suture. The neurocranium is short (*brachykran*) and very high (*hyperhypsikran*). The endocranial surface is showing Pacchionian impressions. Slight calculus formation is apparent on the teeth. The lower right canine is showing slight signs of linear enamel hypoplasia, a physiological stress indicator. The tibias and the femurs are covered in light periosteal appositions of possible inflammatory origin. Some fragments of lumbar vertebras exhibit Schmorl's nodes.

Grave no. 8: 7–8th century, 9–11 years old child. Fragmented skull and postcranial elements are poorly preserved. The upper left lateral incisor carries an enlarged palatal cusp.

Grave no. 9: 7–8th century, 40–60 years old female. The skull is baldy preserved and fragmented, the postcranial elements were not present during the examination. The endocranial surface exhibits Pacchionian impressions and deep blood vessel impressions, which are probably age-related.

Grave no. 10: 7–8th century, 9–11 years old child. Both the skull and the postcranial elements are moderately preserved. The upper permanent incisors are shovel-shaped, the skull shows some other mongoloid morphological features too. The lambdoid suture contains several Wormian bones. In the mediodorsal corners of the parietals, 5 × 3 cm wide patches of porotic new bone structures called *cribra cranii* are observed. An alteration of similar appearance, *cribra orbitalia* is visible in the left orbit. The endocranial surface shows Pacchionian impressions, probably because of increased intracranial pressure. The femurs and tibias exhibit signs of superficial *periostitis*. The alterations observed throughout the skull and the postcranial elements may refer to some kind of infectious disease or nutritional deficiency. The *fossa olecrani* is fenestrated.

Grave no. 11: 7–8th century, 22–30 years old male. Both the skull and the postcranials are moderately preserved; the skull is *post mortem* deformed showing mongoloid morphological features (shovel-shaped incisors, filled *fossa canina*). Pacchionian impressions are observed in the

endocranial surface. Incisors of the mandible carry some tartar. The right tibia exhibits signs of a well-aligned healed fracture. This lesion may be in connection with the *myositis ossificans traumatica* observed on the lower part of the right *linea musculi soleii*, and the 5 mm wide opening seen on the right *tuberositas tibiae*. These signs may be related to abnormal work load having affected the wounded limb. Minor signs of *discus hernia* are observed on the T5, T6 and T11. Thoracic and lumbar vertebras are slightly hypervascularised.

Grave no. 12: 7–8th century, 25–35 years old male. Skull and postcranial elements are moderately preserved. The neurocranium is short (*brachykran*) and very high (*hyperhypsikran, hyperakrokran*). The endocranial surface of the skull is showing Pacchionian impressions and deep blood channels. Both dental arches carry signs of calculus formation. An upper right rib is fractured while the sternal end of the right clavicle implies cartilage anomaly. The femurs and tibias show very light striation. Vertebral bodies are deformed, all their surfaces are wavy and irregular including the end-plates, and the anterior surfaces exhibit fibrous new bone structures. Edges of the vertebral bodies carry osteophytes. T9, T10 and T11 vertebras are connected through a continuous, 1–1.5 cm wide cavernous system (Fig. 1A and 1B). This tube system starts in the upper end-plate of T9, completely penetrates T9 and T10 (Fig. 2), and ends inside T11. It also has approx. 5 mm wide openings on the sides of T10 and T11. T6 and T7 are fused as a block vertebra. These vertebral changes may refer to *osteomyelitis* of infectious origin (TB, *brucellosis*, pyogenic bacteria etc.). Signs of severe *discus hernia* are apparent in the lower left corner of the L5 body at the connection of the vertebral arch.

Grave no. 13: 7–8th century, 19–22 years old male. Fragmented skull and postcranial elements. The *post mortem* deformed skull shows mongoloid morphological features (shovel-shaped incisors, shape of the mandible). The upper incisors carry palatal tubercles. Deep vessel impressions are observed in the endocranial surface. In the anterior surface of the right humeral midshaft, 23 × 8 × 2–3 mm wide elongated irregular depressions is seen probably referring to excessive work-load put to the particular limb. The femurs show slight striation.

Grave no. 14: 7–8th century, 25–30 years old female. Both the skull and the postcranial are fragmented. The *post mortem* deformed skull shows some mongoloid morphological features (shallow *fossa canina*, slight *torus mandibularis*). A considerable part of the dentition has been lost *ante mortem* which is not common for this age at death. The remaining teeth exhibit a lot of carious lesions and some calculus formation. There are 3 abscesses present in the jaws. On the upper right incisors, signs of linear enamel hypoplasia are present. In the dorsal part of the right maxillary sinus, fine periosteal new bone formation is observed referring to *sinusitis*. *Cribra cranii* is present in the mediodorsal corners of the parietals. Entheseal changes are visible in the patellas and calcaneuses possibly in connection excessive work load. The visceral surfaces of ribs show slight layered new bone structures. Vertebras of the lower thoracic and the lumbar region exhibit osteophytes and Schmorl's nodes.

Grave no. 15: 7–8th century, 50–60 years old female. Skull and postcranial elements are poorly preserved. The neurocranium is medium long (*mesokran*) and medium high (*orthokran, metriokran*). The dentition is slightly worn and shows calculus formation. In the anterior part of the right parietal a 17 × 22 mm wide, long-healed symbolic trephination is present, carrying a couple of blood vessel pores in the externals surface (Fig. 3). The endocranial surface shows several different inflammatory and age-related changes, especially under the symbolic trephination and along branches of the *arteria meningea media* (deep, submerging blood vessel impressions, sclerotized surfaces, abnormal radiating vessels along the sagittal sinus) (Fig. 4). Age alone cannot be accounted for all these changes; they must be of infectious origin to some extent. Infectious origin is further supported by the presence of porous new bone deposits in the nasal cavity referring to *rhinitis*. The right *sulcus nervi radialis* and the right *tuberositas deltoidea shows* some *periostitis*.

The epiphysis of the left *acromion* connects through a pseudoarthrosis surrounded with mild inflammation signs.

Grave no. 16: 7–8th century, male over 60 years of age. Both the skull and the postcranials are fragmented. The remains have been united with bones labelled as *grave no. 2*, since they anatomically complement each other representing the same individual. The endocranial surface shows blood vessel impressions and Paccionian marks. The 1st and 2nd metatarsophalangeal joints of the right hand exhibit signs of inflammation. The articular processes of the lumbar vertebras also show signs of inflammation with pores and deformed surfaces. The lumbar vertebral bodies carry minor osteophytes. The entheses are well expressed; the patellas carry comb-like new bone structures.

Grave no. 17: undated, 20–40 years old male. The remains have been found in a crouched position.[3] Both the skull and the postcranials are fragmented. The bones are big and exhibit well expressed entheses.

Grave no. 18: 7–8th century, 50–60 years old female. Skull and postcranial elements are poorly preserved. The skull shows a few mongoloid features, signs of senile *osteoporosis* are also present. The neurocranium is very short (*hyperbrachykran*), high (*hypsikran*), and medium high (*metriokran*). Pacchionian impressions are observed in the endocranial surface. Several teeth contain carious cavities, and there is a pyogenic abscess at the tip of the root of the lower left canine. Calculus formation is very mild. The left sacroiliac joint is partly fused, while the right shows complete closure. The external surface of the right *ala ossis ilii* exhibits rough irregular bone deposits. The femurs and tibias are striated. The vertebras are porotic, the thoracic and the lumbar regions carry osteophytes.

Grave no. 19: 10–11th century, 30–34 years old male. Fragmentary and incomplete skeleton, the skull was not present at the examination. Considerable lesions were not found.

III. Discussion

The 7–8th century remains are well in accordance with formerly known picture of Avar Age bioarchaeology, also in their morphological, metrical and pathological characteristics. Mongoloid features appear very frequently in this era. The joint lesions and the stress indicators more or less match the picture expected for age-at-death of the particular individuals. The community might have led a strenuous lifestyle, where signs of physically stressful life were common symptoms.

There are only a couple of pathological features that stand out from this picture. The infectious (or metabolic) disease of the child in grave no. 10, the severe *discus hernia* and chronic infectious vertebral *osteomyelitis* of the male buried in grave no. 12, the infectious symptoms of the female in grave no 14, and the infectious features occurring along with a symbolic trephination in the female from grave no. 15 are worth mentioning. These cases found in a relatively small sample imply very strong exposure and susceptibility to infectious agents in this community.

Osteomyeilitis of the vertebras is not uncommon in different infectious diseases caused by e.g. *Mycobacteria*, *Brucella*, pyogenic bacteria etc.[4] Atypical pathological features of the individual in grave no. 12, however do not facilitate accurate diagnosis of the infectious agent, and further a DNA or other biomarker tests are needed.

Grave no. 15 is especially important. Signs of systemic infection are not commonly found (or rather, postcranial data in general are not reported and published) in the context of symbolic trephinations. The cranial alterations in this particular case may have been accompanied by headaches, causing extra suffering for the individual. Both the endocranial changes and the symbolic

[3] Medgyesi, oral communication.

[4] Aufderheide–Rodríguez-Martín 1998; Ortner 2003.

trephination seem to have been present since at least several months prior to death, hence it is not groundless to assume the trephination was applied as a ritual healing technique.

Symbolic (or incomplete) trephination is a special form of trephination, where only the external layer of the cranial vault of living subjects was removed for ritual reasons.[5] Mainly adults were affected, two third of them being males. The lesions were found on the top of the head. The 10–20 mm wide superficial carvings were usually round or oval. These artificial wounds may have been intended to provide unearthly communication channels to certain adult members of the community who needed such a connection either for spiritual or medical reasons.[6] However, the exact cause of symbolic trephination is still unknown, but curing headaches is often mentioned in the literature as a possible aim of intervention. Symbolic trephination is very common among 9–11th century pagan Hungarians in the Carpathian Basin, but it also occurs sporadically in other ethnic groups and areas including the Avars. Our current knowledge is not sufficient to decide whether the two ethnic groups transferred this custom between each other in the Carpathian Basin (or somewhere else) or acquired it from a third source (or several sources). With the ongoing research cranial modification techniques we will have to pay more attention these rare and unique cases like Csárdaszállás gr. no. 12 or Pusztapáka-Nádorhalom gr. no. 90[7] where pathological signs are in an actual favour for interpreting symbolic trephinations as healing methods.

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[6] Nemeskéri et al. 1960A, 1965; Grynaeus 1996; Fodor 2003, Bereczki 2013; Bereczki et al. 2015.

[7] Molnár et al. 2014.

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Figure legends

Fig. 1. Csárdaszállás-Hanzély-tanya, grave no. 12, 25–35 years old male. A: Deformed thoracic vertebrae no. 9, 10 and 11 with one of the openings of the osteomyelitic cavernous system; B: X-ray image of the deformed vertebrae with the cavernous system in the middle of the vertebral bodies showing sclerotized walls.

Fig. 2: The irregular, sclerotized surface of thoracic vertebra no. 10 at the middle section of the cavernous system.

Fig. 3: Long-healed symbolic trephination on the right parietal of a 50–60 years old female from grave no. 15.

Fig. 4: Endocranial changes in the skull of a 50–60 years old female from grave no. 15.

Table 1. Metrical data of the skulls from Csárdaszállás-Hanzély-tanya.

Table 2. Metrical data of the postcranial skeletons from Csárdaszállás-Hanzély-tanya.

Figure 1a

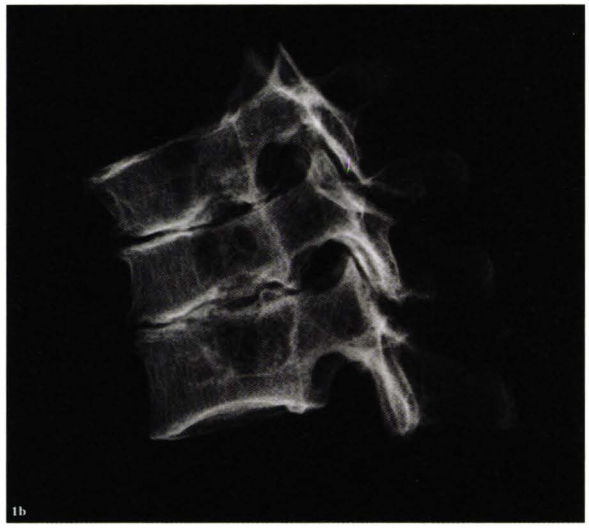

Figure 1b

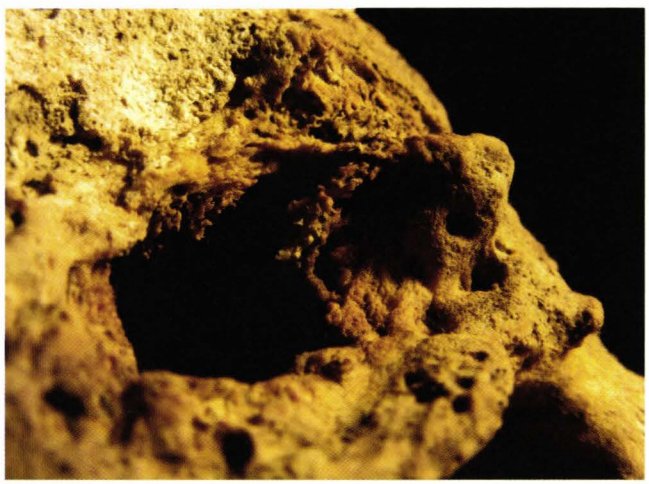

Figure 2

Figure 3

Figure 4

Plate no. I.

Table 1: Cranial data

measurement	males				females			
(mm) or index	#4	#11	#12	#13	#7	#14	#15	#18
1	-	-	165	-	171	-	174	171
5	-	-	-	-	-	-	-	101
8	-	-	135	-	150	-	139	145
9	95	-	90	97	96	-	103	99
17	-	-	-	-	-	-	-	131
20	-	-	112	-	120	-	113	118
40	-	-	-	-	-	-	-	-
45	-	-	128	-	-	-	-	-
46	-	-	91	103	-	-	-	-
47	-	-	121	-	-	-	-	-
48	-	-	69	-	-	-	-	-
51 left	-	-	37	-	-	-	37	-
51 right	-	-	38	-		-	-	-
52 left	-	-	31	-	-	-	32	-
52 right	-	-	-	-	-	-	-	-
54	-	-	-	-	-	-	-	-
55	-	-	48	-	-	-	-	-
62	-	-	-	-	-	-	-	-
63	-	-	-	38	-	-	-	-
65	-	135	112	-	-	126	-	122
66	104	95	96	106	-	-	91	92
69	-	36	36	37	34	32	28	29
70 left	-	69	59	61	-	-	-	59
70 right	-	-	62	60	-	60	58	58
71 left	33	34	31	33	-	30	29	34
71 right	35	35	30	34	32	-	28	34
8:1	-	-	81.81818	-	87.7193	-	79.88506	84.79532
17:1	-	-	-	-	-	-	-	76.60819
17:8	-	-	-	-	-	-	-	90.34483
20:1	-	-	67.87879	-	70.17544	-	64.94253	69.00585
20:8	-	-	82.96296	-	80	-	81.29496	81.37931
9:8	-	-	66.66667	-	64	-	74.10072	68.27586
47:45	-	-	94.53125	-	-	-	-	-
48:45	-	-	53.90625	-	-	-	-	-
52:51	-	-	83.78378	-	-	-	86.48649	-
54:55	-	-	-	-	-	-	-	-
63:62	-	-	-	-	-	-	-	-

Table 2: Postcranial data

measurement	males					females					subadults	
(mm)	#11	#12	#13	#16	#19*	#7	#14	#15	#18	#3*	#6	#10
hum1 right	339	312	-	-	-	-	-	295	296	-	-	-
hum1 left	338	309	-	-	-	-	285	288	298	-	-	238
hum2 right	334	307	-	-	-	-	-	-	292	-	-	-
hum2 left	334	306	-	-	-	-	284	287	294	-	-	-
rad1 right	245	238	-	-	288	-	-	-	226	-	-	-
rad1 left	245	-	-	-	-	-	224	224	-	-	-	-
rad1b right	244	236	-	-	-	-	-	-	223	-	-	-
rad1b left	243	-	-	-	-	-	-	223	-	-	-	-
ulna right	269	-	-	-	-	-	-	-	-	-	-	-
ulna left	265	258	-	-	-	-	-	-	-	-	-	-
fem1 right	474	422	473	-	-	436	411	-	402	-	211	331
fem1 left	470	425	472	-	523	433	421	408	404	326	212	335
fem2 right	470	422	472	-	-	430	408	-	399	-	-	-
fem2 left	455	422	469	-	520	427	418	405	403	321	-	-
tib1 right	383	-	-	371	-	348	337	-	-	-	-	280
tib1 left	373	-	-	375	-	347	340	329	331	-	174	275
tib1b right	383	-	-	365	-	346	336	-	-	-	-	-
tib1b left	373	-	-	370	-	347	340	-	321	-	-	-
fib right	384	-	-	374	-	-	-	-	-	-	-	-
fib left	378	-	-	-	-	-	333	-	335	-	-	-

*Non-Avar skeletons

CAN MICRO-CT AND 3D IMAGING ALLOW DIFFERENTIATING THE MAIN AETOLOGIES OF ENTHESEAL CHANGES?

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Keywords: Paleopathology; entheseal changes; 3D imaging; activity markers; radial tuberosity.

Abstract

Entheseal changes (EC), alterations at insertion sites on the bones, may be related to various causes, including mechanical stress and metabolic disorders such as Forestier's disease (DISH). This preliminary study aims to explore the osseous microarchitecture of the radial tuberosity to identify ECon the basis of their probable aetiology.

We relied on radii belonging to three male adults: (i) one from the Hungarian cemetery of Sárrétudvari-Hízóföld, probably a mounted archer from the Conquest period (Xth century), exhibiting EC; (ii) one from the Hungarian cemetery of Bácsalmás-Homokbánya (XVI–XVIIth centuries), showing EC associated with a DISH condition; (iii) one from the medieval cemetery of Val-de-Reuil (France), with a normal condition and aspect at the entheses. Bicipital tuberosities were micro-CT scanned (15–17µm) and several portions were analysed in order to reconstruct in 3D the canals of the cortical bone.

Differences were observed in their osseous microarchitectural organisation. In particular, canals were preferentially oriented in "mechanical" EC, while an irregular widening and a higher density characterised "metabolic" EC.

Until further analyses will be performed, the results of this study point towards a possible distinction between different aetiologies of EC, which might represent a valuable contribution to the research on lifestyles and activities in past populations.

Introduction

Entheses are the insertion sites of tendons, ligaments and joint capsules on the bone. A distinction can be made between two types of entheses. Fibrous entheses are mainly encountered at the metaphyseal or diaphyseal areas while fibrocartilaginous entheses include the insertions at the

epiphyses and processes of long bones as well as the short bones of hands and feet and several ligaments in the spine.[1]

Entheseal changes (EC) are pathological or non-pathological modifications at the insertion sites.[2] They result in bone alterations visually observable on dry bone. It can take the form of mineralised tissue formation (e.g. irregular surface) and bone formation (raised margin, enthesophyte etc.) as well as surface discontinuity such as fine and macro-porosity, cortical defect, erosive areas, cavitations etc.[3]

EC can be related to age and sex or they can also result from various causes like metabolic or inflammatory disorders, macro-traumas, as well as mechanical stress.[4] Indeed, one of the fundamental roles of an enthesis is stress dissipation, distributing load forces across the bone.[5] Therefore, EC have been considered for decades as occupational stress markers, with the perspective of reconstructing activities and lifestyles of ancient populations.[6] Nevertheless, before the interpretation of EC in terms of possible activities, one might wonder how we can distinguish "mechanical" EC from changes related to other causes. From a direct observation of the changes on the bone, suggesting any specific cause for the observed EC seems to be problematic, especially if there is a lack of information regarding the context and/or the rest of the skeleton.[7]

Entheses and their changes have been extensively studied, with clinical, radiological, histological and osteological methods at the macroscopic and microscopic scales.[8] Recently, the 3D approach has begun to be used as a tool for studying EC, but these researches focus mostly on entheseal surfaces.[9] We chose here the complementary use of micro-tomodensitometry investigation and 3D imaging, which have been little applied to occupational markers so far.[10] In 2015, we already conducted a preliminary exploration of the radial tuberosity of two Neolithic individuals from Mali, in order to perform a first test of the methodology and primary observations of the entheseal microarchitecture.[11] The promising results encouraged us to continue on this path for further studies.

Our aim here is to explore the microarchitecture of the radial tuberosity, in order to identify possible features distinguishing EC on the base of their supposed aetiology. For this exploratory research, we chose to compare EC presumably related to the repeated movement involved in archery, with EC probably caused by a metabolic disorder. Both cases were also compared to the normal aspect of the insertion site. The potential distinction between "mechanical" EC and changes related to other aetiologies would allow us to gain insights into the research on lifestyles and activities in ancient populations.

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[1] Benjamin and Ralphs 1998; Benjamin and McGonagle 2001.

[2] La Cava 1959; Niepel - Sit'aj 1979; Lagier 1991; Benjamin et al. 2002.

[3] Hawkey - Merbs 1995; Robb 1998; Mariotti et al. 2004; Villotte 2009; Villotte et al. 2016.

[4] Dutour 1992; Claudepierre - Voisin 2005; Slobodin et al. 2007; Villotte - Kacki 2009; Jurmain - Villotte 2010; Paja et al. 2010; Milella et al. 2012; Alves Cardoso - Henderson 2013; Henderson - Alves Cardoso 2013; Niinimäki - Baiges Sotos 2013; Villotte - Knüsel 2013; Santana Cabrera et al. 2015; Djukic 2016; Michopoulou et al. 2016.

[5] Benjamin and McGonagle 2001.

[6] Dutour 1986; Hawkey - Merbs 1995; Pálfi 1997; Peterson 1998; Molnar 2006; Villotte et al. 2010b; Baker et al. 2012; Henderson et al. 2016a.

[7] Salmi - Niinimäki 2016.

[8] Cooper - Misol 1970; Resnick - Niwayama 1983; Olivieri et al. 1998; Benjamin et al. 2002; Claudepierre - Voisin 2005; Maffulli et al. 2005; Villotte 2009; Junno et al. 2011; Schlecht 2012; Henderson 2013a; Henderson et al. 2016b; Miszkiewicz - Mahoney 2016.

[9] Pany et al. 2009; Henderson 2013b; Noldner - Edgar 2013; Nolte - Wilczak 2013; Karakostis - Lorenzo 2016.

[10] Djukic et al. 2015; Djukic 2016; Mulder et al. 2016.

[11] Berthon et al. 2015a.

Materials

We focused here on *tuberositas radii*, or the bicipital tuberosity of the radius, which is the insertion site of *biceps brachii*, and one of the fibrocartilaginous entheses.[12] These are the most documented group of entheses in the attempt to reconstruct past activities.[13] *Biceps brachii* is one of the flexor and supinator muscles of the elbow, and changes at *tuberositas radii* were previously interpreted to be linked with occupation.[14] Agricultural and building activities, especially carrying heavy loads, have proved to be a potential cause for EC at this enthesis.[15] Thomas[16] investigated the frequency of entheseal changes in a Neolithic population from the Cerny culture (Paris Basin, France). Among 36 identified adult males, 13 were buried in association with arrowheads. Her results reveal a higher frequency of EC at several insertion sites, with a significant difference for the radial tuberosity in particular, among the group of individuals buried with arrowheads. This is one of the studies suggesting archery as an activity prone to lead to EC at this specific insertion site.

We relied on three pairs of radii, belonging to three male adults (Fig. 1).

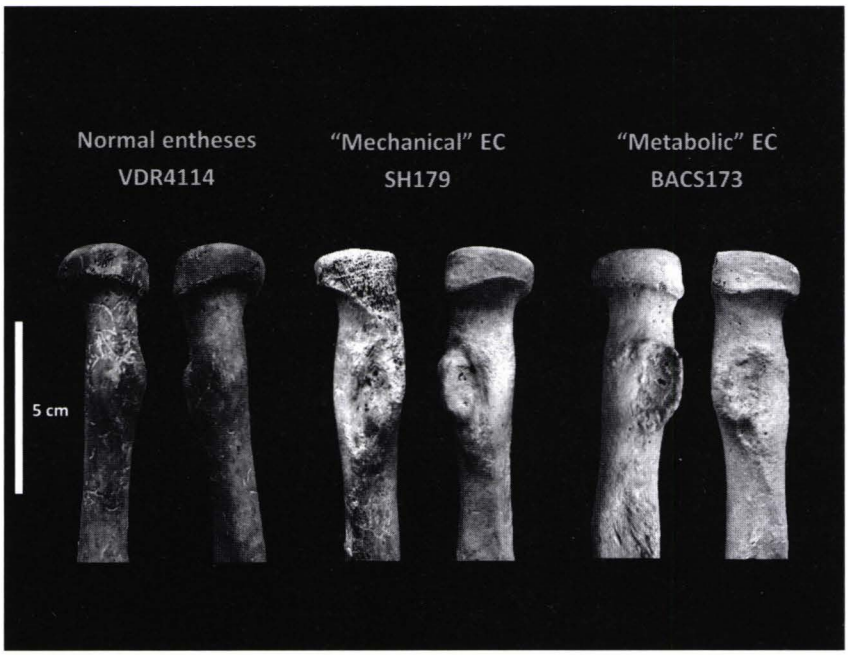

Figure 1.

1) The first individual is presumed to represent the normal aspect of the enthesis. He comes from the Merovingian-Carolingian (VII–Xth Centuries AD) cemetery of Val-de-Reuil "Le Chemin aux Errants", in Normandie, France. The excavation was led by the French National Institute for Preventive Archaeology (INRAP), under the supervision of Yves-Marie Adrian, in 2012. A total of 230 burials were excavated and studied.[17] No evidence for warfare context had been uncovered. The examination of the skeletal remains of the selected individual (VDR4114), a 20–50 years old male,

[12] Benjamin et al. 1986.

[13] Havelková – Villotte 2007; Villotte 2009; Villotte et al. 2010a; Henderson et al. 2013; Villotte – Knüsel 2013; Thomas 2014; Weiss 2015; Henderson et al. 2016c.

[14] Dutour 1986; Hawkey and Merbs 1995; Pálfi 1997; Robb 1998; Molnar 2006; Weiss 2007; Baker et al. 2012; Thomas 2014; Tihanyi et al. 2015.

[15] Commandré 1977; Galera – Garralda 1993; Al-Oumaoui et al. 2004; Havelková et al. 2011; Rojas-Sepúlveda – Dutour 2014.

[16] Thomas 2014.

[17] Beurion 2009; Berthon et al. 2015b.

did not suggest any particular pathological condition or stress prone to lead to bias in the entheseal changes analysis.

Both radial tuberosities showed smooth-rounded contour and smooth and regular surface, despite slight taphonomic alterations likely due to roots in the soil.

2) The second individual is presumed to show activity-related EC at bicipital tuberosities. This mature male comes from the Hungarian Conquest period (Xth Century AD) cemetery of Sárrétudvari Hízóföld. The Hungarian Conquerors of the Xth Century were, according to historical and archaeological data, a population of mounted archers. 263 individuals from that period were excavated under the supervision of Ibolya M. Nepper, between 1983 and 1985, and 58 graves contained weapons, mostly related to archery.[18] This is the case for the selected individual (SH179), with arrowheads and bow elements discovered in association with the skeleton.

The two radiuses of this "presumed archer" exhibited raised margins, bone formation, macro-porosity and fine porosity as well as erosive areas at bicipital tuberosities.

3) The third and last individual has been selected to represent EC probably related to a metabolic condition. This mature male comes from the Hungarian Late Middle Ages – Early Modern time's cemetery (XVI–XVIIth Centuries AD) of Bácsalmás-Homokbánya. This cemetery was excavated in several phases between 1993 and 2003, by Erika Wicker, Zoltán Polgár and László Pintér, and 481 skeletons were unearthed. The archaeological and historical data suggest the presence of a population of farmers, with no evidence for warfare context.[19]

The selected individual (BACS173) was affected by diffuse idiopathic skeletal hyperostosis (DISH) or Forestier's disease. This metabolic disorder is particularly characterised by the calcification and ossification of soft tissues, including ligaments and entheses.[20] The main diagnostic criteria that were observed on the skeleton for this metabolic disorder are: ossification of the right side anterior longitudinal ligament from T2 to L5 (complete and non-complete fusion), with a "candle wax" appearance; normal intervertebral disc spaces; entheseal changes at radii, claviculae, patellae, calcanei or ilii; ossification of rib cartilage and sternocostal ligaments.[21]

The bicipital tuberosities, in particular, were characterised by raised and irregular margins, bone formation, irregular surface and macro-porosity.

Methods

All 6 radii were micro-CT scanned in order to investigate bone microarchitecture of the entheses. Micro-tomodensitometry provides, in a non-destructive way, an insight into the biomechanical properties of bone and the characteristics of bone remodelling through a three-dimensional approach.[22] We applied the micro-computed tomography (micro-CT) acquisitions processing chain[23] developed in research unit PACEA (UMR 5199, CNRS/University of Bordeaux, Pessac, France), including image processing with TIVMI® (Treatment and Increased Vision for Medical Imaging) software. It is based on the HMH (Half Maximum Height) 3D algorithm, allowing the software to automatically identify the optimal limits between each material such as bone and air.[24] The radii were CT scanned at PLACAMAT (UMS 3626, CNRS/University of Bordeaux), Pessac, France, on a GE® Phoenix v|tome|x s, with an isotropic resolution between 15.7 and 17.8 μm. We

[18] Nepper 2002; Tihanyi et al. 2015.

[19] Lovász et al. 2013.

[20] Resnick – Niwayama 1976; Waldron 2009; Holgate – Steyn 2016.

[21] Paja et al. 2010; Paja 2012.

[22] Lespesailles et al. 2006; Coqueugniot et al. 2010; Colombo 2014; Rittemard et al. 2014; Khoury et al. 2015.

[23] Coqueugniot et al. 2011.

[24] Spoor et al. 1993; Dutailly et al. 2009.

focused the acquisitions on the enthesis area. The micro-CTs were operated at 120 kV and 110 µA, with a 500 ms integration time per projection. The data, which are slices in the three plans of space, were then treated with TIVMI® software to obtain 3D reconstructions from their superposition.

Several preliminary steps were required in order to analyse the microarchitecture of the entheses. We realised a primary 3D reconstruction of the whole entheses to globally visualise the entheseal surface for selecting regions of interest (ROIs), on which observations were then performed. In each radius, three portions localised at different height levels (25, 50 and 75%) of the enthesis were selected (Fig. 2). The total height was visually estimated regarding the superior and inferior portions of the margin and considered in terms of number of horizontal slices between these limits. Each bounding box created in this way was 4 mm high, with the medium slice exactly located at the level of interest. The length and width of the boxes depended on the morphology of the bone itself. In general, we selected the ROIs on the medial half of the tuberosity, where biceps brachii's tendon does attach to the bone. We also ensured that these ROIs were long enough to catch a portion on the outside of the entheses, in order to investigate the transition between normal diaphyseal bone (on the medial-posterior face of the bone) and the entheseal area.

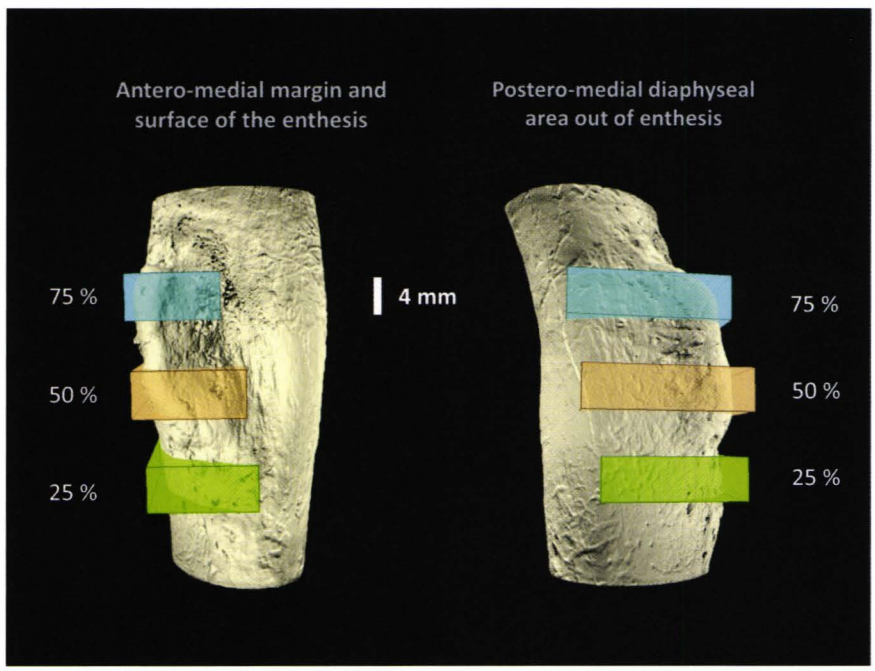

Figure 2.

We then operated segmentation according to the grey level values of each component. It consists of the definition of subsets or materials, in order to make the software able to distinguish bone from empty canals and medullary cavities, external vacuum and sedimentary residues such as sand. Subsequently, a binary image was obtained using a double threshold. It consists of white pixels (the elements we want to keep in the 3D reconstruction) and black pixels (the elements to exclude). Finally, using a HMH algorithm, binary slices were superposed to reconstruct the canal system of the cortical bone, to observe its three-dimensional organisation. This methodology, using micro-CT and 3D reconstructions with TIVMI® software program, has already been performed in a research focusing on trabecular bone microarchitecture during growth, with good repeatability.[25]

[25] Colombo 2014.

Results

From the first observations of the final 3D reconstructions, it appeared that the most relevant level of interest for comparisons between the 3 groups was 50% of the enthesis height. At this medium region of the enthesis indeed, the organisation of the canals of the cortical bone seemed to be less influenced by the morphology of the enthesis itself. At the upper and lower areas, a specific orientation of canals, for example, might be problematic to interpret, because they are transitional locations between a normal flat diaphysis and the most elevated part of the insertion site. Considering this, and in order to make the comparison easier, we decided to present here only the 6 reconstructions performed at this medium level (Fig. 3). The main observations in each case are summarised in Table 1.

The 3D reconstruction of the canal system of the cortical bone, as well as the medullary cavities, concerning a presumed normal enthesis had already been described with more details on a previous case.[26] The "normal" enthesis of this work exhibited a similar type of organisation regarding the canals of the cortical bone. On the medial-posterior face of the shaft, outside the enthesis, we observed a normal Haversian organisation, with thin and longitudinal Havers' canals and a few transversal Volkmann's canals. On the antero-medial margin of the enthesis, the canals, which were thicker, revealed a reticulated organisation with roughly oblique interconnections.

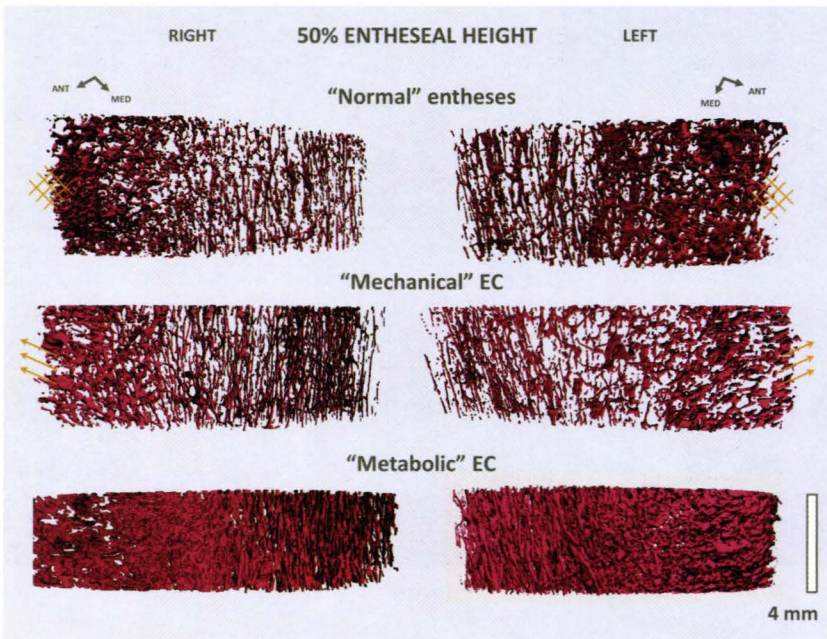

Figure 3.

In the case of the presumed activity-related EC, observed on a probable archer, the medial-posterior face of the diaphysis revealed the same longitudinal and thin organisation of canals. At the antero-medial margin, however, they appeared to be globally oriented toward the same anterior and proximal direction.

The third case, with a DISH condition, showed considerable differences from the two others. Even if the longitudinal organisation was preserved on the diaphysis, the canals were much larger. On the antero-medial face, the reticulation previously observed was not visible anymore. Instead, the organisation appeared to be very irregular, with wide canals present in a higher density.

[26] Berthon et al. 2015a.

	Medial-posterior diaphysis	Antero-medial entheseal margin
"Normal" entheses	Longitudinal; thin	Reticulated; rough
"Mechanical" entheses	Longitudinal; thin	Preferentially oriented
"Metabolic" entheses	Longitudinal; large	Loss of reticulation; irregular; wide; dense

Table 1. Summary of the cortical bone canal organization differences observed between the three groups of entheses

Discussion

Focusing on the organisation of the canal network of the cortical bone, this preliminary exploration of the microarchitecture of an enthesis already allows the identification of variations between the normal condition and EC seemingly related to different causes. We could observe a normal pattern, already identified in a previous work, with a Haversian organisation out of the enthesis and a variation at the entheseal margin. We can assume that the reticulation observed in this case may correspond to the structural adaptation of osteons to normal mechanical constraints, in accordance with Wolff's Law.[27] Concerning the probable archer individual, there is only a slight variation from the normal organisation, involving a bone remodelling with a preferential orientation of the canals. Could we consider this as the reflection of the adaptation to mechanical constraints involved in standardised gestures of specific activities like archery? In this exploratory work, and until further studies will be performed, we can at least support this hypothesis. The last case, with a metabolic disorder, is characterised by the loss of the osteonic organisation inside the entheseal area. This suggests that calcification at entheses resulting from DISH condition might be related to a primary ossification process.

Finally, our preliminary results confirm that the use of micro-CT and 3D imaging can surely enhance our understanding of entheseal changes and their formation. Is it possible to distinguish mechanical and metabolic-related EC? While it is premature to give a definitive answer to this question, we put forward the fact that the observations performed here are promising.

The next steps of this investigation will include larger samples, in order to multiply the observations. The sampling will take into account the numerous biases inherent in studies aiming to reconstruct activities in ancient populations.[28] A better comparative work requires more objective criteriae. We will perform a "skeletonisation" method on smaller regions of the 3D reconstructions. It consists in making an object thinner (1 voxel wide) to keep its basic structure. We obtain in this way a simplified modelling of the cortical microstructure allowing determining qualitative and quantitative parameters.[29] These parameters may be used then to quantify the microarchitecture of normal and changed conditions and to test the interindividual variability. Once the methodology will be well tested and validated, other susceptible aetiologies for EC, such as inflammatory diseases, could be investigated, and various entheses could be analysed. For instance, Djukic in 2016 has been interested in the possible identification of horse riding practice in medieval series, confronting macromorphological observations and micro CT analyses of different EC of the upper and lower limbs.

Besides the methodological aspect of this study, we wish also to build upon the knowledge on the population of Hungarian Conquerors of the Xth Century, in particular about their lifestyles and activities. Questions such as bilateral asymmetry or sex differentiation, analysed under this

[27] Frost 1994; Djukic 2016.

[28] Dutour 1992, 2000; Villotte 2009; Meyer et al. 2011; Jurmain et al. 2012; Milella et al. 2012; Alves Cardoso – Henderson 2013; Perréard Lopreno et al. 2013; Thomas 2014.

[29] Colombo 2014.

framework, might open up some interesting horizons for the research field on activities reconstruction but also regarding various cultural aspects among these tribes.

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List of journal abbreviations

Acta Biol Szeged:	Acta Biologica Szegediensis
Am J Phys Anthropol:	American Journal of Physical Anthropology
Angle Orthod:	The Angle Orthodontist
Anthropol Sci:	Anthropological Science
Bailliere's Clin Rheum:	Bailliere's Clinical Rheumatology
BMSAP:	Bulletins et Mémoires de la Société d'Anthropologie de Paris
Chungará (Arica):	Chungará: Revista de Antropología Chilena
Clin Anat:	Clinical Anatomy
Clin Rheum Dis:	Clinics in Rheumatic Diseases
Coll Anthropol:	Collegium Anthropologicum
Comp Biochem Physiol:	Comparative Biochemistry and Physiology – Part A: Molecular & Integrative Physiology
A Mol Integr Physiol	
Connect Tissue Res:	Connective Tissue Research
HOMO:	HOMO Journal of Comparative Human Biology
Int J Anthropol:	International Journal of Anthropology
Int J Osteoarchaeol:	International Journal of Osteoarchaeology
Int J Paleopathol:	International Journal of Paleopathology
J Anat:	Journal of Anatomy
J Bone Joint Surg Am:	Journal of Bone & Joint Surgery
J Hum Evol:	Journal of Human Evolution
Presse Med:	La Presse Médicale
Rev Rhum:	Revue du Rhumatisme
Semin Arthritis Rheum:	Seminars in Arthritis and Rheumatism

Figures

A HUN AGE BURIAL WITH ARTIFICIAL CRANIAL DEFORMATION FROM SÎNGEORGIU DE MUREŞ -'KEREKDOMB'

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[1]Mureş County Museum

Keywords: Hun Age, artificial cranial deformation, pathology, Sângeorgiu de Mureş.

Abstract

The aim of this publication is to present the results of the anthropological analysis of the only horse grave discovered in Transylvania (Transylvanian Basin). The osteological study resulted in/supplied new information concerning the communities from the 4th and 5th centuries, near/around the Mureş River. The study of funerary rite and ritual (orientation, shape of the grave, place of the skeleton in the pit, type and place of funerary offerings etc.), and the osteological remains show a complex image (cultural syncretism) about the Migration Period in Transylvania.[1]

Introduction

During the construction of the bypass route between Corunca-Ernei (Mureş County, Romania), in the course of preventive excavations, the Archaeological Department of the Mureş County Museum discovered a Roman village and a necropolis dating from the Migration Period (according to the funerary offerings and the funeral rites), near Sângeorgiu de Mureş.

The excavations brought to light 19 houses, 15 pits and 3 single-graves. According to the archaeological material the settlement can be dated to the second half of the 3rd century and the beginning of the 4th century, and the funerary offerings date the cemetery to the end of 4th century and the beginning of 5th century.

The graves were discovered on the upper part of the second terrace of the Terebici River, situated in line. Two graves (grave no. 1 and 2) were located inside the village, the third one (grave no. 3)[2] was found beyond the settlement, in sterile soil.

Material and methods

The subject of this paper, grave no. 1/Cx41 was a horse burial oriented N-S, typical for the Migration Period (Huns, Alans, Goths etc.), located inside the settlement, trenched in a Roman period house. Unfortunately, the grave was robbed and the right side of the human skeleton and a significant part of the horse skeleton was destroyed. The preservation of human remains was

[1] Literary adviser Ünige Bencze.

[2] Completely robbed and destroyed grave, only the cast of the skeleton could be observed.

relatively good. We could make several measurements (craniometry and anthropometry) on the skeleton.

The following studies were made on the skeleton with macroscopic methods: sex determination,[3] age estimation,[4] estimation of stature[5] and pathology.[6]

Additionally, with the help of radiography and CT (Siemens Somatom Sensation 4) we could undertake several additional analyses[7] in Radiant Dicom Wiever for age estimation[8] and pathology.[9]

Results

The skull

The skull has good preservation, fragments from the parietal bones could be observed, as well as from the occipital bone, and frontal bone. Small fragments from the temporal bones were missing, the sphenoid bone was badly fragmented, and the front part of the left zygomathic bone was broken *post-mortem*. The base of the skull was missing.

Analysis of teeth

The dental formula is 2122. The preservation of the teeth is good, caries could not be observed. The mandibular central incisors (I1/1, 2) were abnormally worn (Plate no. I, Figure 3a). Dental plaque was observed in lingual aspect on the premolar and molar teeth. The eruption of a third molar tooth did not happen. The wisdom teeth were frequently impacted because they were the last to erupt in the oral cavity.[10]

D.	S.
1.	2.
M2, M1, PM2, PM1, C, I2, I1	I1, I2, C, PM1, PM2, M1, M2
4.	3.
M2, M1, PM2, PM1, C, I2, I1	I1, I2, C, PM1, PM2, M1, M2

Table I. The dental formula.

Postcranial skeleton

Thoracic area: atlas and axis vertebrae, and four lumbar vertebrae. Upper limbs: left and right clavicles (an anomaly on the left clavicle was identified – possibly a stress marker, Plate no. I, Figure 3b), left and right scapula (lower part of the bones were destroyed *post-mortem*), left humerus (l. of bone ~338mm, diameter of the head 43mm), diaphysis fragments from the right humerus, proximal epiphysis end of the right radius, left radius (l. of bone 254mm), left ulna (l. of bone 273mm). The pelvis girdle was fragmented: fragments from the sacrum (the proximal part is missing), the iliac bones, ischium bones and pubic bone. Lower limbs: Left femur (l. of. bone 460mm, diameter of head 46mm – the *linea aspera* was developed), right femur was in fragments (*post-mortem* cut

[3] Éry et al. 1963, Acsády – Nemeskéry 1970.

[4] Todd 1920, Meindl – Lovejoy – Owen 1985; Szilvássy 1988.

[5] Sjøvold 1990; Rösing Friedrick, W. 1988; Bernert 2005.

[6] Pap – Józsa 2006.

[7] We would like to thank Pávai Zoltán M. D. and Pap Zsuzsa M. D. from the University of Medicine and Pharmacy from Târgu Mureş for their help in CT analysis and radiology.

[8] Nemeskéry János – Harsányi László – Acsádi György 1960.

[9] Czigány 2008; Józsa – Pap 2014.

[10] Miloro et al. 2004.

marks on the femur's head could be identified – possible effect of grave robbery), right tibia (l. of bone 365mm), left tibia (the proximal epiphysis end was missing), the right and left fibula was found without the epiphysis' ends. Feet of the skeleton: right and left calcaneus (l. of bones 78mm), right and left talus, and fragments from the metatarsus bones and phalanges were preserved.

Sex determination

The characteristics of the skull (the external occipital protuberance was accentuated, the *glabella* was robust, the edge of the orbits were rounded, the mastoid process was pronounced, the canine *fossa* was 11mm deep, the angle of the mandible was almost 90 degree), and the elements of the postcranial skeleton (diameter of femurs' head, 46mm, the *linea asperea* was advanced) indicated the parameters of a male.

Age estimation

The ecto- and endocranial sutures (phase no. I),[11] the situation of the teeth, the *facies auricularis ossis ilii* (phase no. II, 25–29 years old)[12] all suggested the signs of an adult male.

Stature

Based on the measurements of the length of the long bones, the defunct had medium-high stature probably 171,5cm.[13]

Martin No.	**Clavicle 1**		**Humerus 1**		**Ulna 1**		**Radius 1**		**Femur 1**		**Tibia 1**		**Calcaneus 1**	
Graves	left	right	left	right	left	right	left	right	left	right	left	right	left	right
41	-	-	338	338	273	273	254	254	460	460	365	365	78	78
	-		175.16cm		172,68cm		170,41cm		170,52cm		167,43cm		-	

Table II. Individual measurements of the long bone, male.

Craniometry[14]

According to the craniometry the defunct had a hyperbrachicran skull. The shape of the skull in *norma verticalis* is sphenoid and in *norma occipitalis* had the shape of a bomb, the convexity of the occipital bone was plaocipital, the shape of the maxillary arch was parabolic, the canine *fossa* was deep,[15] the *spina nasalis anterior* was in phase no. 3,[16] *prognathia alveolaris* was in phase no. 3, the shape of nasal bone was in phase no. 1,[17] the nasal bone in lateral aspect was concave (phase no. 1) and the shape of the *apertura piriformis* was in phase no. 4.

Site no. 4	
Martin No.	Grave no. 41
1	163
5	-
8	160

[11] Meindl – Lovejoy 1985; Nemeskéri et al. 1960.
[12] Lovejoy 1985, 15–28.
[13] Sjøvold 1990, Rösing 1988; Bernert 2005.
[14] Bernert 2005.
[15] Lipták 1980.
[16] Broca 1875.
[17] Broca 1875.

Site no. 4	
Martin No.	Grave no. 41
9	99
10	121
11	147
12	111
17	144
20	142
38	1711
40	74
43	110
45	150
46	100
47	127
48	73
51	41
52	38
54	29
55	57
60	69
61	70
62	-
63	42
65	142
66	104
69	33
70	71
71	39

8:1	98.16
17:1	88.34
17:8	90.00
20:1	87.12
20:8	88.75
9:8	61.88
47:45	84.67
48:45	48.67
52:51	92.68
54:55	50.88
63:62	-

Classification by Alekseev and Debets, males	
Martin No.	41
8:1	hbr
17:1	hhyp
17:8	tap
20:1	hhyp
20:8	hakr
9:8	hsten
38	haris
47:45	eup
48:45	eury

52:51	hhyps
54:55	meso
63:62	-

Table III. Individual cranial measurements and indices, males[18]

Pathology

Through macroscopic analyses three nosological types of pathology could be recognized: trauma (artificial cranial deformation), non-specific stress indicators and stress markers.

Artificial cranial deformation:

The skull was artificially deformed, fact which was detected after its reconstruction (Plate no. I, Figure 1a and 1b). During the procedure the frontal bone and the occipital bone were affected (front-occipital type).[19] The head flattening had several side effects, all the anomalies could be seen with the help of radiography (Plate no. II, Figure 6) and the CT image of the skull according to the method of Jenő Czigány (Plate no. II, Figure 4 and 5):[20]

- *Prognathia alveolaris*
- the frontal bone was higher and longer
- deep blood vessel impressions
- distortion of the mandible (gonial angle)
- deformation of the occipital bone
- the shape of the external auditory canal became ovoid
- the dental occlusion was changed

Non-specific stress indicators:

- *Enamel Hypoplasia*: could be observed on canine and premolar teeth as an effect of nutritional deficiencies and chronic diseases during infancy and childhood (see Plate no. I, Figure 3a). [21]

Skull morphology

According to the morphological analysis elements of a Mongoloid (Eastern-type) skull could be identified: the maxillary central incisors had a trapezoid shape and the third molars were missing (*Hypodontia*).[22]

Stress markers

During the anthropological study few stress markers were recognized with macroscopic methods: the mandibular central incisors were abnormally worn this happened probably because of an often used professional technique (of the tanners, shoemakers – see Plate no. 1), the deceased probably towed leather objects in his mouth. On the left clavicle signs of high muscle attachment could be observed (signs of occupational stress), or marks of a healed trauma (see Plate no. 1).

[18] Bernert 2005.

[19] Dingwall 1931, 31–32; Bartucz 1936, 1966.

[20] Czigány 2008, 8.

[21] Marcsik et al 2011, 93–98.

[22] Dr. Budai Mária PhD Disszertáció, 2007, 20.

Conclusions

The defunct from grave no. 1/Cx41 had died between the ages of 25–35 years, according to the skeleton's characteristics he was a man. During his infancy and early childhood he was suffered several chronic diseases (signs of Enemal Hypoplasia on the teeth). The deceased had medium high stature (171,5cm). Based on the pathology the defunct had an artificially deformed skull (front-occipital type) which was a popular practice in the Migration Period in the communities of Alans, Gots, Huns, and later Hungarians.[23] Macroscopic analysis revealed insights of the deceased's life-style: abnormally worn mandibular central incisors and occupational stress on the left clavicle.

The morphological characters of the skull show signs of a Mongoloid type man, a person with eastern features (the shape of the maxillary central incisors, *Hypodontia*). The head flattening was also another eastern practice in the Migration Period but the archeological material (funerary offerings) indicates the characteristics of a Germanic warrior (gold plated dagger, bone comb, Goth or Gepid pottery and glass etc.) so a kind of cultural syncretism could be presumed in this grave.[24]

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[23] Józsa – Pap 1994, 189–194; Hajdú – Bernert 2007, 327–344

[24] A detalied study of the archaeological material will be published soon by the author.

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Figure legends

Figure 1: Grave no. 1/Cx41 (frontal and lateral view);
Figure 2: Age estimation with radiology and CT;
Figure 3a: Signs of occupational stress on teeth;
Figure 3b: Stress marker on the left clavicle;
Figure 4: 3D image of the artificial deformed skull in Radiant Dicom Viewer;
Figure 5: Modification of the cortical layer caused by head flattening;
Figure 6: Radiographic image of the skull (lateral view).

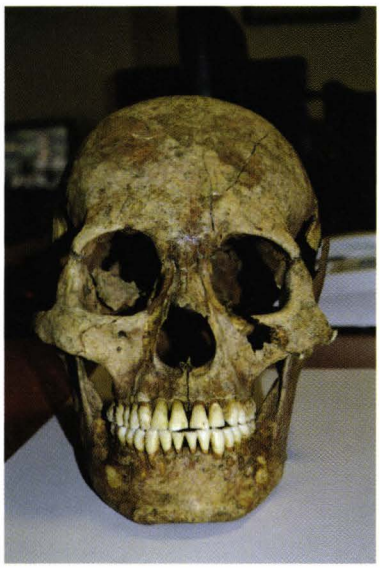


Figure 1a

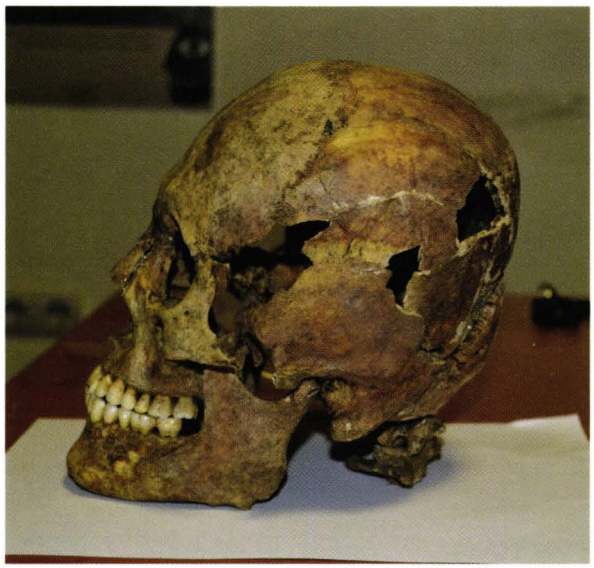
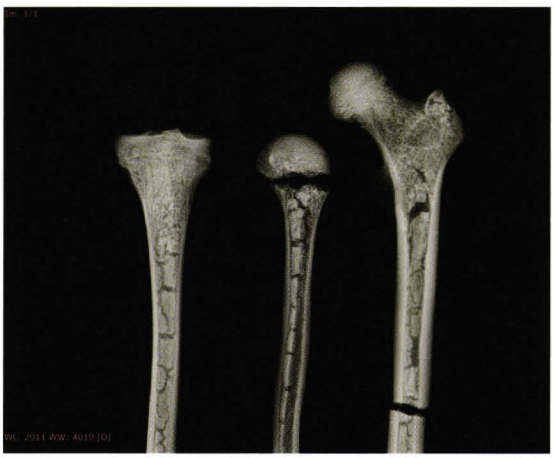


Figure 1b



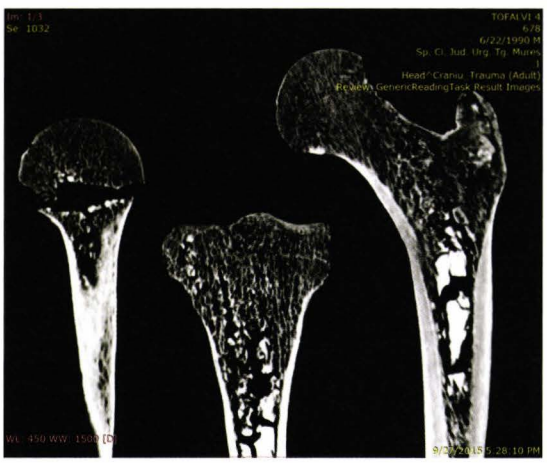


Figure 2

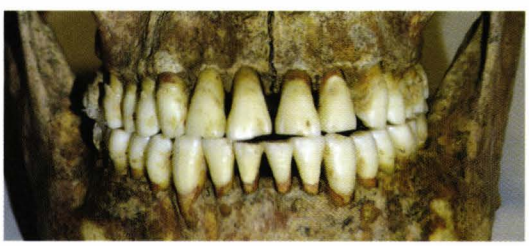


Figure 3a

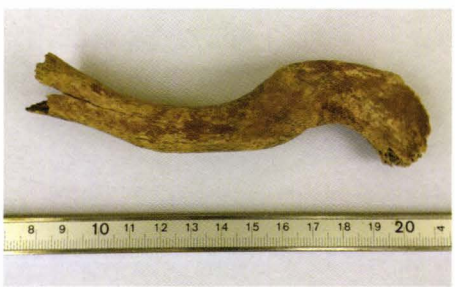


Figure 3b

Plate no. I.

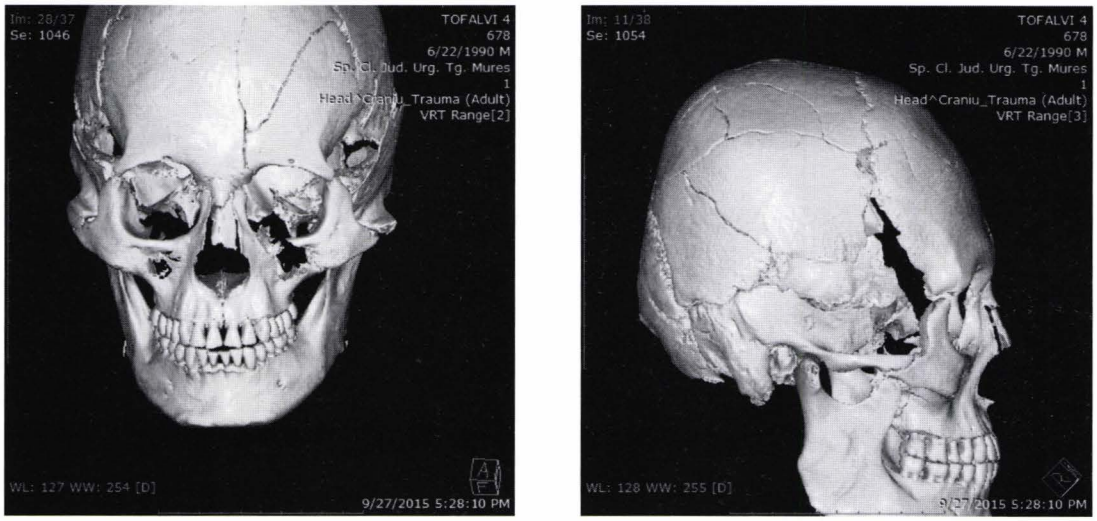


Figure 4

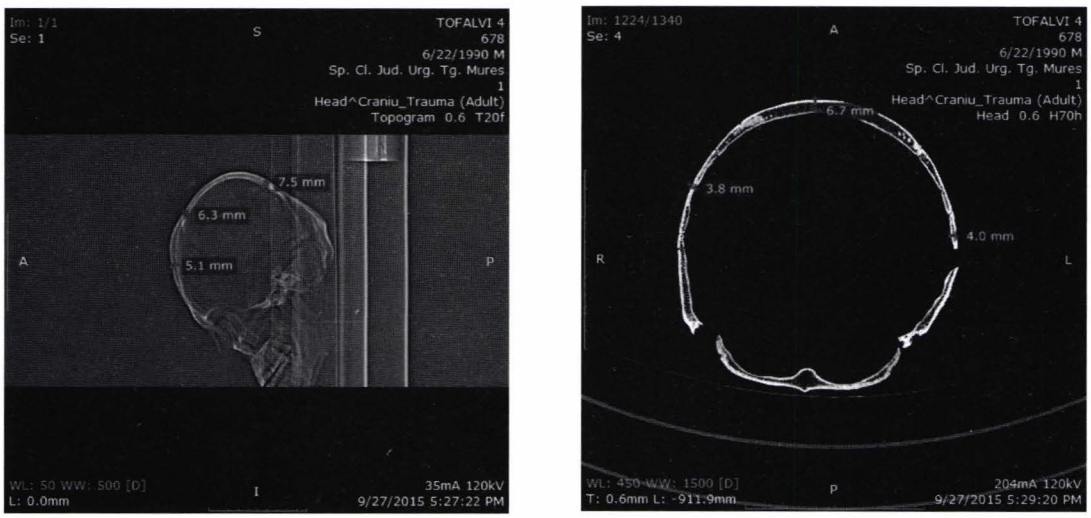


Figure 5

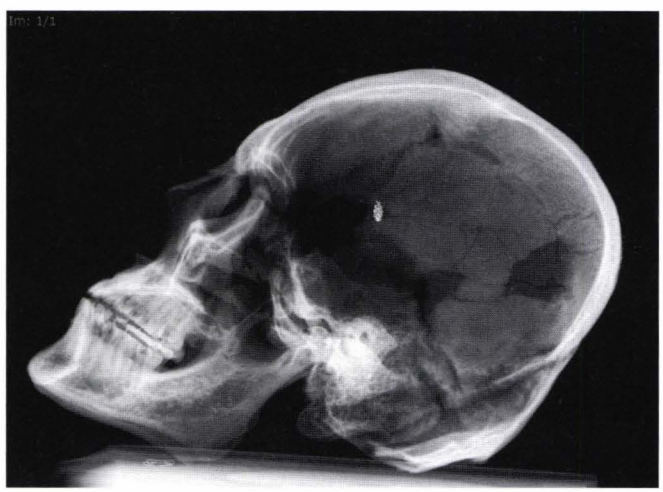


Figure 6

Plate no. II.

AN INSIGHT INTO THE OSTEOLOGICAL MANIFESTATIONS OF DEVELOPMENTAL ANOMALIES

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Abstract

Before the 20th century, developmental anomalies were usually met only as peculiarities of cultural history. Later on, more and more developmental conditions were described and statistically characterized, but mostly in contemporary populations. This field of bioarcheological research has only started gaining more attention in the last decades.

The aim of this current paper is to describe main groups of developmental diseases using examples from osteological series recovered in Southern Hungary. Among the most commonly occurring anomalies some rare conditions will also be explained through cases of significant interest for paleopathology or medical history. We will describe the developmental anomalies of the facial skeleton (facial clefts, primary and secondary palatal clefts), mandibular developmental anomalies (clefted mandible, bifid condyles, hypo- and hyperplasia, torus mandibularis, Stafne's defects), cranial developmental conditions (microcephaly, hydrocephalus, etc.), suture closure anomalies, supernumerary bones or absence of bones, and the anomalies of the cranial base. A few postcranial developmental anomalies will also be presented.

General descriptions and case studies will be completed with statistical data, criteria of differential diagnosis, and/or introduction of accompanying anomalies.

Introduction

Before the 20th century, developmental anomalies were usually met only as peculiarities of cultural history. Later on, more and more developmental conditions were described and statistically characterized, but mostly in contemporary populations. International researchers including Brothwell-Powers (1968), Zimmerman-Kelley (1982), Manchester (1983), Reyman (1983), Gregg-Gregg (1987), Turkel (1989), Kricum (1991), Barnes (1994) began studying this aspect of paleopathology at the end of the 20th century.

Developmental defects are anomalies that start to develop during intrauterine life, and can cause disadvantage to the person (aesthetic, functional, etc.).[1] The general definition of developmental defects includes any type of morphology that in some way differs from a normally expected developmental picture, with variety also in the degree of severity. Whilst some types are only anatomical variants, others can cause secondary pathological problems during the life of the individual. Thus, it is very difficult to predict which defect will later lead to pathological problems. Based on this definition, some researchers have named these defects "congenital defects" or "intrauterine

[1] Barnes, 2012.

defects",[2] because the majority of severe developmental defects can be detected before birth. Most developmental defects are apparent at birth, especially any structural malformation, but some, e.g. those involving organ systems, do not become evident until days, weeks, or even years later.

Developmental defects are influenced by both genetic and environmental factors. Genetic factors include e.g. gene and chromosomal abnormalities, whereas environmental factors may be of chemical, mechanical, nutritional, hormonal or infectious nature. Epigenetic variations are caused by interactions between genetic and environmental factors that explains why some populations are more affected by certain defects than others. It is also not uncommon for many developmental defects to occur in one person, possibly resulting in a syndrome (e.g. Klippel-Feil syndrome). The majority of congenital abnormalities have no known cause. There are some defects that have only been observed in one gender.

Materials and methods

In this study, the osteoarcheological samples used for the analysis of developmental defects were obtained from the osteological collection of the Department of Biological Anthropology, University of Szeged, Hungary. The specimens were taken from historical periods distant from one another such as the Avar Period (6–9th c. AD) and the Middle Ages (11–17th c. AD). We examined the bone remains of more than one thousand individuals (950 well-preserved, 203 fragmented) that originated from 8 different cemeteries and from 2 ossuaries (Table 1.). Investigation of the bone collection was carried out mainly using macromorphological methods. In some instances, where it was necessary (cleft palate, bifid condyle on the mandible, etc.) we carried out a radiological analysis of the bones to determine whether the origin of a particular defect was developmental or a pathological malformation.

Results

I. Cranial vault development and facial anomalies

1. Posterior lingual mandibular depression (PLMD) and similar cavities

Since the first report on a posterior lingual defect by Stafne (1942), many studies have appeared in the clinical, radiological or anthropological literature on the idiopathic bone cavities in the mandible. The shape of these bone cavity can be round or oval, and it varies from 1 to 3 cm in diameter. In the mandibular body they may include posterior buccal (PBMD) and posterior lingual lesions (PLMD, often referred to as Stafne's defect) *(Plate no. I, Figure 1)*, and well as anterior lingual (ALMD) and anterior buccal (ABMD) *(Plate no. I, Figure 2)* types. There may be cavities on the buccal and lingual aspect of the mandibular ramus too. Clinical and dry-bone studies report the incidence of this defect being 7–10 times higher in men than in women.[3] Stafne defect was found at relative frequencies between 0.13%[4] and 0.08%.[5] No PLMD appeared in our material, but we found ALMD in one case in Győrszentiván-Révhegyi tag cemetery. ABMD was found often: 5 cases in Győrszentiván-Révhegyi tag, 2 cases in Csengele-Bogárhát, 7 cases in Csengele-Bogárhát ossuary, 2 cases in Hetényegyháza-Belsőnyír Zana Tanya, 2 cases in Röszke-Kószó Tanya, 23 cases in Szatymaz-Vasútállomás, 2 cases in Téglás-Angolkert, 4 cases in Szegvár-Oromdűlő, which is 47 cases altogether. We also found depression on the ramus of mandibulae in 21 cases: 1 case in Röszke-Kószó Tanya, 19 cases in Szatymaz-Vasútállomás, 1 case in Téglás-Angolkert.

[2] Turkel, 1989; Barnes, 1994.

[3] Mann, 2001.

[4] Assaf et al 2014.

[5] Sisman et al 2012.

2. Bifid mandibular condyles (BMC)

Bifid condyle *(Plate no. I, Figure 3)* is a rare anatomic variation of the mandibular condyle. No definite etiologic factor has been identified behind it. The first cases were reported by Hrdlicka (1941). The morphological variance ranges from a shallow groove to two condylar heads with separate necks, oriented mediolaterally or anteroposteriorly. Anteroposterior bifid condyle could be a developmental anomaly, while mediolateral orientation may be of traumatic origin. In rare instances, they may be associated with temporomandibular joint ankylosis. There is no definite predisposition in either sex or any race. Szentpétery et al. in their study of 1990 found the incidence of BMC to be 0.48%. Sahman et al. in 2011 found 0.52%, while Sahman et al. in 2012 found 1.82%, so our result is similar to the latter report. The data of Haghnegahdar et al. in 2014 signal a somewhat higher incidence rate at population in Southern Iran (3.5%). However, much lower than average frequency (0,018%) may also occur, as it was the case in a Brazilian population investigated by Menezes et al. in 2008. Our examinations show 1.54%. We found 8 cases of BMC in our sample: 1 case in Győrszentiván-Révhegyi tag, 2 cases in Heténye gyháza-Belsőnyír Zana Tanya, 1 case in Röszke–Kószó Tanya, 3 cases in Szatymaz-Vasútállomás, 1 case in Szegvár-Oromdűlő.

3. Hypoplasia and/or hyperplasia in different parts of the mandible

Mandibular hypo- or hyperplasia may occur on the coronoid process, the condylar process or the ramus of the mandibule.[6] These are genetically programmed, progressive, slowly developing processes originating from the ossification centres. Their prevalence is very low without any sexual difference. In case of condylar hypoplasia, the mandibular condyle is smaller than normal, confirmed by the measurement of the maximum width, and may be unilateral or bilateral. Coronoid hyperplasia is recorded where one or both coronoid processes were elongated. We found 1 case of the ramus hypoplasia on right side at Győrszentiván-Révhegyi tag cemetery. Processus coronoideus hypoplasia appeared in a Csengele-Bogárhát ossuary in 1 case. Processus coronoideus hyperplasia appeared also in 1 case at Téglás-Angolkert cemetery.

4. Clefts of the primary and the secondary palate

The most common defects of the skull and face are cleft lip and cleft palate.[7]

Cleft lip is a separation of the upper lip, usually just below the nose. Cleft palate is a split in the roof of the mouth resulting in a passageway into the nasal cavity. Cleft lip and cleft palate often occur together. Clefting of the primary palate most often occurs between the primary and secondary palates at the incisive foramen, near the lateral incisors and the canine teeth. Cleft of the secondary palate spans from anterior to posterior, beginning at the incisive foramen and concluding with uvular fusion .[8] Children born with oral clefts have been shown to have higher mortality rates, especially in the presence of other birth defects.[9] These lesions are very rare in osteoarchaeological samples. A number of publications have already reported several cases from Hungary: Berndorfer (1962) mentioned a female skull, Lipták-Farkas (1967) found bilateral premaxilla deficiency on a subadult skull, Marcsik (1976) described a male skull from the 10th century, Kocsis and Marcsik (1979) diagnosed of 6–7 years old child skull with a cleft palate, Hegyi and Kocsis (2001) reported a premaxilla cleft. In our material we found cleft of the primary palate in

[6] Barnes, 1994.

[7] Venkatesh 2009.

[8] Smith et al., 2012.

[9] Carlson et al., 2013.

Csengele-Bogárhát ossuary and Szatymaz-Vasútállomás cemetery, and cleft of the secondary palate in Heténygyháza-Belsőnyír Zana tanya.

5. Wormian bones

Wormian bones are little extra bones occurring within a suture in the cranium.[10] They are abnormal ossicles that develop from extra ossification centres within the cranium. However, they are often referred to as simple anatomical variants. They are most frequently located in the lambdoid suture (Plate no. II, *Figure 5)* or the coronal suture *(Plate no. II, Figure 6)*, and have also been seen in fontanelles, particularly the posterior fontanelle. It is yet unclear how or why they are exactly formed, although genetic as well as environmental factors have been proposed. Separate name is used for os epiptericum *(Plate no. II, Figure 7)*, os bregmaticum, os apicis, os asterion in distinct locations, but physiologically they are also Wormian bones. Large Wormian bones in the lambdoid sutures are is often called os incae *(Figure 8)*. Brothwell in 1959 studied the incidence of Wormian bones in different populations and found a prevalence of 55% in an Anglo-Saxon population and 80% in a Chinese population. Wormian bones were observed in 73.1% of the Indian population ([11]). In our series the incidence of Wormian bones was 50–60% that correlates with literature data. We also found associations between the appearance of Worm bones and other developmental defects. We found 30 cases of Wormian bone in the lambda suture in Győrszentiván-Révhegyi tag cemetery, 5 cases in Csengele-Bogárhát cemetery, 5 cases in Csengele-Bogárhát ossuary, 15 cases in Heténygyháza-Belsőnyír Zana Tanya, 8 cases in Röszke-Kószó, 49 cases in Szatymaz-Vasútállomás, 12 cases in Téglás-Angolkert and 28 cases in Szegvár-Oromdűlő, altogether 152 cases. We found 1 case of os incae in Győrszentiván-Révhegyi tag and 3–3 cases in Heténygyháza-Belsőnyír Zana Tanya and Szatymaz-Vasútállomás.

8 cases of os epiptericum was found in Győrszentiván-Révhegyi tag cemetery, 1 case in Csengele-Bogárhát, 1 case in Csengele-Bogárhát ossuary, 4–4 cases in Heténygyháza-Belsőnyír Zana Tanya, Röszke-Kószó and Szatymaz-Vasútállomás, 1 case in Téglás-Angolkert, and 11 cases in Szegvár-Oromdűlő, altogether 27 cases.

3 cases of os apicis was found in Győrszentiván-Révhegyi tag cemetery, 2 cases in Heténygyháza-Belsőnyír Zana Tanya, and 4 cases Szegvár-Oromdűlő.

6. Sutural variations (agenesis or extra sutures)

6.1. Craniosynostosis

Craniosynostosis is premature fusion of one or more calvarial sutures, which causes a characteristic skull deformity due to decreased growth in a direction perpendicular to the closed suture.[12] Craniosynostosis has several types depending on which suture is fused:

a) Sagittal craniosynostosis (scaphocephaly) is the most common form of craniosynostosis,[13] which occurs with premature fusion of the sagittal suture *(Plate no. III, Figure 9)*. In our material the incidence of scaphocephaly was 0.2–1% that correlates with literature data. Most cases are isolated within the series. We found 1 case of scaphocephaly in Győrszentiván-Révhegyi tag (young adult female), 1 case in Csengele-Bogárhát ossuary (subadult), and 1 case in Szatymaz-Vasútállomás (subadult).

b) Coronal craniosynostosis is the second most common type. It may be bilateral causing a short and broad skull (brachycephaly), or unilateral causing a diagonal skull deformity (plagiocephaly).

[10] Marti et al, 2013.

[11] Marti et al., 2013.

[12] Pásztor, 2007.

[13] Kabbani et al., 2004.

Coronal craniosynostosis is commonly associated with facial and extracranial anomalies within the context of Pfeiffer-, Saethre-Chotzen-, Carpenter-, or Apert syndromes.[14]

c) Premature closure of the frontal suture results in a growth restriction of the frontal bones, which leads to a skull malformation known as trigonocephaly.[15]

6.2. Extra sutures

The frontal suture dividing the frontal bone of subadults usually fuses during early childhood but often remains in place throughout life *(Plate no. III, Figure 10)*. The adult form of the persistent frontal suture is called metopic suture.[16] 2 cases of metopic suture were found in Győrszentiván-Révhegyi tag cemetery, 3–3 cases in Csengele-Bogárhát and Hetényegyháza-Belsőnyír Zana Tanya, 1 case in Röszke-Kószó Tanya, 6 cases in Szatymaz-Vasútállomás, 4 cases in Téglás-Angolkert, 7 cases in Szegvár-Oromdűlő.

II. Vertebral column anomalies

1. Hemivertebrae

These anomalies include wedge-shaped vertebrae and therefore can cause angular deformity of the spine (such as kyphosis, scoliosis, and lordosis).[17] No cases of hemivertebra have been found in our material.

2. Cleft vertebrae

Sagittal cleft of vertebrae when the two halves of vertebral bodies or arches fail to fuse.[18] Cleft atlas is a good example of the phenomenon. In this case, the two lateral masses of the anterior (or the posterior) arch fail to fuse. Cleft anterior arch often appears accompanied by a cleft posterior arch. Cleft vertebrae appeared in our material very rarely, mostly affecting the first cervical vertebra *(Plate no. III, Figure 11)*. 1 case of cleft atlas was found in Csengele-Bogárhát, 1 case in Csengele-Bogárhát ossuary, 2 cases in Hetényegyháza-Belsőnyír Zana Tanya, 3 cases in Röszke-Kószó Tanya, 2 cases in Szatymaz-Vasútállomás.

3. Block vertebrae

Block vertebra is a type of vertebral anomaly where the separation of two or more adjacent vertebral bodies fails.[19] We can differentiate between single or multiple block vertebrae. It usually appears as a familial trait, mostly within the cervical spine, especially with C2–C3 or C3–C4 vertebrae. Occasionally, it appears in the lumbar region. Multiple blocks of vertebrae are often accompanied by other vertebral developmental disturbances, especially hemivertebrae, ventral and/or lateral hypoplasia. Only 1 case of this anomaly appeared in our material in Hetényegyháza-Belsőnyír Zana Tanya (subadult).

4. Sacralisation – Lumbarisation

Sacralisation and lumbarisation of the sacrum and the base of the spine are very common. The L5 segment is said to be sacralised, when it is fused or semi-fused to the sacrum *(Plate no. III, Figure 12)*. In case of lumbarisation, the first sacral segment fails to fuse to the rest of the sacrum.

[14] Ko, 2016.
[15] Barnes, 2012.
[16] Barnes, 2012.
[17] Barnes 2012.
[18] Barnes, 2012.
[19] Barnes, 2012.

Lumbarisation and sacralisation were observed in our material with very different frequency. Both sacrum abnormalities appeared in three cemeteries (Hetényegyháza-Belsőnyír Zana Tanya (3 sacralisations, 1 lumbarisation), Röszke-Kószó Tanya (2 sacralisations, 1 lumbarisation), Szatymaz-Vasútállomás (8 sacralisations, 5 lumbarisations)). Only sacralisation was found in the Csengele-Bogárhát ossuary in 2 cases and the Győrszentiván-Révhegyi tag cemetery in 12 cases. None of the abnormalities appeared in the Téglás-Angolkert cemetery.

III. Sternum anomalies and variations

Suprasternal ossicles, manubrium-mesosternal joint fusion, mesosternal hypoplasia/aplasia, and caudal sternal clefting belong to the developmental abnormalities of the sternum. One of the most frequent developmental abnormalities is sternal foramina (SF), which is located mainly in the inferior portion of the sternal body and the xyphoid process. *(Plate no. III, Figure 13)*.[20] Their formation is a result of incomplete fusion of the sternal ossification centers. However, no cases of sternal anomalies have been found in our material.

IV. Lower limbs anomalies

This group includes all types of hip dysplasia, and the abnormalities of the femur, patella, tibia, fibula and foot. Clubfoot (talipes equinovarus) is one of the most common member of this group, being a complex childhood deformity that is marked primarily by a deformed talus and calcaneus.[21] The foot and the ankle are twisted out of shape and position. In case of a usual clubfoot, the hind foot and the ankle is oriented down and inward, and the forefoot is twisting inward. Sometimes the foot only appears abnormal because it was held in an unusual position in the uterus (positional clubfoot), but the reason can also be hereditary. Palaeopathological evidence for clubfoot is rare in the literature, but cases from Hungary have already been reported.[22] Clubfoot did not appear in our material.

Summary

Serious developmental abnormalities are easily observable, and cause aesthetical or functional disadvantage in the individual's life, thus, numerous medical and anthropological research projects are targeted to their investigation. Minor developmental abnormalities are generally asymptomatic and less visible, but their examination would also be important. Minor abnormalities can be observed using radiography in contemporary populations, but they can also be easily identified in skeletal populations. In spite of this, there are still only a small number of bioarcheologists who investigate developmental defects. The publications in this field usually focus on individual cases or a single skeletal series. Our case reports and prevalence data provide new information for this field of research and may aid the biological re-evaluation of ancient populations.

Developmental abnormalities may affect isolated or multiple regions of the skeleton, or involve a specific tissue system. In most cases, they do not appear solitarily but co-occur with other developmental abnormalities or as part of developmental syndromes. In general, the prevalence of developmental defects is low, but the less severe forms may appear more often. Some developmental abnormalities affect both sexes equally, some others may be more common in one of the sexes. Prevalences, sex ratios and sidedness (left or right) in our investigation was usually similar to those reported in the literature.

[20] Barnes, 2012.

[21] Barnes, 2012.

[22] Hegyi et al., 1999.

In the future, we intend to conduct a systematic, descriptive, and statistical evaluation of developmental defects observed in bigger composite samples, in order to gain a more comprehensive picture on developmental defects affecting the historical populations of the Carpathian Basin.

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Figure legends

Figure 1: PLMD (posterior lingual mandibular defect), Szarvas Grexa Téglagyár 423, Maturus (40–60 yrs) male.

Figure 2: ABMD (anterior buccal mandibular defect), Győrszentiván Révhegyi tag S-0442, Maturus (40–60 yrs) female.

Figure 3: Bilateral bifid mandibular condyles (BMC), Győrszentiván Révhegyi tag S-0475, Maturus (40–60 yrs) male.

Figure 4: Coronoid process hyperplasia, Csongrád-Ellés 241, Adultus (20–40 yrs) female.

Figure 5: Wormian bone within the lambdoid suture, Győrszentiván Révhegyi tag S-0446, Adultus (20–40 yrs) female.

Figure 6: Wormian bone within the coronal suture, Győrszentiván Révhegyi tag S-0626, Adultus (20–40 yrs) male.

Figure 7: Os epiptericum, Hetényegyháza-Belsőnyír Zana tanya 31, Adultus (20–40 yrs) female.

Figure 8: Os incae, Győrszentiván Révhegyi tag S-0082/S-0306, Adultus (20–40 yrs) male.

Figure 9: Scaphocephaly, Győrszentiván Révhegyi tag S-0471, female.

Figure 10: Metopic suture, Győrszentiván Révhegyi tag S-0435, Infantia I (0–6 yrs).

Figure 11: Cleft atlas, Csongrád-Ellés ossuary, Adultus (20–40 yrs) indeterminable.

Figure 12: Sacralisation, Csongrád-Ellés 20. A/1, Adultus (20–40 yrs) female.
Figure 13: Sternal foramina, Győrszentiván Révhegyi tag S-0443, Juvenis (14–20 yrs) male.

Cemetery	**Historical period**	**Number of individuals**
Csengele-Bogárhát	13–14th century AD	31
Csengele-Bogárhát ossuary	13–14th century AD	13
Csongrád-Ellés	Arpadian Age, 11–14th century AD	198
Csongrád-Ellés ossuary	Arpadian Age, 11–14th century AD	16
Győrszentiván Révhegyi tag	Arpadian Age, 11–14th century AD (57 individual) Bronze age (1 individual)	58
Hetényegyháza-Belsőnyír Zana tanya	Arpadian Age, 11–14th century AD	47
Röszke – Kószó tanya	14–15th century AD	51
Szatymaz-Vasútállomás	Arpadian Age, 11–14th century AD	222
Szegvár-Oromdűlő	6–7th century AD	261
Téglás-Angolkert	Arpadian Age, 11–14th century AD	53
Summary	-	**950**

Table 1: Data of the examined cemeteries.

Figure 1. PLMD (posterior lingual mandibular depression)

Site: Szarvas Grexa Téglagyár, Hungary
Inventory number: 423
Age at death: Maturus (40-60 yrs)
Sex: Male

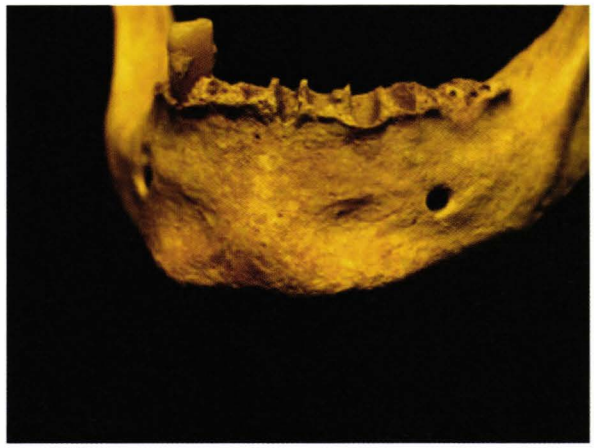

Figure 2. ABMD (anterior buccal mandibular depression)

Site: Győrszentiván Révhegyi tag, Hungary
Inventory number: S-0442
Age at death: Maturus (40-60 yrs)
Sex: Female

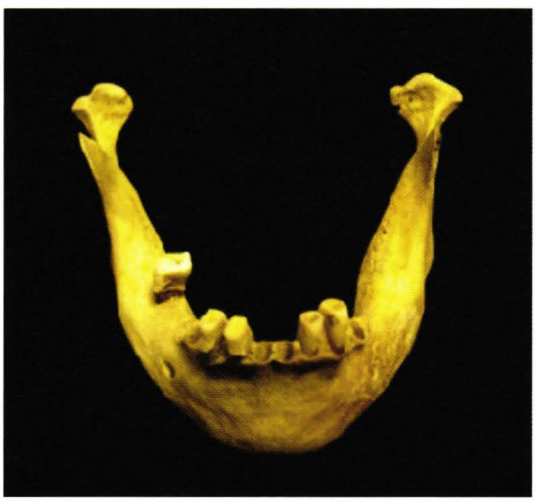

Figure 3. Bilateral bifid mandibular condyles (BMC)

Site: Győrszentiván Révhegyi tag, Hungary
Inventory number: S-0475
Age at death: Maturus (40-60 yrs)
Sex: Male

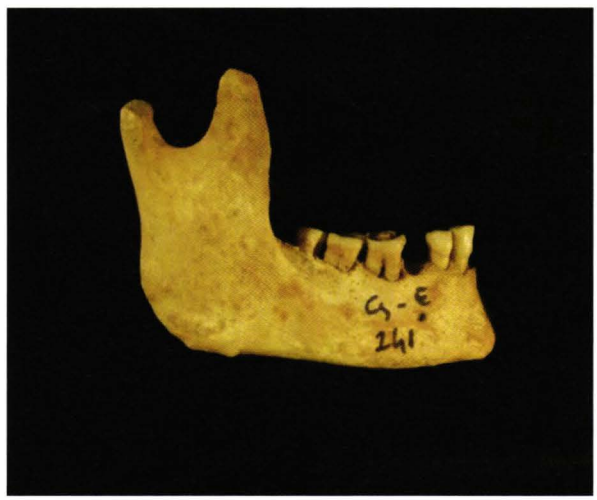

Figure 4. Coronoid process hyperplasia

Site: Csongrád-Ellés, Hungary
Inventory number: 241
Age at death: Adultus (20-40 yrs)
Sex: Female

Plate no. I.

Figure 5. Wormian bon within the lambdoid suture

Site: Győrszentiván Révhegyi tag, Hungary
Inventory number: S-0446
Age at death: Adultus (20-40 yrs)
Sex: Female

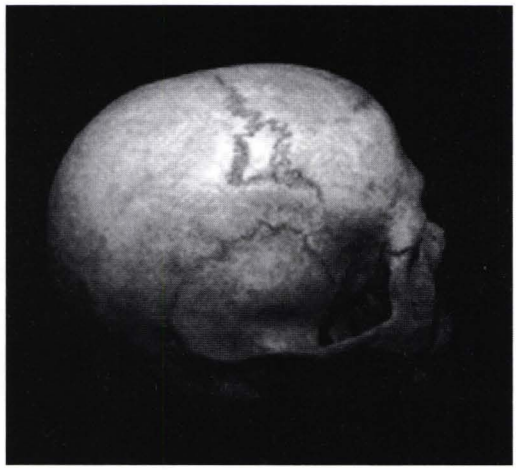

Figure 6. Wormian bone in the coronal suture

Site: Győrszentiván Révhegyi tag, Hungary
Inventory number: S-0626
Age at death: Adultus (20-40 yrs)
Sex: Male

Figure 7. Os epiptericum

Site: Hetényegyháza-Belsőnyír
Zana tanya, Hungary
Inventory number: 31
Age at death: Adultus (20-40 yrs)
Sex: Female

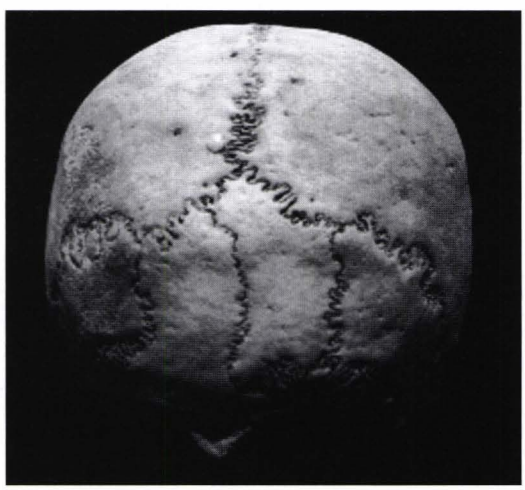

Figure 8. Os incae

Site: Győrszentiván
Révhegyi tag, Hungary
Inventory number: S-0082/S-0306
Age at death: Adultus (20-40 yrs)
Sex: Male

Plate no. II.

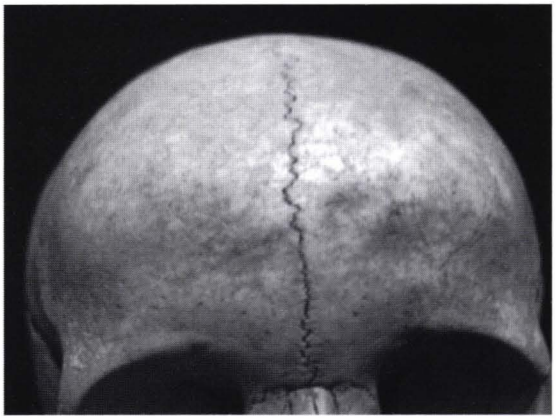

Figure 9. Metopic suture

Site: Győrszentiván Révhegyi tag, Hungary
Inventory number: S-435
Age at death: Infantia I. (0-6 yrs)

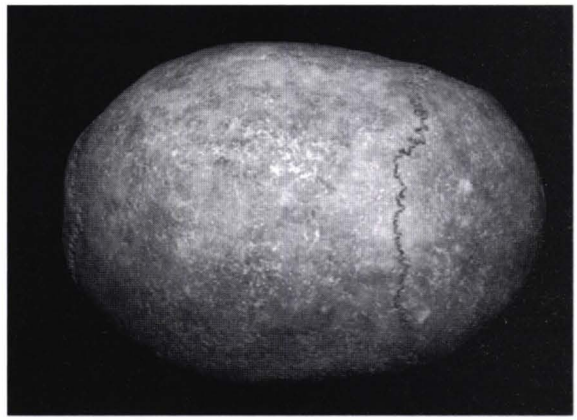

Figure 10. Scaphocephaly

Site: Győrszentiván Révhegyi tag, Hungary
Inventory number: S-0471
Age at death: Adultus (20-40 yrs)
Sex: Female

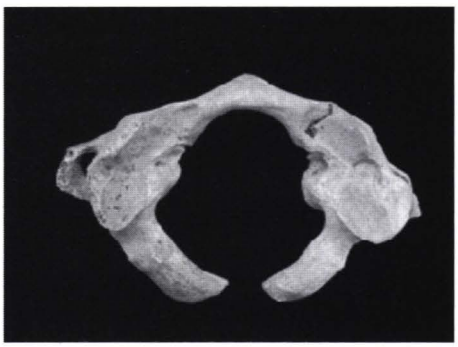

Figure 11. Cleft atlas

Site: Csongrád-Ellés, Hungary
Inventory number: -, ossuary
Age at death: Adultus (20-40 yrs)
Sex: Indeterminable

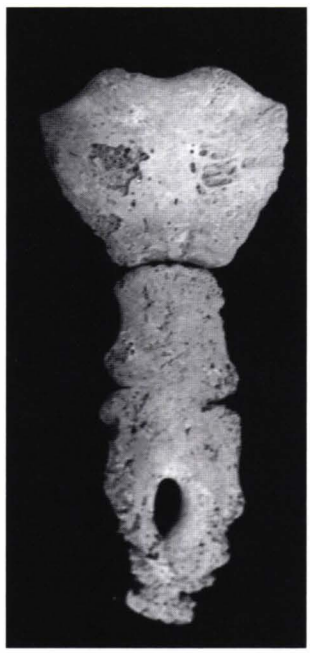

Figure 13. Sternal foramina

Site: Győrszentiván
Révhegyi tag, Hungary
Inventory number: S-0443
Age at death: Juvenis (14-20 yrs)
Sex: Male

Figure 12. Sacralisation

Site: Csongrád-Ellés, Hungary
Inventory number: 20. A/1
Age at death: Adultus (20-40 yrs)
Sex: Female

Plate no. III.

ANTHROPOLOGICAL ANALYSIS OF AN AVAR AGE CEMETERY FROM THE DUNA-TISZA INTERFLUVE (HAJÓS-CIFRAHEGY)

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Keywords: Duna-Tisza interfluve, Avar Age, anthropological and pathological analyses

Abstract

The present study is intended to contribute to the knowledge of typological and pathological characteristics of the Avar Age population through the description of the anthropological profile of a cemetery in the Duna-Tisza interfluve.

The excavation of the Hajós-Cifrahegy cemetery (7–9th century AD) was carried out between 1978 and 1984. From the uncovered 169 graves 135 individuals were possible to identify. During the anthropological and pathological analysis standard methods were applied.

The number of subadults was lower (34) than the number of adults (101). The sex ratio revealed a higher proportion of females at 59% compared to 41% of males. Based on the results of the metric analysis the population was relatively homogeneous for both sexes. From a taxonomic point of view, the population was mostly Europid, only two individuals revealed exclusively Mongolid cranial features. According to the results of the pathological analyses the presence of leprosy in two cases is worth to mention.

The studied anthropological series could be related to the Europid populations from the southern part of Transdanubia and the Danube Tisza interfluve.

Material and methods

The excavation was carried out under the directon of Mihály Kőhegyi between 1978 and 1984. In addition to the remains of a Sarmatian Settlement, 169 graves of an Avar Age (7–9th century AD) cemetery were uncovered.[1] Unfortunately, the anthropological material was stored in different places, which led to issues with the identification of grave numbers, impossible in some cases.

Nevertheless, it was possible to identify 135 individuals (127 skulls and 135 postcranial skeletons). The skeletons were predominantly well preserved, although in some cases the state of preservation was less satisfactory.

The anthropological analysis of the remains of these 135 individuals were performed applying standard methods. The estimation of age at death and the sex determination were based on the work of Acsádi and Nemeskéri (1970). For the analysis of the metric and morphological traits, the methods of Martin-Saller (1957) and Aleksejev-Debec (1964) were used. The stature estimation was carried out using the method developed by Sjøvold (1990). The taxonomic analysis was based

[1] Szentpéteri, 2002.

on the works of Lipták (1959, 1983), while the paleopathological analysis on the works by Barnes (1994) and Ortner (2003) using applied macromorphological methods.

General description of the anthropological material

Age at death and sex distribution are summarized in Table 1. The number of subadults was lower than the number of adults, particularly in the Juvenis age group. The sex ratio revealed a higher proportion of females at 59% compared to 41% of males, excluding 29 indeterminate skeletal remains.

Based on the average of absolute values, the maximal cranial length of male individuals was medium, the cranial base length (ba-n) and the maximum cranial breadth were broad, while the minimum frontal breadth was medium and the basion-bregma height was low. The basion-prosthion length was also medium, as well as the bizygomatic diameter, the zygomaxillary breadth, the upper facial and the total facial height (Table 2).

The orbital breadth of male individuals was small, the orbital height was medium, the maximum height and the minimum breadth of the mandiular ramus were medium. Unfortunately the nasal bones, the hard palate, and some regions of the mandible were not suitable for metric analysis due to their very fragmented state (Table 2).

Based on the average of indices of male individuals, the cranial index was short (brachycran) and the breadth-height index was low (tapeinocran). All other indices were medium (length-height index – orthocran, transversal frontoparietal index – metriometop, morphological and upper facial indices – mesoprosop and mesen, orbital and nasal indices – mesoconch and mesorrhin (Table 2).

The mean value of the stature – estimated from the long bone measurements of 29 males – was 169 cm. The values ranged from 162 to 181 cm.

Based on the average of absolute values, the maximal cranial length of females was great, the cranial base length (ba-n) was medium, the maximum cranial breadth was broad, and the basion-bregma height was low. The basion-prosthion length, the bizygomatic diameter, the zygomaxillary breadth, the upper facial and the total facial height were all medium (Table 3).

The orbital breadth of female individuals was small, the orbital height was medium. All measurements of the nose, the hard palate, as well as the mandible were medium except for the maximum height of the mandibular ramus, which was great (Table 3).

Based on the average of indices of female remains, the cranial index was medium (mesocran), however the value is near to the minimum value of the next category (brachycran). The length-height index was medium (orthocran), the breadth-height index was low (tapeinocran), the transversal frontoparietal index is broad (eurymetop), the morphological and the upper facial indices were medium (mesoprosop, mesen). The orbital index is high (hypsiconch), the nasal index is medium (mesorrhin), while the palatal index is broad (brachystaphyline) (Table 3).

The mean value of the stature – estimated from the long bones measurements of 34 females – was 159 cm. The values varied between 149 and 168 cm.

Morphological characteristics

The occipital profile was usually rounded (*curvoccipital*), while occipital flattening or occipital bun (chignon) were not typical. The lower margin of the nasal aperture was mainly *anthropin,* the occurence of prenasal fossa was very rare. In most cases the canine fossa was shallow, although a total lack of the canine fossa was observed in some cases, linked to the extreme projection of the malar bones. Alveolar prognathism was most often moderate or did not occur at all.

The most commonly observed anatomical variations were Wormian bones. Other variations, such as metopic sutures, epipteric bones or palatal tori (bony protrusion of the palate) were much rarer. The presence of an incomplete Inca bone (or interparietal bone) on a female skull (Grave No. 5) is worth mentioning as this is a relatively rare phenomenon. In addition, moderate expression of mandibular tori (bony growth on the internal suface of the mandible) could also be detected in two cases (Graves No. 15 and 22).

Taxonomic analysis

The skeletal remains of 31 male and 31 female individuals were suitable were suitable for taxonomic analysis.

The remains were overwhelmingly Europid (59 individuals), regardless of sex. Within Europids, the main groups represented were the Cromagnoid-B, the undeterminable Brachycranic and the gracile Mediterranean groups, withthe presence of Cromagnoid-A group not significant.

In several cases, the cranial base was remarkably low (chamaecranic) (Plate no. I, Figures 1–2). Archaeomorph characteristics (strongly slopped forehead, tent-shaped occipital area, robust postcranial skeleton) were observed in some male individuals (Graves No. 17, 21 and 49).

Within the *Europid* group, some individuals also showed a few mongoloid characteristics (nine individuals in total, including six males and three females. These were mostly morphological traits (e.g lack of canine fossa, flat face, wide nasal root, less prominent nasal bones, wide nasolacrimal canal and infraorbital foramen, prenasal fossa, mandibular tori (Plate no. I, Figure 3),[2] with only a few metrical traits also present.

The presence of Mongolids was limited to two individuals (Grave No. 89 – male and Grave No. 107 – female) (Plate no. II, Figures 4–5).

Pathological alterations

The pathological alterations of the Hajós series were grouped as follows:

Developmental anomalies

Developmental anomalies are defects that develop in utero and manifest themselves more noticeably at birth or in the following infant growth phase. They affect the soft tissues and/or the skeletal system occurring in embrional life. These abnormalities fall out of the range of normal anatomical variations and cause some kind of (aesthetical, functional, etc.) impairment in a person's life. The defects may range from slight to severe and can sometimes result in natural miscarriages if the abnormality also affects fetal development. The vast majority of congenital anomalies are caused by intrinsic genetic factors with mutation(s) occurring from genetic traits, being hereditary in nature, or being extrinsic environmental factors.[3]

The incidence of the slight malformations (harmless anomalies, causing no serious problem) was quite low in the examined material. These consisted of one case of bifid vertebrae (*spina bifida*) in anadult female, three cases of addititional segments of the sacrum (*sacralisation*) and (two females and one male), and one case of bifurcation of the first rib.

In addition, one case of a more severe developmental anomaly was observed on a female individual. The acetabular cavity was shalow and elongated (extended upwards) with marginal ossifications. The femoral head displayed a flattened appearance (Figure 6). These changes are typical symptoms of congenital hip subluxation. Due to the underdevelopment of the acetabulum, the femoral head had moved posteriorly creating the above described lesions.

[2] Lipták 1959, 1983.

[3] Barnes, 1994; Dobszay 1969.

Trauma

Trauma is shown by bone lesions caused by external physical impacts. These types of pathological alterations are one of the most frequently occurring recorded pathologies within archaeological assemblages. However, trauma was very rare in the Hajós cemetery. Signs of healed fractures were observed in three cases and skull injuries in two, all described below.

The left *radius* of an adult male (Grave No. 8) and the left *ulna* of an another male (Grave No. 162, Plate no. II, Figure 7) revealed healed parry fractures. On another adult male (Grave No. 138), of an traces of suppuration (probably caused by posttraumatic infection) could also be observed beside a healed fracture near the acromial end of the right clavicle (Plate no. II, Figure 8). Posterior cleft of a lumbal vertebra (*spondylolysis)* was present in an adult male (Grave No. 18).

Healed skull injuries on the parietal bone were observed in two adult females (Grave No. 7 and 57; Plate no. II, Figure 9).

Degenerative Joint Diseases

Degenerative joint diseases (DJD) are non-inflammatory diseases involving the degeneration of bone at the synovial joints. This is the most common type of joint diseases, affecting usually the hands, feet, spine, and large weight-bearing joints, such as the hips and knees. The severity of DJD appear to be dependent on factors such as age, genetic predisposition, climate, weight, and sex.[4] The single most contributive factor to the development of DJD, however, is mechanical stress and physical activity.[5] While the likelihood of DJD increases around the mid–40s, because it is ultimately a stress-induced phenomenon, there is considerable variation in age at onset among populations, with some young adults showing manifestations of the degenerative disease.[6]

In the studied material, DJD, both in its vertebral and extravertebral localizations, affected predominantly the members of maturus and senium age groups. Degenerative alterations in the spinal region were detected in 18 cases (eleven male and seven female), mainly in the lumbar and thoracic regions. Regarding extravertebral lesions, degenerative alterations of the clavicle are worth mentioning, appearing on four mature males and one adult female. Secondary (post-traumatic) degenerative alteration could be detected in an adult male (Grave No. 138). The degenerative changes of the right shoulder joint could be regarded as a consequence of the shortening of the fractured clavicle.

The severity of the detected lesions were mild or medium in all detected cases.

Specific infections

Specific infections can be observed through bony lesions for which, by distribution or physical attribute, the causative micro-organism is known. Because it is necessary for infection to become a chronic condition within the host, only a handful of diseases are known to produce skeletal manifestations (e.g. mycobacterial diseases).

In this sample, leprosy was the only specific infection to be diagnosed, with two cases. The skull of a young adult female (Grave number unknown, andpostcranial bones missing) revealed severe lesions in the rhinomaxillary region (Plate no. II, Figure 10) and on the mandible. Rounding and enlargement of the pyriform aperture due to bone atrophy (Plate no. II, Figure 11) and slight resorption (4 mm long) of the alveolar process between the two maxillary central incisors (in continuation of the intermaxillary suture) could be observed. The bony part of the nasal septum was

[4] Larsen 1997; Roberts and Manchester 2007.

[5] Larsen 1997.

[6] Larsen 1997.

almost completely absorbed. Strong porosity and perforations of the hard palate were seen. The most striking perforation – 15 mm long and 4 mm wide – was detected on the right palatinal process of the maxilla (Plate no. III, Figure 12).

Thick periosteal apposition on both surfaces of the mandibular corpus (Plate no. III, Figure 13) was also recognized. Additionally, the presence of bilateral cribra orbitalia (porotic type) was also noted.

Severe pathological alterations were observed on the incomplete postcranial skeleton and on the well-preserved skull of a young (18–22 years old) female (Grave No. 81). Similarly to the previous case, the lesions affected the rhinomaxillary region of the skull. The edge of the piriform aperture was enlarged and rounded with a "puffed" appearance, the anterior nasal spine was almost completely resorbed with the bony part of the nasal septum slightly less so (Plate no. III, Figure 14). Betwen the two upper central incisors, vertical bone loss (approximately 7 mm long) of the alveolar process of the maxilla was observed (Plate no. III, Figure 15). The inferior edges of the nasal bones were ragged, revealing traces of atrophy and inflammation. Symmetrical hypertrophy in the cortical bone around the edges of the nasal aperture was observed, particularly visible in the area between the lateral and the inferior edge. The hard palate was strongly porotic, showing multiple perforations (especially next to the palatal bone) and traces of inflammation (Plate no. III, Figure 16). Remarkable enlargement of the incisive foramen was also noted.

This individual's postcranial bones were in a good state of preservation, although some elements, e.g. the carpals, tarsals, metacarpals, metatarsals and phalanges of foot and hand, were almost completely missing. The remaining bones of the foot and hand revealed no pathological alterations. Slight periosteal apposition of the distal humerus and proximal ulna could be recognized. Severe periostitis and vascular grooves were observed along the diaphysis of both tibias (Plate no. III, Figure 17). Furthermore, lesions suggestive of ulcer were noted on the medial surface of the right tibial diaphysis (Plate no. III, Figure 18). The above described alterations were identified as osteological manifestations of leprosy.

Nonspecific infections

Beside lesions highly suggestive of specific infections (leprosy in this sample), other rather nonspecific infectious alterations were also recognized in a few cases. The endocranial changes observed in a young female (Grave No. 20) may refer to increased intracranial pressure possibly due to meningeal reactions induced by intrathoracic infection. Bony sign of mastoiditis could be seen in a mature female, a consequence of a probable middle ear infection. Symmetrical periostitis of unknown origin was also noticed on the tibia and fibula of a young female.

Metabolic and hematologic disorders

Osteoporosis is one of the most frequently reported metabolic diseases mentioned in the paleopathological literature. In our material, these changes affected exclusively the elderly age groups (maturus, senium). Osteoporotic vertebral deformations were observed in four cases (Graves No. 21, 38, 83, 113).

Porosities in the outer table of the cranial vault (porotic hyperostosis) and orbital roof (cribra orbitalia) are among the most frequent pathological lesions seen in ancient human skeletal collections. Although cranial vault and orbital roof porosities are sometimes conflated under the term porotic hyperostosis, paleopathological and clinical evidence suggests they often have different etiologies. Reconsidering the etiology of these skeletal conditions has important implications for current interpretations of malnutrition and infectious disease in earlier human populations.[7]

[7] Stuart-Macadam 1989; Walker et al, 2009.

In this sample, all observed anomalies (graves no. 47, 51, 111, 151, 66 – subadults; grave no. 6, unknown grave number– males; graves no. 27, 69 – females) belonged to the mildest form (porotic type) of porotic hyperostosis, with only one exception.

Others

During the pathological analysis, an extremely rarely diagnosed pathological phenomenon among historical populations was discovered, published previously.[8] The right calcaneus of a young adult (23–30 years old) female (Grave No. 113) was significantly shorter compared to the left side. On the basis of the observed lesions, it was possible to establish a diagnosis of calcaneal apophysitis. This disease is an inflammation of the growth plate in the heel of growing children, who exercise intensively.

Similarly to calcaneal apophysitis, femoral head epiphysiolysis also occurs in adolescents. This condition affects the physis of the proximal femur. During adolescence, the growth cartilage of the femoral head is extremely active and influenced by growth hormones. In certain situations, when the mechanical „stress" on the femoral head exceeds the resilience of cartilage growth, a separation can occur between the head and the neck of the femur. The head may „slip" back and down. This situation is called epiphysiolysis of the upper femur and profoundly alters the mechanics of the hip. It is likely that the severe deformation of the left hip of an adult male (Grave No. 134) was a consequence of femoral head epiphysiolysis. The shape and form of the femoral head were abnormal with widening and flattening clearly visible. Moreover, the head of the femur had slipped along the femoral neck, below the level of the greater trochanter. Secondary degenerative changes of the acetabular cavity could also be observed (Plate no. III, Figure 19).

Summary

Based on the results of estimation of age-at-death and sex the demographic profile of the examined series appears slightly disproportionate. The inadequate storage conditions that resulted in post mortem loss may have therefore affected the results.

The results of the metric analysis suggest a relatively homogeneous population for both sexes. Metric parameters mostly belong to the medium category, except for the value of the height of the skull which was low (tapeinokran). The average stature reflected sexual dimorphism with a 10 cm difference between females and males (169 compared to 159 cm), and was about 2 cm higher than the average stature observed in Avar age series (167.75 cm for males and 157.44 cm for females.[9]

The morphological analysis of the series revealed a relatively uniform picture: the skeletons were usually gracile with the exception of three robust male individuals. No notable differences could be observed based on the occurence of anatomical variations either.

From a taxonomic point of view, the population was almost exclusively Europid (brachycranic: cromagnoid-B and undeterminable Brachycranic; dolichocranic/mesocranic: gracile Mediterranean and cromagnoid-B), frequently showing a remarkably low cranial base (chamaecranic). Only two individuals revealed mostly Mongolid cranial features, although Mongoloid features in addition to Europid characteristics were observed in several cases.

Pathological alterations linked to development anomalies were identified, in particular with a case of hip subluxation. This anomaly probably resulted in severe claudication and caused secondary degenerative changes.

[8] Hegyi et al, 2005.

[9] Éry 1998.

The number of traumatic lesions, including bone fractures, was surprisingly low. No signs of traumatic injuries commonly associated with interpersonal violence were found. Considering the localization of the fractures, these lesions may have been related to daily activities (e.g. clavicle fracture).

Skeletal markers of occupational stress (degenerative joint diseases and enthesopathies) were not indicative of strenuous physical activity. Although their incidence was moderate to high, no severe cases were observed and they mainly affected elderly age groups.

Among mycobacterial diseases, no evidence for the presence of tuberculosis in any forms (advanced, atypical, or early stage forms of bone and joint tuberculosis) was detected. However, rhinomaxillary lesions in the skeletal remains of two individuals confirmed the presence of leprosy. Leprosy is known from the Avar Age cemeteries of Kiskundorozsma-Kettőshatár and Kiskundorozsma-Daruhalom in the Danube Tisza Interfluve.[10] Examination of the Hajós series highlights confirmed previous theories that this severe infectious disease was more widespread in the Danube Tisza interfluve.

Based on the lack of bone lesions suggestive of nutritional deficiencies (e.g. enamel hypoplasia, scurvy, severe forms of porotic hyperostosis), the diet of the Hajós population would seem to have been appropriate.

The taxonomic composition of the series (Europid characteristic) differs from the predominantly Mongolid populations of the Danube Tisza interfluve (e.g Kiskőrös-Cebepuszta, Kiskőrös-Vágóhíd, Csengele-Feketehalom, Madaras-Téglavető dűlő, Kunszállás-Fülöpjakab;[11] Kunpeszér-Felsőpeszéri út;[12] Felgyő-Ürmös tanya;[13] Kunszállás-Fülöpjakab;[14] Hetényegyháza-Mária),[15] although in the southern regions of Transdanubia and the Danube Tisza Interfluve surrounding Hajós, some Avar Age populations show almost exclusively Europid characteristic (Kiskőrös-Város alatt, Kiskőrös-Pohibuj-Mackó dűlő, Kecel I, Homokmégy-Halom, Mélykút-Sáncdűlő, Sükösd-Ságod, Szekszárd-Palánk, Szebény, Kékesd;[16] Stara Moravica-Bácskossuthfalva;[17] Pécs-Nagyárpád, Boros utc;[18] Sükösd-Ságod).[19]

The studied anthropological series could be related to the Europid populations from the southern part of Transdanubia and the Danube Tisza interfluve, especially to those who are characterized by significant brachycrany (Kiskőrös-Város alatt, Kecel I, Homokmégy-Halom, Sükösd-Ságod).

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List of journal abbreviations

Act Biol Szeg – Acta Biologica Szegediensis
Am J Phys Anthropol – American Journal of Physical Anthropology
Ann Eug – Anals of Eugenics
Annls hist.-nat. Mus. natn. hung – Annales historico-naturales Musei nationalis hungarici
Annu Rev Anthropol – Annual Review of Anthropology
Anthrop Hung – Anthropologia Hungarica
Arch Cum – Archaeologia Cumanica
Arch Hung – Archaeologia Hungarica
Biol Kozl – Biológiai Közlemények
Bull Mém Soc Anthr – Bulletin de la Société d'Anthropologique de Paris
Hum Evol – Human Evolution
Hum. Biol. Budapestiensis – Humanbiologia Budapestiensis
MFMÉ – MA – Móra Ferec Múzeum Évkönyve – Monumenta Archaeologica
Rad VM – Rad vojvođanskih muzeja

Figure legends

Figure 1: Chamaecranic skull – frontal view (Grave No. 156, adult, female)
Figure 2: Chamaecranic skull – lateral view (Grave No. 156, adult, female)
Figure 3: Mandibular tori (Grave No. 89, mature, male)
Figure 4: Skull with Mongoloid features – frontal view (Grave No. 107, adult, female)
Figure 5: Skull with Mongoloid features – lateral view (Grave No. 107, adult, female)
Figure 6: Unilateral hip subluxation (left side) (Grave No. 136, adult, female)
Figure 7: Healed parry fracture – left ulna (Grave No. 162, mature, male)
Figure 8: Cloaca in the fracture of the clavicle indicating infection – inferior view (Grave No. 138, mature, male)
Figure 9: Healed skull injury – right parietal bone (Grave No. 57, senile, female)
Figure 10: Widened piriform aperture with rounded margins (without Grave No., adult, female)
Figure 11: Resorption of the edge of the piriform aperture (left side) (without Grave No., adult, female)
Figure 12: Abnormal porosity and perforations of the hard palate (without Grave No., adult, female)
Figure 13: S evere periosteal apposition of the mandible (without Grave No., adult, female)
Figure 14: Resorption of the anterior nasal spine and widened piriform aperture (Grave No. 81, juvenile, female)
Figure 15: Resorptive lesion between the two upper central incisors (Grave No. 81, juvenile, female)
Figure16: Severe porosity and destructive focus of the hard palate (Grave No. 81, juvenile, female)
Figure 17: Periosteal apposition on the right tibia (Grave No. 81, juvenile, female)
Figure 18: Periostitis and destructive lesion probable related to a skin ulcer of the left tibia (Grave No. 81, juvenile, female)
Figure 19: Epiphysiolysis capitis femoris (Grave No. 134, senile, male)

Tables

Age group / Sex	Inf. I.	Inf. II.	Juvenis	Adultus	Maturus	Senium	Indet	Altogether
Male	-	-	2	10	25	7	-	44
Female	-	-	5	36	9	9	3	62

Indet	17	8	2	-	-	-	2	29
Altogether	17	8	9	46	34	16	5	135
Subadults 34 (25%)				Adults 101 (75%)				**135**

Table 1: The age at death and sex distribution

Males									
Martin No	1	5	8	9	17	40	45	46	47
n	31	24	27	32	24	22	18	23	16
v_{max}	195	117	155	107	135	111	142	122	126
v_{min}	161	90	133	85	116	89	125	85	108
$\bar{x}$	181	101	146	96	129	99	135	99	118
s	7,5	5,4	5,5	5,3	5,5	5,5	4,5	7,3	5,9

Martin No	48	51	52	54	55	66	69	70	71
n	27	26	27	28	26	25	28	14	14
v_{max}	76	44	37	30	59	119	42	70	40
v_{min}	63	37	30	22	41	90	30	54	30
$\bar{x}$	70	40	34	26	52	106	34	63	34
s	1,6	1,6	1,9	1,7	4,4	7,5	2,7	5,5	3,2

Indices	8:1	17:1	17:8	9:8	47:45	48:45	52:51	54:55
n	26	24	22	25	13	19	25	22
v_{max}	87,0	81,3	97,8	75,3	94,7	60,7	92,5	65,8
v_{min}	76,8	60,1	75,8	60,8	79,0	48,5	71,4	42,9
$\bar{x}$	80,4	71,2	88,7	66,0	86,7	52,6	83,3	50,4
s	2,4	4,3	5,0	3,7	3,8	3,3	5,1	4,8

Table 2: Metric data – males

Females										
Martin No	1	5	8	9	17	40	45	46	47	48
n	37	29	35	42	29	24	25	26	19	26
v_{max}	194	115	150	101	135	100	135	102	128	74
v_{min}	153	85	130	86	113	83	117	81	101	58
$\bar{x}$	176	96	140	94	124	93	125	93	110	67
s	7,7	6,1	4,2	4,2	5,9	5,0	5,0	4,9	6,7	4,2

Martin No	51	52	54	55	62	63	65	66	69	70	71
n	29	29	28	27	16	14	24	27	33	18	20
v_{max}	41	38	27	54	48	42	125	105	40	68	39
v_{min}	32	28	20	44	41	33	97	85	26	52	26
$\bar{x}$	38	33	24	49	44	38	113	93	30	60	31
s	2,0	2,3	1,7	2,7	1,9	3,1	5,9	5,2	2,7	4,0	3,0

indices	8:1	17:1	17:8	9:8	47:45	48:45	52:51	54:55	63:62
n	34	27	26	33	18	23	29	25	13

v_{max}	92	83	97	74	97	60	119	60	98
v_{min}	72	63	77	59	82	48	77	39	77
$\bar{x}$	79	71	89	69	88	53	88	50	88
s	3,7	4,1	5,0	3,1	4,4	3,1	7,7	4,9	5,8

Table 3: Metric data – females

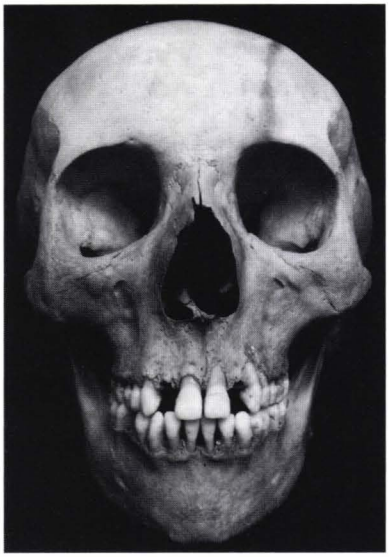


Figure 1

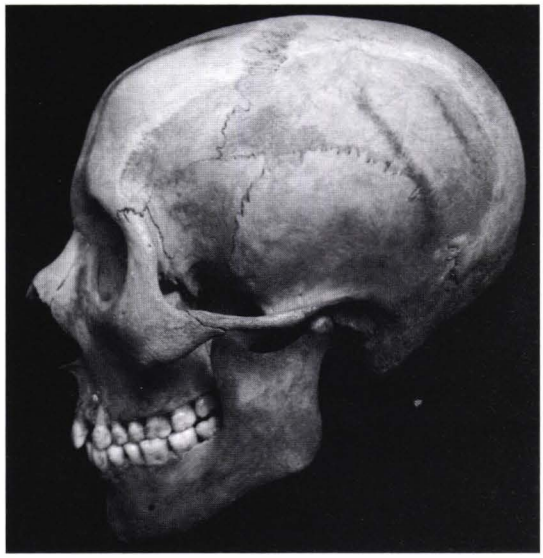
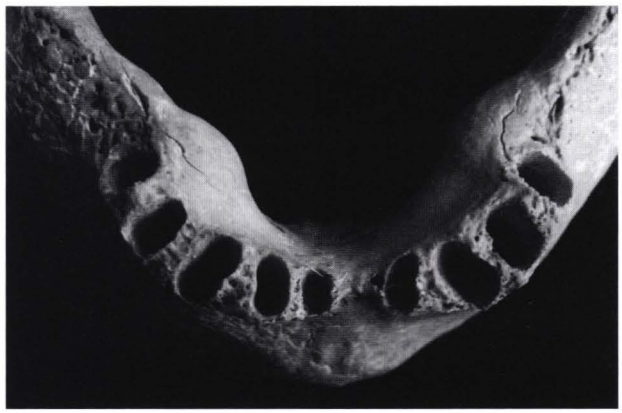


Figure 2



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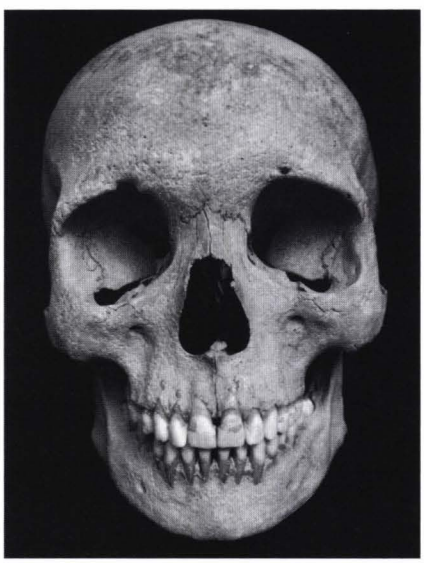


Figure 4

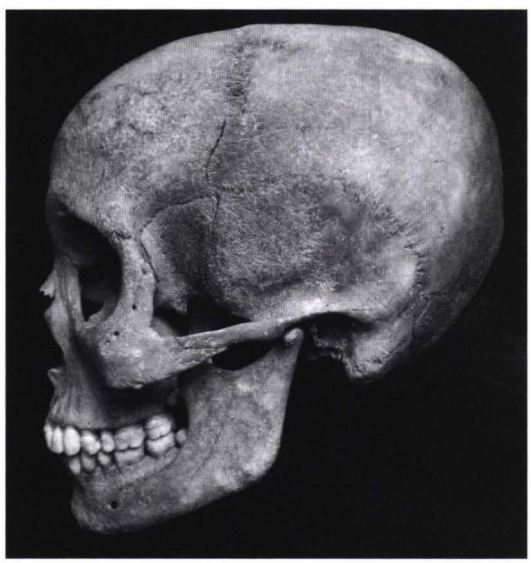
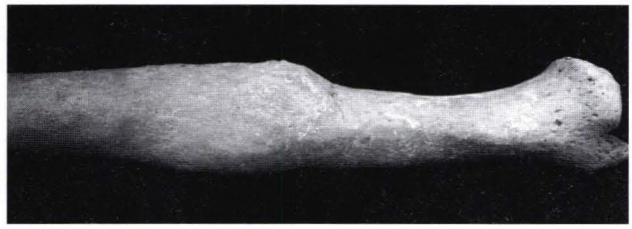


Figure 5

Plate no. I.

Figure 6



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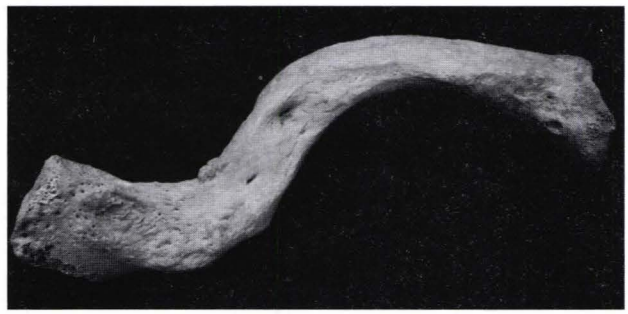


Figure 8

Figure 9

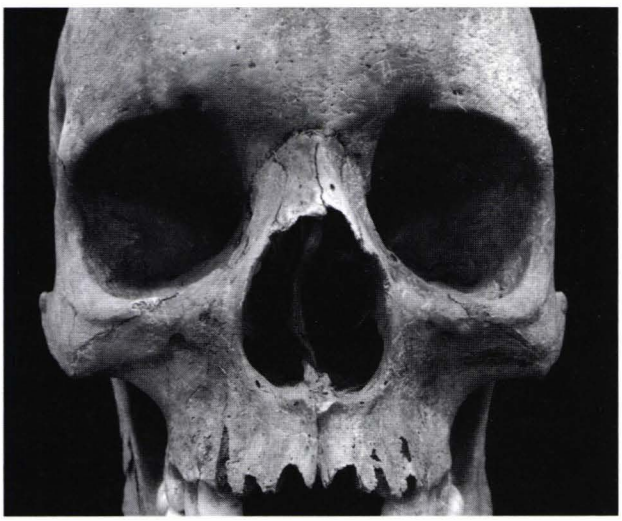


Figure 10

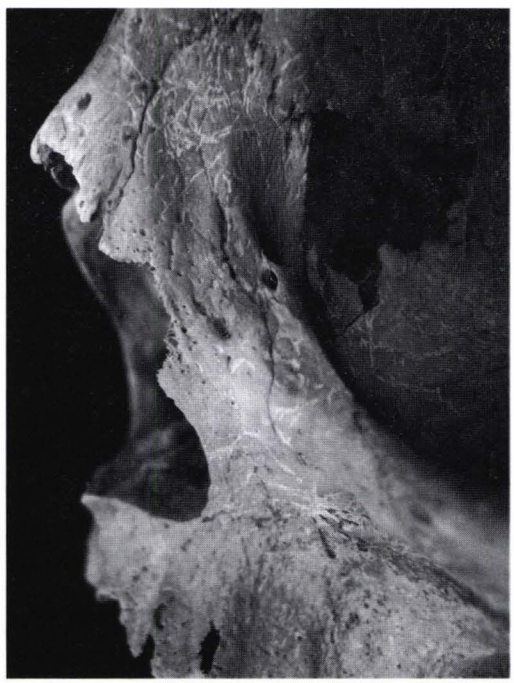


Figure 11

Plate no. II.

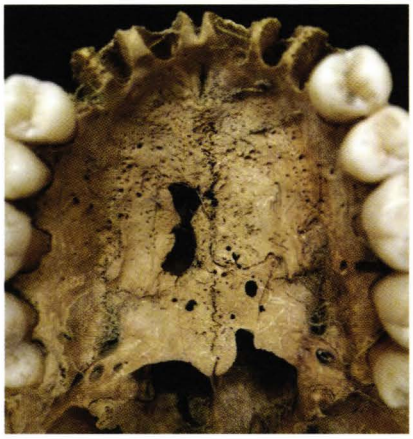


Figure 12

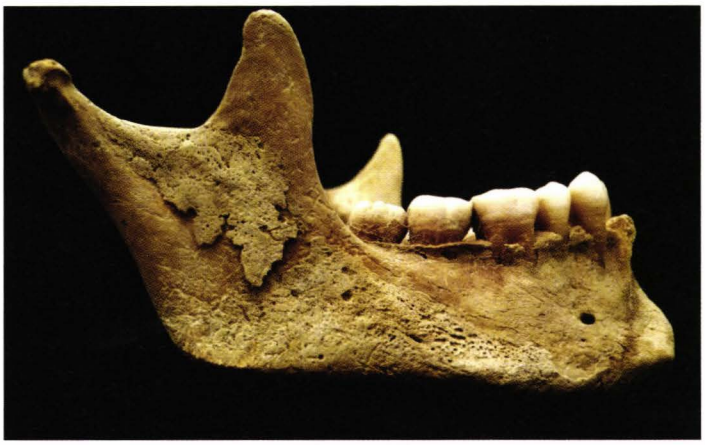


Figure 13

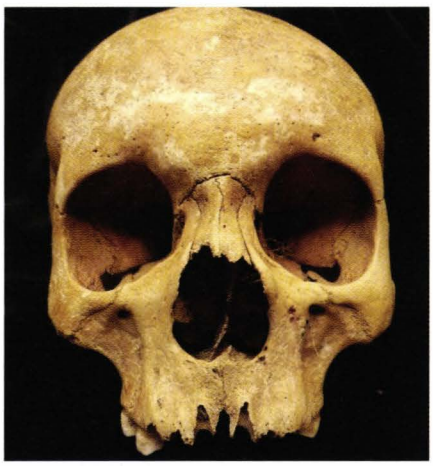
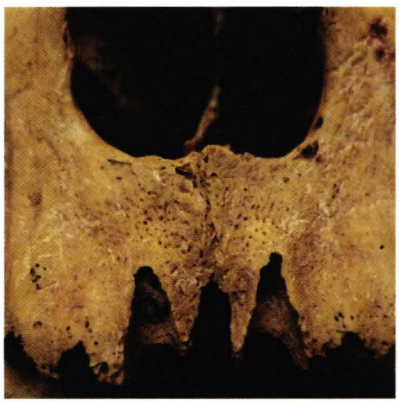


Figure 14



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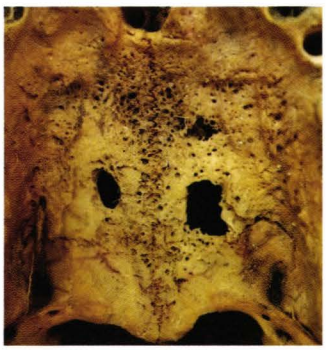

Figure 16

Figure 17

Figure 18

Figure 19

Plate no. III.

TUBERCULOSIS PALEOPATHOLOGY RESEARCH IN THE SZEGED ANTHROPOLOGICAL COLLECTION: NEW DATA FROM THE AVAR AGE

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Short title: Avar Age tuberculosis from the Szeged Anthropological Collection
Keywords: paleopathology, paleomicrobiology, skeletal tuberculosis, *Mycobacterium tuberculosis*, Avar Age, Szeged Anthropological Collection, Hungary

Abstract

Tuberculosis (TB) paleopathology research in the Szeged Anthropological Collection (SAC) has begun in the 1970's; several typical skeletal TB cases were identified and published in the last decades of the 20th century. Between 2009 and 2015 the Hungarian National Scientific Research Fund (OTKA) provided us with funding for a new comprehensive research project on the paleopathology and paleoepidemiology of TB. During these studies in the SAC we have studied skeletal materials belonging to 51 archaeological sites from the Neolithic Period to the Late Middle Ages, totaling 11001 individuals.500 cases of possible skeletal TB (typical/advanced-stage TB: 53; atypical/early-stage TB: 447) were found. 57/ 112 samples furnished positive a DNA and/or lipid biomarker results for the presence of the Mycobacterium tuberculosis complex.

As the first TB case identified in the SAC was found in an Avar Age osteological series and as TB-like pathological conditions were always frequently observed in our Avar Age anthropological material, a particular attention was given to the study of TB occurrence in this archeological period. In this study we briefly summarize new results of the Avar Age TB paleopathology research in the SAC, and we give more detailed presentation of two new studies from this field. First, we present the complex

macromorphological and paleomicrobiological case study of a very special Avar Age skeletal TB from the "Csongrád-Felgyő, Ürmös-tanya" anthropological material. In the second part of our paper we detail our most complete series-level morphological and biomolecular ancient TB analysis in the 8th century AD "Bélmegyer-Csömöki domb" series.

Introduction

Tuberculosis is one of the oldest known infectious diseases, which is caused by a group of closely related bacteria of the *Mycobacterium tuberculosis* complex, most often by *M. tuberculosis* and *M. bovis*.[1] The most common form of the disease is pulmonary TB, but in the minority of the cases (approximately 15–20%) the bacteria spread from the initial site of infection to other compartments of the human body, including the skeletal system, resulting in extra-pulmonary TB. Data indicate that about 3–5% of patients with chronic tuberculosis suffer from osseous involvement.[2]

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 twothirds of the vertebral changes are associated with other TB alterations.After vertebral involvement, the second most frequent alteration in TB is arthritis of the large, weight-bearing joints.[3] Beside the classic bone alterations, there are also several early-stage or minor osseous lesions that can be indicative of tuberculosis, such as superficial vertebral changes, including hypervascularisation, endocranial alterations, proliferative rib changes and diffuse periosteal new bone formation.[4]

In the paleopathological diagnosis of ancient TB cases, complementary analyses, especially biomolecular techniques, might provide invaluable help since they can detect ancient pathogen remains and therefore support the osteological diagnosis.[5] Since 2000, the identification of skeletal tuberculosis in ancient human remains has been facilitated by the confirmation of atypical/early-stage TB lesions by new biomolecular methods based on mycobacterial a DNA and lipid biomarker analyses.[6]

In Hungary, the paleopathological investigation of skeletal remains from various archaeological periods has an almost a century year old history. The study of specific infectious diseases, such as tuberculosis has been specially emphasized.

TB paleopathology research in the osteoarchaeological collection of the Department of Biological Anthropology, University of Szeged, Hungary ('Szeged Anthropological Collection – SAC', which represents more than 30 thousand skeletons actually) has begun in the 1970's. The

[1] Ortner, 2003; Gutierrez et al., 2005; Hershkovitz et al., 2008; Donoghue, 2009; Comas et al., 2013; Baker et al., 2015; Donoghue et al., 2015; Pálfi et al., 1999, 2015.

[2] Ortner, 2003; Donoghue, 2009.

[3] Resnick and Niwayama, 1988; Ortner, 2003.

[4] Roberts et al., 1994; Baker, 1999; Schultz, 1999; Hershkovitz et al., 2002; Pálfi, 2002; Maczel, 2003; Masson et al., 2013.

[5] Spigelman et al., 1993; Donoghue et al., 1998, 2015; Guernay et al., 1999; Mays et al., 2001; Zink et al., 2001; Minnikin et al., 2012.

[6] Haas et al., 2000; Zink et al., 2007; Hershkovitz et al., 2008; Masson et al., 2013, 2015

mostly morphological research results of the first 25 years have been summarized in a paper by Pálfi and Marcsik[7] in 1999 that also served as the first publication in this field with an epidemiological approach to the topic (32 archaeological sites, 5848 individuals, 31 identified typical TB cases).

At the turning of the millennia, TB research in Szeged became more intensive with a new focus on registering atypical TB lesions and including more molecular diagnostics in our research portfolio with the help of international cooperation. Between 2009 and 2015 the Hungarian National Scientific Research Fund (OTKA) provided us with funding for a new comprehensive research project on the paleopathology and paleoepidemiology of TB.

The first TB case identified in the SAC was found in a 7–8th century AD Avar Age osteological series and was published in 1972 by Antónia Marcsik.[8] This case was a typical form of chronical *spondylitis tuberculosa* (Pott's disease) with serious spinal deformity and ankylosis (Plate no. I, Fig. 1 and 2) and was subject of CT- and molecular examinations in 1999.[9] As a relatively high number of different TB-related pathological conditions were observed in our Avar Age anthropological material during the next decades,[10] in the above mentioned OTKA-project a particular attention was given to the study of TB occurrence in this archeological period.

In this current paper we briefly summarize the results of the Avar Age TB paleopathology research in the SAC from the 1970's to the present day, and we give more detailed presentation of two new studies from this field. First, we present the complex macromorphological and paleomicrobiological case study of a very special Avar Age skeletal TB from the "Csongrád-Felgyő, Ürmös-tanya" anthropological material. In the second part of our paper we detail our most complete series-level morphological and biomolecular ancient TB analysis in the 8th century AD "Bélmegyer-Csömöki domb" series.

The ancient TB studies in the Szeged Anthropological Collection (SAC) and the Avar Age TB research

Between 2009 and 2015 the Hungarian National Scientific Research Fund (OTKA) provided us with funding for a new comprehensive research project on the paleopathology and paleoepidemiology of TB.

During the 5 years of the mentioned "*THE PALEOEPIDEMIOLOGY OF TUBERCULOSIS*" OTKA research project (OTKA no K–78555; 2009–15) in the SAC we have studied skeletal materials belonging to 51 archaeological sites from the Neolithic Period to the Late Middle Ages (Plate no. I, Fig. 3), totalling 11001 individuals.

The chronological distribution of this important amount of human skeletal material is presented in Fig. 4. The Avar Age skeletons (2057) represented approximately 20% of the total amount.

By our morphological studies, 500 cases of possible skeletal TB (typical/advanced-stage TB: 53; atypical/early-stage TB: 447) were found. The morphological studies were completed by different biomolecular analyses. 57 samples furnished positive aDNA and/or lipid biomarker results for the presence of the *Mycobacterium tuberculosis* complex. The important fees of the aDNA and lipid biomarker studies did not allow the realization of systematic paleomicrobiological analyses: samples from 112 individuals could be tested. 57 aDNA and/or lipid biomarker positive cases were found among the tested 112 samples. These biomolecular results complete (or make stronger) the results of our previous macromorphological research.

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[7] Pálfi and Marcsik, 1999.

[8] Marcsik, 1972.

[9] Haas et al., 1999; Marcsik et al., 1999.

[10] Pálfi, 1991; Pálfi et al., 1992; Marcsik and Pálfi, 1999.

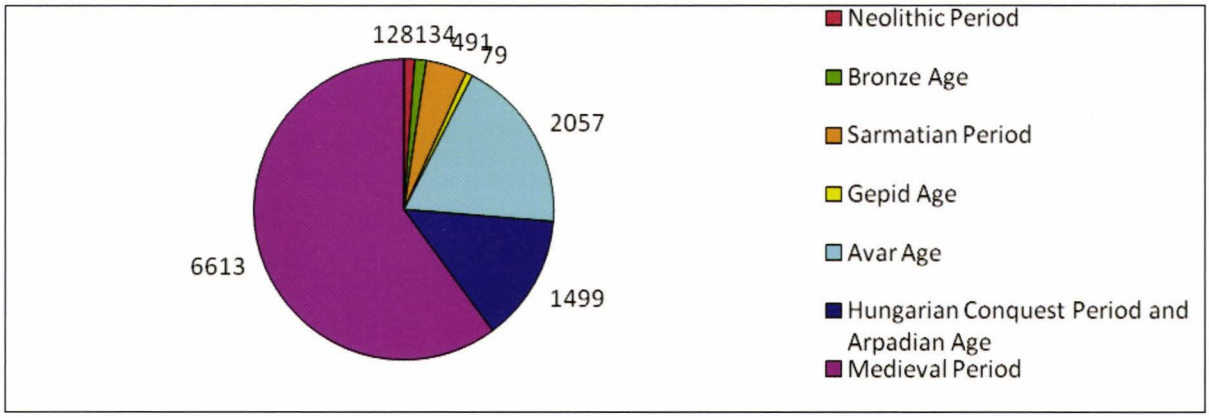

Fig. 4.

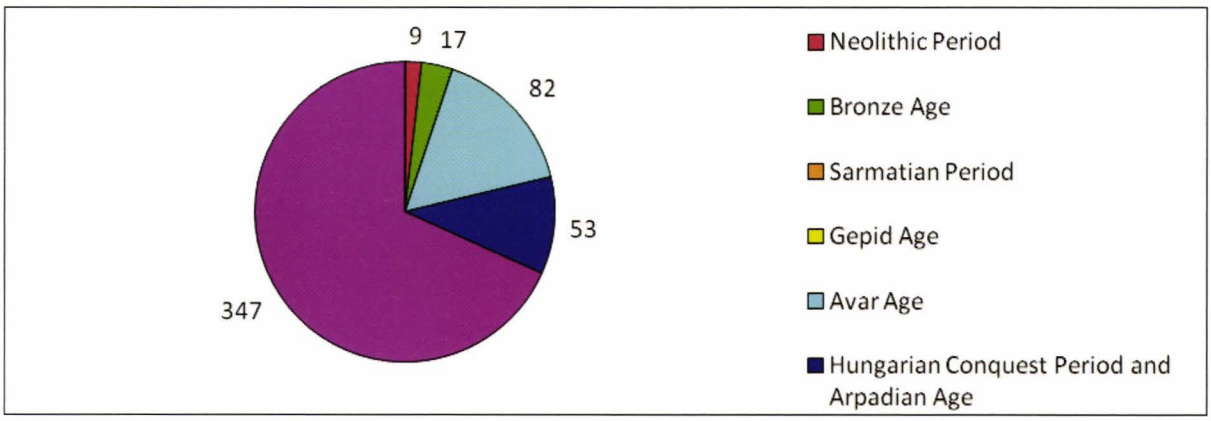

Fig. 5.

As for the Avar Age material, the morphological studies furnished 82 possible cases (Fig. 5) of skeletal TB (among them 15 advanced-stage / chronical forms). 38 cases were tested by paleomicrobiology, among them 25 cases furnished positive aDNA and/or lipid biomarker results for the presence of the *Mycobacterium tuberculosis* complex.

NEW RESULTS FROM THE AVAR AGE SKELETAL MATERIAL IN THE SZEGED ANTHROPOLOGICAL COLLECTION

Case-study: Paleopathological / paleomicrobial analysis of an atypical vertebral TB in an Avar Age skeleton (Csongrád-Felgyő, Ürmös-Tanya, Hungary)

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 biomolecular analyses.

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.[11] 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.[12] Although the skeletal remains of 219 individuals had been recovered from the 216 excavated gravesof 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.[13] 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.[14] This was the name used in publications presenting the occurrence of skeletal TB from different parts of this site.[15] 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".

Methods

The morphological studies

By utilizing standard sexing[16] and aging[17] methods, individual no. 205 was identified as a female adult, approximately 20–30 years old.

[11] Balog, 2010.
[12] Balog, 2010.
[13] Marcsik 2010.
[14] László, 1965.
[15] Pálfi and Marcsik, 1999; Maczel et al., 2009.
[16] Bass, 1995.
[17] Acsádi and Nemeskéri, 1970; Lovejoy, 1985.

The skeleton was subjected to a detailed paleopathological analysis at the Department of Biological Anthropology, University of Szeged. The detection of pathological changes was principally based on standard gross morphological analysis,[18] 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.

The 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.[19] 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 analysiscarried 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.[20] Following centrifugation for 15 min at 3000 g, 0.5 ml of the supernatant was removed and 1ml guanidine isothiocyanate solution and diatomaceous earthwere added.[21] After incubation on a rotatory mixer for another 2 hours, 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. 1µl 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 20–40 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

[18] Resnick and Niwayama, 1988; Ortner, 2003; Roberts and Manchester, 1995; Aufderheide and Rodriguez-Martin, 1998.

[19] Spigelman and Lemma, 1993; Haas et al., 2000; Zink et al., 2007; Pósa et al., 2013.

[20] Boom et al., 1990.

[21] Evison et al., 1997.

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

For the specific amplification of mycobacterial DNA a primer pair targeting a 123 base pair (bp) segment of the repetitive sequence IS6110 of the *M. tuberculosis* complex was used, which covers *M. tuberculosis, M. bovis, M. microti, M. africanum* and *M. canettii*.[22] The PCR reaction mix contained 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl2, 200 µM of each deoxynucleotide trisphosphate (Amersham Pharmacia, Uppsala, Sweden), 1 µM of each primer, 0.025 U/µl AmpliTaq Gold (PE Biosystems, Foster City, CA) and 0.5 µL extracted DNA to a final volume of 20 µl. PCR-conditions were as follows: 10 min at 95°C followed by 45 cycles of 94°C for 1 min, 66°C for 1 min and 72°C for 1 min. After the final cycle another 8 min at 72°C were added.

– Amplification of human DNA

To test whether amplifiable DNA was present in the sample and to ascertain that the PCR was not inhibited, a 202 bp segment of the human b-actin gene was amplified in parallel. The PCR mixture was prepared as described above. The following amplification protocol was used: 10 min at 95°C, 45 cycles of 94°C for 1 min, 60°C for 1 min and 72°C for 1 min, and final extension at 72°C for 8 min. As a further amplification control, segments of the amelogenin gene, which is located on the human sex chromosomes, was amplified. The amplification product of the amelogenin gene is a 112bp fragment from the Y-chromosome and a 106bp fragment of the X-chromosome.[23] Therefore males will show two PCR products, while females give a single amplification product. The amplification protocol for the amelogenin gene was as follows: 10min at 95°C, 45 cycles of 94°C for 1 min, 60°C for 1 min, 72°C for 1 min, and a final extension at 72°C for 8 min.

– Detection of PCR and digestion products

The PCR products were separated by electrophoresis on a 4% agarose gel and visualised on a UV-screen after staining with ethidium bromide. PCR products of the amelogenin gene amplification were additionally separated on a 15% polyacrylamide gel and visualised by silver staining.

– Sequence analysis of PCR products

The nucleotide sequences of the PCR products were determined by direct sequencing: after electrophoresis on a 4% low-melting-point agarose gel, the respective fragment of the PCR reaction was eluted with a purification kit (Freeze'n Squeeze, Bio-Rad, Hercules, CA). With the eluted DNA, cycle sequencing was performed with a dye terminator cycle sequencing kit (PE Biosystems). Automatic sequencing was performed on an ABI PRISM 310 Genetic Analyzer (PE Biosystems).

– Complementary paleomicrobial study carried out at the a DNA Laboratory, EURAC, Bolzano

In order to confirm the first a DNA results in an independent laboratory to complement the first results, a second series of analysis was carried out at the ancient DNA Laboratory of the EURAC Institute for Mummies and the Iceman, Bolzano, Italy. Sample preparation and DNA extraction was performed in a dedicated pre-PCR area following the strict procedures required

[22] Eisenach et al., 1990; Soolingen (van) et al., 1997.

[23] Nakahori et al., 1991.

for studies of ancient DNA: use of protective clothing, UV-light exposure of the equipment and bleach sterilization of surfaces, use of PCR workstations and filtered pipette tips. Within a designated sample preparation room the outer surface of the bone samples was mechanically removed by using a Dremel speed rotary tool. Cleaned samples were pulverized using a Retsch mixer mill.

The following three different skeletal samples from individual no.205 were further subjected to DNA extraction and PCR-based analysis: a rib fragment (EURAC-ID 1288), the spongiosa of a fibula (EURAC-ID 1289) and a vertebra fragment (EURAC-ID 1290). DNA extraction was performed with 250 mg of bone powder using the silica-based DNA extraction technique described by Rohland and colleagues with minor modifications (Rohland et al, 2009). The presence of TB DNA was assessed by applying a PCR-based assay targeting the MTBC multicopy IS6110 region.[24] The PCR reaction contained 10 mM tris-HCl (pH 8.3), 50 mM KCl, 1.875 mM MgCl2, 200 μM of each deoxynucleotide trisphosphate, 0.5 μM of each primer, 0.1 mg/ml Bovine serum albumin, 0.05 U/μl AmpliTaq Gold (Applied Biosystems, Foster City, CA), and 4 μl of extracted DNA to a final volume of 50 μl. The PCR reaction was carried with the following conditions: 10 min at 95°C followed by 45 cycles of 95°C for 1 min, 68°C for 1 min and 72°C for 1 min. After the final cycle another 8 min at 72°C were added. Amplification products were further subjected to sequencing and sequence analysis via NCBI blastN.[25] To further diagnose and possibly subtype MTBC bacteria, spoligotyping was used on the IS6110 positive specimen. The method was performed as described by Kamerbeek et al.with minor modifications.[26]

Results and discussion

The morphological findings

The macromorphological study of the postcranial skeleton revealed multiple lytic lesions on the thoracic and lumbar vertebral bodies (Plate no. II, Figures 6a-b). The round lytic areas, which varied in size from 2 to 40 mm, were characterized by smooth marginal zones and space-occupying mass appearance (Plate no. II, Figures 6a). Except for the seventh cervical vertebra, the cervical area did not seem to be affected. The considerable loss of spongy bone in the thoracolumbar vertebral bodies, especially between T10 and L3, had resulted in angular deformity and fusion of the affected vertebrae (T12-L3; Plate no. II, Figure 6b).

The same type of osteolytic lesions was detected in the posterior part of the vertebral bodies, the vertebral processes and on the head and neck of the ribs. Signs of a pathological process were visible in the pronounced woven aspect of the posterior part of the sternum. Furthermore, a slight unilateral sacroiliitis was also observed on the right pelvic bone. However, neither the tubular bones (long or short), nor their joints showed any pathological changes.

Regarding the skull, slight abnormal blood vessel and small granular impressions were observed on the endocranial surface of the frontal and parietal bones. Additionally, a bilateral *cribra orbitalia* accompanied the aforementioned alterations.

To ascertain our macromorphological diagnosis, we carried out X-ray and CT analyses of the affected vertebrae and ribs, which revealed abnormal bony structures and cystic zones of destruction (Plate no. II, Figures 7a-b). The lesions were not clearly bordered in all instances by areas of increased density. However, a significant remodelling of the osseous structure could be seen, with

[24] Eisenach et al., 1990; Soolingen (van) et al., 1997.

[25] Altschul et al., 1997.

[26] Kamerbeek et al., 1997.

a secondary new bone layer covering the majority of the osteolytical lesions and remodelled vertebral surfaces.

The pathological changes in individual no.205 can mostly be characterized as typical of TB in the spinal angulation of the lower thoracic and upper lumbar vertebrae, with the bone destructive process more pronounced than the bone reparative process. The observed spinal lesions therefore corresponded to a morphology described as skeletal TB.

However, other features observed in this individual were unusual and could also be attributed to other diseases.[27] The atypical nature of this case consisted of the fact that more than four vertebrae were involved, posterior vertebral elements were also affected (transverse processes), and the vertebral endplates seemed to have been spared by the disease process. Kaufmann described a similar case of vertebral TB belonging to the anatomopathological Museum of Bâle.[28] An illustration from Billroth and von Winiwarter's 19th-century medical book (Plate no. II, Figure 8) also showed multiple lytic TB lesions in a spinewith tubercular caries extending over the ribs, exactly like the case of individual no.205.[29] In this instance, the authors recommended to consider, other diseases such as actimomycosis in the differential diagnosis.

In fact, bone changes similar to those of tuberculosis can result from a considerable number of diseases, several of which could be excluded in the case of individual no.205. Conditions that were considered in the final differential diagnosis for TB in this case study included diseases with potential for destructive spinal changes and possible extension, such as chronic pyogenic osteomyelitis, brucellosis, mycotic infection (esp. blastomycosis), eosinophilic granuloma and metastatic bone tumors.[30] However, the large degree of vertebral destruction, gibbus formation and lack of sequestra eliminated the possibility of *vertebral osteomyelitis*. There was no evidence of considerable new bone proliferation characteristic of *brucellosis*. The smooth-walled, often coalesced lesions observed in this case were also in direct opposition to the isolated, irregular lesions with fronts of resorption characteristic of *blastomycosis*. In spite of the fact that the space occupying mass appearance was characteristic of *eosinophilic granuloma*, the lack of 'geographic shaped' destructive lesions on the skull, mandible or flat bones also led to the exclusion of *histiocytosis X*. Since cortical remodelling and vertebral fusion were not a feature of *metastatic cancer*, this condition was also excluded.

Conversely, the macromorphological aspect of the endocranial changes observed on the skull highly resembled other cases of TB meningitis in the paleopathological record.[31] While the differential diagnosis of the pathological changes observed macromorphologically therefore favoured tuberculosis, some uncertainty remained regarding the assignation of these alterations to this disease due to their unusual features.

The biomolecular findings

To confirm the osteological diagnosis of possible TB infection, DNA was extracted from affected vertebral remains and was subjected to biomolecular analysis. In search of demonstrative proof for TB infection, the amplification procedure proved to be positive for the targeted gene segments of *Mycobacterium tuberculosis* complex DNA. A 123bp fragment of the insertions sequence IS6110 was successfully amplified in two samples from affected vertebral bodies (Figure 9a). These samples

[27] Kelley and El-Najjar, 1980; Zimmerman and Kelley, 1982; Resnick and Niwayama, 1988; Ortner, 2003; Roberts and Manchester, 1995; Aufderheide and Rodriguez-Martin, 1998.

[28] Kaufmann, 1903.

[29] Billroth and Winiwarter (von), 1887.

[30] Hershkovitz et al., 1998.

[31] Schultz, 1999, 2001; Hershkovitz et al., 2002; Pálfi et al., 2012.

were also found to be positive for human aDNA (amelogenin and beta-actin). All blank controls were negative. The direct sequencing of amplified DNA of the IS6110 insertion sequence in these cases provided a mycobacterial sequence confirming the presence of *M. tuberculosis complex* DNA.

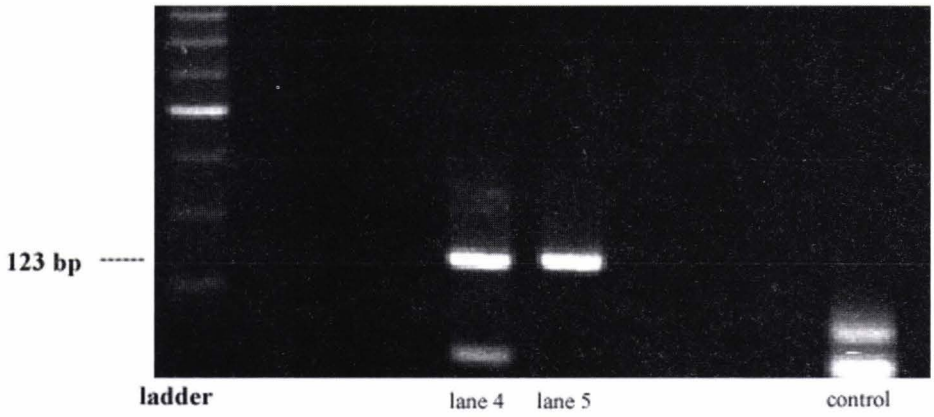

Fig. 9a

In order to confirm the results of the first biomolecular study and to specify the species of the infectious agent, a second DNA analysis was carried out at the ancient DNA laboratory of the EURAC Institute for Mummies and the Iceman, Bolzano, Italy. DNA was extracted from three different skeletal sites (rib, fibula and vertebra) of the individual and further subjected to PCR-based detection of MTBC DNA. The vertebra sample proved to be positive for the IS6110 repetitive element thereby complementing the results of the first laboratory.

Fig. 9b.

Moreover, to further subtype the MTBC strain involved in the infection we subjected the DNA of the vertebra to spoligotyping analysis. The retrieved patchy spoligotyping pattern displayed in Figure 9b supported the results of the presence of MTBC DNA in the specimen. Interestingly, the occurrence of the spoligotyping spacers 42 and 43 indicated the presence of bacteria belonging to the *M. tuberculosis* lineages and excluded the infection with *Mycobacteria* belonging to the *M. microtii/M.bovis* lineage, the latter lacking spacers 39 to 43.[32] However, these results could only offer a rough initial estimation. In order to investigate the MTBC lineage affiliation further, it was decided that the analysis would be extended in the future to the detection of the absence or presence of a *M. tuberculosis* specific deletion (TbD1) as well as the loss of region of difference 9 (RD9), characteristic of *M. africanum, M. microti*, and *M. bovis* strains.[33]

[32] Smith et al., 2006, 2009.
[33] Zink and Nerlich, 2004.

SERIES-STUDY: PALEOPATHOLOGICAL / PALEOMICROBIAL ANALYSES OF TB IN 8TH CENTURY AD SKELETONS FROM BÉLMEGYER-CSÖMÖKI DOMB, HUNGARY

Macromorphological analysis of skeletons, from 20 selected graves of the 8th century AD Bélmegyer-Csömöki domb, revealed 19 cases of possible skeletal tuberculosis. Biomolecular analyses – aDNA and lipid biomarker studies – provided general support for such diagnoses, including an individual without pathology, but the data did not show coherent consistency over the range of biomarkers examined. Our series-study gives some details of this complex research work.

Former ancient TB studies in the Bélmegyer-Csömöki domb series

In 1990, the first paleopathological analyses of the 8th century AD Bélmegyer-Csömöki domb series were essentially based on macromorphological and radiological examinations, biomolecular methods were used only in a few cases. From a macromorphological point of view, those investigations only considered classical TB alterations.[34] An advanced-age female skeleton from the grave No. 65 showed severe osteolytic lesions of the anterior portion of the thoracic and lumbar vertebral bodies, causing an unequal collapse, which led to angular kyphosis (Plate no. III, Fig. 10a-b).[35]

Mycobacterial DNA targets IS*6110* and the 65-kDa antigen gene of the *Mycobacterium tuberculosis* complex (MTBC) were found in samples from this specimen.[36]

In another case, of a mature male individual (grave No. 90), the pathological remodelling and fusion of the lumbosacral region and the irregular *ante-mortem* erosion on the ventral surface of the sacrum, support the diagnosis of a lumbosacral tuberculous involvement with cold abscess. In addition, the severe destruction both of the left hip bone and proximal femur is suggestive of tuberculous arthritis or *coxitis tuberculosa* (Plate no. III, Fig. 11a-b).[37] The diagnosis of skeletal TB was confirmed by biomolecular results, the identification of the DNA-fragment (65-kDa antigen gene) of the MTBC was successful.[38]

In a further case, the complete ankylosis of the right knee indicated *gonitis tuberculosa* of an elderly male individual from grave No. 215.[39] Marcsik and co-workers published two further classical TB cases in 2007. A young female skeleton from grave No. 38 exhibited signs of probable tuberculous arthritis (*coxitis tuberculosa*) of the right hip joint. Skeletal remains of an adult male individual (grave No. 189) presented complete ankylosis of the 9th and 10th thoracic vertebrae and fusion of the 1st and 2nd and the 3rd and 4th lumbar vertebrae. In addition, new bone formation and osteophytes were found on the ventral surfaces of all lumbar vertebral bodies. These alterations suggest the diagnosis of spondylitis tuberculosa.[40]

The above mentioned former investigations of the series from the Bélmegyer-Csömöki domb have provided interesting paleopathological cases of skeletal tuberculosis. However, the complete skeletal material has never been analysed systematically for both classical and early-stage TB lesions, and biomolecular analyses had been undertaken only in a few cases. The recent development of diagnostic criteria in the field of paleopathology of TB and biomolecular methods for

[34] Pálfi and Csernus, 1990; Pálfi, 1991; Pálfi et al., 1992.
[35] Pálfi, 1991.
[36] Haas et al., 2000.
[37] Pálfi et al., 1992.
[38] Haas et al., 2000.
[39] Pálfi and Csernus, 1990.
[40] Marcsik et al., 2007.

detection of the MTBC encouraged us to perform a re-examination of the series in the OTKA project "Paleoepidemiology of Tuberculosis".

Material and Methods

Archaeological background

The skeletal material for this study derives from the archaeological site of the Bélmegyer-Csömöki domb, rising about three kilometres south-east of the village Bélmegyer, in South-Eastern Hungary. During a long-running excavation (1985–1989), skeletal remains of 240 individuals were unearthed. On the basis of the grave goods found in the completely excavated cemetery, it was used for burials between 670–800 AD during the late Avar Age.[41]

Our research strategy was to combine different diagnostic methods in order to get independent verification using different biomarkers. First we conducted the morphological analysis of the skeletal series. Next, bone samples were taken from the skeletal remains of the suspected TB cases. Small pieces from the same rib were selected and sent to separate centres for the aDNA and lipid biomarker analyses.

Macromorphological analysis

The paleopathological examination of the mostly well-preserved skeletal remains of the 240 individuals (95 males, 72 females, 73 undeterminable) was carried out in the Department of Biological Anthropology, University of Szeged, Hungary. These investigations were performed using macromorphological methods, focussing on previously detailed classical[42] and atypical TB alterations.[43]

Mycobacterial aDNA analysis

– Mycobacterial DNA extraction

Possible cases of skeletal TB, defined according to skeletal morphological alterations, were examined for the presence of aDNA from the *Mycobacterium tuberculosis* complex (MTBC). These researches were carried out in the Centre for Clinical Microbiology, University College London, London, UK. Recommended protocols for aDNA work were followed[44] with separate rooms and equipment for different stages of the process. Well-established methods were employed for a DNA extraction and amplification,[45] as it was recently detailed by Donoghue and co-workers.[46] The approach used was of a slow but thorough period of sample disruption, one aliquot treated with N-phenacylthiozolium bromide (PTB), to cleave any covalent cross-links thus facilitating DNA strand separation and amplification.[47] Subsequently samples were treated with guanidium thiocyanate, followed by sample and bacterial cell disruption, using boiling and snap-freezing in liquid nitrogen. All fractions of the sample were used in the extraction. DNA was captured with silica and the pellets washed and dried. Silica supernates from PTB-negative samples were also processed by removal of protein followed by DNA precipitation with isopropanol (-20°C). Dried samples were re-hydrated with elution buffer and used immediately or stored at -20°C. Negative extraction controls were processed in parallel with the test samples.[48]

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[41] Medgyesi, 1991, 1998.

[42] Ortner 2003.

[43] Roberts et al., 1994; Baker, 1999; Hershkovitz et al., 2002; Pálfi, 2002; Dutour, 2008.

[44] Taylor et al., 2010.

[45] Eisenach et al., 1990; Poinar et al., 1998; Abu Al-Soud and Rådström, 2000;Taylor et al., 2003, 2007; Donoghue et al., 2005; Évinger et al., 2011; Hajdu et al., 2012.

[46] Donoghue et al., 2015.

[47] Poinar et al., 1998.

[48] Donoghue et al., 2015.

– DNA amplification and detection

Two specific regions of the *M. tuberculosis* complex were targeted – the repetitive elements IS*6110* (1–25 copies/cell) and IS*1081* (6 copies/cell). The IS*6110* primers used for conventional PCR had a target region of 123 bp[49] and the IS*1081* primers produce an amplicon of 113 bp.[50] Later, specific *M. tuberculosis* primers and a fluorescent probe were used[51] to enable shorter DNA fragments to be detected in a real-time PCR reaction.

– The PCR conditions

The PCR mix included 2mM bovine serum albumin to reduce PCR inhibition (Abu Al-Soud and Rådström, 2000) and 2.0mM $MgCl_2$. PCR assays were initially run at an annealing temperature of 58°C and amplified DNA was examined by agarose gel electrophoresis.[52] Subsequently, amplification was performed in a final volume of 25µl using a RotorGene© 3000 (Qiagen) real-time platform[53] to enable the detection of DNA using SYBR Green and melt analysis or specific primers with fluorescent probe. Annealing was at 60°C. A hot-start *Taq* polymerase was used to minimize non-specific primer and template binding. Negative DNA extraction and PCR controls were processed alongside the test samples.

Lipid Biomarker Analysis – Analysis of mycolipenate and mycocerosates

The initial analyses were performed at Bristol University, using a Thermo Finnigan MAT95 XP-Trap mass spectrometer, fitted with a Phenomenex Zebron ZB–5 (5% phenyl, 95% dimethylpolysiloxane) capillary column (30 m × 0.25 mm i.d. × 0.25 µm film thickness) using He as carrier gas (constant flow mode 1 ml min–1) and ammonia as the CI reagent gas. A GC oven temperature gradient from 200 to 300°C at 6.7°C min–1 was used, the final temperature being held for 20 min. The ion source temperature was 250°C, the injector 300°C and the transfer line 300°C. Selected ion monitoring (SIM) was used for mycocerosate ions at m/z 367.6311 (C24), 395.6844 (C26), 409.7111 (C27), 437.7645 (C29), 451.7911 (C30), 479.8445 (C32), 493.8712 (C33) and 507.8978 (C34); additionally, m/z 407.6952 was monitored for the presence of C27 mycolipenic acid.

Later studies were carried out at Swansea University with the same Phenonemex Zebron ZB–5 column, using He as carrier gas. PFB esters, on NICI-GCMS, fragment to produce negative carboxylate [M – H]- ions, which can be detected at high sensitivity. Selected ion monitoring (SIM) was used to search for mycocerosate carboxylate ions at m/z 367.6311 (C24), 395.6844 (C26), 409.7111 (C27), 437.7645 (C29), 451.7911 (C30), 479.8445 (C32), 493.8712 (C33) and 507.8978 (C34). Additionally, m/z 407.6952 was monitored for the presence of the C27 mycolipenate carboxylate ion.[54] Partial racemisation of mycocerosates during the alkaline hydrolysis leads to the formation of diasteroisomers, which resolve on gas chromatography to give characteristic doublets; in contrast, mycolipenates are singlets as they cannot racemise.[55]

Specimens were hydrolysed by heating with 30% potassium hydroxide in methanol (2 ml) and toluene (1 ml) at 100°C overnight.[56] In parallel, standard biomass of *M. tuberculosis* and *M. leprae* was processed. Long-chain compounds were extracted as described previously[57] and the extract was treated with pentafluorobenzyl bromide, under phase-transfer conditions to convert acidic

[49] Eisenach et al., 1990.
[50] Taylor et al., 2003.
[51] Évinger et al., 2011.
[52] Hajdu et al., 2012.
[53] Taylor et al., 2007.
[54] Lee et al., 2012.
[55] Lee et al., 2012.
[56] Hershkovitz et al., 2008; Lee et al., 2012.
[57] Lee et al., 2012.

components into pentafluorobenzyl (PFB) esters. Subsequent separation on an Alltech 209250 (500 mg) normal phase silica gel cartridge gave fractions containing non-hydroxylated fatty acid PFB esters, mycolic acid (MA) PFB esters and free phthiocerols. The MA PFB esters reacted with pyrenebutyric acid fi(PBA) to produce PBA-PFB MA derivatives, which were purified on an Alltech 205250 (500mg) C_{18} reverse phase cartridge.[58] The PBA-PFB mycolates were analysed by reverse phase HPLC, as described previously. The non-hydroxylated PFB ester fractions were refined on an Alltech 205250 (500 mg) reverse phase silica gel cartridge, using a water-methanol/methanol/methanol-toluene elution sequence. A fraction enriched in mycocerosic acid and other longer chain (> C20) PFB esters was eluted by 100% methanol with the more usual C_{12} to C_{20} esters eluting in the earlier water/methanol fractions. The fractions containing possible mycocerosates, were analysed by negative ion chemical ionization gas chromatography mass spectrometry (NICI-GCMS), as previously described.[59]

Results

Macromorphological analysis

During the macromorphological analysis of the skeletal material of the Bélmegyer-Csömöki domb series, 19 cases of probable skeletal tuberculosis were detected. Classical TB changes were observed in the five cases detailed above, while atypical or early-stage TB lesions were observed in a further 14 cases. It is clear, therefore, that these atypical or early TB changes occurred significantly more often than the classical alterations. (Among the cases used for biomolecular studies, only grave No. 86 showed no macromorphological evidence of tuberculosis – Table 1).

Table 1. Ancient TB data for material investigated from the Bélmegyer-Csömökidomb Avar Age skeletal series.

CLASSICAL TB CASES									
Gr No	Sex	Age at death	Macromorphology			aDNA	Lipid biomarkers		
			ST	CT	GT		MA	ML	MC
38	F	16–18	–	+	–	–	+	+?	+?
65	F	Maturus	+	–	–	–	+?	+	+
90	M	57–62	+	+	–	–	+	+	+?
189	M	25–28	+	–	–	+	+	+	+?
215	F	55–60	–	–	+	–	+?	+	+

ATYPICAL (EARLY-STAGE) TB CASES										
Gr No	Sex	Age at death	Macromorphology					aDNA	Lipid biomarkers		
			SVC	RP	EL	LBP	CO		MA	ML	MC
12	M	33–39	–	+++	–	+	–	+	+?	++	+?
17	M	22–25	+	+	+	+	+	+	+?	++	+?
22	undet.	16–18	+	–	+	–	–	–	+	++	+
33	M	40–45	+	–	+	+	+	+	–	–	–
48	M	55–60	+	+	–	+ (DP)	–	+	+	+	+
66	F	61–67	+	+	–	–	–	+	–	+	+?

[58] Hershkovitz et al., 2008; Lee et al., 2012.

[59] Lee et al., 2012.

ATYPICAL (EARLY-STAGE) TB CASES										
Gr No	Sex	Age at death	Macromorphology					aDNA	Lipid biomarkers	
			SVC	RP	EL	LBP	CO		MA ML MC	
86	M	59–64	–	–	–	–	–	–	+	++ +
88	F	40–45	–	+++	–	–	–	+	+	+ +++
92	M	20–25	+	+++	–	+	–	+	+	+? +?
116	F	25–30	–	+++	+	+++ (DP)	–	–	+	+? +?
134	F	16–18	+	–	–	–	+	–	++	+ +
154	M	20–24	+	–	–	–	+	+	–	+? –
188	undet.	7	+	–	–	–	–	–	–	++ +
212	M	18–20	+	+	–	–	–	–	–	– –
233	F	23–25	–	–	+	–	–	–	+	+? +?

Gr No = grave No; **F** = female; **M** = male; **undet.** = undeterminable sex; **ST** = *spondylitis tuberculosa*; **CT** = *coxitis tuberculosa*; **GT** = *gonitis tuberculosa*; **SVC** = superficial vertebral changes; **RP** = rib periostitis; **EL** = endocranial lesions; **LBP** = long bone periostitis; **CO** = *cribra orbitalia*; **DP** = diffuse periostitis; **MA** = mycolates; **ML** = mycolipenate; **MC** = mycocerosates.

The most frequent lesions were periosteal reactions on the visceral rib surfaces and abnormal vertebral vascularisation. Ten cases of superficial vertebral changes were detected (Table 1). With the exception of three specimens (two mature males and one elderly female), the affected individuals belong to younger age groups: one Infants II, three juveniles and three young adult males. Eight individuals exhibited hypervascularisation of the anterior aspect of vertebral bodies, while lytic vertebral lesions were revealed in only two cases.

As for rib changes (Plate no. III, Figure 12), eight individuals (one juvenile, four adults, two mature and one elderly) showed signs of periosteal appositions on the visceral costal surfaces (Table 1).

In the majority of the cases, rib periostitis showed a woven-remodelled character, indicating a less active process generating these pathological changes. In two other cases (grave No. 17 and grave No. 212) it was noticed that the visceral surfaces of ribs had a roughened texture.

Endocranial alterations were revealed in five individuals only (Table 1). Except for a mature male specimen (grave No. 33), the affected individuals represent younger age groups: one juvenile and three young adults (one male and two females). Concerning lesion morphology, abnormal blood vessel impressions on the internal surface of the skull were observed in three of the five cases, though the endocranial lamina of grave No. 22 exhibited small granular impressions similar to those described by Schultz[60] and in the skeleton of a young adult female individual (grave No. 233) *serpens endocrania symmetrica* (SES) was also identified.

Several cases of superficial vertebral changes and/or vertebral hypervascularisation were observed (Plate no. IV, Figure 14a). With the exception of two cases (grave No. 88 and grave No. 188), an association of different alterations could be detected. Atypical or early-stage TB changes were accompanied by the so-called 'stress factors' in a number of cases: *cribra orbitalia* (mainly the porotic form) was observed in seven cases, while diffuse long bone periostitis occurred in six cases (Table 1). Long bone periostitis appeared mostly on femora (Plate no. IV, Figure 14b) and tibiae, but in three cases the long bones of the upper extremities were also affected.

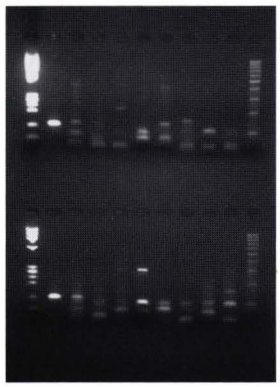
[60] Shultz, 2001.

Biomolecular Analyses

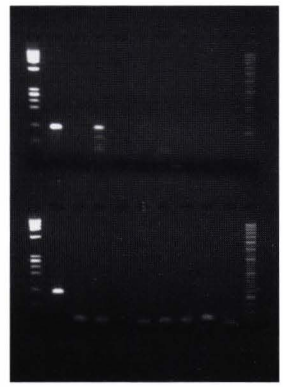
aDNA results:

The aDNA amplification studies gave positive results for nine of the 20 graves investigated, but for only one of the four "classical TB cases" (Table 1).

Data of the aDNA analysis are detailed in Figures 15–18:



Gel with IS*6110* PCR products



Gel with IS*1081* PCR products

Figure 15: Single-stage PCRs with outer primer pairs

Key to abbreviations:

EC = negative extraction controls = silica supernate (fluid left in 2 ml tubes after silica spun down, normally short aDNA fragments); LVs = large volume silica supernate (fluid left in 9 ml lysis buffer tubes after silica spun down, short aDNA fragments); wb = water blank negative control in PCR.

Lanes (left to right): 1: Phi X–174 *Hae* III markers; 11: 20bp and 100bp molecular markers

Top row: 2: +vecontrol; 3: BC–12s; 4: BC–12 LVs; 5: wb1; 6: BC–17s; 7: BC–17 LVs; 8: BC–22s; 9: wb2; 10: BC–22 LVs;

Bottom row: 2: +vecontrol; 3: BC–33s; 4: BC–33 LVs; 5: BC–38s; 6: ECs; 7: BC–38 LVs; 8: BC–48s; 9: BC–48 LVs; 10: EC LVs;

Figure 15 – Conclusions:

IS*6110*: possible weak positives with BC–12 LVs and BC–17 LVs. Positive with BC–33s. Non-specific bands from BC–12s, BC–22s, BC–33 LVs, and BC–48 LVs. Others negative.

IS*1081*: positive with BC–12 LVs. All other samples (except positive controls) were negative

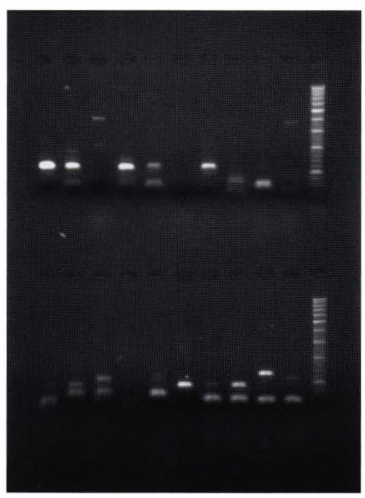


Figure 16: Single-stage IS1081 PCRs using inner primers (113 bp)

Lanes (left to right): 11: 20bp and 100bp molecular markers
Top row: Lane 1: +vecontrol; 2: BC–12; 3: wb5; 4: BC–12+; 5: BC–17; 6: wb6; 7: BC–17+; 8: BC–22; 9: BC22+; 10: wb7.
Bottomrow: Lane 1: BC–33; 2: BC–33+; 3: BC–48; 4: wb8; 5: BC–48+; 6: EC; (lanes 7–10: different samples and another PCR)
Figure 16 – Conclusions:
Positives from samples BC–12, BC–12+, BC–17, BC–17+, BC–48.
Doubtful results from BC–22, BC–22+ (very faint trace) and BC–48+.
Negativesfrom BC–33 and 33+, and all waterblanks.
BC–51 was examined separately for MTB *IS1081* but was negative.

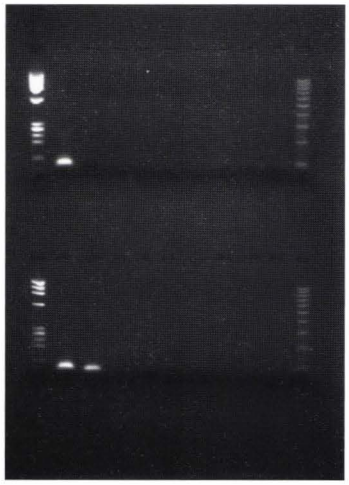


Figure 17: Nested IS6110 PCRs using inner primer pair

Samples loaded in the same order as above, using the stage 1 PCR products that werere amplified for a further 25 cycles.
Conclusions:
Positive and negative controls were satisfactory. Only BC–33s was positive.
Real-time experiments were also carried out with the same primers and melt analysis. Results are summarized at the end of the document.

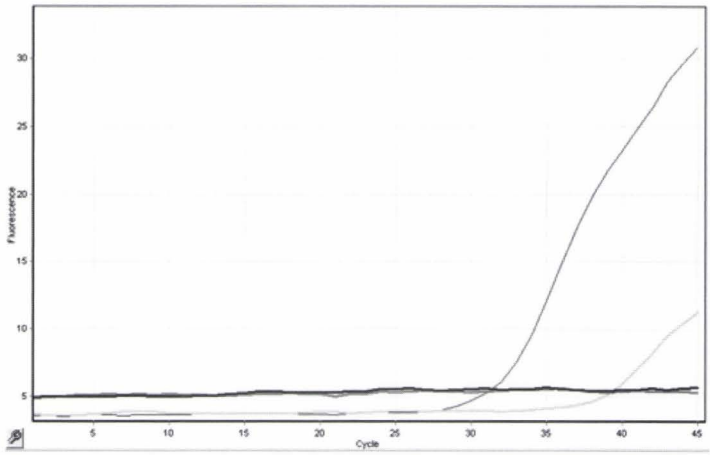
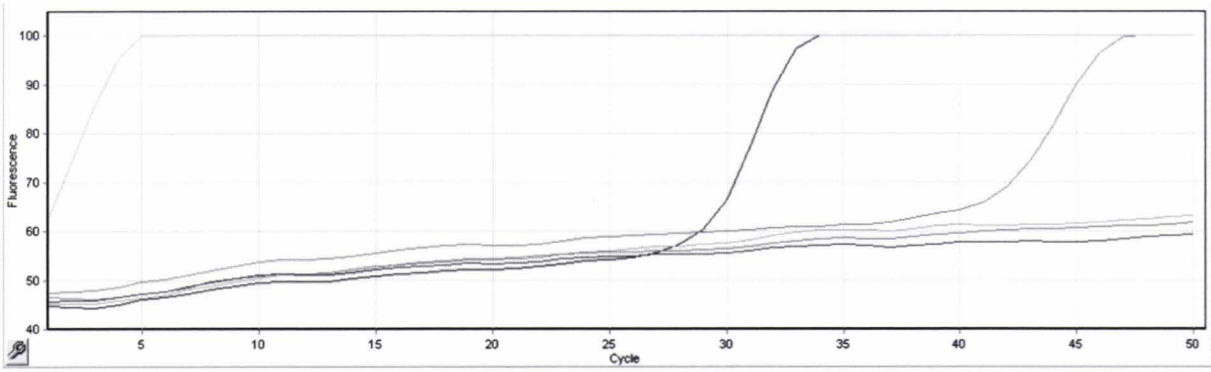


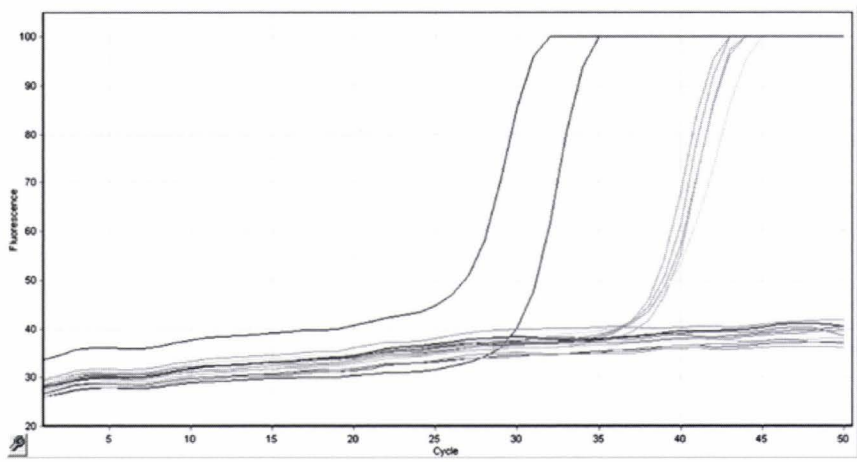
Figure 18a-b-c: Real-time PCR with IS1081 primers and probe

A) The lower the cycle threshold (C_t) the greater the quantity of target aDNA in the sample. In this image, the positive samples in order of their $C_t$was as follows:
Positive control (a 1/10 dilution of extract from a Vác mummy) C_t 32 cycles

BC–189+ (+ indicates the DNA was extracted using PTB) C_t 39 cycles
Negatives were obtained from BC–12, BC–22, BC–116+, BC–116, BC–134+, BC–134, BC–134s, BC–188+, BC–188, BC–189, BC–215, wb1, wb2, wb3, wb4, EC, EC1, EC+, ECs



B) In this experiment a nested PCR was performed on the PCR product from BC–189+ which explains the high level of signal at the start of the reaction. The positive control had a C_t of 28 and sample BC–92 had a C_t of 41.
Negatives were obtained from BC–22, BC–65+, BC–65, BC–66+, BC–66, BC86+, BC–86, BC–88+, BC–88, BC–90+, BC–90, BC–92+, BC–154+, BC–154, wb1, wb2, wb3, wb4, EC+, EC.



C) The positive control had a C_t of 28.6, BC–66s: 36.6, BC–86s: 36.9, BC–92: 36.0, BC–92s: 36.3, and BC–154: 36.1. Individual screenshots are available for each positive sample.
Negative results were obtained from BC–65s, BC–86s, BC–90s, wb, EC and ECs.
These results were confirmed by agarose gel electrophoresis.

Overall findings for M. tuberculosis complexaDNA in these samples:

Positives with one or both target regions:
BC–12, BC–17, BC–33, BC–48, BC–66, BC–88, BC–92, BC–154, BC–189
Negative (but cannot exclude poor preservation):
BC–22, BC–38, BC–65, BC–86, BC–90, BC–116, BC–134, BC–188, BC–212, BC–215, BC–233

Lipid biomarker results

Total mycolic acid (MA) profiles are recorded in Figure 19 (plate no. V) that also includes a summary of the overall lipid biomarker and aDNA results. All the MA profiles were too weak to allow further diagnostic analyses, by sequential normal and reverse phase HPLC.[61]Material from five graves (Nos. 33, 66, 154, 188, 212) yielded no MAs. Figure 20 (plate no. VI) shows three representative profiles of mycolipenic (ML) and mycocerosic (MC) acids.

The results of the lipid biomarker analyses could be placed into 6 groups (Table 1, Figs. S3–5). Group 1 (grave Nos. 22, 86, 88, 134) had clear evidence of all three MA, ML and MC lipid biomarker classes; grave No. 22, however, also included C_{33} and C_{34} mycocerosates, indicative of leprosy. The major Group 2 (grave Nos. 12, 17, 48, 65, 90, 189, 215) was characterised by the presence of a clear signal for mycolipenate (ML), with less convincing evidence for the other MA and MC classes. Group 3 (grave Nos. 66, 188) had two representatives with good ML, weak MCs, but no MA; a single member of Group 4 (grave No. 154) had only a poor ML signal. Four representatives in Group 5 (grave Nos. 38, 92, 116, 233) provided weak inconclusive evidence for ML and MC biomarkers. Final Group 6 (grave Nos. 33, 212) lacked any evidence of mycobacterial lipid biomarkers. A close correlation with the aDNA data was not observed. Only one (grave No. 88) of four in the best Group 1 lipid class gave amplified DNA. Correlation was better for the Group 2 lipid class with four of seven having aDNA. In the less strong or negative lipid Groups 3–6, only one grave in each group had a positive aDNA result.

Discussion and conclusions

During our five-year research project'Paleoepidemiology of Tuberculosis' (OTKA Grant No. K78555; 2009–15),11001 human skeletons have been examined or re-examined in the Szeged Anthropological Collection (SAC).500 cases of possible skeletal TB were found, indicating a one/nine ratio (53/447) between 'classical' and 'atypical' skeletal TB cases. These results underline the importance of further researches on the early stage skeletal TB manifestations, and that of the re-examination of the osteoarchaeological series previously studied. In the 20th century, the diagnosis of TB in osteoarchaeological samples focused only on these classical/advanced-stage TB lesions, representing a fairly developed stage of tuberculosis. As TB may have affected many individuals without classical pathological changes, thus patients died in an earlier stage of tuberculosis long before these symptoms could have developed. The early-stage TB is not recognizable on the basis of classical TB alterations, so if we consider only individuals with visible TB-related lesions, it is likely this will significantly underestimate the prevalence of tuberculosis in the examined historical population.

The study of 2057 Avar Age human skeletons has furnished 82 cases of probable tuberculosis. Our results indicate an important TB infection level at this historical period. However, we have to continue the systematic complex paleopathological studies of these series, including more complete biomolecular investigations.

In our paper we presented a complex morphological and biomolecular study of a special Avar Age skeletal TB case (Csongrád-Felgyő), and by a 'series-study' we gave more details about TB paleoepidemiology in a 8th century AD Avar Age population (Bélmegyer-Csömöki domb).

The pathological lesions observed in skeleton no. 205 of Csongrád-Felgyő indicated that this young adult female might have suffered from long standing skeletal tuberculosis and had developed unusual bone lesions in addition to the spinal angulation typical of TB. The involvement

[61] Hershkovitz et al., 2008; Lee et al., 2012.

of the spine, ribs and sternum might have resulted from the spread of the infection directly from the thorax to the adjacent skeletal elements (which may have been related to a large prevertebral tuberculous abscess) and/or from a systemic spread of the bacteria through the blood stream. The latter could explain the endocranial changes observed on the frontal and parietal bones, suspected to have resulted from tuberculous meningitis. The vertebral alterations observed in this individual provided a clear example of the great morphological variability of spinal TB lesions. During the 20th century, paleopathological studies frequently regarded Pott's disease as the only typical form of infection from tuberculous origin. However, spinal TB lesions have been demonstrated to mimic numerous pathological conditions, such as malignous conditions.[62] In paleopathology, TB has been uncovered as the origin of less typical vertebral alterations on new as well as previously analysed skeletal material.[63]

In this case study, the presence of MTBC DNA in the skeletal remains was confirmed independently by biomolecular analysis in two different laboratories, clearly supporting the osteological diagnosis of skeletal TB as the most probable aetiology of the alterations observed on this individual. Moreover, the first spoligotyping results have already excluded bacteria belonging to the *M. microtii/M. bovis* lineage as possible causative agents, providing the opportunity to carry out a detailed typing of this MTBC strain in the near future.

The morphological study of the 240 skeletons from the Bélmegyer-Csömöki domb series has furnished a rich TB paleopathology: 5 classical and 14 atypical cases were identified. These 19 skeletons and a morphologically negative case were used for sampling for the different biomolecular analyses. Only nine of the 20 graves yielded *M. tuberculosis* aDNA on amplification. Lipid biomarker evidence for *M. tuberculosis* was discerned in all of the specimens, but the strength and conclusiveness of the lipid signals could be allocated to five levels (Groups 1–5) (Plate no. VI, Figure 20). Taken by themselves, the weak total mycolic acid profiles (Plate no. VI, Figure 20) cannot be regarded as positive evidence for ancient tuberculosis. The constituents of the profiles are significantly smaller than those of standard *M. tuberculosis*, suggesting either considerable degradation or the presence of environmental mycobacteria. The former alternative is favoured, as the two specimens (grave Nos. 33, 212) that lacked any evidence of mycolipenate (ML) and mycocerosate (MC) biomarkers (Table 1), showed no evidence of any mycolates (Plate no. I, Fig. 2). Given that assumption, the MA profiles provide background support for mycobacterial infection.

The most positive evidence for the presence of tuberculosis resides in the MLs, which were found to be usually, as strong as, or stronger than the MCs, an exception being grave No. 88 with an excellent MC profile. Indeed in grave No. 154 the only lipid biomarker evidence is a very weak ML signal; this is probably genuine as aDNA was amplified from this sample.

Of five classical Bélmegyer tuberculosis cases (Table 1) with skeletal alterations characteristic for advanced stage TB, one only (grave No. 189) was positive for MTBC DNA with clear lipid biomarker support (Plate no. VI, Figure 20; Table 1). Four of the diagnosed classical TB cases were DNA negative. However, in three of these negative cases (grave Nos. 65, 90, 215) the diagnosis of skeletal tuberculosis was confirmed by lipid biomarker analysis with quite strong evidence (Plate no. VI, Figure 20). For grave No. 38, lipid biomarker data were weak. For cases, showing atypical or early-stage TB lesions (Table 1), many of the biomarker results were inconsistent. The best lipid profiles (Group. 1) were recorded for grave Nos. 22, 86, 88 and 134, but only the very fragmented material from grave No. 88 was supported by aDNA. Interestingly, grave No. 22 appeared to be a co-infection with tuberculosis and leprosy, the former being confirmed with a strong mycolipenate

[62] Tan et al., 2010; Thawani, 2011; Gosal et al., 2012.

[63] Baker, 1999; Maczel, 2003; Ortner, 2003; Pálfi et al., 1999, 2012.

peak and the latter by C_{33} and C_{34} mycocerosates (Plate no. VI, Figure 20). The next Group 2 lipid biomarker level, with clear ML backed up by MCs in seven graves (Nos. 12, 17, 48, 65, 90, 189, 215) was supported by a DNA in three atypical cases (grave Nos. 12, 17, 48) in addition to the classical case in grave No. 189 (Table 1). Only one (grave No. 66) of the two Group 3 biomarker level specimens had aDNA support, but both graves (Nos. 66, 188) had good ML backed up by weak but clear MCs. Dropping down to the single lipid biomarker Group 4 representative (grave No. 154), as mentioned above, aDNA amplification was supported by a weak but clear ML. The four graves (Nos. 38, 92, 116, 233) assigned to lipid Group 5 had only minimal ML and MC evidence but a DNA was obtained from No. 92. Although grave No. 33 was MTBC DNA positive, negative lipid profiles were recorded. A juvenile male (grave No. 212.) was the only specimen showing atypical or early-stage TB lesions, where the biomolecular analyses gave negative results for the presence of both MTBC DNA and lipid biomarkers.

Morphological assessment, detection of ancient DNA and demonstration of *M. tuberculosis complex* cell wall lipid markers proves there was widespread TB infection in this 8th century population. In this Bélmegyer Avar Age material, a variety of lesions at different stages of development were observed. The biomolecular studies confirmed the presence of tuberculosis and lipid analysis also indicated a TB/leprosy coinfection. Our study highlights the difficulties of demonstrating TB in these individuals from over 1300 years ago and the importance of using different methods is very clear. The relative success of lipid biomarkers compared with aDNA is probably due to their greater stability over time.

Our results underline the complementarity of morphological, aDNA and lipid biomarkers analyses in the diagnosis of ancient TB infections.

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Figure legends:

Figure 1: Skeletal reconstruction of a typical Pott's disease from grave no.19 of the 7–8th century AD Avar Age cemetery of Sükösd, Hungary (Marcsik, 1972; Marcsik *et al*, 1999).

Figure 2: 3D CT reconstruction of the Pott's disease presented if Fig. 1 (Marcsik *et al*, 1999).

Figure 3: Geographical distribution of the examined cemeteries.
Neolithic period – red, Bronze Age – green, Sarmatian period – orange, Gepid Age – yellow, Avar Age – light blue, Hungarian Conquest period and Arpadian Age – dark blue, Middle Ages – purple.

Figure 4: Number of the examined skeletons by archaeological periods; $\Sigma = 11001$.

Figure 5: Number of possible+probable+confirmed TB cases / archeological periods; $\Sigma = 517$.

Figure 6a: Multiple lytic lesions on the spine of individual no.205 of the Csongrád-Felgyő Avar Age cemetery (CsoF 205). The extensive involvement of thoracic vertebral bodies and transverse processes and the sparing of vertebral endplates are unusual features in skeletal TB.

Figure 6b: Angular spinal deformity generated by the collapse and fusion of lower thoracic and upper lumbar vertebrae (T10-L3, CsoF 205). The kyphosis and ankylosis are characteristic alterations in the healing stage of spinal tuberculosis.

Figure 7a-b: Abnormal bony structures and cystic zones of destruction revealed by the X-ray and CT analyses (CsoF 205).

Figure 8: A 19th-century illustration of vertebral tuberculosis originating from Billroth and von Winiwarter's work (1887), representing multiple lytic lesions in spinal TB, with tubercular vertebral caries extending over the ribs.

Figure 9a: Biomolecular demonstration of mycobacterial DNA from vertebral remains CsoF 205. The presence of the 123 bp segment of the IS6110 insertion sequence (lane 4 and 5), specific to the *Mycobacterium tuberculosis* complex, was unambiguously confirmed by the paleomicrobiological analysis (Institute of Pathology, Munich).

Figure 9b: Spoligotyping pattern of the vertebra sample of individual CsoF 205(ID 1290) in comparison with the spoligotyping pattern of *M. tuberculosis* H37v and *M. bovis* BCG.

Figure 10a: Tuberculous spondylitis healed with gibbus formation (L1-L3) – Grave No. 65 of the Avar Age cemetery of Bélmegyer-Csömöki domb (BC 65).

Figure 10b: Severe destruction of the 3rd lumbar vertebral body (inferior view; BC 65).

Figure 11a: Lumbosacral tuberculosis: severe erosion of the ventral sacral surface (traces of cold abscess) – Grave No. 90 of the Avar Age cemetery of Bélmegyer-Csömöki domb (BC 90).

Figure 11a: *Coxitis tuberculosa*: complete destruction and remodelling of the acetabulum (BC 90).

Figure 12: Atypical/early stage TB changes in the Bélmegyer-Csömöki domb series – periosteal apposition on the visceral costal surface (Grave No. 88, BC 88).

Figure 13: Atypical/early stage TB changes: endocranial changes (Grave No. 233, BC 233).

Figure 14a: Atypical/early stage TB changes: abnormal vertebral vascularisation – Grave No. 92 of the Avar Age cemetery of Bélmegyer-Csömöki domb (BC 92).

Figure 14b: Atypical/early stage TB changes: traces of diffuse periostitis on the femur (BC 92)

Figure 15: Single-stage PCRs with outer primer pairs

Figure 16: Single-stage IS1081 PCRs using inner primers (113 bp)

Figure 17: Nested IS6110 PCRs using inner primer pair

Figure 18: (a, b, c) Real-time PCR with IS1081 primers and probe

Figure 19: Reverse phase fluorescence HPLC of total mycolates.
The grave numbers are accompanied (in brackets) by the amount of sample analysed (mg). The "Lipid" column indicates the diagnostic power of mycolate (MA), mycolipenate (ML) and mycocerosate (MC) lipid biomarkers: ++++ (group 1), clear evidence of MA, ML and MC; +++ (group 2), clear ML signal with less strong MA and MC; ++ (group 3), good ML, weak MC and no MA; + (group 4), only a clear weak ML peak; +? (group 5), weak inconclusive ML and MC with some MA support; – (group 6), no evidence of mycobacterial lipids; ++++*, strong M. tuberculosis lipid signals with additional MC indicating M. leprae. The "aDNA" list records the presence of amplified DNA fragments.

Figure 20: Representative selected ion monitoring (SIM) negative ion-chemical ionization gas chromatography-mass spectrometry (NI-CI GC-MS) profiles of mycolipenate and mycocerosates.
A, C, E grave Nos. 88 (Bristol University), 134 and 22 (Swansea University); **B, C** *M. tuberculosis* standard recorded at Bristol and Swansea, respectively; **F**, *M. leprae* standard recorded at Swansea. Ions monitored are exemplified by C_{27}*m/z* 407 and C_{27}*m/z* 409, representing C_{27} mycolipenate and C_{27} mycocerosate, respectively. Relative intensities **(bold in brackets)** are shown normalized to the major component **(100)**.

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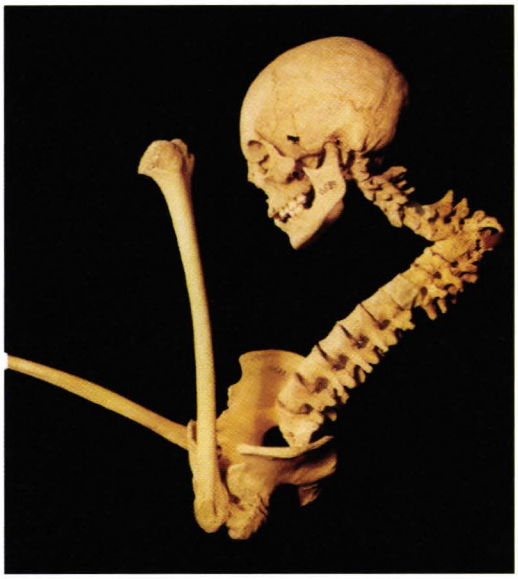

Figure 1

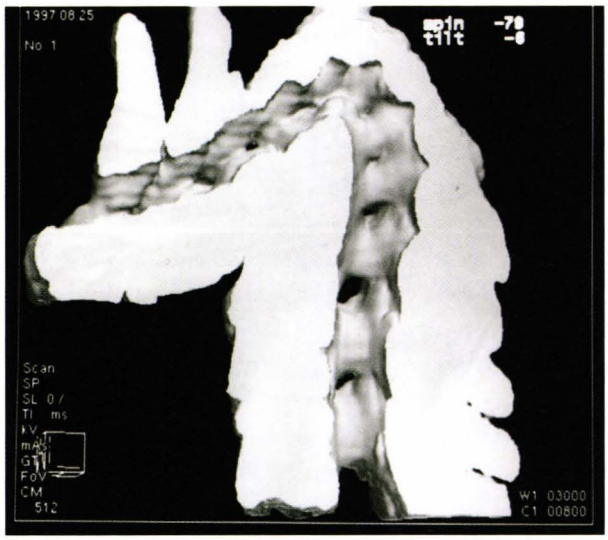

Figure 1

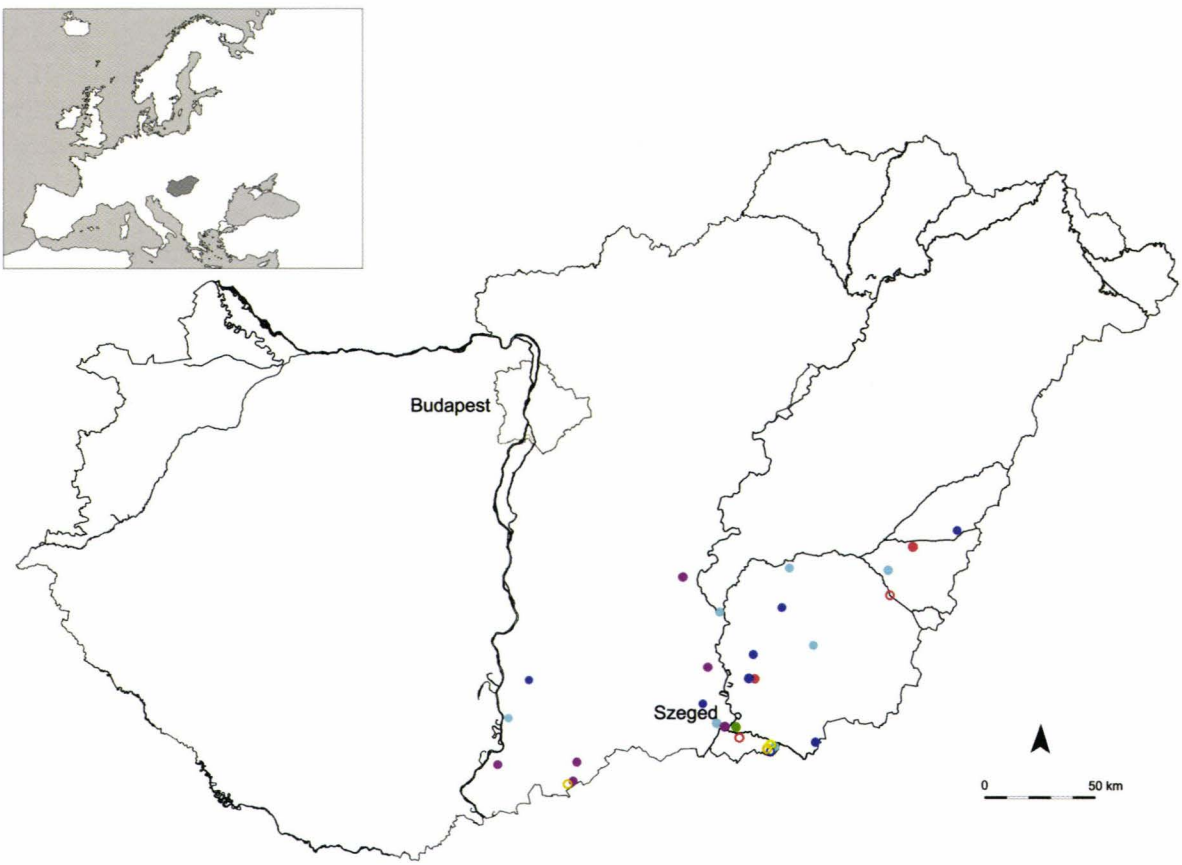

Figure 3

Plate no. I.

Figure 6a

Figure 6b

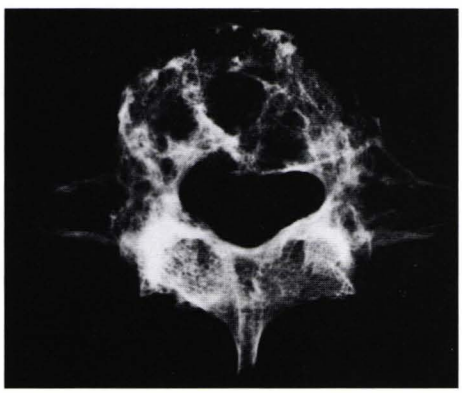

Figure 7a

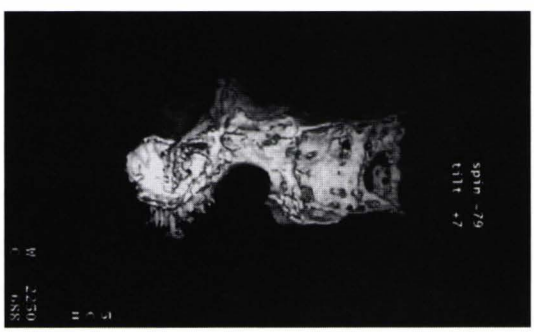

Figure 7b

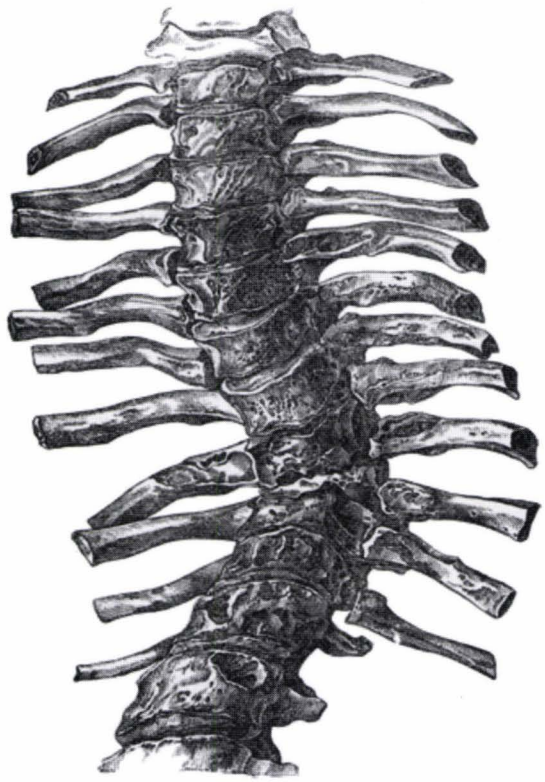

Figure 8

Plate no. II.

Figure 10a

Figure 10b

Figure 11a

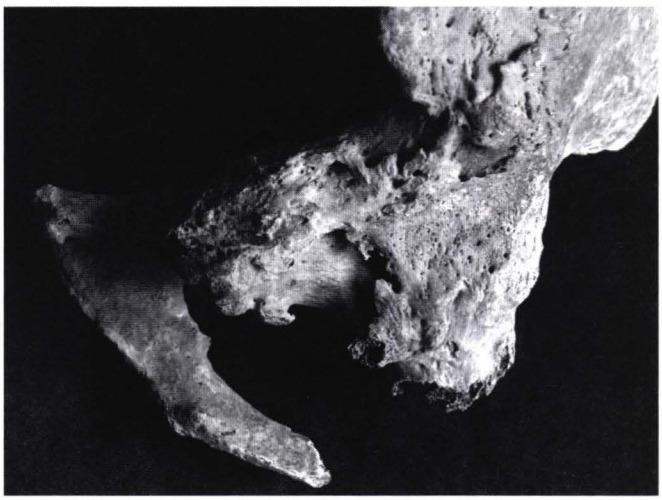

Figure 11b

Figure 12

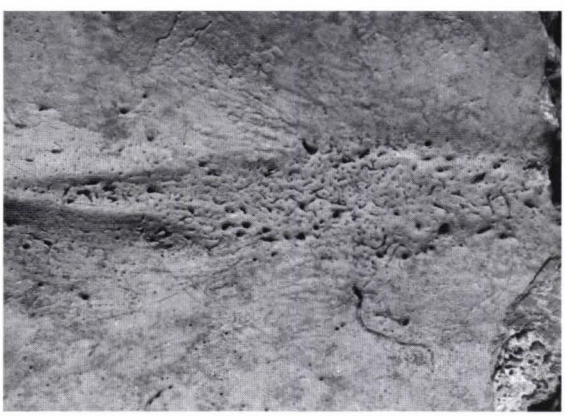

Figure 13

Plate no. III.

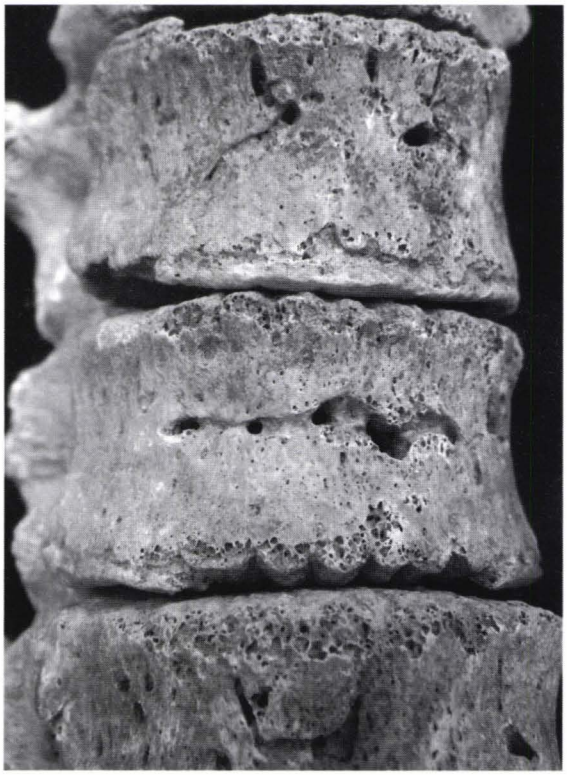

Figure 14a

Figure 14b

Plate no. IV.

A SCAPHOCEPHALY CASE FROM SAINT SAVA CEMETERY, BUCHAREST

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Keywords: Scaphocephaly, radiocarbon data, Saint Sava cemetery.

Introduction

During the years 2010–2011 the rescue excavations of the southern area of Saint Sava church (located in the Bucharest downtown) unearthed a number of 676 graves dated in 16th–19th centuries.[1]

Anthropological analysis of the 624 human skeletons revealed that there are 491 adults and 133 sub-adults; by sexes there are 149 females, 236 males and 239 are undetermined.[2]

The skeleton from the grave 423 has a skull with typical scaphocephaly modification. Archaeological description: it was found in trench XVI, square 1D. The skeleton (found as a package of bones) was deposited in a pit with dimensions of 0.54 × 0.48 × 0.17 meters. A bottle of glass was found as inventory. The radiocarbon data (Poz–72760, 170±30 BP) has indicated the year of 1803±119 AD (calibrated with CalPal).

Methods

Sex determination is based on the evaluation of the cranial visual features (glabella area, orbital edge, mastoid process, mental eminence, nuchal crest), followed by calculation of discriminant functions who indicate the probability to be male or female of the skull.[3] Age estimation is based on the cranial sutures closer,[4] but for scoring was excluded the sagittal one.

Martin's system was used for skeleton measurements and calculation of indices.[5] Also, next indices were added: index 29/30/31:1 = the ratio between frontal/parietal/occipital chord and maximum cranial length; index 8+17:1 = the ratio between maximum cranial breadth plus *basion-bregma* height and maximum cranial length; 29/30/31:17 = the ratio between frontal/parietal/occipital chord maximum cranial breadth; 29/30/31:8 = the ratio between frontal/parietal/occipital chord and maximum/minimum????? cranial breadth.

For paleopathology was recorded: *cribra cranii* and *cribra orbitalia*; the number of permanent erupted teeth positions, the number of permanent teeth present, number of permanent teeth with

[1] Mănucu-Adameşteanu & al. 2012, 181–191; Velter&Mănucu-Adameşteanu 2013, 169–193.

[2] Soficaru & al. 2015, 229–255.

[3] Soficaru 2014, 464–475.

[4] Buikstra&Ubelaker1994, 32–38.

[5] Bräuer 1988, 160–232.

caries, the number of the ante mortem teeth lose, number de abscesses; mandibular/maxillary canines/incisors with hypoplasia; recording of the degenerative joint disease presence; the presence of osteoperiostitis on the long bones.

Study

The scaphocephaly was recognized using follow criteria: "*premature fusion of sagital suture, the calvaria is very elongated and narrow, forehead and occiput may be very prominent.*"[6]

The individual with scaphocephaly, M 423 (Plate no. I), was buried in the cemetery of the Saint Sava church as the other ordinary people. The grave was situated in the southern side of the church at about 2 m from its wall. As pathology the skeleton displays a carious lesion, enamel hypoplasia on the mandibular and maxillary canines, osteoarthrosis of some joints, stature of 173.68 cm[7] in comparison with the average of the males' skeletons from same site, 167.96 cm.

Dimension (mm), Martin number	***M, 33–42***	***University Plaza. males***				
	M 0423	**Average**	**Minimum**	**Maximum**	**Sigma**	**No.**
1. Maximum cranial length (g-op)	193.00	182.28	167.00	192.50	5.65	46
3. Length glabella-lambda (g-l)	184.00	176.49	161.00	186.00	5.75	45
5. Cranial base length (n-ba)	105.00	102.88	90.27	111.00	4.89	26
7. Foramen magnum length (ba-o)	42.50	36.20	32.04	46.00	3.17	30
8. Maximum cranial breadth (eu-eu)	117.00	144.11	130.00	159.00	6.52	46
9. Minimum frontal breadth (ft-ft)	100.47	97.36	89.00	106.57	4.04	74
11. Biauricular breadth (au-au)	120.00	125.50	111.00	135.00	6.49	35
16. Foramen magnum breadth	28.54	31.14	27.54	35.47	2.00	35
17. Basion-bregma height (ba-b)	144.00	135.69	127.00	144.00	5.27	26
*19a. Mastoid length right	31.44	29.59	23.01	39.69	3.60	99
*19a. Mastoid length left	31.77	29.04	21.06	38.51	3.50	86
29. Frontal chord (n-b)	102.74	108.47	11.96	124.00	16.05	82
30. Parietal chord (b-l)	130.63	111.32	91.91	126.63	6.14	68
31. Occipital chord (l-o)	105.85	96.58	84.84	109.79	5.32	69
38d. Cranial capacity (Lee-Pearson)	1389.54	1481.47	1332.53	1616.35	65.63	22
40.Basion-prosthion length (ba-pr)		97.45	89.20	105.79	4.84	20
43. Upper facial breadth (fmt-fmt)	107.74	104.07	95.41	115.28	4.03	66
45. Byzigomatic diameter (zy-zy)		128.92	122.00	140.00	5.08	13
47. Total facial height (n-gn)		113.64	63.78	126.00	14.29	19
48. Upper facial height (n-pr)	65.12	70.04	57.50	79.68	5.09	34
50. Interorbital breadth (mf-mf)	97.07	95.45	87.99	101.52	3.40	37
51. Orbital breath (mf-ek) (right)	41.98	39.07	35.37	42.74	2.19	25
51. Orbital breath (mf-ek) (left)	37.91	39.04	35.10	42.97	2.23	35
52. Orbital height (right)	34.97	33.78	27.77	41.66	2.78	26
52. Orbital height (left)	35.27	34.06	28.38	38.51	2.48	35
54. Nasal breadth	23.14	24.87	19.80	34.67	2.51	43
55. Nasal height (n-ns)	56.64	51.24	44.23	61.30	4.03	29
60. Maxillo-alveolar length (pr.-alv.)		53.37	39.16	94.75	9.99	45
61. Maxillo-alveolar breadth (ekm-ekm)		64.20	54.62	129.00	11.49	39
65.Bycondilar breadth (kdl-kdl)		118.18	95.03	135.00	7.71	40
66. Bigonal width (go-go)		100.05	66.94	113.37	10.24	58

[6] Cohen & MacLean 2000, 161–162.

[7] Breitinger's method.

Dimension (mm), Martin number	*M, 33–42*	*University Plaza. males*				
	M 0423	Average	Minimum	Maximum	Sigma	No.
68. Mandibular length	80.00	80.77	67.00	96.00	6.27	71
69. Chin height (id-gn)	28.63	31.66	23.78	39.08	3.48	100
69(1). Height of mandibular body right		31.57	22.95	38.06	2.79	95
69(1). Height of mandibular body left	29.30	31.53	20.37	38.55	3.14	94
69(3). Thickness of mandibular body right		12.78	8.75	18.67	1.88	120
69(3). Thickness of mandibular body left	12.36	12.58	8.95	17.74	1.75	120
70. Maximum ramus height	60.00	61.54	49.00	70.00	4.98	71
71a. Minimum ramus breadth right		32.47	25.32	70.49	5.30	93
71a. Minimum ramus breadth left	35.40	31.83	24.38	38.42	3.04	85
71(1). Maximum ramus breadth right		44.29	33.95	51.96	3.34	68
71(1). Maximum ramus breadth right	46.63	44.24	35.85	51.78	3.39	57
79. Mandibular angle	125.00	124.20	110.00	141.00	6.98	71
I. 1. Cranial index (8:1)	60.62	79.13	70.65	91.62	4.73	37
I. 2. Vertical index (17:1)	74.61	74.74	67.20	82.04	3.59	26
I. 3. Transvers-vertical index (17:8)	123.08	94.08	84.21	105.88	5.83	22
I. 13. Transversal fronto-parietal index (9:8)	85.87	67.98	59.84	74.51	3.21	38
I. 33. Index of foramen magnum (16:7)	67.15	86.47	75.10	97.63	6.73	29
I. 38. Total facial index (47:45)		78.69	0.00	95.05	28.26	10
I. 39. Facial superior index (48:45)		53.21	45.15	59.62	3.84	12
I. 42. Orbital index (52:51) right	83.30	85.19	75.56	93.70	5.46	24
I. 42. Orbital index (52:51) left	93.04	87.08	77.45	101.44	6.12	33
I. 48. Nasal index (54:55)	40.85	48.12	36.87	55.95	5.10	27
I. 54. Maxillo-alveolar index (61:60)		124.38	98.08	155.99	15.13	29
I. 60. Gnaticindex (40:5)		94.65	84.30	100.00	4.11	20
I. 62. Mandibular index (68:65)		68.91	55.56	82.12	7.13	39
I. 69. Longitudinal cranio-facial index (40:1)		53.56	48.22	58.77	2.94	20
I. 70. Vertical cranio-facial index (45:8)		89.93	82.99	97.69	4.82	13
I. 72. Fronto-biorbitalindex (9:43)	93.25	93.38	85.18	104.17	3.36	63
Index (8+17:1)	135.23	150.85	74.73	173.65	17.63	23
Frontal chord-max. length index (29:1)	53.23	61.80	55.67	66.85	2.55	46
Parietal chord-max. length index (30:1)	67.68	61.44	53.05	67.36	3.13	45
Occipital chord-max. length index (31:1)	54.84	53.27	45.86	59.02	2.66	42
Frontal chord-ba-br height index (29:17)	71.35	82.93	73.55	94.39	4.89	26
Parietal chord-ba-br height index (30:17)	90.72	82.99	70.70	93.80	5.12	25
Occipital chord-ba-br height index (31:17)	73.51	71.89	64.12	83.98	5.00	25
Frontal chord-eu-eu index (29:8)	87.81	77.84	67.18	87.94	4.55	42
Parietal chord-eu-eu index (30:8)	111.65	77.89	64.32	90.46	5.50	42
Occipital chord-eu-eu index (31:8)	90.47	65.63		80.14	11.76	39

Table I. Cranial measurements of the skull with scaphocephaly compared with males' skulls from University Plaza cemetery.

Clavicle	Right	Left
1. Maximum length		136.00
4. Vertical diam. midshaft	10.06	9.17
5. Sagittal diam. midshaft	14.20	12.12
4:5. Midshaft index	70.85	75.66

Scapula	**Right**	**Left**
1. Anatomical breadth		
2. Anatomical length		
12. Glenoid height	41.24	
13. Glenoid breadth		38.48
2:1. Scapular index		
Humerus	**Right**	**Left**
1. Maximum length	336.00	
4. Epicondylar breadth		
5. Max. diam. midshaft		
6. Min. diam. midshaft		
9. Head vertical diameter		51.83
10. Head sagittal diameter		
6:5. Diaphysis index		
9:10. Head index		
Radius	**Right**	**Left**
1. Maximum length	251.50	250.00
4. Trans. diam. midshaft	16.39	16.37
5. Sag. diam. midshaft	12.98	12.24
5(6). Dist. max. breadth	36.00	36.00
5:4. Diaphysis index	79.19	74.77
Ulna	**Right**	**Left**
1. Maximum length		
*2a. Physiological length		
3. Min. circumference	35	
11. Dorso-volar diameter		
12. Transversal diameter		
11:12. Diaphysis index		
Femur	**Right**	**Left**
1. Maximum length	485.00	482.50
2. Bicondylar length	481.00	
6. Sag. midshaft diameter	30.61	28.67
7. Trans. midshaft diameter	26.29	27.86
8. Midshaft circumference	87.00	88.00
9. Trans. mubtroch. diam.	30.00	31.93
10. Sag. subtroch. diam.	26.42	26.45
18. Head vertical diameter	49.05	49.62
19. Head sagittal diameter	49.78	50.38
6:7. Pilastric index	116.43	102.91
10:9. Platymeric index	88.07	82.84
18+19:1	49.15	49.72
6+7:1	30.66	28.73
Tibia	**Right**	**Left**
1. Tibia length	388.00	381.00
1a. Medial length	398.00	392.00
3. Max. prox. epiph. breadth		
6. Max. dist. epiph. breadth	59.00	57.00
8. Sag. diam. midshaft	34.54	34.55
8a. Sag. diam.f. n.	39.22	40.79
9. Trans. diam. mid.	22.90	23.61
9a. Trans. diam. f. n.	25.82	26.30

10a. Circum. at f. n.	102.00	105.00
9:8. Diaphysis index	66.30	68.34
9a:8a. Cnemic index	65.83	64.48

Table II. Postcranial measurements of M 423.

Discussion

The person buried in the cemetery of Saint Sava church displays cranial modification caused by scaphocephaly. Despite of visual and pregnant differences of the skull (narrow frontal, elongated parietal bones, and short occipital) the postcranial measurements and pathological conditions incorporate him, as person, in the normal people buried in that cemetery.

Acknowledgements

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Figure 1

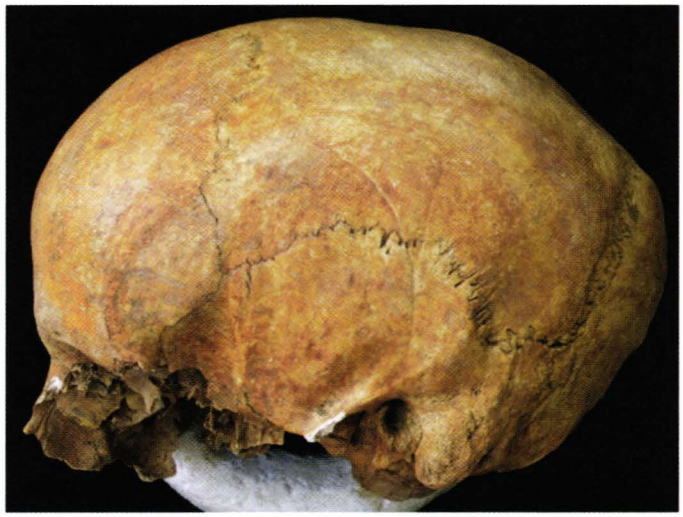

Figure 1

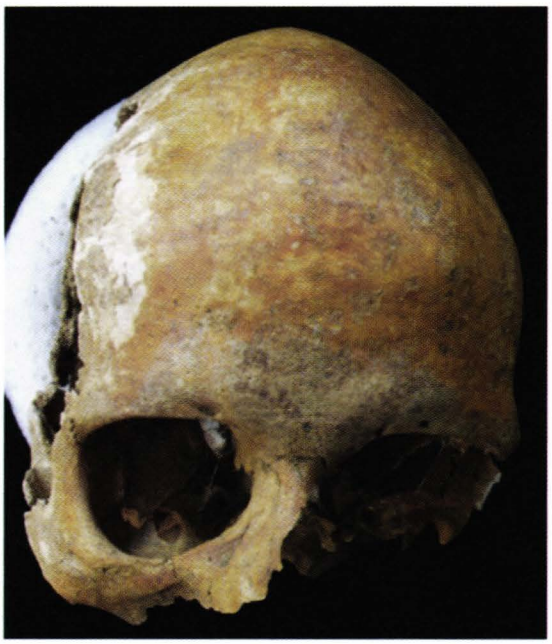

Figure 3

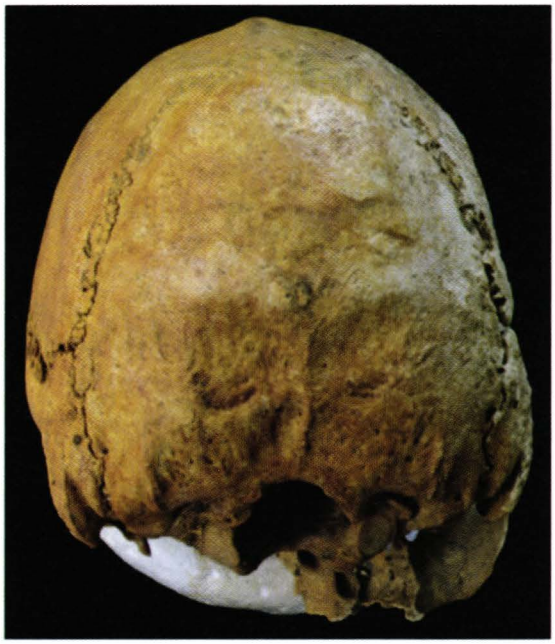

Figure 4

Plate no. I. Figure 1: Frontal view ; Figure 2: Lateral left view;
Figure 3: Posterior view; Figure 4: Superior view

THE HUNGARIAN CONQUEST PERIOD ARCHERY AND ACTIVITY-INDUCED STRESS MARKERS – A CASE STUDY FROM THE SÁRRÉTUDVARI-HÍZÓFÖLD 10TH CENTURY AD CEMETERY

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Keywords: Hungarian Conquest Period; Sárrétudvari-Hízóföld; archery; bioarchaeology; activity-induced skeletal changes

Abstract

In this paper, we present a bioarchaeological case study of archery-related stress markers in two skeletons from a Hungarian Conquest Period cemetery. According to both historical and archaeological data, the bow was a commonly used weapon in this period. The classical archaeological research of the Hungarian Conqueror army and the application of archery on the basis of the armed and unarmed graves are problematic since the presence and lack of weapon-founds do not directly refer to life activities. On the other hand, activity-related skeletal changes may develop in the bones as a result of archery induced physical stress, and therefore biological/physical anthropological investigations can give many research possibilities. As a part of a bigger bioarchaeological project, macroscopic analysis was performed on the scapulae claviculae, humeri, radii and ulnae of the unarmed grave 65 and armed grave 66 of the Sárrétudvari-Hízóföld (Hungary)10th century AD cemetery. We found hypertrophy at the attachment of a wide scale of upper limbs' muscles that are usually involved in the shooting process. As a result of our analyses, we can state that the complex analysis extends our knowledge concerning the Hungarian Conquest Period archery and burial customs.

Introduction

Archaeological and historical sources clearly state that warriors took a very important part in the Hungarian society in the 10th century AD, but we still have basic questions – e.g. the total size of the Hungarian army that cannot be answered with the utilization of classical archaeological and historical methods.

The historians have set up a couple of hypotheses about the total number of the warriors and/or the conquering Hungarian population.[1] These models are usually based on the Persian explorer

[1] Tóth 2010.

and geographer, Ibn Rustah's work. According to him, the ruler of the Hungarians usually moved out with twenty thousand riders.[2] New investigations highlighted an important issue: it is not possible to estimate the size of the whole Hungarian army and the total number of the conquering Hungarian population since Ibn Rustah's description is related only to the core of the army.[3]

On the other hand, the interpretation of the archaeological findings has its own limits too: it is always a controversial issue whether the grave-founds are the mirrors of life.[4] During his investigations on German and Anglo-Saxon materials, Heinrich Härke found out that the grave-founds have a fragmented and conceptual nature, and primarily do not reflect the occupation and life of the dead.[5]

Most of the archaeological founds from the Hungarian Conquest Period are connected to graves and cemeteries. In the case of the Hungarian warriors it means that these grave goods are provided by the family and the community, thus they reflect wealth, tradition, and religious beliefs of those who laid the dead to rest. Someone may have been a warrior in his life, although has no weapons in his grave. Moreover, one could get a weapon in the grave without using any in his entire life. This phenomenon warns us that the estimation of the military force of a population by the number of armed and unarmed graves is not an appropriate way.

However, the weapon set of the Hungarians in the 10th century AD consisted of axes, sabres, swords and spears too, according to the written sources and archaeological findings, mounted archers were the core of the Hungarian army and the bow was the common weapon in that era.[6] This statement is very important from the bioarchaeological point of view, since shooting the bow has a complex and unique physiological process in the background and it affects numerous anatomical areas (see Plate I, Fig. 1).

According to the literature[7], shooting the bow loads the torso and the upper extremities, and a wide scale of muscles are usually involved in the movement from the core muscles of the trunk to the muscles of the arms and hands. The repetitive physical load may develop special, activity-induced skeletal changes, and therefore can be investigated with classical biological anthropological methods. Paleopathologists started to use these markers to reconstruct past life activities, although the link between the actual activity and the skeletal markers is not clear yet.[8]

In Hungary, some scholars have already targeted enthesopathies of historical series in their research[9]; furthermore, in the case of grave 183 from the 10th century AD cemetery of Sárrétudvari–Hízóföld (Hajdú-Bihar county, Hungary) György Pálfi and his colleagues suggested a link between some lesions of the elbow and archery.[10]

We have started to carry out systematic research of the activity-induced skeletal changes of the Hungarian archers recently, and published our first results about the complex archaeological and physical anthropological investigation on the series of the 10th century AD cemetery of Sárrétudvari–Hízóföld. As a final result we could state that it is possible to identify the archers on the basis of the archaeological context and the activity-induced skeletal markers; also investigating the possible traces of archery, only comparing the archers with the unarmed individuals is not a

[2] HKÍF 1995.

[3] Szabados 2011.

[4] Härke 1997.

[5] Härke 1997.

[6] Kovács 1986, Révész 1996.

[7] Axford 1995, Miltényi 2008.

[8] Dutour 1992; Robb 1998; Jurmain 1999; Pearson – Lieberman 2004; Villotte 2008; Jurmain et al. 2012; Thomas 2014.

[9] Józsa et al. 1991, 2004; Józsa – Pap 1996, Pálfi – Dutour 1996.

[10] Pálfi et al 1996.

sufficient way of examination since there were more archers (without equipment) in the cemetery.[11] The most obvious way to present this dual issue is to compare the activity-related skeletal changes of individuals from the armed and unarmed groups.

In this paper, we would like to give the case study of the graves 65, and 66 of the Sárrétudvari–Hízóföld 10th century AD cemetery. First, we summarise the archaeological and anthropological characterisation of the two cases relying on the data of earlier archaeological and anthropological analyses. Then we describe activity-markers and discuss the possible evaluation of the results.

Material and methods

Since the excavation of the cemetery between 1983 and 1985, both anthropological[12] and archaeological[13] studies have been published on the material.

In the grave 65, a skeleton of a middle adult male was found. The dead was laid on his back in an extended position, with northwest-southeast orientation (275,6°–95,6°) and without any sign of post-depositional abnormality or robbery. His items were found at the skull, two silver penannular banded rings were found around the areas of the left and right ears. Weapons or grave-goods in association with military activity were not recorded.[14]

In the grave 66, a middle adult male individual was laid on his back (extended position), with southwest – northeast orientation (241,9°–61,9°) and without any signs of robbery and abnormality. The individual had multiple grave-goods, a silver penannular banded ring was found at the right side of the skull around the area of the ear, while two arrowheads were found at the left side of the skull. There was a little iron knife at the right side of the pelvis. A sabre and an antler plate of a compound bow were found on the opposite side of the body.[15] In summary, the Sárrétudvari cemetery can be dated to the 10th century[16] and these two graves fit in the gaps. Both of our chosen individuals belong to the mid-adult category and none of them show skeletal signs of DISH or other metabolic disorders that would exclude them from the evaluation.

During our macroscopic morphological analysis, the scapulae, the claviculae, the humeri, the radii, and the ulnae were systematically checked for activity-induced changes. Muscle attachment sites were in the focus of the analysis, but we also recorded the traces of traumas and other pathological changes. The scoring of entheseal changes was binary, and related to their presence and absence, but on the basis of the referential material and scoring method of Valentina Mariotti and her colleagues, with the minimum of 1c robusticity level.[17]

Results

In general, the two skeletons are affected by post-mortem damages and erosions, but there is no doubt of the high robusticity of the bones. The activity markers described below are presented bilaterally, both on the left and the right bones, but they show severe asymmetry.

The scapulae of the Individual 65 are highly fragmented and eroded. Traces of hypertrophy can be observed along the *margo lateralis* at the attachment of *musculus (m.) latissimus dorsi, m. teres major, m. teres minor* and especially at the attachment of the *m. triceps brachii caput longum* (see Plate I, Fig. 2a).

[11] Tihanyi et al 2015.
[12] Oláh 1990; Pálfi 1992;Pálfi 1993; Pálfi et al. 1996.
[13] M Nepper 1994; M Nepper 2002.
[14] Nepper 2002.
[15] Nepper 2002.
[16] Nepper 2002.
[17] Mariotti et al 2004, 2007.

The claviculae' state of preservation is more satisfying. At the site of *ligamentum costoclaviculare* (Fig. 3a) a strongly depressed area with porosity and well-defined margins can be observed. At the attachment of *ligamentum conoideum* (Fig. 3b) there is tubercle-like raised and elongated area with rough surface. The insertion area of the *ligamentum trapezoideum* (Fig. 3c) is rugose and highly raised. The anterior profile of the claviculae is interrupted by a rugose prominence at the insertion site of the *m. deltoideus* (see Plate I, Fig. 3d).

The humeri were robust but post-mortally eroded. At the proximal end of the bone, the attachments of the rotator muscles (*m. subscapularis, m. supraspinatus, m. infraspinatus, and m. teres minor*) were healthy but marked, especially at the right humerus. Although the insertion areas of the *m. pectoralis major* (see Plate I, Fig. 4a) are strongly eroded, the remains of the raised crests still can be seen bilaterally, just like in the case of the *m. latissimus dorsi* (see Plate I, Fig. 4b), *m. teres major, m. deltoideus* (see Plate I, Fig. 4c) and *m. triceps brachii caput mediale*. On the distal end, the insertion of the *m. brachioradialis* (see Plate I, Fig. 4d) presents a strongly developed and anteriorly curved crest, the *epicondylus lateralis humeri* is also affected.

The surfaces of the radii are strongly eroded, but it is clearly visible that they are massive and robust, especially at the insertion area of *m. biceps brachii* (see Plate II, Fig. 5a). The attachment site of the *m. pronator teres* (see Plate II, Fig. 5b) presents obvious rugosity, especially on the surface of the right side bone. Although the insertion of the *membrana interossea* is highly eroded, it shows widening and signs of rugosity.

On the ulnae, activity-related changes can be seen at five areas. The posterior and superior surfaces of the olecranon form a right angle and evident muscle markings can be seen as a result of the *m. triceps brachii* attachment sites' involvement (see Plate II, Fig. 5c). The insertion surface of the *m. supinator* (see Plate II, Fig. 5e) forms a crest with a rugose tail oriented to the posterior-inferior direction. The insertion zone of *m. brachialis* (see Plate II, Fig. 5d) is very rugose, the elevated margins are enclosing a depressed centre area. The insertion of the *membrana interossea* is well-developed at the ulnar site too (see Plate II, Fig. 5f).

The skeleton of the grave 66 is better preserved, it is also affected by post-mortem damages. Three sites of the scapulae show clearly the effect of physical stress. An osteophytic margin can be seen around the *cavitas glenoidalis* (see Plate II, Fig. 6a). The hypertrophy at the *margo lateralis* (the site of the attachment of *m. latissimus dorsi, m. teres major, m. teres minor,* and *m. triceps brachii caput longum*) is very characteristic (see Plate II, Fig. 6b), strongly developed crests of the attachment of *m. subscapularis* can also be registered (see Plate II, Fig. 6c).

On the claviculae, the sites of *ligamentum costoclaviculare* are affected: strongly depressed area can be observed with well-defined margins, porosity can be seen too on this surface (see Plate III, Fig. 7a). The *ligamentum conoideum* appears in the form of a well-developed, rough-surfaced tuberculum (see Plate III, Fig. 7b). Although the acromial ends of the claviculae are fragmented, a raised and rugose surface can be registered at the *ligamentum trapezoideum* insertions sites (see Plate III, Fig. 7c). The well-developed prominence at the insertion site of the *m. deltoideus* strongly interrupts the anterior profile of the claviculae (see Plate III, Fig. 7d).

The humeri are well-preserved and very robust. The attachments sites of the rotator muscles (*m. subscapularis, m. supraspinatus, m. infraspinatus, and m. teres minor*) show superficial irregularity and margins, especially at the right side. *M. pectoralis major* (see Plate III, Fig. 8a), *m. latissimus dorsi, and m. teres major* insertions are very well-developed and characterized with crests and longitudinal fossas (see Plate III, Fig. 8b). The tubercle of the *m. deltoideus* is highly raised (see Plate III, Fig. 8c), altering the profile of the bone. Advanced hypertrophy can be registered at the sites of the *m. triceps brachii caput mediale* (see Plate III, Fig. 8d). The attachment of *m. brachioradialis* presents a developed crest curved anteriorly (see Plate III, Fig. 8e), and the *epicondylus lateralis humeri* shows clear margins.

On the right radius, *m. biceps brachii* attachment site is prominent, the emerged area is especially clearly visible at the medial margin is of the site (see Plate III, Fig. 9a). Unfortunately, the area of the left side enthesis is fragmented and cannot be analyzed. Both the left and right *m. pronator teres* sites present "herring-bone" rugosity and are slightly raised (see Plate III, Fig. 9c). The *membrana interossea* insertions are flattened and thickened with rugosity (see Plate III, Fig. 9b).

The insertions of *m triceps brachii* on the proximal ulna are well-developed on both sides (see Plate III, Fig. 9d), similarly to the crested and tailed *m. supinator* sites (see Plate III, Fig. 9e). The *m. brachialis* insertion is depressed and surrounded by pronounced margins (see Plate III, Fig. 9f). The attachments of *m. pronator quadratus* show developed margins with a longitudinal fossa next to them (see Plate III, Fig. 9h). The right ulnar insertion of *membrane interossea* is also affected (see Plate III, Fig. 9g), a thickened margin can be observed (the same area on the left ulna is fragmented).

Discussion and conclusions

It is clearly visible that both individuals were muscular and well-trained during their life. If we compare the observed markers of the attachment sites of the two skeletons, we can see similarities resulting characteristic pattern. According to the activity-related skeletal changes of the upper limbs, we can state that they have practiced strong physical activity during their life resulting anatomically complex alterations that involved the muscles of the torso, the shoulders and the arms simultaneously.

The tendency of our earlier results concerning the armed graves of the cemetery perfectly correlates with the registered entheseal changes of the two cases: hypertrophic sites and entheseal alterations appear at characteristic attachment sites of the clavicle (attachments of *ligamentum costoclaviculare, m. deltoideus,* and *m. trapezius)* on the proximal/ mid humerus (attachment of *m. teres major, m. pectoralis major, m. latissimus dorsi, m. deltoideus)*, at the distal humeral end, where the common flexors and extensors attach (*epicondylus medialis and lateralis and crista supraepicondylaris lateralis*), on the radius (attachment of *m. biceps brachii* and at the site of margo interosseus) and on the ulna (attachment of *m. brachialis).*[18]

Site	Muscles
Body	*m. serratus anterior, m. pectoralis minor and major, m. rhomboideus minor and major, m. latissimus dorsi, m. trapezius, m. levator scapulae*
Shoulder	*m. deltoideus, m. supraspinatus, m. infraspinatus, m. teres minor and major, m. subscapularis*
Arm	*m. biceps brachii, m. brachialis, m. triceps brachii*
Forearm	*m. flexor digitorum, m. flexor digitorum profundus, m. flexor pollicis longus*

Table 1.

On the other hand, if we compare the affected muscles with the muscles usually involved in archery (Table 1), we can see many similarities as well. We have to know that work load of the muscles involved in archery is very different. These muscles overlap each other, some of them do not even attach to the bone surfaces, and therefore not all the muscles have their own observation sites on the bones. Also, the different technical implementations may occur in alteration of the muscle work and in variation of developing skeletal changes (e.g. using different fingers with different techniques of archery).

[18] Tihanyi et al 2015.

In the case of the grave 66, the archaeological and bioanthropological data do certify each other, and we can state that the individual was a potential archer in his life, and the weapons in his grave were not just the symbols of the wealth of his family.

The bioanthropological data of the grave 65 skeleton do extend the archaeological data since the activity-related changes are the same as markers of the armed individuals of the Sárrétudvari-Hízóföld cemetery. According to this statement, he might have practiced the same activity, but those who laid him rest did not put the weapon in the grave, or pieces of his weaponry were completely destroyed post-depositionally.

However, hypertrophies of such attachments sites as the *m. pronator teres, m. supinator*, or *m. pronator quadratus* of the forearm may refer that these individuals practiced additional activities besides archery, while some other entheseal changes (e.g. at the *m. biceps brachii)* are not specific enough to draw conclusion.[19]

The complex anthropological and archaeological investigation extended our knowledge and revealed the real groups of archers and non-archers are not parallel with the armed and unarmed groups. Therefore „archer", "warrior" and „non-archer" terms must be handled with care and further investigations of the Hungarian Conquest Period series is necessary.

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[19] Tihanyi et al 2015.

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Abbrevations of journals

Acta Biol Szeged – Acta Biologica Szegediensis
Am J Phys Anthropol – American Journal of Physical Anthropology
Ann Histor-Nat Mus Nat Hung – Annales Historico-Naturales Musei Nationalis Hungarici

Bull et Mémo de la Soc d'Anthrop de Paris	– Bulletins et mémories de la Société d'Anthropologie de Paris
Coll. Antropol	– Collegium Anropologicum
Int J Anthropol	– International Journal of Anthropology
Int J Osteoarchaeol	– International Journal of Osteoarchaeology
Yearb Phys Anthropol	– Yearbook of Physical Anthropology

List of figures and tables

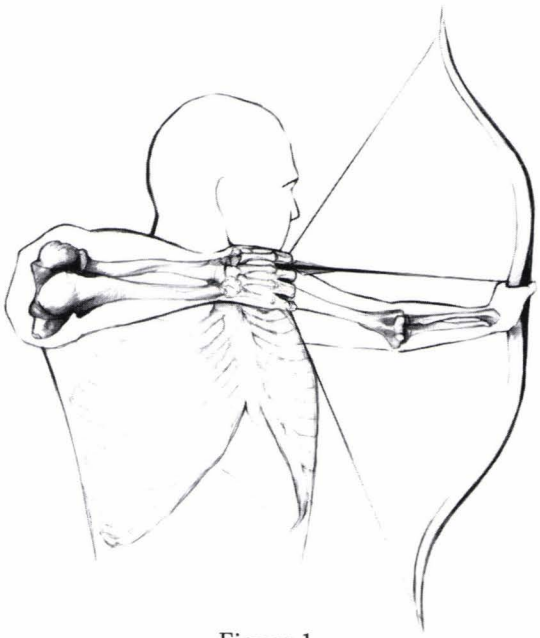


Figure 1

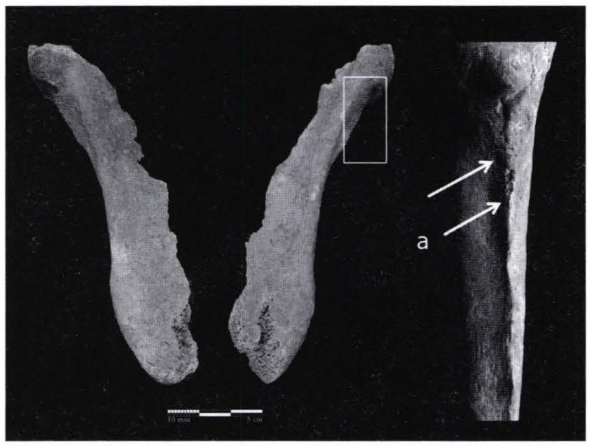
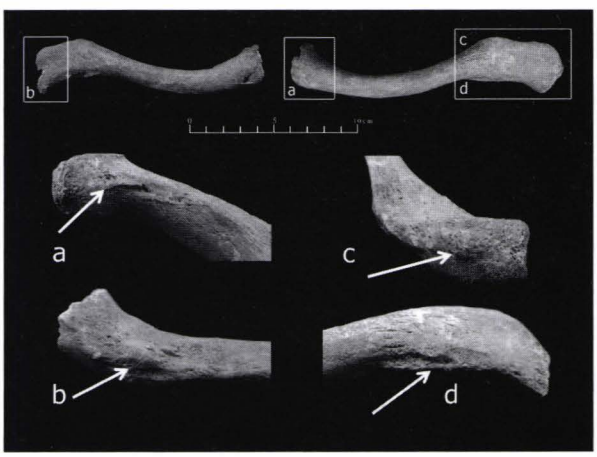


Figure 2



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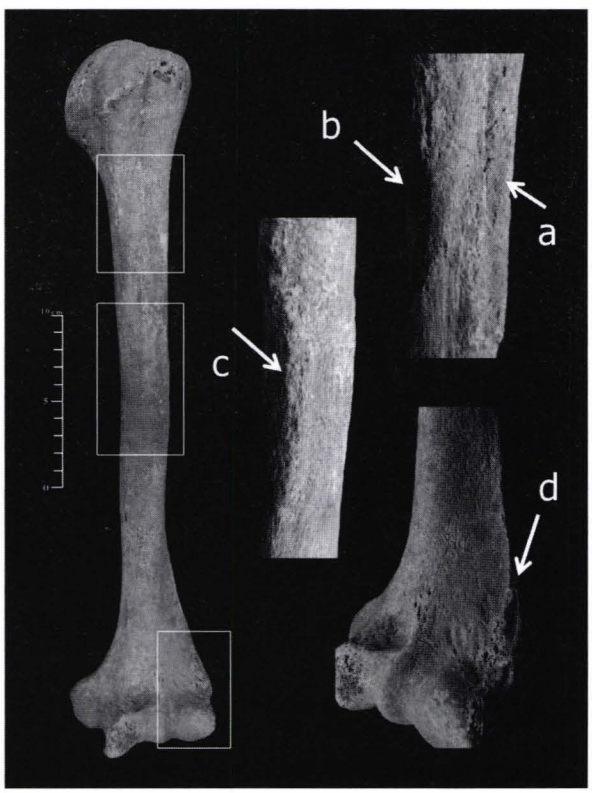

Figure 4

Plate no. I.

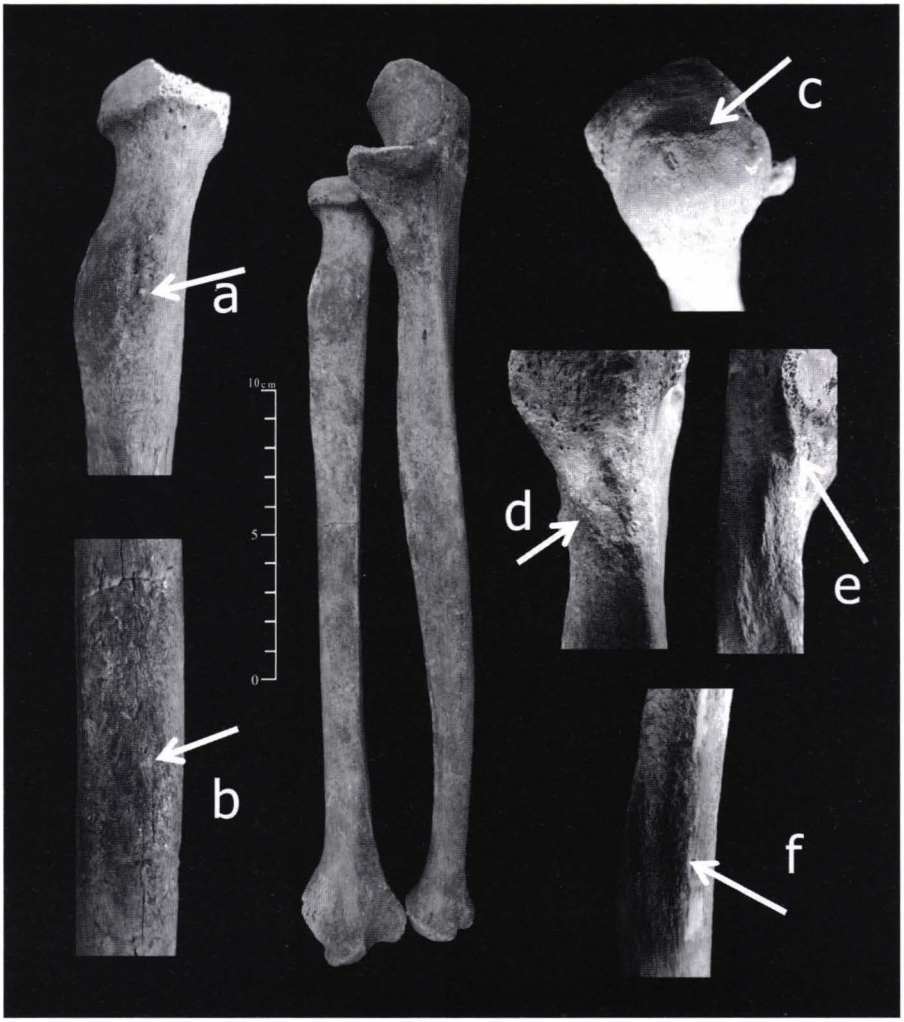

Figure 5

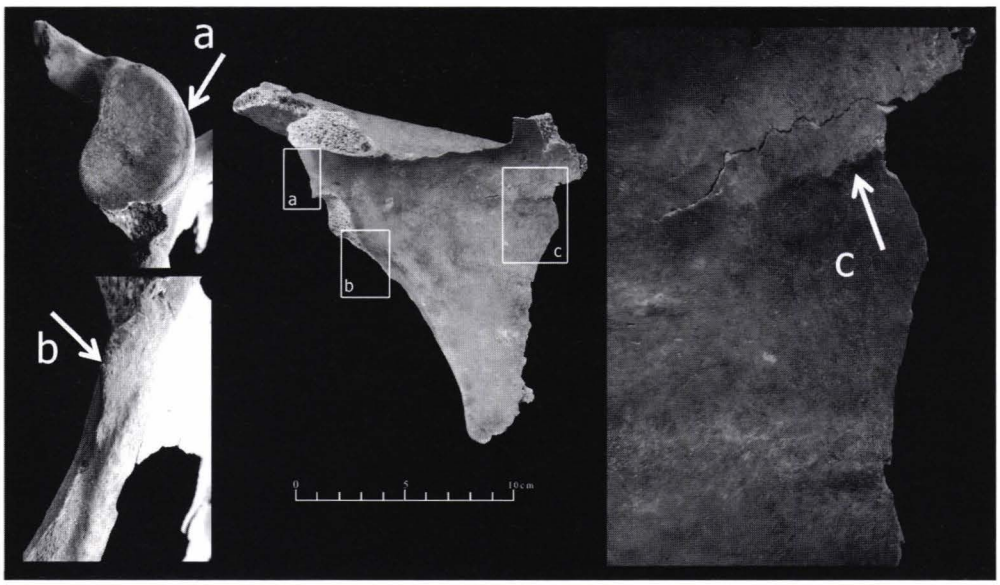

Figure 6

Plate no. II.

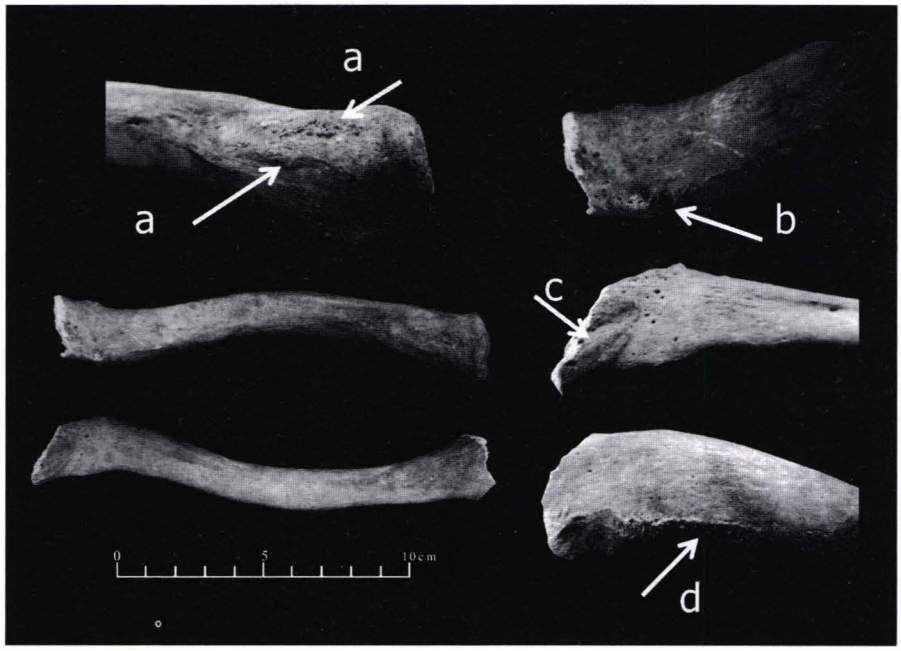

Figure 7

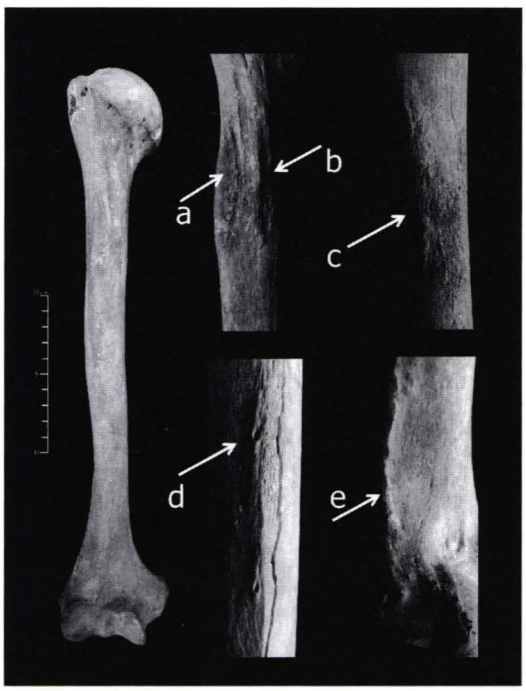

Figure 8

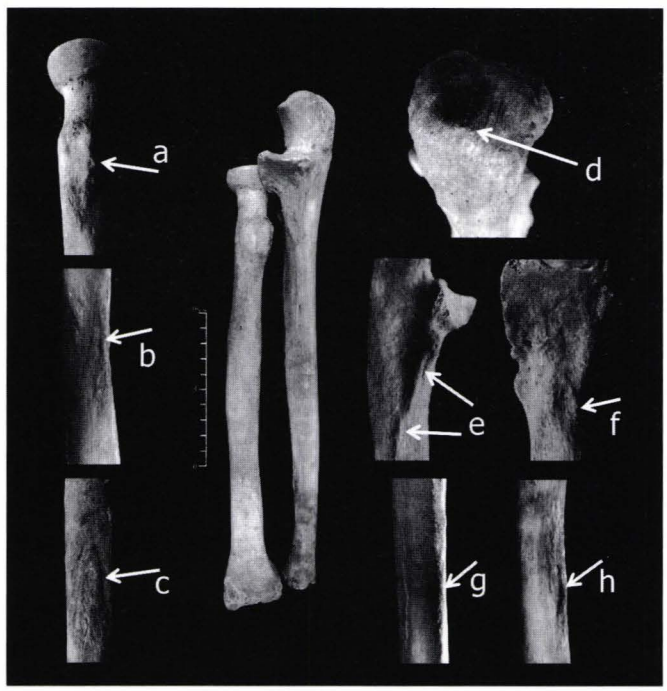

Figure 9

Plate no. III.

CASES OF TUBERCULOSIS INFECTION VERIFIED BY LIPID BIOMARKER ANALYSIS IN HUNGARIAN ARCHAEOLOGICAL SAMPLES

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Abstract

Since tuberculosis still causes more deaths than any other infectious disease, better understanding of the pathomechanism and the evolution of its infectious agent is surpassingly important. The disease is caused by members of Mycobacterium tuberculosis complex including several species, but the major infectious agent is M. tuberculosis.

Several methods exist in the clinical practice to diagnose this causative agent. One of them is an integrated protocol based on the unique lipid rich cell wall structure of Mycobacteria. The applied lipid biomarkers can be detected by HPLC and GC-MS. This protocol can also be used with a great efficiency in palaeopathological investigations.

The Department of Biological Anthropology at the University of Szeged has coilaborated with David E. Minnikin's research group in the School of Biosciences at the University of Birmingham since 2012 in the investigation of archaeological samples from the viewpoint of tuberculosis infection. In this review, we give a short summary of the published results of the collaborative research work, which are good examples for the excellent application possibilities of lipid biomarker analysis. The reviewed samples were taken from three cemeteries in Hungary within a wide time range, from the Late Neolithic to the 7th–8th centuries AD.

Introduction

Nowadays, tuberculosis (TB) caused by bacteria belonging to the *Mycobacterium tuberculosis* complex (MTBC) is responsible for more deaths than any other infectious disease.[1] This complex includes several species, but the main infectious agents among them are *Mycobacterium tuberculosis* strains. The *Mycobacterium* genus is the member of the *Mycobacteriaceae* family belonging to the *Actinomycetales* ordo.[2] One of the most common features among *Mycobacteria* is their unique staining that requires special dyes (e.g.carbolfuchsin) because of the complex structure of their cell envelope containing lipid-rich molecules in high concentration.[3] The identification of these

[1] WHO, 2016.
[2] Somosköví, 2007.
[3] Minnikin, 1982; Minnikin et al., 2002.

infectious agents in archeological samples has a great importance, and besides DNA analyses, the detection of characteristic cell wall components of the causative microbes can also provide reliable diagnostic possibilities.

I.1. Mycobacterial cell wall structure

Mycobacteria have a remarkably complex cell wall structure, described by the dual membrane model,[4] where a mycobacterial inner and outer membrane (MIM and MOM) can be distinguished, and according to David E. Minnikin and his colleagues,[5] there might be an extensive "periplasmic space"between them. Furthermore, there is a suspected peptidoglycan-galactan layer in the cell wall, which canprovide anchorage points for the arabinanmoieties. Mycolic acids(MAs) are esterified with these arabinan moietiesin the MOM, which also contains other unusual free lipidsinteracting with mycolic acids such as mycocerosic and mycolipenic acids.[6]

We can use several components of this cell wall structure as lipid biomarkers in the identification of *Mycobacterium* species both in anthropology and in clinical practice. In this review the possibilities of analytical detection of lipid biomarkers are summarized through a few bioarchaeological samples investigated during the course of collaboration between the Department of Biological Anthropology at the University of Szeged and the laboratory of David E. Minnikinin the School of Biosciences at the University of Birmingham.

I.2. Lipid biomarkers

I.2.1. Mycolic acids

MAs are integral part of mycobacterial cell envelopes, with a remarkable biomarker value both in identification and classification.[7] The MAs in *M. tuberculosis* can be classified into three principal groups, and all of them have a range of homologues with different chain lengths.[8] The three main types of MAs are α -, methoxy- and keto-mycolates, which can be distinguished from each other in a simple normal phase chromatographical separation. [9]The amount of α-mycolatesis the highest, approximately 50 percent of allMAs.[10] Furthermore, chemical sub-classes can be differentiated within both methoxy- and keto-mycolates based on presence of alternative methyl branched trans-cyclopropane or cis-cyclopropane moieties, but the separation of them is not possible by normal phase chromatography.[11] MA-groups can be separated chromatographically from each other on normal stationary phase,[12] but it is still not diagnostic for the MTBC because a lot of other mycobacterial species also produce these three groups of MAs. Fortunately, separation within the three major groups of MA components is possible based on their chain length and polarity using reverse phase HPLC after a normal phase separation.[13] After this, computerized comparison with an appropriate standard seems to be adequate for diagnostic purposes.[14] The applicability of this method has been proved also in archaeological materials, for example in the case of samples

[4] Christensen et al., 1999; Minnikin, 1982.
[5] Minnikin et al. 2015.
[6] Minnikin, 1982; Minnikin et al., 2002.
[7] Butler &Guthertz, 2001; Dobson et al., 1985.
[8] Minnikin, 1982; Minnikin&Polgar, 1967a, 1967b; Watanabe et al., 2001, 2002.
[9] Dobson et al., 1985; Minnikin, 1993.
[10] Watanabe et al., 2001; 2002.
[11] Donoghue et al., 2010a; Gernaey et al., 1998, 2001; Hershkovitz et al., 2008; Watanabe et al.,2001.
[12] Dobson et al., 1985; Minnikin, 1993.
[13] Minnikin, 1993; Qureshi et al., 1978; Steck et al., 1978.
[14] Butler &Guthertz, 2001.

collected from Atlit Yam, a circa 9000 years-old archaeological site.[15] The unique lipid biomarker pattern of the members of the MTBC is usually complemented with DNA analysis[16] providing excellent possibility to conduct complex paleoepidemiological investigations with a broad time window in human prehistory due to the MAs' particular stability.[17] Furthermore, it is important to consider that some *Mycobacteria* can also be found in the soil and can contaminate the bone samples, but these are also distinguishable by their lipid biomarker pattern.[18]

One of the most popular lipid biomarker based method used in anthropological practice was developed by David E. Minnikin and his colleagues.[19] They published a multistep procedure for the detection of characteristic lipids.[20] This protocol was built from different previous methods and rigorously optimised to diagnose TB infection originally in clinical samples (sputum).The procedure starts with a saponification and a methylation step, followed by different liquid-liquid extractions, and a 9-anthrylmethyl derivatisation. After this, a solid phase extraction is carried out on C–18 cartridges and the derivatised MAs are separated at first with normal phase HPLC (Plate no. I, Fig. 1A). The anthrylmethyl-esters are detected via fluorescence detector, and the three MA groups are fractionated. The collected fractions are separated according to their chain length and polarity with reverse phase HPLC[21] (Plate no. I, Fig. 1B).To examine archaeological samples, this method was supplemented further with an initial reverse phase HPLC separation before the normal phase HPLC in case of the samples from a Byzantine basilica in the Negev desert at Karkur[22] (Plate no. I, Fig. 1C). In this site a calcified pleura fragment was found in the grave of a 35–45 years old male dated approximately to1400 BP. The sample was taken from this pleura fragment.

The exact method was published by Gernaey and colleagues in 1998.[23] For the examination of biomarker patterns the samples were collected from the burial ground of the Newcastle Infirmary. This institute was used actively between 1753 and 1845 and in most cases the burials were well documented.

The lipid biomarker method was renewed in 2008,[24] when samples for both DNA analysis and lipid biomarker detection were taken from the skeletal remains of a woman and an infant, buried together at the approx. 9000 years old archaeological site of Atlit Yam. The earlier method[25] was changed at the derivatisation phase. Pentafluorobenzyl (PFB) was introduced instead of methylanthryl derivatisation,[26] because the derivatives were relatively instable.[27] Mycolate-PFB esters were purified by solid phase extraction (the purification was done on normal phase silica) and esterified further with pyrenebutyric acid (PBA).[28]

I.2.2. Mycocerosic and mycolipenic acids

Mycocerosic and mycolipenic acids are further groups of lipids in the MOM, which can be applied as lipid biomarkers to diagnose TB infection. These are so-called "free" lipids, which are

[15] Hershkovitz et al., 2008.
[16] Donoghue et al., 1998, 2010a; Gernaey et al., 2001; Hershkovitz et al., 2008.
[17] Gernaey and Minnikin, 2000; Redman et al., 2009.
[18] Gernaey et al, 1999.
[19] Donoghue et al., 1998.
[20] Minnikin et al., 1993b.
[21] Minnikin, 1993b.
[22] Donoghue et al., 1998.
[23] Gernaey et al., 1998.
[24] Hershkovitz et al., 2008.
[25] Gernaey et al., 1998.
[26] Hershkovitz et al., 2008.
[27] Minnikin et al., 2012.
[28] Hershkovitz et al., 2008.

associated with anchored mycolic acids to form the outer myco-membrane.[29] Mycocerosic acids are long-chain multimethyl-branched-chain fatty acids belonging to phthiocerol dimycocerosate waxes.[30] Different mycocerosate types and their distribution among a range of *Mycobacterium* species has already been defined.[31] The pattern of C29, C30 and C32 mycocerosates is the most characteristic for the MTBC (Redman et al., 2015). Mycolipenates are members of pentaacyltrehalose glycolipids, and among them the C27 mycolipenate is specific to *M. tuberculosis*.

According to Larsson and colleagues,C32 mycocerosates can be detected by GC-MS[32] from 5-day cultures of sputum samples collected from tuberculosis patients. The analysisis carried out using selected ion monitoring (SIM) negative ion chemical ionisation gas chromatography mass spectrometry (NI-CI GC-MS). When SIM is used, only the target analytes are monitored, thus the analytical sensitivity improves. The method, which can also be used in paleopathological research, was described by Redman and colleagues in 2009[33] during the analysis of samples from the Coimbra Collection dated to the 19th–20th centuries AD. This collection consists of the remains of more than 500 individuals who died in the Coimbra University Hospital. For this special investigation 49 individuals' bone samples were used. The cause of death was probably tuberculosis in the case of about 50% of the examined individuals.

The GC-MS method starts with similar steps like the HPLC protocol from the saponification to the derivatisation procedures.[34] After derivatisation, the mycocerosic acid PFB esters are purified by solid phase extraction using normal phase SPE cartridges before HPLC separation. To define the retention time of the sought mycocerosic acid esters on the normal phase,decafluorobenzhydrol and decafluorobenzophenoneco-markersare used. A range of decafluorobenzhydrol esters of long-chain fatty-acids are eluted before mycocerosate, while decafluorobenzophenoneis eluted after them.The mycocerosic peak is collected and examined by selected ion monitoring (SIM) negative ion chemical ionisation gas chromatography mass spectrometry (NI-CI GC-MS).

II. Detection of *M. tuberculosis* infection from Hungarian samples on the basis of lipid biomarker patterns

The Department of Biological Anthropology at the University of Szeged had already investigated specific infectious diseases in archaeological series since the 1970's.[35] The most outstanding cases are discussed in this review grouped by excavation sites.

II.1. Szeged-Kiskundorozsma, Daruhalom-dűlő

During the course of former investigations at the Department of Biological Anthropology mainly macromorphological and aDNA techniques were used,[36] but from 2010 collaboration was built with the laboratory of David E. Minnikin, opening new possibilities in the identification of TB infections with the application of lipid biomarker pattern analysis. One of the first cooperations was the examination of bone material from grave no. 517(KD 517) in the Szeged-Kiskundorozsma, Daruhalom-dűlő archaeological site.[37] The cemetery is dated to the Avar Age (7th century AD),

[29] Minnikin, 1982; Minnikin et al., 2002.
[30] Minnikin et al., 1983, 1985a, 1985b, 2002.
[31] Daffé&Lanéelle, 1988; Minnikin et al., 1985a; Minnikin et al., 1993a.
[32] Larsson et al., 1981.
[33] Redman et al., 2009.
[34] Redman et al., 2009.
[35] Marcsik, 1972; Pálfi–Molnár, 2009.
[36] Haas et al., 2000;Pósa et al., 2015;Zink et al., 2007.
[37] Lee et al., 2012.

where 94 skeletons had been excavated.[38] This material is special for the high number of individuals who probably suffered in mycobacterial infection. Eight of them showed macromorphological features of *M. leprae* infection in different phases.The person who rested in grave KD 517 was a 35–40 years old male.[39] In the rhinomaxillary region considerable remodelling can be noticed. There is slight alveolar resorption, the ospalatinus shows abnormal porosity and the nasal spine is completely obliterated. Furthermore, there are slight changes on the tibia and the fibula. Bilateral porotic *cribra orbitalia* was also observed. To support the diagnosis, aDNA and lipid biomarker analysis were performed. A scraped sample was taken earlier from the nasal region and aDNA investigation was done by Donoghue and colleagues.[40] The residuals of this samples were used for the lipid biomarker examination.[41] Symptoms in other skeletons from this site implied leprosy and/or tuberculosis, so KD 517 was tested for both *M. tuberculosis* and *M. leprae*. The HPLC profiles of total mycolates from KD 517 showed a closer similarity to *M. tuberculosis* than to *M. leprae* on reverse phase, which could mean the predominance of tuberculosis infection. Moreover, methoxy-mycolate fraction was recognized on the normal phase HPLC chromatogram. It suggested the presence of *M. tuberculosis*, as this kind of MAs are absent in the of *M. leprae*.[42] The second reverse phase profiles of the three main mycolate types suggested the presence of both *M. tuberculosis* and *M. leprae*.[43]

Mycocerosic acid content of this sample was also investigated with NI-CI-GCMS[44] and their four major characteristic components,C29, C30, C32 and C34 were detected. The high amount ofC34 components suggested *M. leprae* infection, while the enhanced amount of C29 mycocerosates confirmed the presence of *M. tuberculosis,* implying a case of leprosy-TB coinfection.

II.2. Hódmezővásárhely-Gorzsa

Hódmezővásárhely-Gorzsawasa Late Neolithic tell settlement belonging to the early Tisza culture, which was located in southern Hungary.[45] The most interesting symptoms were found in the fragmentary bones of a probably19–20 years old male(grave no. 64, code HGO–53). According to the results of radiocarbon analysis of bone fragments, this individual can be dated back to the start of the fifth millennium BC. During the macroscopical observation the following pathological changes were noticed: light *cribra orbitalia* and *cribra cranii*, small patch of periostitis on the mandible. Cavitations were also found on the fragments of vertebral bodies. On the ventral surface of the heads of left ribs, active diffuse periostitis was noticed, heads of right ribs were not recovered. Other rib fragments from indeterminable sides also showed active diffuse periostitis, and one of them showed focal lytic lesion with reactive new bone surface. Signs of widespread active periosteal new bone formation were found along the shafts of the long bones, which was strikingly symmetrical both on the upper and the lower limbs. These periosteal changes can refer to HPO (Hypertrophic Pulmonary Osteopathy). On the foot bones, the signs of bilateral periostitis were also recognised.

After macromorphological observations, aDNA and lipid biomarker analysis were conducted.[46] First the IS1081 DNA region had been tested showing positive results. However, the application

[38] Molnár et al., 2006; Pálfi and Molnár, 2009.

[39] Lee et al., 2012; Molnáret al2006; Pálfi and Molnár 2009.

[40] Donoghue et al., 2005; Molnár et al., 2006.

[41] Lee et al., 2012.

[42] Minnikin et al., 1985.

[43] Lee et al., 2012.

[44] Lee et al., 2012.

[45] Masson et al., 2013.

[46] Masson et al., 2013.

of IS6110 complex-specific insertion sequence did not refer to the presence of *Mycobacterium* species. The detection of lipid biomarkers was more successful, since the mycolate fractions on the first reverse phase HPLC separation indicated the presence of long-chain mycolic acids. Although the profile was weak, it correlated with the standard profile for *M. tuberculosis*. The normal phase HPLC of the total mycolatefraction gave only a small peak for α-mycolates, but the preservation of methoxy- or ketomycolates was not sufficient enough for the diagnosis. In contrast to this, the mycocerosic and the mycolipenic profiles confirmed the presence of the ancient tuberculosis infection measured by NI-CI-GC-MS.

Four additional cases were also examined later from this site with complex aDNA/HPLC/GC-MS strategy.[47] At first, these individuals were diagnosed as probable cases of tuberculous infection on the basis of marcromorphological examination, later also confirmed by molecular and chemical methodologies.HGO-08 involved in this work was a young, 17–22 years old female. On her skull light bilateral *cribra orbitalia* was recognised and other lesions were found in the thoracal region such as resorptive lesions on the anterior side of all thoracal vertebrae from the T5 to the T12 and also on two lumbar vertebrae. Furthermore, Schmorl's nodes were also visible on each thoracal vertebra from the T7 to the T12 (Plate no. I, Fig. 2). On the inferior side of the L1 and the superior side of the L2, an extensive lesion was observed with adjacent osteophytes. Following the marcromorphological identification of the disease, strong and clear mycocerosate- and mycolate profiles were also found indicating MTBC infection.

The next examined case was HGO–10, a male individual in the beginning of his twenties.[48] Hypervascularisation was observed on the anterior side of five successive thoracal vertebrae and two lumbar ones. Slight hypervascularisation was found on the visceral surface of the ribs. Furthermore, linear enamel hypoplasia was also found on the remains of this individual. The result of the lipid biomarker analysis also gave confirmation of a MTBC infection similarly to the previous case.

The third examined case was HGO–21, a female in her early twenties.[49] On her skulls mall endocranial pits and *cribra orbitalia* were found. Resorptive lesions were observed on the T9 vertebra. Symptoms were also observed on her ribs such as increased vascularisation on the ventral side of one rib and light periostitis on the external surface of two fragments of other ribs towards their sternal end. The mycolate profile was weak, however, the positive aDNA result and the strong mycocerosate profile gave good confirmation of the infection.

The last examined individual from this site, HGO–48 was a young adult female. Abnormal blood vessel impressions were visible mainly on the frontal endocranial surface with a SES-like pattern(*serpensendocraniasym metrica*) (Plate no. I, Fig. 3). These signs refer to meningitis, possibly caused by infectious diseases including tuberculosis. A large round depression (circa 1 cm in diameter) on theendocranial surface of the right parietal might also be a tuberculous lesion. Slight *cribra orbitalia* was also recognised in the left orbit by Masson and colleagues. The mycolate profile was weak, however the strong mycocerosate profile and the result of the aDNA analysis confirmed the infection.

II.3. Bélmegyer–Csömökidomb

The Bélmegyer–Csömökidomb archaeological site contained the remains of 240 individuals. The cemetery was used between 670 and 800 AD during the Late Avar Age.[50] Nineteen tuberculosis

[47] Masson et al., 2015.
[48] Masson et al., 2015.
[49] Masson et al., 2015.
[50] Molnár et al., 2015.

infected individuals had been found based on earlier macroscopic and radiological examinations, including both classical and atypical/early stage TB cases.

The remains of a 40–60 years old female excavated from the grave no. 65 showed osteolytic lesions on the anterior aspect of the thoracal and lumbar vertebral bodies.[51] The lesions led to collapse of vertebras causing angular kyphosis of the spine.[52] The aDNA analysis gave positive result for TB, which was also confirmed by the lipid biomarker analysis targeted to both mycolipenic- and mycocerosic acids, but the mycolic acid profile was weak.

The bones of a 57–62 years old male from gave no. 90 showed pathological remodeling and fusion of the lumbosacral region.[53] Furthermore, irregular *ante mortem* erosion was visible on the ventral surface of the sacrum suggesting earlier presence of cold abscess (Plate no. II, Fig. 4). Probable signs of *coxitistuberculosa* or tuberculous arthritis of the left hip was observed involving the left innominate and the femur (Plate no. II, Fig. 5).The aDNA analysis,[54] the mycolic acid and the mycolipenic acid profile all confirmed the MTBC infection, but the mycocerosic acid profile gave a less convincing evidence.[55]

In the case of the elderly male resting in grave no. 215, complete ankylosis of the right knee was reported.[56] This lesion strongly indicated the *gonitis tuberculosa*.The aDNA results were negative, the mycocerosic acid investigation was not effective enough, and the mycolic acid profile was also weak, but the mycolipenic detection diagnosed the *M. tuberculosis* infection.

Probable *coxitistuberculosa* of the right hip joint was found in the case of a 16–18 years old female individual from grave no. 38.[57] The aDNA analysis did not give conclusive results, nor did the mycolic acid separation, and the mycolipenate and the mycocerosate profiles recorded by NI-CI-GCMS were also only giving a weak signal.

The last case with classical TB changes is an adult male from the grave no. 189.[58] In this case, the main pathological changes affected the vertebrae suggesting *spondylitis tuberculosa* and ankylosis of the T9-T10 and the L1-L4 vertebrae. Furthermore, osteophytes and new bone formation were detected on the ventral surface of all lumbar vertebral bodies and long bone periostitis was detected on both femurs and tibias. The aDNA analysis along with the mycolipenic and mycocerosic analyses gave positive results, but the mycolic acid profile was not clear enough.

Further 14 examined individuals exhibited mainly atypical or early-stage macromorphological TB alterations and one of them did not show any lesion.[59] Most of the affected individuals belonged to younger age cohorts.8cases out of 14 showed rib periostitis (Plate no. II, Fig. 6) and10 cases showed superficial vertebral changes. Long bone periostitis was found in the skeleton of 6 individuals, from which two showed diffuse periostitis. Endocranial lesions were observed in 5 cases. The presence of *cribra orbitalia* was observed on skulls of four individuals. The aDNA analysis gave positive result in 8 cases of out 14.[60]

Molnár and colleagues classified the individuals from Bélmegyer–Csömökidomb site into 6 groups on the base of lipid biomarker results. The first group includes the cases where

[51] Pálfi, 1991.

[52] Haas et al., 2000; Molnár et al., 2015.

[53] Molnár et al., 2015; Pálfi et al., 1992.

[54] Molnár et al., 2015; Haas et al., 2000.

[55] Molnár et al., 2015.

[56] Molnár et al., 2015; Pálfi–Csernus, 1990.

[57] Marcsik et al., 2007; Molnár et al., 2015.

[58] Marcsik et al., 2007; Molnár et al., 2015.

[59] Molnár et al., 2015.

[60] Haas et al., 2000; Molnár et al., 2015.

evidence of TB was found based on the mycolic, mycolipenic and mycocerosic acid profiles as well.[61] This group consists of 4 cases, among which the remains of a 16–18 years old individual excavated from grave no. 22exhibited signs of leprosy-tuberculosis co-infection. The second group consists of 7 cases, where the presence of mycolipenate was clear, but the mycolic and mycocerosic acid signals gave less convincing evidence. This group included some individuals with classical TB changes. The third group contained 2 individuals with complete mycolipenate profiles, but the mycocerosic patterns were weak, while the mycolic acid examination gave no result. The fourth group included only one case, where even the mycolipenate signal was poor. The fifth group with 4 members showed weak and inconclusive evidence for both mycolipenic and mycocerosic acids. 2 samples were placed in the last group, where no mycobacterial lipid biomarker was detected. It is also important to mention that the individual, who did not show any classical or atypical TB associated alteration, was classified as a member of the first group.

III. Discussion

It is important to understand the evolutionary history of *Mycobacterium tuberculosis* because tuberculous infections are becoming very frequent nowadays. In this question, examinations of archaeological samples may have a crucial role and could provide significant new information. The application of mycobacterial lipid biomarkers in the identification of *Mycobacterium tuberculosis* infections has a long history. However, this field is still developing nowadays considerably. The method developed by David E. Minnikin and his colleagues, can be used successfully in clinical practice and biological anthropology as well. Based on the formerly introduced examples, the method works well in a long time scale and in the case of leprosy co-infections as well. The application of lipid biomarker examination and a DNA analysis together with macromorphological methods provides a very effective and successful combination of diagnostic tools that will hopefully aid further important new discoveries in the future.

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[61] Molnár et al., 2015.

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Figures

Fig. 1: HPLC PBA-PFB esters of mycolic acids extracted from skeletons from Atlit Yam and standard *M. tuberculosis*. A. Reverse phase HPLC of total mycolates; B. Normal phase HPLC of total

mycolates, collected from the former reverse phase HPLC measurement; C. Reverse phase HPLC of α-mycolate, methoxymycolate and ketomycolate classes. Taken from Hershkovitz et al. (2008), modified by the authors.

Fig. 2: Schmorl's node on the 12th thoracic vertebra. Hódmezővásárhely-Gorzsa, inv. no.: HGO-08, 17–22 years old female.

Fig. 3: SES-like pattern. Hódmezővásárhely-Gorzsa, inv. no.: HGO–48, young adult female.

Fig. 4: Pathological remodeling and fusion of the lumbosacral region. Further irregular *ante- mortem* erosion on the ventral surface of the sacrum. Bélmegyer-Csömökidomb, gr. no.: 90, 57–62 years old male.

Fig. 5: Destruction on the left hip bone and on the left proximal femur. Bélmegyer-Csömökidomb, gr. no.: 90, 57–62 years old male.

Fig. 6: Periostitis on a right rib fragment. Bélmegyer-Csömökidomb, gr. no.: 12, 33–39 years old male.

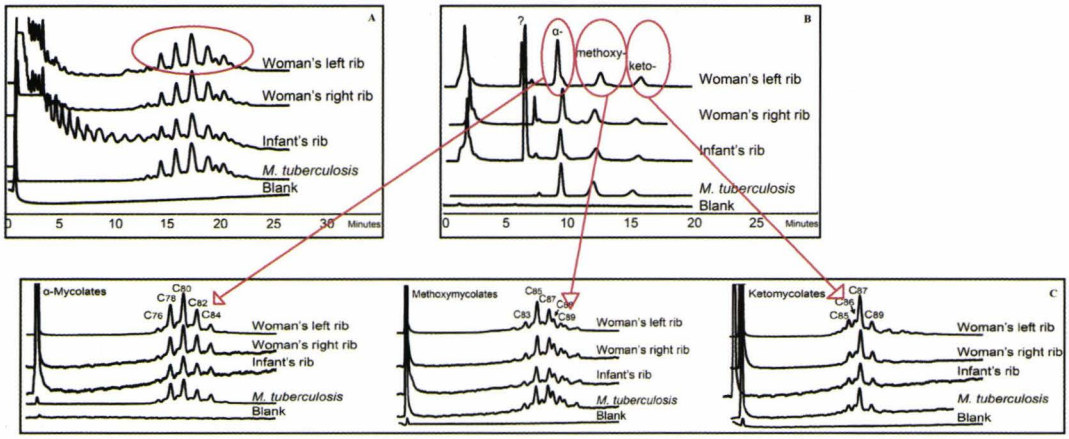


Figure 1

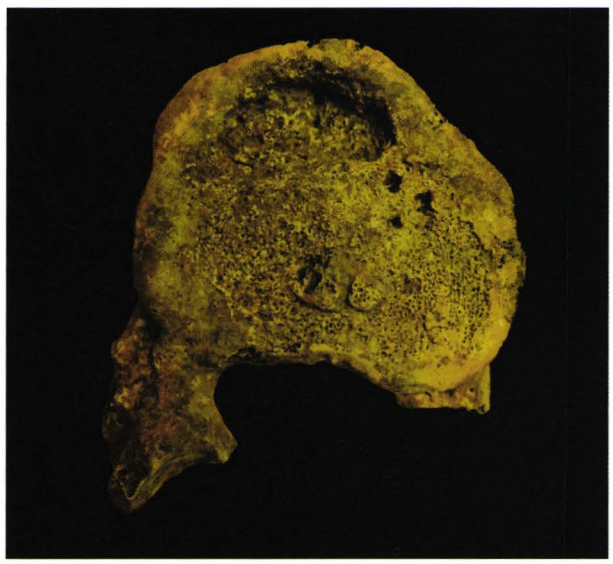


Figure 2

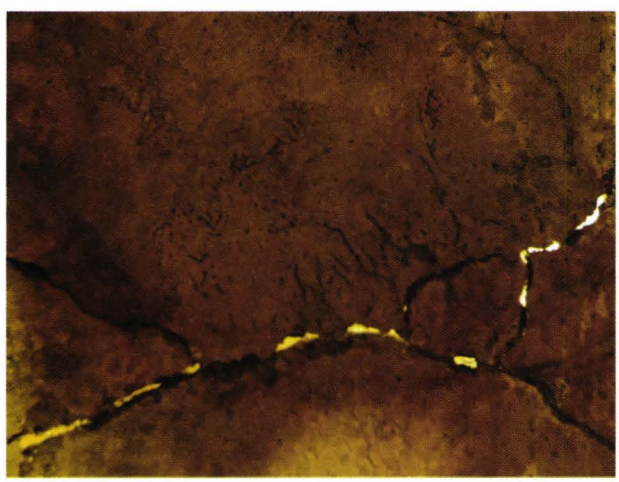


Figure 3

Plate no. I.

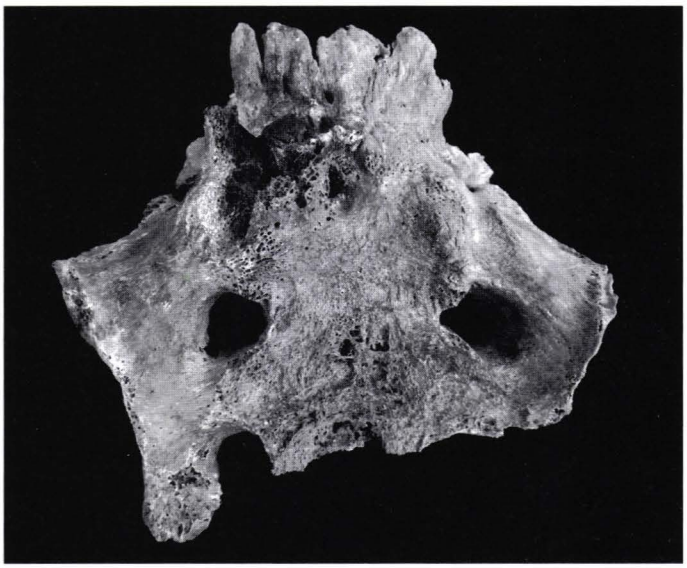


Figure 4

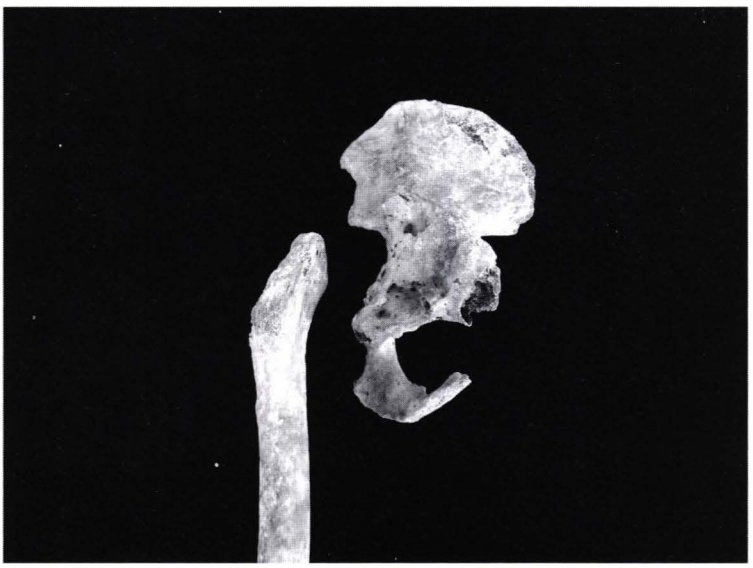
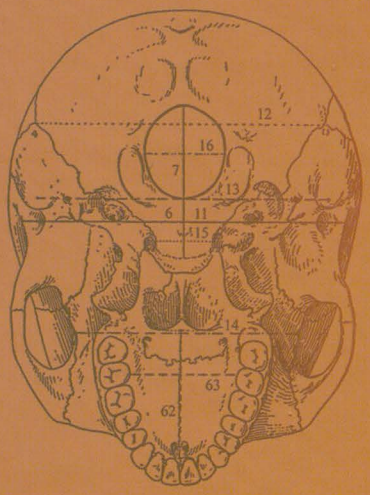
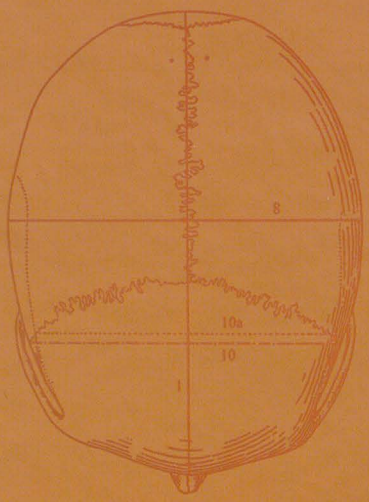


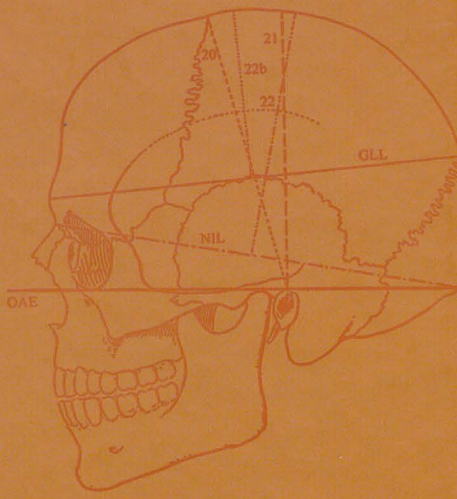
Figure 5

Figure 6

Plate no. II.







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