

Research Article

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Odontological analysis of medieval archaeological samples from Krešića greblje near Čitluk, Bosnia and Herzegovina - An auxiliary method to genetic analysis

Selma Zukić^{1*}, Edin Bujak², Edita Vučić³

¹University of Sarajevo, Faculty of Dentistry with Dental Clinical Center, Department for Dental Morphology, Anthropology and Forensics, Sarajevo, Bosnia and Herzegovina

²University of Sarajevo, Faculty of Philosophy, Department of Archaeology, Sarajevo, Bosnia and Herzegovina

³University of Mostar, Faculty of Philosophy, Mostar, Bosnia and Herzegovina

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Abstract

*Correspondence

E-mail: szukic@sf.unsa.ba

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Previous research on archaeological skeletal remains in Bosnia and Herzegovina rarely included odontological analysis. The objective of this research was biological profiling through odontological analysis (dental morphological characteristics, estimation of sex and dental age at death) in medieval archaeological skeletal remains samples from Krešića greblje in Paoča (Čitluk municipality, Bosnia and Herzegovina). After the excavations of the archaeological site, odontological analysis was performed before genetic analysis. The sample comprised 22 permanent teeth from 6 individuals. Odontological analysis included odontometric estimation of sex, estimation of age at death by two morphological methods, and an analysis of dental traits and dental pathology. Genetic analysis was used for sex determination. The results of odontological analysis classified all individuals as adults, with estimated age at death 20-25 in one individual, 30-35 in three individuals, 35-45 in one individual, and one individual older than 45 years of age. Odontometric sex estimation was in high concordance with sex determination by genetic analysis, and correctly classified five individuals' sex (four females and one male). In one individual odontometric sex was assessed as female while genetic analysis revealed male. Morphological dental traits and dental pathology were investigated as well, adding to a better understanding of the odontological characteristics of the medieval population in the area of Bosnia and Herzegovina. Sex and dental age estimation given by odontological analysis provide fast and cost-effective information, and should be performed as an auxiliary before genetic analysis.

Introduction

There are several research conducted on archaeological human skeletal remains in Bosnia and Herzegovina (Klonowski, 2012; Zukanović et al., 2012; Zukić and Bujak, 2020; Džehverović et al., 2022; Jusić et al., 2022; Džehverović et al., 2023), from various historical periods, and with different methods of analysis applied. Among those, only a few included odontological analysis (Zukanović et al., 2012; Zukić and Bujak, 2020; Jusić et al. 2022). In previous research on medieval archeological human skeletal samples (Jusic et al., 2022), genetic analysis was superior to anthropological and odontological analysis in sex determination. Alongside with sex estimation, this research aimed to include several other aspects of biological profiling within odontological analysis, such as dental age estimation, morphological dental traits, dental pathology, etc. Teeth are very resistant to degradation processes and therefore play an important role in the analysis of human skeletal remains (Vodanović et al., 2007), especially of remains in bioarchaeology (Vodanović et al., 2007; Larsen, 2002). The specific composition of highly mineralized dental tissues provide protection to both nuclear and mitochondrial DNA available for identification (Higgins and Austin, 2013). In genetic analysis of archaeological samples teeth seems to be more suitable source of DNA compared to bones due to less porosity.

In 2021, a part of Krešića greblje site in Paoča, Čitluk municipality was archaeologically investigated, which is also the first archaeological investigation of graves under the stećak (stećci pl.) in the mentioned area. (Vučić and Lukačević, 2022) The archaeological survey was preceded by the mapping of the necropolis, the creation of technical documentation, and obtaining basic information about 18 stećci tombstones that are located in the necropolis. A four stećci were dislodged by the machine thus creating the conditions for the beginning of archaeological investigations of the supposed graves. During the

excavations, a total of four graves and two burials were recorded, three of which contained the skeletal remains of one individual each, while the skeletal remains of three individuals were found in the grave marked as G2. The graves were buried at a relative depth of 0.83 to 1.35 meters.

Grave G1 was an ordinary oval-shaped earth pit, without any structure. The deceased was laid in the grave in an anatomical position on the back, with arms bent at the elbow and placed on the stomach and chest. Graves G2 and G3 each had a construction in the form of a rectangular stone chest formed by vertically placed untreated limestone slabs (footboards, headboards and side slabs), then one larger and a series of smaller lid slabs, and stones to fill the cavities between them. In grave G2 the mutilated remains of three individuals were recorded (a, b and c), one of which was in an anatomical position on the back with arms extended by the body (the youngest burial). The postcranial parts of previously buried individuals were located on the lower extremities, i.e. on the central left and right side of the skeleton in anatomical position, while their skulls were found on the left and right side of the skeleton's skull in anatomical position. C14 analysis of the osteological material of the deceased found in anatomical position resulted in the dating of the burial in the time range from 1470 to 1640. Grave G3, discovered northeast of G1 was not marked with a stećak. In the burial construction in the shape of a stone chest, the osteological material of a person in an anatomical position on his back was recorded. Based on the results of the C14 analysis, the grave was dated to the time range from 1420 to 1470. In Grave G4 the skeletal remains of an individual in an anatomical position on the back with arms resting next to the body were found in a shallow, oval-shaped earthen pit, surrounded on the left and right sides by unworked stones.. Based on the results of the C14 analysis, the grave was dated to the period from 1320 to 1430. (Vučić and Lukačević, 2022).

The aim of this research was an assessment of the biological profile through dental morphological

characteristics, estimation of sex and dental age at death in medieval archaeological skeletal remains samples from Krešića greblje (Čapljina municipality, Bosnia and Herzegovina).

Material and methods

The sample for odontological analysis comprised 22 permanent upper and lower teeth from 6 individuals. Material (teeth) was categorized in plastic test tubes and labeled "sample" with the corresponding letter and number. Teeth of the individual from grave 1 (G1) were labeled A1/03-21. Teeth of the first individual from grave 2 (G2) were labeled A2/03-21, of the second individual were labeled A3/03-21, and of the third individual from the same grave (G2) were labeled A4/03-21. Recovered teeth of the individual from grave 3 (G3) were labeled A5/03-21, and of the individual from grave 4 (G4) were labeled A6/03-21. The samples were pre-washed, cleaned, and categorized by a forensic anthropologist. All teeth were photographed. Analysis was performed by visual inspection, a PPC camera with 10x magnification, and by measurements with digital veneer caliper (with precision $\square$ 0,01 mm). All visible anthropological features were noted and compared to ASUDAS (Arizona State University Dental Anthropology System) referenced plaques (Turner et al, 1991)

Age at death was assessed by the Brothwell system (Brothwell, 1989) based on tooth abrasion, and the Lamendin method (Lamendin et al., 1992) based on root dentin transparency and periodontium regression. Sex of the individuals was assessed by odontometric analysis, based on a comparison of tooth measurements to average tooth dimensions of the contemporary population in Bosnia and Herzegovina (Konjhodžić, 1978; Vuković et al. 2013).

Included teeth measurements were mesiodistal (MD), buccolingual (BL), mesiodistal at cement-enamel level (MDC) crown dimension, and root length. Other dental dimensions, such as cervicoocclusal measurement of the tooth crown and tooth length, were excluded to avoid false

results. All odontological analyses were performed by a single researcher (SZ), according to the methodology proposed by Vodanović (Vodanović et al. 2007).

After the dental age and sex were estimated, the results were compared to sex determination derived by genetic analysis of specimens. The results of the genetic analysis were unknown to the researchers at the time of odontological analysis.

Sex determination using genetic analysis

To determine the sex of individuals, the detection of amelogenin STR marker was performed by using PowerPlex Fusion and PowerPlex Y23 amplification kit following the manufacturer's instructions (Promega Corporation 2016, 2017). Before detection, DNA was isolated according to the slightly modified protocol for organic extraction (Dzehverovic et al., 2021) preceded by teeth cleaning and grinding. To prevent contamination and thus ambiguous results, extraction was repeated in two temporally separated reactions in pre-PCR room previously irradiated with UV lights for 72 hours (MacHugh et al., 2000; Willerslev & Cooper, 2005). Additionally, cross-contamination was checked for every single sample against negative extraction and PCR controls. Sex determination detection was performed by detecting peaks at AMEL locus, with a single peak indicating XX genotype (female), and two peaks XY genotype (male). The male genotype was confirmed by generating Y-STR profile using sex-linked STR markers.

Results

Results of odontological analysis

Derived data are shown in Table 1, and results of odontological sex and dental age estimation are presented in Table 2. Regarding the results of dental anthropological analysis, an enamel extension grade 1 (Photo 1) was found on 2 teeth

of one specimen (A2), and in one case (A1), a shovel-shaped grade 3 upper incisor was found. Other findings include enamel hypoplasia in almost all teeth, followed by dental abrasion.

Dental caries was found in 5 out of 6 specimens (5 teeth out of 22 examined). In one specimen (A5) there was no hypoplasia, no abrasion, and no caries.

Table 1. Raw data derived by odontometric analysis of tooth size

Specimen	Tooth number (FDI)	MD (mm)	BL (mm)	MDC (mm)	Root length (mm)
A1/03-21	21	8,06	6,89	5,40	13,33
	22	6,44	5,89	4,41	14,01
	23	7,84	8,25	5,37	17,53
	24	6,60	8,52	4,21	13,51
	25	6,56	8,87	4,20	13,63
	26	9,86	11,67	7,55	14,08
	27	9,31	10,62	6,70	13,08
A2/03-21	23	6,02	7,06	4,11	13,01
	36	11,27	10,23	9,22	12,09
	47	11,16	10,23	9,22	12,09
A3/03-21	16	10,17	10,8	7,85	13,16
	26	9,82	10,76	7,35	12,21
	27	8,73	8,89	7,71	14,19
A4/03-21	13	7,38	11,96	5,36	13,85
	16	9,73	11,96	7,98	13,85
	26	9,77	11,70	8,11	13,54
A5/03-21	33	6,23	7,35	4,65	16,95
	36	10,67	10,47	8,77	11,70
	38	9,43	9,27	7,9	10,28
A6/03-21	13	6,23	7,8	4,65	16,85
	16	10,83	11,56	8,13	15,37
	46	8,73	10,21	9,17	14,09

Table 2. Results of dental age and sex estimation with level of confidence

Specimen	Estimated dental age		Estimated sex by odontometrics	Level of confidence*
	Brothwell system (years)	Lamendin method (years)		
A1/03-21	25-35	30	Male	4
A2/03-21	25-35	34,6	Female	2
A3/03-21	25-35	35,1	Female	2
A4/03-21	35-45	40	Female	2
A5/03-21	17-25	25	Female	2
A6/03-21	>45	42	Male	2

*Scale of confidence: 1- not possible to determine the sex, 2- sex determination based on 1-3 teeth, 3- sex determination based on 4-8 teeth, 4- sex determination based on more than 8 teeth (Jusić et al., 2022)



Figure 1. Enlarged photo of the tooth with enamel extension along cervical line and linear enamel hypoplasia.

Results of genetic analysis

For all 6 samples partial autosomal DNA profiles were generated (Table 3). Good partial profile was generated only for sample A2 with 20 out of 24 amplified loci. For the other five samples, less than 13 out of 24 analyzed STR loci were amplified. Determined genotypes for all samples was XX but for A5 and A6 samples male sex could not be excluded due to the small quantity of DNA presented as partial DNA profiles. Additional Y-STR analysis confirmed the male sex for the A6 sample.

Table 3. Results of genetic analysis

STR LOKUS	A1/03-21	A2/03-21	A3/03-21	A4/03-21	A5/03-21	A6/03-21
D3S1358	16 -	15 17	16 17	15 15	14 15 18	17 -
D1S1656	-	17 18.3	12 17.3	12 16	-	-
D2S441	11 -	11 11.3	-	11 14	10 -	-
D10S1248	12 -	16 -	-	-	-	-
D13S317	-	8 11	-	11 -	-	-
Penta E	-	-	-	-	-	-
D16S539	-	12 14	11 12	12 -	9 12	11 13
D18S51	13 14	14 15	13 16	14 18	16 22	12 14
D2S1338	-	18 -	-	-	20 24	-
CSF1PO	-	12 -	-	-	-	10 -
Penta D	-	12 -	-	-	-	-
TH01	9 9.3	8 9.3	7 9	6 9.3	8 9	7 -
vWA	-	16 18	16 18	18 18	18 -	17 18
D21S11	29 -	27 30.2	30 -	30.2 32.2	-	32.2 -
D7S820	-	8 12	-	-	-	-
D5S818	-	13 -	-	-	-	-
TPOX	-	12 -	-	-	-	-
DYS391	-	-	-	-	-	-
D8S1179	11 14	12 14	14 -	13 15	12 13	13 14
D12S391	-	21 23	-	18 18.3	15 19	20 23
D19S433	-	14 -	-	13 14.2	13 -	-
FGA	-	22.2 24	-	19 -	-	-
D22S1045	-	-	-	-	-	-
Amelogenin	X X	X X	X X	X X	X -	X -

Discussion

The main findings of this research gained through odontological analysis showed that all archaeological samples from Krešića greblje site were adult individuals, with estimated age at death 20-25 in one individual, 30-35 in three individuals, 35-45 in one individual, and one individual older than 45 years of age. According to the odontometric analysis, four individuals (A2, A3, A4 and A5) were females and two individuals (A1 and A6) were male. The odontological sex assessment was confirmed by genetic analysis for samples A2, A3, A4, and A6. For sample A5 genetic analysis assesses the female sex, confirming the odontological sex estimation, but without certainty to exclude the male sex due to partial DNA profile. This is in concordance with the results of similar research on the medieval archaeological sample (Jušić et al., 2022) when concordance of sex estimation by odontological and genetic analysis occurred in 4 out of 17 cases. Interestingly enough, in that same research, genetic analysis of sex was not possible due to the inability to generate genetic profiles in three cases, when odontological sex assessment was in concordance with anthropological sex estimation. The obvious limitation of most research on archaeological samples, therefore in this one as well, is the number of individuals. This limitation is understandable, especially when it comes to the medieval and prehistoric populations where only a limited number of skeletal remains with different grade of preservation is available for analysis and identification. Genetic assessment of the sex is superior to other sex assessment methods, with the highest level of accuracy and confidence (Capitaneanu et al., 2017; Buonasera et al., 2020), but limited by the ability to derive genetic profile. Teeth are a good source of different information in biological profiling due to their resilience to degradation during time and the fact that they are a source of DNA. Age at death in our research was

assessed with two methods, the Brothwell system according to tooth wear, and the Lamendin method based on periodontal regression and dentin transparency. Both of these methods have their limitations when applied to archaeological samples. Brothwell system is developed for archaeological samples when all molar teeth are present (Mays et al. 2022). On the other hand, the Lamendin method is more accurate in one-rooted teeth but limited by a difference in root dentin transparency extension in teeth of archaeological origin (Megyesi et al. 2006). Dental age is only approximative and therefore is given in an age range.

Even though it cannot be tested and/or confirmed by genetic analysis it is valuable information in an archaeological context. All odontological analyses applied in this research were non-invasive in order to preserve archeological samples as much as possible. It is important to point out here that in parallel with the archaeological research, anthropological analyses of the osteological material provided information about the age of the deceased, gender, and pathological changes in the teeth and bones. Changes in the bone material suggest that the deceased lived in difficult living conditions (infectious diseases, unhygienic lifestyle, anemia), which had a negative impact on their health, confirmed by odontological analysis. Findings of enamel hypoplasia, dental wear, and dental caries are in concordance with other research on medieval populations worldwide (Djuric-Srejac et al., 1998; Gamble et al., 2017; Vodanović et al, 2003; Esclassan et al. 2009) explained by lifestyle and food consumption deficiency in that historical period. The results of the DNA analysis of the dental material (molars and canines) of the skeletons indicate the need to question the previous assumptions that necropolises with a smaller number of stećaks are treated as the eternal resting places of one family. Archaeological investigation provide information

about the architecture of graves and the position of the graves in relation to stećci, insight into burial practices as well as the chronological and cultural attribution of graves. Further research on the biological characteristics of the medieval population from different archaeological sites in Bosnia and Herzegovina will provide more information about the ancestors of the contemporary population in this region. Dental characteristics, such as shovel shaping and enamel extensions as seen at this archaeological site, provide an insight into anthropological trends within one population. Having in mind the hypothesis of heritability of dental variants described by ASUDAS, further genetic research on specific dental characteristics like shovel-shaped incisors could help in understanding genetic relatedness among populations. The research on the contemporary population in Japan investigated the correlation of mesiodistal (MD) diameter of the first upper incisors, shovel shaping, and genes on sex chromosomes, emphasizing the power of DNA technology in understanding the connection between genotypes and phenotypes in different dental traits' expression. (Kimura et al., 2009) The possibility of using ASUDAS dental traits as proxies for genetic data is intriguing, both in archaeological investigations when genomic markers are not available and in research of contemporary populations for understanding inferring population structure (Irish et al., 2020; Rathman et al., 2023).

Conclusion

Developmental enamel defects (hypoplasia) and pathological conditions (caries) are present in the individuals buried at this location. This speaks in favor of the poor nutrition of the population in the Middle Ages at the location of Krešića greblje. The occurrence of dental-anthropological characteristics (enamel extensions and shovel-shaped incisors) on the teeth of these

archaeological samples could be the markers for further genetic analysis. Sex and dental age estimation given by odontological analysis provide fast and cost-effective information, and should be performed as an auxiliary before genetic analysis.

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Conflict of interest

Authors declare no conflict of interest.

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