Molekularna analiza arheoloških ljudskih ostataka iz Zagajaca (Hrvatska)

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The paper presents molecular analysis of archaeological human remains from Zagajci near Belišće in Croatia. Bone sample from a single human cranium and two tooth samples obtained from teeth in a scanty "set" of remains found in farther vicinity (all secondary finds) were employed in DNA typing. DNA was analysed for autosomal short tandem repeat (STR) loci and the amelogenin – sex typing locus. The aim was obtaining STR loci profiles in order to investigate possible molecular relatedness between examined materials. Another aim was molecular identification of unknown sex of an individual represented by scanty remains, and confirming of the sex of the individual whose skull was estimated female by morphology. We obtained either low/partial STR loci allelic profiles for tooth samples, or a complete STR loci dropout for a bone sample. There was a single allele (17) of the D3S1358 locus shared among tooth samples. However, molecular sex identification confirmed the presence of a female individual in a scanty "set" of remains in accordance with archaeological data. Major or complete loci STR allele dropout is suggestive of a severe DNA degradation. A complete allelic loci dropout in a bone sample is also suggestive of the lack of contamination with modern DNA.

Key words: DNA typing, STR loci matching, Amelogenin, Zagajci, Croatia.

Rad predstavlja molekularnu analizu arheoloških ljudskih ostataka iz Zagajaca kraj Belišća u Hrvatskoj. U određivanje DNK uključeni su uzorak kosti iz pojedinačnog nalaza ljudske lubanje i dva zubna uzorka dobivena od zubā iz oskudnih ostataka pronađenih nešto dalje (svi su nalazi sekundarni). DNK smo analizirali po autosomnim kratkim udvojenim sljedovnim (STR) lokusima i amelogeninskom lokusu za spol. Cilj je bio utvrditi profile STR lokusa u smislu ispitivanja moguće molekularne sličnosti proučavanog materijala. Drugi cilj bio je molekularno utvrđivanje nepoznatog spola osobe predstavljene oskudnim ostacima, te potvrđivanje spola osobe koja je morfološki, na osnovi lubanje, određena kao žena. Iz zubnih smo uzoraka dobili oskudne/djelomične profile STR lokusa, dok je koštani uzorak iznjedrio potpuni izostanak STR lokusa. Samo se jedan alel (17) na lokusu D3S1358 pojavio u oba zubna uzorka. Međutim, molekularnim ispitivanjem po spolu potvrđena je nazočnost ženske osobe u oskudnim koštanim ostacima sukladno arheološkom nalazu. Znatni ili potpuni izostanak alela na STR lokusima upućuje na izraženo propadanje DNK. Potpun izostanak alela na svim lokusima u koštanom uzorku upućuje, također, na odsustvo onečišćenja suvremenom DNK.

Ključne riječi: određivanje DNK, podudarnost STR lokusa, amelogenin, Zagajci, Hrvatska.

INTRODUCTION

It is already fairly known that using of DNA typing in bioarchaeology became widely employed in analyzing "ancient" DNA in old human bones and teeth. Molecular approach has been particularly crucial in terms of investigation of relatedness within and between burial sites (Keyser-Tracqui et. al 2003: 247–260), i.e. possible kinship relationships either among the deceased from primary skeletal inhumations in a single burial site (Boljunčić 2007: 536–546), or from a disturbed collective burial site (Schultes et al. 2000: 37–44). The same is the case with investigation of kinship

UVOD

Već je razmjerno dobro poznato kako je određivanje DNK u bioarheologiji naišlo na široku primjenu kad je riječ o analiziranju "stare" DNK u ljudskim kostima i zubima iz starijih razdoblja. Molekularni pristup pokazao se naročito bitnim pri istraživanjima odnosa unutar i između pojedinih grobnih nalazišta (Keyser-Tracqui et. al 2003: 247–260), u smislu mogućeg srodstva između pokojnika iz primarnih kosturnih ukopa unutar istoga grobnog nalazišta (Boljunčić 2007: 536–546), ili iz poremećenoga skupnog ukopa (Schultes et al. 2000: 37–44). Isti je slučaj s istraživanjem

relationships in double burials (Clisson et al. 2002: 304–308; Adachi et al. 2003: 347–363; Boljunčić 2007: 536–546). Although, there is a general lack of articles on ancient DNA analysis in Croatian bioarchaeology, there are also tendencies that DNA typing becomes more employed in studies of archaeological human remains beside standard anthropological methods. This is substantiated by increasingly employed DNA analysis in studies of archaeological human remains from Croatian territory reported by Boljunčić (2007: 536–546), Hincak et al. (2007: 1135–1141), Bečić et al. (2011: 144), Ljubković et al. (2011: 144).

DNA typing from ancient, i.e. very old samples is faced with various problems such as DNA degradation (Benecke 1997: 186) and contamination, i.e. the presence of DNA polymerase inhibitors (Sutlović et al. 2005: 556–562). Damaged DNA templates in ancient, i.e. very old bones/teeth with conserved minute amount of cells could occasionally generate a single or complete allelic loci dropout with non-reproducible results (Benecke: 1997: 186). In the post-mortem period DNA has a limited life depending on various conditions – environmental and possible (other) destructive factors (Bär et al. 1988: 59–70; Woodward et al. 1994: 244–247). This is particularly significant in terms of ancient or old bones and teeth.

Molecular analysis of bioarchaeological samples requires using of short sized loci amplified by polymerase chain reactions (PCR) with short tandem repeat (STR) loci. The suitability of autosomal STR loci as markers for ancient DNA typing lies in their small size and "capacity" of recognition of sample contamination by modern DNA (Hummel et al. 2000: 15–21; Boljunčić 2007: 537). Short tandem repeat markers are polymorphic DNA loci containing a repeated nucleotide sequence from two to seven nucleotides in length. Alleles are marked with numbers in accordance with a number of repetitive units (Hincak et al. 2007: 1139).

Human gender identification, i.e. sex determination based on the amelogenin gene has an important implication in forensic casework, DNA databasing (Abebe 2004: 1) as well as in the study of bioarchaeological human remains. The amelogenin gene, located on the X and Y chromosomes in humans produces a protein important in the development of the tooth enamel matrix (Abebe 2004: 1).

The present study reports on DNA typing of a bone sample from a single human cranium (with no enclosures associated) and two tooth samples obtained from teeth in a scanty "set" of human remains found in a farther vicinity. All listed finds were secondary. Bone fragments and teeth were discovered in association with grave enclosures (jewellery) originating probably from a disturbed female burial. The remains were all discovered accidentally by local villagers in Zagajci near Belišće (Osječko-Baranjska County). They are stored at the Museum of Belišće.

In the present paper ancient DNA was analysed for nine autosomal short tandem repeat (STR) loci and the amelogenin – sex typing locus. The aim was obtaining STR loci profiles in terms of investigation of possible relatedness (population or other) between the examined materials. Another important aim was molecular identification of unknown sex

srodstvenih veza u dvostrukim ukopima (Clisson et al. 2002: 304–308; Adachi et al. 2003: 347–363; Boljunčić 2007: 536–546). Iako u hrvatskoj bioarheologiji uglavnom nedostaje članaka o analizama stare DNK, jednako se tako uočava namjera da se određivanje DNK pridruži standardnim antropološkim metodama u istraživanju arheoloških ljudskih ostataka. Kao dokaz tomu može poslužiti sve učestalija uporaba analize DNK u proučavanjima arheoloških ljudskih ostataka iz Hrvatske, što je vidljivo iz radova Boljunčić (2007: 536–546), Hincak et al. (2007: 1135–1141), Bečić et al. (2011: 144) i Ljubković et al. (2011: 144).

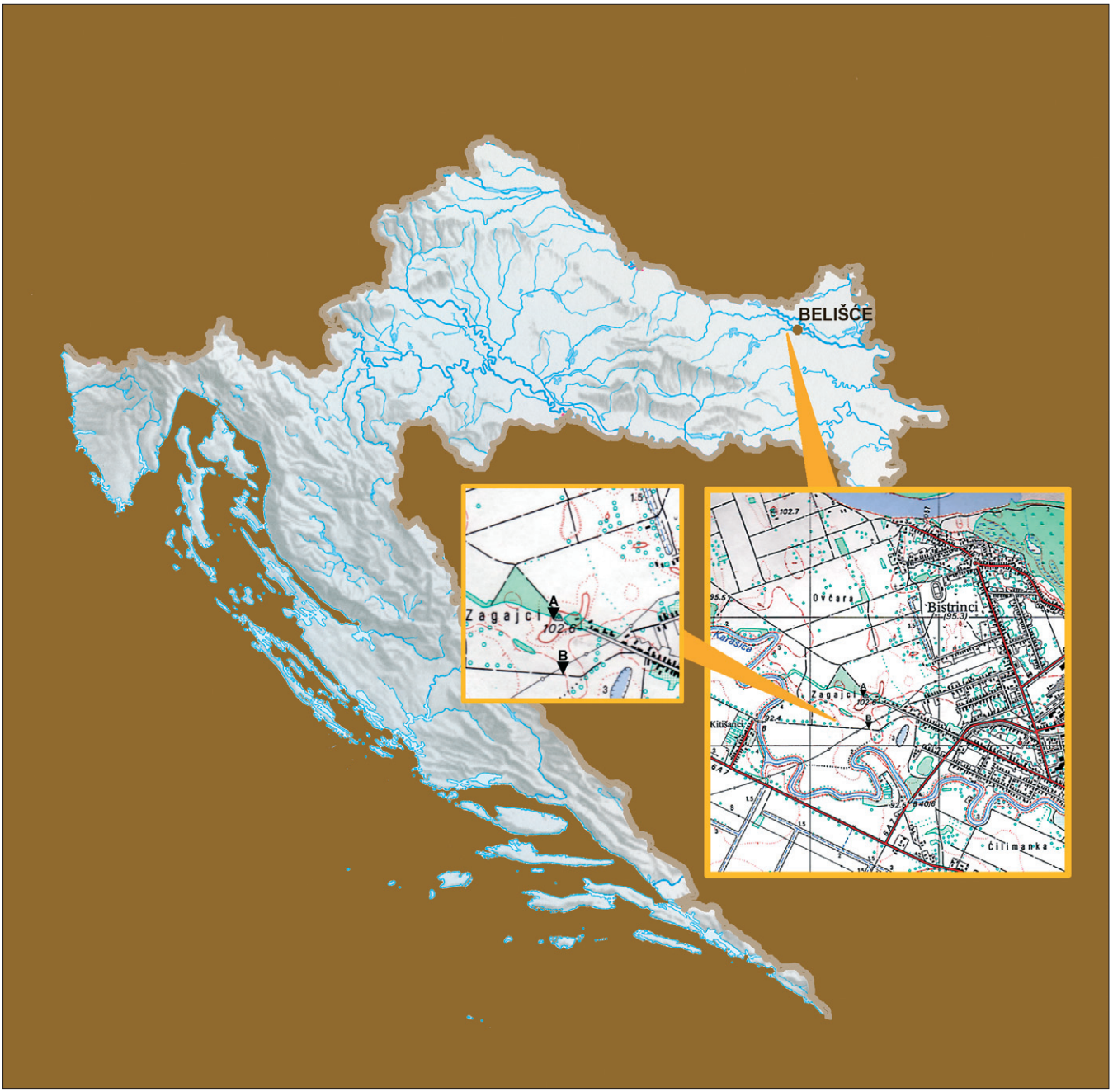
Određivanje DNK u starim ili vrlo starim uzorcima suočeno je s raznovrsnim problemima poput propadanja DNK (Benecke 1997: 186) ili onečišćenja, odnosno prisutnosti inhibitora DNK polimeraze (Sutlović et al. 2005: 556–562). Oštećeni DNK predlošci u starim, odnosno vrlo starim kostima/zubima sa sačuvanom minimalnom količinom stanica mogu ponekad iznjedriti izostanak pojedinačnog alela ili potpun izostanak alela na lokusima, s rezultatima koje nije moguće reproducirati (Benecke 1997: 186). U razdoblju nakon smrti trajnost DNK je ograničena na različite načine – okolišnim te mogućim (drugim) razarajućim čimbenicima (Bär et al. 1988: 59–70; Woodward et al. 1994: 244–247), što naročito dolazi do izražaja kad je riječ o kostima i zubima iz starijih razdoblja.

Molekularna analiza bioarheoloških uzoraka iziskuje uporabu kratkih lokusa umnoženih lančanim reakcijama polimeraze (*Polimeraze Chain Reaction*, PCR) s kratkim udvojenim sljedovnim (*Short Tandem Repeat*, STR) lokusima. Prikladnost autosomnih STR lokusa kao indikatora za određivanje stare DNK temelji se na njihovoj maloj veličini te "sposobnosti" prepoznavanja onečišćenja uzorka suvremenom DNK (Hummel et al. 2000: 15–21; Boljunčić 2007: 537). Kratki udvojeni sljedovni markeri su polimorfni DNK lokusi koji sadrže ponavljajuće slijedove dužine od dva do sedam nukleotida. Aleli su označeni brojevima sukladno broju ponavljajućih jedinica (Hincak et al. 2007: 1139).

Rodno određivanje, odnosno određivanje spola u čovjeka na osnovi gena za amelogenin ima važnu ulogu u forenzičkim slučajevima, stvaranju baza podataka DNK (Abebe 2004: 1), kao i u istraživanjima bioarheoloških ljudskih ostataka. Gen za amelogenin, smješten u ljudi na kromosomima X i Y, proizvodi protein važan za razvoj matriksa zubne cakline (Abebe 2004: 1).

U ovom su radu predstavljeni rezultati određivanja DNK u uzorku kosti uzetog iz pojedinačnog nalaza ljudske lubanje (bez pridruženih grobnih priloga) te dva zubna uzorka koji potječu od zubā iz oskudnoga skupnog nalaza ljudskih ostataka pronađenih nešto dalje. Svi navedeni nalazi otkriveni su u sekundarnom arheološkom kontekstu. Ulomci kostiju, kao i zubi otkriveni su zajedno s grobnim prilozima (nakit) te, po svemu sudeći, potječu od poremećenog groba žene. Sve su ostatke slučajno pronašli mještani sela Zagajci kraj Belišća (Osječko-baranjska županija), a pohranjeni su u Muzeju grada Belišća.

Analiza DNK u predmetnom je radu provedena na devet autosomnih kratkih udvojenih sljedovnih (STR) lokusa te amelogeninskom lokusu za spol. Cilj je bio utvrditi profile



Map 1 Map of Croatia with enlarged details – topographic maps of location of Zagajci near Belišće (Osječko-Baranjska County). Human remains were discovered at spots marked with letters A (a scanty "set") and B (a single cranium); both below topographic elevations marked with black triangles (created by: Boljunčić and Krajcar). Basic topographic maps are provided here courtesy of the Museum of Belišće.

Karta 1Karta Hrvatske s uvećanim detaljima – topografskim kartama s položajem nalazišta Zagajci kraj Belišća (Osječko-baranjska županija). Ljudski ostaci otkriveni su na položajima označenima slovima A (oskudni skupni nalaz) i B (pojedinačni nalaz lubanje); oba ispod kota označenih crnim trokutićima (osmislili: J. Boljunčić i I. Krajcar). Osnovne topografske karte priložene su ljubaznošću Muzeja Belišće.

of an individual represented by a scanty "set" of very poor and fragmentary remains (possibly female according to archaeological data), and confirming of the sex of an individual whose remains (a single partial skull) were estimated female by morphology.

It is important to point out that we were not authorized to carry out "bone to bone" genetic "matching", i.e. of the bone fragment taken from a single cranium to bone fragments from a scanty "set" of remains, apparently from a single individual. In addition, in terms of a scanty "set" of

STR lokusa u smislu ispitivanja moguće povezanosti (populacijske ili druge) proučavanog materijala. Drugi važan cilj bio je molekularnim putem utvrditi nepoznati spol osobe predstavljene oskudnim skupnim nalazom slabo očuvanih i ulomljenih ostataka (arheološki podaci upućuju na ženski spol), te s druge strane potvrditi spol osobe čiji su ostaci (djelomice očuvan pojedinačni nalaz lubanje) sukladno morfologiji upućivali na pripadnost ženskom spolu.

Važno je naglasiti kako nismo bili ovlašteni provesti analizu genetičke podudarnosti "bone to bone" ("kost po

human remains, apparently there was no reasonably sufficient/reliable material for successful ancient DNA typing other than teeth. Regardless of the latter, teeth are proved to be most reliable in ancient DNA typing. Likewise, due to poor/fragmented bone nature there was a possibility of obtaining complete STR loci profiles typing failure. Finally, regardless of their status, bones from a scanty "set" of human material would have been permanently lost as a record – crushed during DNA procedure.

MATERIAL AND METHODS

Description of the site and discovery of finds

In 1992 a scanty "set" of secondary archaeological human finds – two teeth and a few very poor and fragmentary bone elements, was found in association with several grave enclosures (jewellery) in Zagajci near Belišće (Osječko-Baranjska County).

Bones and teeth were found in a sandy pit – at the place marked on the enclosed map (Map 1) with the letter A, 3.5 metres bellow the topographic elevation at the altitude of approximately 102.6 metres. The pit was left over after mechanical works, i.e. setting installations for the pipeline and/or draining system in the local area. The jewellery and scanty human remains were discovered accidentally by a local villager Zoran Cesar and Stjepan B. Komaromi – local schoolteacher. At the time no additional skeletal finds and jewellery were discovered in the nearby area. Apparently the jewellery – a ring, bracelet and fibula originated from a female burial dated to the period of 4th cent. BC (Early Iron Age). The same type of the fibula that was found in Zagajci was also found in Sanski Most (Bosnia and Herzegovina); the only difference laid in the fact that the fibula from Belišće lacked triangled lamellas – pendants.[1]

On the other hand, in 1996 a partially preserved secondary skull find was discovered at the same locality by local villager Rudolf Sopić. It was found some 100 metres from where the jewellery and scanty bones and teeth were previously found. The skull was apparently unearthed from a sandy Eolian dune nearby the country road Belišće – Zagajci – Kitišanci. It was found in a sandy pit, at the place marked on the enclosed map (Map 1) with the latter B – 1.5 metres bellow the topographic elevation at the altitude of approximately 99.6 metres. Likewise it was discovered accidentally when a sandy dune was dug by a backhoe during the setting of installations for the pipeline/and or drainage system in the local area. No additional enclosures were found in association with the cranium. Archaeological and bioarchaeological materials are stored at the Museum of Belišće with no inventory numbers/designations assigned to archaeological human remains.[2]

At first a single cranium from Zagajci was delivered from

1 Data on both the site of Zagajci and discovery of bioarchaeological and archaeological finds quoted in this section are obtained from the Archive of the Belišće Museum. They are provided here courtesy of the Museum of Belišće.

2 Data on the site of Zagajci site and discovery of bioarchaeological find (the skull) quoted in this section are also obtained from the Archive of the Belišće Museum. They are provided here courtesy of the Museum of Belišće.

kost"), odnosno usporediti ulomak kosti s lubanje sa svim ulomcima kostiju iz oskudnoga skupnog nalaza koji, po svemu sudeći, pripada jednoj jedinoj osobi. K tomu, kad je riječ o oskudnom skupnom nalazu, čini se kako nije bilo dovoljno drugoga pouzdanog materijala za uspješnu analizu DNK osim zubā. No, neovisno o tomu, zubi su ionako dokazano najpouzdaniji materijal kad je riječ o ispitivanju stare DNK. Osim toga, zbog loše očuvanosti i fragmentarne prirode kostiju postojala je mogućnost da određivanje profila STR lokusa bude u cijelosti neuspješno. Konačno, neovisno o njihovu stanju, kosti iz oskudnoga skupnog nalaza zauvijek bi nestale, jer bi se zdrobile tijekom postupka DNK analize.

MATERIJAL I METODE

Opis nalazišta i otkriće nalaza

Tijekom 1992. godine u Zagajcima kraj Belišća (Osječko-baranjska županija) pronađen je oskudni skupni nalaz – dva zuba te nekoliko loše očuvanih i ulomljenih kostiju. Nalaz je pronađen s grobnim prilozima (nakit) u sekundarnom arheološkom kontekstu.

Kosti i zubi pronađeni su u pješčanoj jami – na mjestu označenom slovom A na priloženoj karti (karta 1) 3,5 m ispod kote na nadmorskoj visini od otprilike 102,6 m. Jama je preostala nakon mehaničkih radova, tj. postavljanja instalacije za vodovodne cijevi i/ili sustav odvodnje (kanalizacija) u tom kraju. Nakit i oskudne ljudske ostatke slučajno su otkrili mještanin Zoran Cesar te mjesni školski učitelj Stjepan B. Komaromi. Tom prilikom u obližnjem području nisu pronađeni nikakvi drugi kosturni ostaci ili nakit. Po svemu sudeći, nakit – prsten, narukvica i fibula, potječe iz ženskog groba iz 4. st. pr. Kr. (starije željezno doba). Isti tip fibule poput ove iz Zagajaca pronađen je i u Sanskom Mostu (Bosna i Hercegovina); jedina razlika jest u tomu što na fibuli iz Belišća nedostaju trokutaste lamele – privjesci.[1]

S druge strane, na istom je nalazištu mještanin Rudolf Sopić 1996. godine pronašao pojedinačni, djelomice očuvani sekundarni nalaz lubanje. Otkrio ju je nekih 100 metara od mjesta gdje su prethodno pronađeni nakit te oskudni ostaci kostiju i zubā. Čini se kako je lubanja iskopana iz pješčane eolske dine pokraj seoske ceste Belišće – Zagajci – Kitišanci. Pronađena je u pješčanoj jami, na mjestu označenom slovom B na priloženoj karti (karta 1), 1,5 m ispod kote na nadmorskoj visini od otprilike 99,6 m. Otkrivena je slučajno pri iskopu pješčane dine bagerom tijekom polaganja instalacija za vodovodne cijevi i/ili lokalni sustav odvodnje (kanalizaciju). Tom prilikom uz lubanju nisu pronađeni nikakvi grobni prilozi. Arheološki i bioarheološki materijal pohranjen je u Muzeju grada Belišća, a arheološki ljudski ostaci nisu označeni bilo inventarnim brojem bilo oznakom.[2]

Muzej grada Belišća je Institutu za arheologiju u Zagrebu najprije dostavio na analizu lubanju iz Zagajaca. Nakon što smo dobili dodatne podatke o pohrani oskudnoga bioarheološkog materijala s istog nalazišta u tom muzeju

1 Podaci o nalazištu u Zagajcima, kao i o otkriću bioarheoloških i arheoloških nalaza koji se navode u ovom dijelu članka, potječu iz arhive Muzeja grada Belišća (ljubaznošću Muzeja Belišće).

2 Podaci o nalazištu u Zagajcima te o otkriću bioarheološkog nalaza (lubanja) koji se navode u ovom dijelu članka, također potječu iz arhive Muzeja grada Belišća (ljubaznošću Muzeja Belišće).

Fig. 1 Lateral aspects of the cranium from Zagajci. The left lateral view (on the right) shows the cranium after taking a bone for DNA sampling. No inv. no. assigned (photo: I. Krajcar).

Sl. 1 Postranični prikazi lubanje iz Zagajaca. Lijevi postranični prikaz (desno) pokazuje lubanju nakon uzimanja uzorka kosti za analizu DNK. Bez inventarnog broja (fotografija: I. Krajcar).

the Museum of Belišće to the Institute of Archaeology in Zagreb for the analysis. After obtaining an additional information about the storage of a scanty bioarchaeological material from the same locality at the Museum of Belišće (Zdravko Pavlović, personal communication), we suggested the delivery of additional material to the Institute of Archaeology for further analysis. Then official authorization was requested for sampling bone from the skull and teeth for DNA typing.

Skeletal material

Skull find: taphonomy, sex and age-at- death

A single secondary skull find was in a well state of preservation except for lacking almost the entire viscerocranium. Bone color was medium-dark. There was also a minor damage to the neurocranium associated with minor post-mortal changes to the bone cortex (Fig. 1). In addition there was a presence of a bone discoloration bilaterally on the cranium and the frontal bone. Such discoloration stems most likely from metal – the jewellery which is supposed to have been once originally attached to the dead (silver annulets?) or from other metal objects (?) from a disturbed original burial (?), which could have been leaned to the skull. The postcranium of the individual was completely lacking. The preserved bones of the neurocranium were as follows: the frontal bone with certain damage to the orbits, both parietals, the occipital bone, both temporal bones, both zygomatic bones with a damage to zygomatic arches (only the right arch was partially preserved). The nasal region and sphenoid bones were also damaged. According to preserved cranial bones, the sex of the individual could be determined only by following standards for cranial morphology, after Krogman and Işcan (1986) as well as Bass (1987). Likewise, age-at-death of the individual could be estimated only by employing standards for ectocranial suture closure, after Meindl and Lovejoy (1985: 57–66). Based on partially available sex and age criteria, the individual was identified as a female apparently in her "thirties", i.e. 30–40 (±) years old**.**

(usmeno priopćenje, Zdravko Pavlović), predložili smo da se i preostala građa dostavi u Institut za arheologiju u cilju daljnje analize. Zatim je zatraženo službeno ovlaštenje za uzorkovanje kosti lubanje te zubā u cilju određivanja DNK.

Kosturni materijal

Nalaz lubanje: tafonomija, spol i starost u trenutku smrti

Pojedinačni sekundarni nalaz lubanje dobro je očuvan, osim što mu gotovo u cijelosti nedostaju kosti lica. Boja kostiju je srednje tamna. Također postoji manje oštećenje neurokranija povezano s promjenama kortikalne kosti nakon smrti (sl. 1). Osim toga, na lubanji su postrance obostrano, kao i na čeonoj kosti, vidljive promjene u boji kostiju. Takve promjene, po svemu sudeći, potječu od metala – nakita koji je nekad mogao ukrašavati pokojnu osobu (srebrne karičice?) ili drugih metalnih predmeta (?) iz razorenoga izvornog ukopa (?) koji su možebitno bili prislonjeni uz lubanju. Postkranij pokojne osobe posve nedostaje. Sačuvane su sljedeće kosti neurokranija: čeona kost s ponešto oštećenim očnicama, obje tjemene kosti, zatiljna kost, obje sljepoočne kosti, obje jagodične kosti s oštećenjem jagodičnih lukova (jedino je desni luk djelomice sačuvan). Nosna regija i klinaste kosti također su oštećene. Na osnovi sačuvanih kostiju lubanje spol osobe bilo je moguće odrediti samo sukladno standardima za morfologiju lubanje, prema autorima Krogman i Işcan (1986), te Bass (1987). Također, starost osobe u trenutku smrti bilo je moguće procijeniti samo na osnovi propisanih standarda za stupanj sraštavanja vanjskih lubanjskih šavova, prema Meindl i Lovejoy (1985: 57–66). Na temelju djelomice dostupnih pokazatelja spola i dobi utvrđeno je kako ostaci pripadaju ženskoj osobi u dobi od 30 do 40 (±) godina, odnosno u tridesetima.

Nalazi oskudnih ljudskih ostataka: tafonomija, spol i starost u trenutku smrti

Oskudni skupni nalaz ljudskih ostataka, pronađen zajedno s nakitom (prsten, narukvica i fibula), osim dva zuba – pretkutnjaka gornje čeljusti (sl. 2) te kutnjaka donje čeljusti (sl. 3), kao i nekoliko krajnje ulomljenih (neprepoznatljivih)

Fig. 2 Distal and mesial view of the premolar from Zagajci. No inv. no. assigned (photo: I. Krajcar).

Sl. 2 Distalni i mezijalni prikaz pretkutnjaka iz Zagajaca. Bez inventarnog broja (fotografija: I. Krajcar).

Scanty human remains' finds: taphonomy, sex and age-at-death

In a scanty "set" of human remains found in association with jewellery (a ring, bracelet and fibula), beside the presence of two teeth – maxillary premolar (Fig. 2) and mandibular molar (Fig. 3) as well as a few extremely fragmented (unrecognizable) bone fragments, there were also a few other recognizable fragments. They were as follows: small fragment of the left parietal with severe changes to the bone cortex, the right occipital condyle with hypoglossal canal, small fragment of the atlas – the right lateral mass with superior/inferior articular facets and a part of the transverse process with the foramen, small fragment of the left mandibular ramus with the head and coronoid process, also preserved partially down to the lingula, and a few small rib fragments in a very poor state. The same was the case with a very small fragment of a long bone (radius?) and a few phalanxes' fragments (apparently from hand) with marked discoloration stemming probably from a metal ring. Small skull fragment containing the right occipital condyle could be perfectly matched with the right lateral mass of the atlas. Among other, the latter suggested the presence of a single individual in a scanty "set", beside the presence of another individual from the same site, found in farther vicinity. This was also supported by the colour of the bone "set" – all elements were medium dark, except for a severely changed cortex of one skull (parietal) fragment and discolorated phalanxes' fragments. Morphological sex of the individual was impossible to establish. Likewise, as to the assessment of age, the individual was only roughly aligned into adults.

DNA ANALYSIS

DNA analysis included sample preparation, DNA extraction, purification and quantification as well amplification by polymerase chain reaction (PCR) for short tandem repeat loci, using AmpFlSTR Profiler™ PCR Amplification Kit. "Ancient" DNA was analysed for nine autosomal short tandem repeat (STR) loci and the amelogenin – sex typing locus.

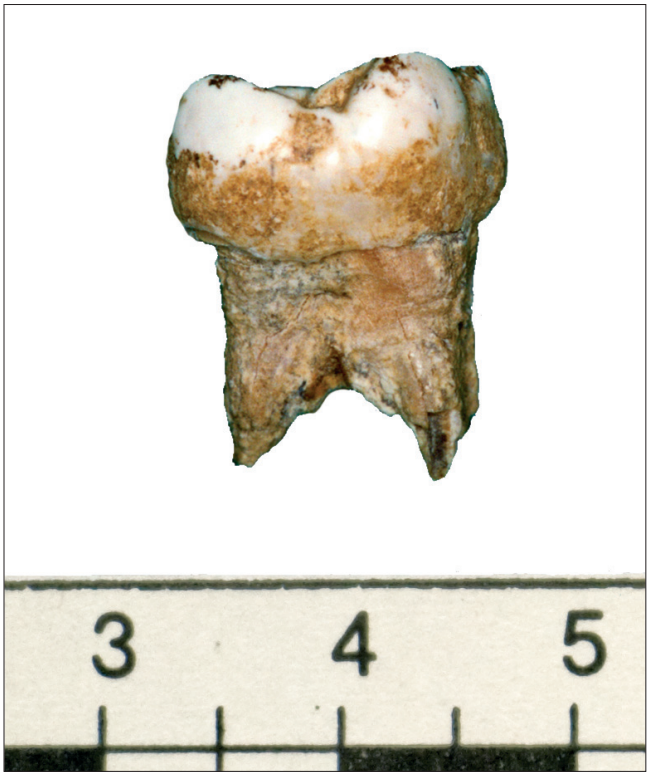


Fig. 3 Polyradicular tooth from Zagajci (buccal side). No inv. no. assigned (photo: I. Krajcar).

Sl. 3 Višekorijenski zub iz Zagajaca (bukalna strana). Bez inventarnog broja (fotografija: I. Krajcar).

ulomaka kostiju, sadržavao je i nekoliko drugih prepoznatljivih ulomaka. Riječ je o sljedećim ulomcima: mali ulomak lijeve tjemene kosti sa znatnim promjenama na kortikalnoj kosti, desni zatiljni kondil s hipoglosalnim kanalom, mali ulomak atlasa – desna postranična masa s gornjom/donjom zglobnom plohom i dijelom poprečnog nastavka s nazočnim otvorom, mali ulomak lijeve grane donje čeljusti s glavicom i koronoidnim nastavkom, također djelomice sačuvanim do lingule (jezičca), te nekoliko manjih i vrlo loše očuvanih ulomaka rebara. Isti je slučaj i s vrlo malim ulomkom neke duge kosti (palčana kost?) te nekoliko ulomaka članaka (po svemu sudeći sa šake) sa zamjetnom diskoloracijom što, po svemu sudeći, potječe od metalnog prstena. Mali ulomak lubanje s desnim zatiljnim kondilom savršeno pristaje uz desnu bočnu masu atlasa. Ovo, između ostaloga, govori u prilog tomu kako je u oskudnom skupnom nalazu kostiju bila zastupljena jedna jedina osoba kojoj nije bilo moguće pridružiti ostatke druge osobe s istog nalazišta, pronađene nešto dalje. Navedenomu u prilog ide i boja kostiju iz skupnog nalaza – svi su elementi srednje tamni, osim znatnih promjena na korteksu lubanjskog ulomka (tjemena kost) te na ulomcima članaka koji pokazuju diskoloraciju. Spol pokojne osobe nije bilo moguće utvrditi morfološkim putem. Također, s obzirom na dob, osoba je samo općenito uvrštena u grupu odraslih osoba.

ANALIZA DNK

Analiza DNK sastojala se od pripreme uzoraka, izdvajanja, pročišćavanja i kvantifikacije DNK, kao i njezina umnažanja lančanom reakcijom polimeraze (PCR) za STR lokuse, uporabom AmpFlSTR Profiler™ PCR amplifikatora. „Stara" DNK ispitana je na devet autosomnih STR lokusa te na ame-

Following loci were included in the analysis: D3S1358, vWa, FGA, TH01, TPOX, CSF1PO, D5S818, D13S317, D7S820 and AMEL – sex typing locus.

Sample preparation

Two teeth – the only ones present in a scanty "set" of bioarchaeological material were used for sampling. Samples (Tab. 1) were obtained from the premolar (Fig 2.) and molar (Fig. 3). Aside from the fact that the mentioned teeth were the only ones from the scanty "set" of remains, teeth are usually used for sampling due to the fact that they are the best sources of DNA. Namely dental enamel – the hardest human tissue a human body protects the DNA rich pulp and dentin by ensuring a good quality of isolated DNA (Schwartz et al. 1991: 979; Pötch et al. 1992: 139–143; Boljunčić 2007: 538). Teeth taken for sampling were thoroughly cleaned following the standard procedure. All traces of tissue were removed from dental cavities by razor blades. Teeth surfaces were cleaned – abraded with a grinding tip and sandpaper. Finally they were crushed into small fragments which were stored in sterile polypropylene tubes at –20°C until analyzed (Boljunčić 2007: 538). Further tooth preparation and DNA extraction followed standards provided by Alonso et al. (2001: 260–266).

Bone fragment for sampling was taken from the left posterior parietal of a single cranium (Fig. 1, on the right). Bone surface was also cleaned as tooth surfaces and abraded with a grinding tip and sandpaper. This was followed by the same procedure as already described for tooth samples. Further bone preparation as well DNA extraction followed the same protocol as in teeth sampling and DNA extraction proposed by Alonso et al. (2001: 260–266).

DNA quantification

Data in the present paper were collected by using the ABI PRISM 7000 Sequence Detection System (Applied Biosystems, Foster City, CA, USA). The obtained data were analysed with ABI PRISM 7000 Sequence Detector Software (SDS) version 1.0 to generate the individual standard curves from each experiment and calculate the amount of DNA from each unknown sample. The total DNA was evaluated using agarose gel electrophoresis (ethidium bromide staining) and absorbencies at 260 and 280 nm were measured by spectrophotometer (Ultrospec 2000. Pharmacia Biotech). The A_{260}/A_{280} ratios were used to evaluate the quality of the extracted DNA (Burgi 1997; Boljunčić 2007: 539). Reactions without templates served as negative controls.

The exclusion of the possible inhibitors of Taq polymerase in the preparations followed the procedure from the manufacturer's protocols described in detail in the previous study by Boljunčić (2007: 539).

DNA (PCR) amplification and typing

Polymerase chain reaction (PCR) is a method where a certain region of DNA is copied (amplified) producing at optimum a billion copies completely identical to the original (Primorac et al. 2000: 34). DNA amplification was performed on the Perkin-Elmer Thermal Cycler 9600 employing the AmpFlSTR Profiler™ PCR Amplification Kit (Applied Biosystems, Foster City, CA, USA), following the recommended protocol. Typing of polymerase chain reaction products

logeninskom lokusu za spol. Analiza je obuhvatila sljedeće lokuse: D3S1358, vWa, FGA, TH01, TPOX, CSF1PO, D5S818, D13S317, D7S820 te AMEL – lokus za spol.

Priprema uzoraka

Za uzorkovanje su upotrijebljena dva zuba – bili su to jedini zubi prisutni u oskudnom skupnom bioarheološkom nalazu. Uzorci (tab. 1) su izdvojeni iz pretkutnjaka (sl. 2) i kutnjaka (sl. 3). Osim spomenute činjenice da su ti zubi bili jedini sačuvani zubi u oskudnom nalazu, oni se i inače najčešće koriste za uzorkovanje jer predstavljaju najbolje izvore DNK. Naime, zubna caklina kao najčvršće tkivo u ljudskome tijelu, štiti pulpu i dentin bogate DNK-om osiguravajući dobru kvalitetu izdvojene DNK (Schwartz et al. 1991: 979; Pötch et al. 1992: 139–143; Boljunčić 2007: 538). Zubi uzeti za uzorkovanje temeljito su očišćeni prema standardnom postupku. Svi tragovi tkiva odstranjeni su iz zubnih šupljina pomoću skalpela. Zubne su površine očišćene – izbrušene brusilicom i brusnim papirom. Naposljetku, zubi su izmrvljeni u male komadiće koji su pohranjeni u sterilne polipropilenske cjevčice na –20 °C do analize (Boljunčić 2007: 538). Sljedeće faze procesa pripremanja uzoraka i izdvajanja DNK provedene su prema standardima opisanima u Alonso et al. (2001: 260–266).

Ulomak kosti za uzorkovanje uzet je sa stražnjeg dijela lijeve tjemene kosti pojedinačnog nalaza lubanje (sl. 1, desno). Površina kosti očišćena je poput površina zubā, te izbrušena brusilicom i brusnim papirom. I ovdje je postupak tekao kao i pri opisanom uzorkovanju zubā. Daljnja priprema kosti i izdvajanje DNK načinjeni su prema jednakom postupku kao i kod uzorkovanja zubā i izdvajanja DNK, sukladno Alonso et al. (2001: 260–266).

Kvantifikacija DNK

Podaci u ovom radu prikupljeni su pomoću uređaja ABI PRISM 7000 Sequence Detection System (Applied Biosystems, Foster City, CA, USA), a za analizu je rabljen ABI PRISM 7000 Sequence Detector Software (SDS) verzija 1.0, koji proizvodi pojedinačne standardne krivulje za svaki pokus te izračunava količinu DNK u svakom nepoznatom uzorku. Ukupna DNK procijenjena je pomoću elektroforeze u agarskom gelu (bojanje etidij bromidom), a apsorpcije su izmjerene spektrofotometrom (Ultrospec 2000. Pharmacia Biotech) na 260 i 280 nm. Omjeri A_{260}/A_{280} korišteni su za procjenu kvalitete izdvojene DNK (Burgi 1997; Boljunčić 2007: 539). Reakcije bez predložaka služile su kao negativna kontrola.

Isključenje prisutnosti mogućih inhibitora Taq polimeraze u pripravcima provedeno je sukladno postupku koji je propisao proizvođač, a detaljno ga je opisala Boljunčić u prethodnom radu (2007: 539).

(PCR) umnažanje i određivanje DNK

Lančana reakcija polimeraze (PCR) je metoda tijekom koje se kopira (umnaža) određena regija/dio DNK, stvarajući optimalno milijardu kopija potpuno jednakih izvorniku (Primorac et al. 2000: 34). Umnažanje DNK izvedeno je na uređaju Perkin-Elmer Thermal Cycler 9600, korištenjem AmpFlSTR Profiler™ PCR amplifikatora (Applied Biosystems, Foster City, CA, USA), prema preporučenom postupku. Određivanje proizvoda lančane reakcije polimeraze načinjeno je pomoću uređaja ABI Prism 310 Genetic Analyzer (Applied Biosystems). Kao interni standard korišten je Rox-350 (Butler

No. of sample	Type of analyzed sample	Year of discovery	Morphological age/sex	Molecular sex
1	bone fragment[1] (parietal)	1996	adult/ female	unknown
2	tooth[2] (premolar)	1992	adult/ unknown	female
3	tooth[2] (molar)	1992	adult/ unknown	female[?]

[1]Bone obtained from a single skull (secondary find) lacking associated grave enclosures.

[2]Teeth obtained from a poor/scanty „set" of human remains (secondary finds) associated with grave enclosures from 4th cent. BC, found in farther vicinity.

Bioarchaeological material was from the Belišće Museum (Osječko-Baranjska County).

Tab. 1 Bone and tooth samples used for DNA typing at 10 short tandem repeat (STR) loci obtaned from archaeological human remains from Zagajci near Belišće (Croatia).

Br. uzorka	Vrsta analiziranog uzorka	Godina otkrića	Morfološka dob/ spol	Molekularni spol
1	Ulomak kosti[1] (tjemena kost)	1996.	odrasla dob/ ženski	nepoznat
2	zub[2] (pretkutnjak)	1992.	odrasla dob/ nepoznat	ženski
3	zub[2] (kutnjak)	1992.	odrasla dob/ nepoznat	ženski[?]

[1] Kost uzeta iz pojedinačnog nalaza lubanje (sekundarni nalaz) bez pripadajućih grobnih priloga.

[2] Zubi uzeti iz loše očuvanog i oskudnoga skupnog ljudskog nalaza (sekundarni nalaz) s pridruženim grobnim prilozima iz 4. st. pr. Kr., pronađenih nešto dalje.

Bioarheološki materijal potječe iz Muzeja grada Belišća (Osječko-baranjska županija).

Tab. 1 Koštani i zubni uzorci korišteni za određivanje DNK na 10 kratkih udvojenih sljedovnih (STR) lokusa dobivenih iz arheoloških ljudskih ostataka iz Zagajaca kraj Belišća (Hrvatska).

was performed on ABI Prism 310 Genetic Analyzer (Applied Biosystems). The internal standard was Rox-350 (Butler et al. 1994: 271-280; Burgi 1997; Alonso et al. 2001: 260–266; Boljunčić 2007: 546).

RESULTS

STR genotyping from samples obtained from archaeological human remains from Zagajci near Belišće, generated either a complete STR loci dropout (bone sample: 1), or low/ partial STR loci allelic profiles (tooth samples: 2 and 3) (Tab. 2). In terms of a bone sample (sample 1) obtained from a single (female) cranium (Fig. 1) we failed to obtain both autosomal and sex typing profiles during PCR procedure, i.e., as already mentioned there was a complete loci dropout. A locus dropout is the failure to detect an allele within a sample or to amplify an allele (Boljunčić 2007: 540). Such failure of detecting any DNA in the bone sample occurred in spite of the repeated (3 times) DNA extraction and purification (Tab. 2).

In terms of a tooth sample (sample 2) obtained from the premolar (Fig. 2) stemming from a scanty "set" of archaeological human remains, DNA typing after the repeated DNA extraction and purification generated a low/partial STR loci profile (Tab. 2). The DNA was completely successfully amplified only at two autosomal loci – D3S1358 (alleles 16;17) and D5S818 (alleles 10;12), also with generated clear result for the sex typing locus – amelogenin. The result obtained for the amelogenin contained only one peak from the X chromosome. It was in accordance with typical amelogenin sex test results for the female sex as shown by Abebe et al. (2004: 1). This was also in accordance with archaeological data i.e. grave enclosures (jewellery) associated with human remains which were too poor (scanty) for obtaining morphological sex of the individual.

In terms of another tooth sample (sample 3) obtained from the molar (Fig. 3) stemming from the same "set" of scanty human material apparently from the same individual, there was also a low/partial STR loci profile obtained. This refers to partially successful allelic amplification only at three autosomal loci as follows: D3S1358 (allele 17), et al. 1994: 271–280; Burgi 1997; Alonso et al. 2001: 260–266; Boljunčić 2007: 546).

REZULTATI

Određivanje STR genotipova iz uzoraka dobivenih iz arheoloških ljudskih ostataka iz Zagajaca kraj Belišća, s jedne je strane generiralo potpun izostanak STR lokusa (koštani uzorak: 1), dok je s druge strane rezultiralo oskudnim/djelomičnim profilima alela STR lokusa (uzorci zuba: 2 i 3) (tab. 2). U slučaju koštanog uzorka uzetog iz pojedinačnog nalaza (ženske) lubanje (sl. 1), tijekom PCR postupka nismo uspjeli dobiti ni autosomni ni spolni profil, odnosno, kako rekoh, svi su lokusi izostali. Izostanak lokusa (*locus dropout*) je termin koji označava neuspjeh u otkrivanju alela u uzorku ili nemogućnost umnažanja alela (Boljunčić 2007: 540). U koštanom uzorku nismo uspjeli otkriti tragove DNK unatoč tomu što je izdvajanje i pročišćavanje DNK provedeno u nekoliko navrata (triput) (tab. 2).

Glede zubnog uzorka (uzorak 2) dobivenog iz pretkutnjaka (sl. 2), koji pripada oskudnom skupnom nalazu arheoloških ljudskih ostataka, određivanje DNK nakon ponovljenog izdvajanja i pročišćavanja DNK generiralo je oskudni/ djelomični profil STR lokusa (tab. 2). DNK je u potpunosti uspješno umnožena samo na dva autosomna lokusa – D3S1358 (aleli 16;17) i D5S818 (aleli 10;12), dajući ujedno i jasan rezultat za određivanje amelogeninskog lokusa za spol. Rezultat dobiven za amelogenin sadržavao je samo jedan vrh ("*peak*") svojstven X kromosomu, što je u skladu s tipičnim rezultatima amelogeninskog testa za ženski spol, kao što su prikazali Abebe et al. (2004: 1). To se također poklapa s arheološkim podacima, tj. grobnim prilozima (nakit), pridruženim ljudskim ostacima koji su bili loše očuvani te nedostatni za morfološko određivanje spola osobe.

Drugi zubni uzorak (uzorak 3), dobiven iz kutnjaka (sl. 3) koji također potječe iz oskudnoga skupnog nalaza ljudskih ostataka, po svemu sudeći od iste osobe, također je dao oskudni/djelomični profil STR lokusa. To se odnosi na djelomice uspješno umnažanje alela na samo tri autosomna lokusa: D3S1358 (alel 17), vWa (alel 16) i D13S317 (alel 8) (tab. 2). Rezultati za lokus za spol sadrže dva vrha – jedan svojstven

Success of DNA amplification			
No. of DNA isolations	3	2	2
Loci/sample	1	2	3
D3S1358	–*	16;**17**[1]	**17**[1]
vWa	_	_	16
D5S818	_	10;12	_
D13S317	_	_	8
Amelogenin– sex typing locus	_	**X;X**	**X;X**[?]

*Unsuccessful DNA amplification.
[1]Shared allele 17 at the locus D3S1358.
[?] Unclear result for the amelogenin – however, atypical for male sex (probably female).
The rest of STR profiles of bone and tooth samples is not shown due to unsuccessful amplification.

Tab. 2 Results of DNA analysis of bone and tooth samples from Zagajci with AmpFlSTR ProfilerIM PCR Amplification Kit.

Uspješnost umnažanja DNK			
Br. izolacija DNK	3	2	2
Lokusi/uzorak	1	2	3
D3S1358	–*	16;**17**[1]	**17**[1]
vWa	_	_	16
D5S818	_	10;12	_
D13S317	_	_	8
Amelogenin – lokus za spol	_	**X;X**	**X;X**[?]

*Neuspjelo umnažanje DNK.
[1] Zajednički alel 17 na lokusu D3S1358.
[?] Nejasan rezultat za amelogeninski lokus – međutim, netipičan za muški spol (po svemu sudeći, ženski).
Preostali STR profili uzorka kosti te uzoraka zubā nisu prikazani zbog neuspješnog umnažanja.

Tab. 2 Rezultati analize DNK na uzorcima kostiju i zubā iz Zagajaca pomoću AmpFlSTR ProfilerIM PCR amplifikatora.

vWa (allele 16) and D13S317 (allele 8) (Tab. 2). The results for the sex typing locus yielded two peaks – from the X chromosome, and another atypical "supposedly from the Y chromosome". However, the obtained peaks – from the X chromosome and the Y chromosome were not within their expected size range, i.e. they were not typical for the male sex. This particularly refers to the second peak which was not in accordance with the typical peak expected from the Y (amelo) chromosome.

There was only one shared allele – allele 17 of the D3S1358 locus among tooth samples 2 and 3 (Tab. 2).

DISCUSSION

Of three samples examined in the present study for autosomal short tandem repeat loci and the amelogenin – sex typing locus, we obtained a completely successful DNA amplification only at two autosomal loci – D3S1358 (alleles 16;17) and D5S818 (alleles 10;12) in addition to AMEL – the sex typing locus in one case. Low/partial STR loci allelic profiles obtained for tooth samples, or even a complete loci dropout (for a bone sample), suggests that the ancient DNA in analyzed remains was poorly conserved. Such an issue usually stems either from a severe ancient DNA degradation/ contamination from soil, or from handling of the bioarchaeological material/samples. In our case there was an extremely cautions handling of the material prior to DNA typing and analysis performed after repeated DNA extraction and purification like in previous studies (Boljunčić 2007: 543). In addition a complete loci dropout obtained from aforementioned bone sample is also suggestive of the absence of possible contamination with modern DNA or any other DNA processed in other analyses in the laboratory. The latter usually refers to possible contamination either from the staff /investigators or personnel who are not part of a staff elimination database and therefore undetected (Gill, Kirkham 2004: 2; Boljunčić 2007: 543), which was not the case.

kromosomu X, te dodatni drugi, "za koji bi se moglo pretpostaviti kako potječe od kromosoma Y". Međutim, dobiveni vrhovi – za kromosom X i Y nisu bili unutar referentnih vrijednosti za muški spol. To se naročito odnosi na drugi vrh koji nije odgovarao standardnim vrijednostima za (amel) kromosom Y.

Zubni uzorci 2 i 3 imali su samo jedan zajednički alel – alel 17 na lokusu D3S1358 (tab. 2).

RASPRAVA

Od tri uzorka u ovom radu ispitana glede autosomnih STR lokusa i amelogeninskog lokusa za spol, uspjeli smo umnožiti DNK samo na dva autosomna lokusa – D3S1358 (aleli 16;17) i D5S818 (aleli 10;12), te na jednom amelogeninskom (AMEL) lokusu za spol. Oskudni/djelomični profili alela STR lokusa koje su iznjedrili uzorci zubā, zajedno s potpunim izostankom lokusa za koštani uzorak, upućuju na lošu očuvanost stare DNK u analiziranim ostacima. Takav se problem obično pojavljuje zbog znatnog propadanja stare DNK ili onečišćenja iz tla, te prilikom rukovanja bioarheološkim nalazima/uzorcima. U našem se slučaju izrazito pažljivo rukovalo materijalom prije određivanja DNK, kao i pri analizama izvršenim nakon ponovljenih izdvajanja i pročišćavanja DNK, sukladno prethodnim istraživanjima (Boljunčić 2007: 543). K tomu, potpun izostanak lokusa u spomenutom koštanom uzorku upućuje, također, na odsustvo mogućeg onečišćenja suvremenom DNK ili bilo kojom drugom DNK koja se obrađivala tijekom drugih analiza u laboratoriju. Potonje se obično odnosi na moguće onečišćenje ili od strane osoblja/istraživača, ili osoba koje ne sačinjavaju dio eliminacijske baze podataka koja postoji za osoblje, pa se tim putem ne bi mogle otkriti (Gill, Kirkham 2004: 2; Boljunčić 2007: 543), što ovdje nije bio slučaj.

Do znatnog propadanja DNK koje dovodi do potpunog izostanka lokusa ili generiranja oskudnih/djelomičnih profila STR lokusa moglo je doći zbog oštećenih predložaka

se in our study.

Severe DNA degradation producing a complete loci dropout or low/partial STR loci profiles, could be due to damaged/limited DNA templates in old bones/teeth which according to Benecke (1997: 181) could lead eventually to such major or even total elimination of all alleles. In the present case DNA degradation, i.e. damage of the DNA templates in the bone could possibly stem from post-mortal changes of the skull, even though there were no visible morphological signs of any significant damage to the bone cortex. However, one also has to take into account archaeological age of the examined material.

In terms of teeth DNA degradation could stem either from somewhat worn out (ante-mortal) tooth enamel of the examined premolar, or somewhat (post-mortal) damaged roots of the molar. The fact is that there is faster pulp cells degradation in damaged teeth with an exposed opened pulp (Pfeiffer et al. 1999: 143) than in teeth the pulp of which is protected by enamel in a good state of preservation. On the other hand, among other, the study of Pfeifer et al. (1999: 143) also showed that isolated teeth even with high degree of destruction (which was not the case in our study) were lucrative source of DNA. Regardless of the mentioned, the processed teeth were the only teeth in the "set" of scanty archaeological human remains to deal with.

Another problem is possible contamination from soil. However Taq Polimerase inhibitors which are fairly known to be particularly significant when DNA is extracted from old/ ancient material, were not detected in the examined samples. The absence of DNA polymerase inhibitors from humic acid was confirmed in all preparations during performed QRT-PCR according to the manufacture's protocols – Quantifiler ™ human DNA quatification Kit, user's manual.

In conclusion the performed molecular analysis for autosomal STR loci in three samples (one bone sample from a single female cranium and two tooth samples from a scanty "set" of remains of another – supposedly female individual) yielded altogether a successful amplification only at two loci, and a clear result for amelogenin – the sex typing locus, in one case. In other case the sex-typing was ambiguous. The amplification of the rest of autosomal loci yielded either low partial STR profiles (tooth samples), or a complete loci dropout (bone sample). There was only one allele shared among tooth samples – allele 17 of the D3S1358 locus. Such result has no significance in possible "genetic matching" of two analyzed teeth.

However, there is a significance of DNA typing for amelogenin obtained from one tooth – the premolar associated with small bone fragments and grave enclosures (jewellery). Molecular analysis for the sex-typing locus confirmed the presence of a female individual in a scanty "set" of bioarchaeological material in accordance with archaeological data. Such multidisciplinary approach is particularly important in bioarchaeology when researchers often deal only with limited bioarchaeological material. Otherwise possible erroneous gender DNA identification could appear even in modern cases such as described by Abebe et al. (2004: 1–2), in which DNA could be processed again as well as compared to an individual karyotype. In bioarchaeology we could relay for a possible "control" of molecular sex, either on bioarchaeological data (when available) or we could reasonably explain possible ambiguous sex-typing results in reference to associated (bio)archaeological data.

DNK, odnosno njihove ograničene količine u starim kostima/zubima, što bi prema Benecke (1997: 181) moglo u konačnici dovesti do znatnog ili čak potpunog uklanjanja svih alela. U ovom slučaju propadanje DNK, tj. oštećenje predložaka DNK u kosti, moglo bi potjecati od promjena na lubanji nakon smrti, iako nije bilo vidljivih morfoloških pokazatelja ikakvih znatnijih oštećenja na korteksu. Međutim, valja uzeti u obzir i arheološku starost ispitivanog materijala.

Kad je riječ o zubima, propadanje DNK moglo je potjecati od ponešto (zaživotno) istrošene zubne cakline ispitivanog pretkutnjaka, ili od donekle oštećenih (nakon smrti) korijena kutnjaka. Činjenica je da stanice pulpe propadaju brže u oštećenih zuba s otvorenom i izloženom pulpom (Pfeiffer et al. 1999: 143) nego kod zuba u kojima je pulpa zaštićena dobro očuvanom caklinom. S druge strane, između ostalog, Pfeiffer et al. (1999: 143) su također pokazali kako izolirani zubi čak i pri visokom stupnju oštećenja (što nije bio slučaj u našem radu) mogu predstavljati bogat izvor DNK. Neovisno o tomu, kako rekoh, obrađeni zubi bili su jedini sačuvani zubi u oskudnom skupnom nalazu arheoloških ljudskih ostataka.

Drugi problem obično predstavlja moguće onečišćenje iz tla. Međutim, inhibitori Taq polimeraze, za koje je dobro poznato kako mogu biti značajan čimbenik onečišćenja kad je riječ o izdvajanju DNK iz starog/drevnog materijala, nisu otkriveni u ispitivanim uzorcima. Odsustvo inhibitora DNK polimeraze, iz humusne kiseline, potvrđeno je u svim pripravcima tijekom provođenja QRT-PCR prema protokolu proizvođača sukladno priručniku za korisnike opreme za kvantifikaciju ljudske DNK Quantifiler™.

U zaključku, provedena molekularna analiza glede autosomnih STR lokusa na tri uzorka (jedan koštani uzorak iz pojedinačnog nalaza ženske lubanje te dva uzorka zuba iz oskudnoga skupnog nalaza, koji je pripadao drugoj, po svemu sudeći, ženskoj osobi) dovela je do ukupnoga uspješnog umnažanja samo na dva lokusa, te je u jednom slučaju dala jasan rezultat za amelogeninski lokus za spol. U drugom je slučaju određivanje spola bilo dvojbeno. Umnažanje ostatka autosomnih lokusa generiralo je oskudne i djelomične STR profile (zubni uzorci) ili potpuni izostanak lokusa (koštani uzorak). Samo je jedan alel bio zajednički obama zubnim uzorcima – alel 17 lokusa D3S1358. Takav rezultat nema značenja u smislu moguće "genetičke podudarnosti" dvaju analiziranih zuba.

Međutim, važnost određivanja DNK ovdje leži u amelogeninskom lokusu dobivenom iz jednog zuba – pretkutnjaka koji je pronađen u oskudnom skupnom nalazu ulomaka kostiju zajedno s grobnim prilozima (nakit). Molekularna analiza za lokus za spol potvrdila je nazočnost ženske osobe u oskudnom skupnom bioarheološkom nalazu sukladno arheološkim podacima. Takav multidisciplinarni pristup posebno je važan u bioarheologiji kad istraživači često imaju na raspolaganju samo ograničenu količinu bioarheološkog materijala. Inače, prema autorima Abebe et al. (2004: 1–2), pogrešno određivanje DNK po spolu može se dogoditi čak i kad je riječ o suvremenim slučajevima, gdje se DNK može ponovno ispitati te usporediti s kariotipom osobe. U bioarheologiji se za moguću "kontrolu" molekularnog spola možemo osloniti na bioarheološke podatke (kad su dostupni) ili možemo razumno obrazložiti moguće dvojbene rezultate određivanja spola u skladu s pridruženim (bio)arheološkim podacima.

U konačnici, iako zbog propadanja DNK u starom bio-

Finally, although containing limited molecular results apparently due to degraded DNA in old bioarchaeological material, we hope that the present study will supplement a database on DNA typing in Croatian bioarchaeology. It points out to the problems with which bioarchaeologists are often faced when dealing with old and scanty/damaged, i.e. limited material available for DNA analysis.

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LIST OF ABBREVIATIONS

AMEL – Amelogenin
CSF1PO – protooncogene for CSF1 receptor gene
DNA – deoxyribonucleic acid
FGA – human alpha fibrinogen
PCR – polymerase chain reaction
QRT-PCR – quantitative real time – polymerase chain reaction
STR loci – short tandem repeat loci
TAQ polymerase – Thermus aquaticus polymerase (thermostable DNA)
TH01 – tyrosine hydroxylase, intron 1
TPOX – human thyroid peroxidase gene
vWA – von Willebrand factor, intron A

Finally, although containing limited molecular results apparently due to degraded DNA in old bioarchaeological material, we hope that the present study will supplement a database on DNA typing in Croatian bioarchaeology. It points out to the problems with which bioarchaeologists are often faced when dealing with old and scanty/damaged, i.e. limited material available for DNA analysis.

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POPIS KRATICA

AMEL – amelogenin
CSF1PO – protoonkogen za CSF1 receptor
DNA – deoksiribonukleinska kiselina
FGA – ljudski alfa fibrinogen
PCR – lančana reakcija polimeraze
QRT-PCR – lančana reakcija polimeraze u stvarnom vremenu
STR loci – kratki udvojeni sljedovni lokusi
TAQ polimeraza – polimeraza iz bakterije Thermus aquaticus (termostabilna DNK)
TH01 – tirozin hidroksilaza, intron 1
TPOX – gen za tiroidnu peroksidazu
vWA – von Willebrandov faktor, intron A

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