

Frequency of Main Western-Euroasian mtDNA Haplogroups and Paleolithic and Neolithic Lineages in the Genetic Structure of Population of Northeastern Bosnia

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ABSTRACT

Mitochondrial DNA (mtDNA) variations were analyzed in a sample of 245 individuals of Bosnian-Herzegovinian population from the area of Northeastern Bosnia (also known as Tuzla region). Haplogroup affiliation was determined using RFLP method (Restriction Fragment Length Polymorphism) analyzing haplogroup-specific markers of mtDNA coding region, characteristic for the main Western-Eurasian haplogroups. Additional analyses of two sequenced hypervariable segments (HVSI and HVSII) of mtDNA control region were performed in order to identify U subhaplogroups. The study revealed that 95.51% of the analyzed individuals belonged to the typical Western-Eurasian haplogroups: H, I, J, K, T, U, V, W or X. The most frequent haplogroup in the analyzed population was the haplogroup H (52.65%) which, due to its increased frequency, represents a marking haplogroup of the population of Northeastern Bosnia. The results of intergroup genetic analysis showed that Bosnian-Herzegovinian population is genetically closer to previously studied populations of Herzegovinians (part of Bosnia and Herzegovina), Slovenians and Croats in relation to other neighboring populations located in Southeastern Europe. Our study also suggests that population genetic structure of Tuzla region is dominated by mutations that are classified as »Paleolithic«. These mutations were probably brought to the area of Northeastern Bosnia through waves of prehistoric and historic migrations, but the impact of any pre-Neolithic, Neolithic or some »later« migrations, with a slightly lower contribution to the genetic structure of this population, also can-not be neglected.

Key words: mtDNA haplogroups, intergroup population variation, Paleolithic, Neolithic

Introduction

Mitochondrial DNA (mtDNA) polymorphisms are often used today in research studies of intrapopulation and interpopulation genetic diversification of human populations, pointing to a genetic origin of populations in the maternal line and the processes of human colonization^{1–14}. Modern classification of European mtDNA variations based on the combined data on polymorphisms of hypervariable segments I and II (HVSI and HVSII) of the control region (CR)^{9,10,15} and the coding region of mtDNA^{15,16} shows that in the evolution of mitochondrial gene flow groups of phylogenetically related haplotypes occur, which are classified into appropriate mtDNA ha-

plogroups¹⁷. The European populations are stratified into nine general haplogroups: H, I, J, K, T, U, V, W and X, designated as the Western-Eurasian haplogroups^{9,12,15,16}. In the former studies, following haplogroups were also observed: pre-HV, pre-V, HV, N1a, N1b, F and a few Asian and African haplogroups in European populations^{5,14,16–23}. Prior research on mtDNA variations of Southeastern Europe population lineages^{3,24}, to which Bosnia and Herzegovina also belongs indicated the presence of characteristic Western-Eurasian mtDNA haplogroups, with a small portion of Asian and African lines, as well as a significant impact of historical population migrations on

forming their genetic structure. The history of Bosnia and Herzegovina is very complex due to its territorial exposure to different routes of migratory movements from East and West. Existing archaeological data from central Bosnia (ca.100,000 years) and northern Bosnia (ca. 50,000 years) point to the fact that Bosnia and Herzegovina was inhabited back in the Paleolithic age²⁵. In the region of Tuzla, which covers the northeastern part of Bosnia (Figure 1), numerous Paleolithic sites were registered. As suggested by archaeologists, they probably originate from 20,000 to 30,000 years ago, pointing to the assumption about the existence of human populations in this area even back in the Old Stone Age²⁶. Although some stone artifacts found in these parts of Bosnia originate from Paleolithic times, the known prehistoric period of civilization paths dates from the Neolithic²⁷, as evidenced by numerous archaeological investigations, emphasizing Tuzla region as an area in Bosnia-Herzegovina with the most famous artifacts from the Early Stone Age. Throughout the history, the territory of Tuzla region and the rest of the Bosnian and Herzegovinian area was populated by the Illyrians, Romans, Gauls, Egyptians, Germans, Iranians and many other nations^{25,28}. Historically, because of its outstanding mineral wealth, this area attracted immigrants from all over the world, which probably influenced the genetic structure of this part of Bosnian-Herzegovinian population through mixing with the indigenous population. During history, this territory was mostly an immigration area, and the population settled in from different regions, mostly from Herzegovina, Montenegro and Serbia.

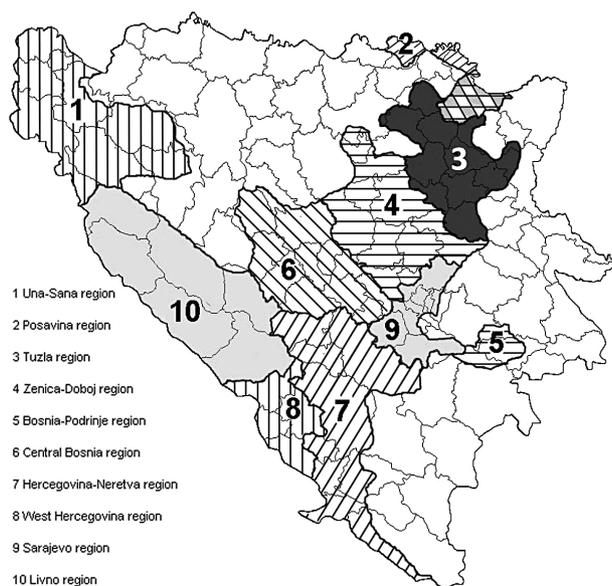


Fig. 1. Geographical position of Tuzla region in the map of Bosnia and Herzegovina.

This work presents the first extensive population study of mtDNA variations in the area of northeastern Bosnia, where we describe (1) the portion of represented Western-Eurasian mtDNA markers in the population of

Tuzla region, (2) identify the position of the analyzed population compared to the observed southeastern European populations, and (3) estimate the contribution of Paleolithic and Neolithic mtDNA mutations to the gene flow and forming of specific genetic structure of the analyzed population.

Materials and Methods

Population samples

A total of 245 buccal samples have been collected from unrelated individuals in the area of Northeastern Bosnia. Sampled population consisted of persons from all municipalities of Tuzla region (area of Northeastern Bosnia): Tuzla, Živinice, Srebrenik, Gradačac, Kalesija, Kladanj, Gračanica, Teočak, Banovići, Sapna, Lukavac, Doboj-Istok and Čelić. Prior to sampling, all participants provided written informed consent for the collection of samples and subsequent analyses. For the purpose of comparative analysis, we included data from previous studies on populations from Bosnia and Herzegovina^{3,24}, Southeastern Europe^{3,24}, Central and Eastern Europe²⁹, as well as Northern Europe¹⁶.

MtDNA analysis

Genomic DNA was extracted from dried swab samples of buccal mucosa, using salting out method³⁰. The method was slightly modified in order to optimize the extraction of DNA from buccal swab³¹. Total extracted DNA was further used for the PCR (*Polymerase Chain Reaction*) and RFLP (*Restriction Fragment Length Polymorphism*) analyses. Determination of mtDNA haplogroups was carried out by analysis of enzyme restriction, applying 14 haplogroup-specific polymorphic sites of mtDNA coding region, using primer pairs and amplification coordinates as described by Torroni et al.¹⁶. Relevant polymorphic sites used for determination of nine Western-Eurasian mtDNA haplogroups (H, I, J, K, T, U, V, W and X) were: -7025 *AluI* (haplogroup H); -1715 *DdeI*, +8249 *AvaII*, +10028 *AluI* (haplogroup I); -13704 *BstOI* (haplogroup J); -9052 *HaeII*, +12308 *HinfI* (haplogroup K); +13366 *BamHI*, +15606 *AluI* (haplogroup T); +12308 *HinfI* (haplogroup U); -4577 *NlaIII* (haplogroup V); +8249 *AvaII*, -8994 *HaeIII* (haplogroup W) and -1715 *DdeI* (haplogroup X). Each of the twelve PCR segments (with haplogroup-specific polymorphic sites for a specific mtDNA haplogroup) was subjected to a separate enzyme reaction with the appropriate set of restriction endonucleases. Working concentrations of all enzymes used were 2 u/μL and digestion was performed overnight at 37 °C, except for the enzyme *NlaIII*, where the used working concentration was 1 u/μL. The success of an enzyme reaction was checked by the process of separation of the obtained restriction fragments on 2% or 3% agarose gel in 1xSB (Sodium Borate) buffer, depending on the expected size of restriction products. Parallel with the samples, a size standard ΦX174DNA / BsuRI (*HaeIII*) marker 9, 72–1353bp was separated. Sizes of obtained restriction fragments were estimated using EDAS

software (Electrophoresis Documentation and Analysis System). In an additional analysis, hypervariable segments I and II (HVS I and HVS II) of the mtDNA control region (CR) were amplified and sequenced for 23 samples, in order to subtype U haplogroup. The set of primers F15971/R16410 was used for PCR reaction of the HVS I region sequence, while for HVS II region we used the F15/R484 primer set. PCR products were sequenced within facilities of the Macrogen Korea Inc. as their regular capillary DNA sequence services. Sequence analysis was performed with BioEdit software³² and compared in relation to the consensus, Cambridge Reference Sequence (CRS)^{33,34}. On the basis of observed differences analyzed with mtDNA Manager software³⁵, the sequences were classified into appropriate mtDNA haplogroups.

Population-genetic analyses

Estimation of genetic variability of mitochondrial genomes within analyzed population of Tuzla region and its comparison with the observed European and South-eastern European populations were done using intra- and intergroup genetic indices. The haplogroup diversity as a parameter of intergroup population variation was calculated according to Nei³⁶. For the assessment of intergroup genetic differentiation pairwise F_{ST} analysis³⁷ was used. The genetic distance between populations was calculated according to Reynolds³⁸. As an additional test of interpopulation differentiation we used exact-test³⁹ based on haplogroup frequencies. For analyses of the portion of molecular-genetic variations of the observed markers at individual, intragroup and intergroup levels we performed AMOVA test⁴⁰. All abovementioned analyses were implemented within ARLEQUIN ver. 3.11 software⁴¹. Principal Component (PC) analyses were performed on mtDNA haplogroup frequencies implemented by PAST software⁴². The phylogenetic networks based on

HVS I and HVS II sequences for haplogroup U were constructed by use of a median-joining algorithm⁴³ as implemented in the Network 4.6.1.2. software (<http://www.fluxus-technology.com>).

Results

In a group of 245 unrelated individuals from Northeastern Bosnia (Tuzla region) we identified all nine typical Western-Eurasian haplogroups. The majority of examined individuals (95.51%) were affiliated to the following haplogroups: H, V, T, I, J, K, U, X and W. Frequencies of the identified haplogroups and subgroups are given in Table 1. The haplogroup U was typed to ten sub-haplogroups. Twenty three different mtDNA haplotypes of the haplogroup U were observed (Table 2). The phylogenetic network for mtDNA haplotypes of haplogroup U is shown in Figure 2. The most frequent mtDNA haplogroup in the population of Tuzla region is haplogroup H (52.65%) and undoubtedly it is the most frequent haplogroup in Europe^{5,44–46}. The second most frequent haplogroup (with all the determined subgroups) was the haplogroup U (9.39%). In subcluster diversification of this most ancient haplogroup in Europe⁹, we determined the component of the early Upper Paleolithic (45 kya) (45 thousand years ago), haplogroup U5, and the components of the middle Upper Paleolithic (26 kya), haplogroups U1, U2, U4^{5,9}. Haplogroup J, the representative component of the gene flow which was brought to Europe by Neolithic expansion from the Near East^{2,5,9}, was present in our study with the share of 7.75% variations. The noted frequency of this haplogroup fits in the range of maternal genetic variation in populations of Southeastern Europe (Table 3). Haplogroup T, also, representative Neolithic component of the early farmers in the modern European populations^{5,9}, was observed in a somewhat lower frequency,

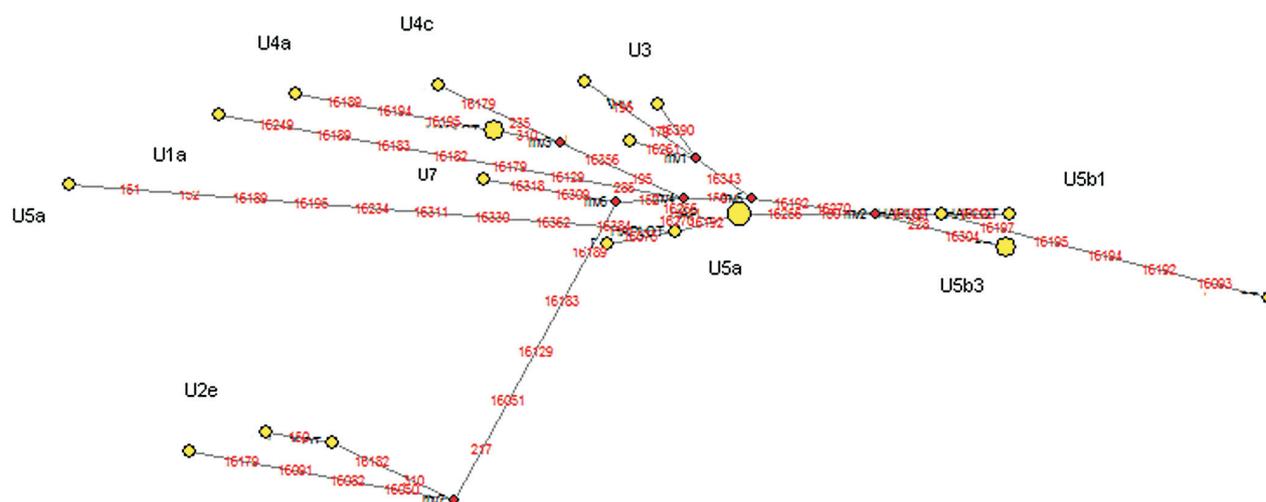


Fig. 2. The phylogenetic network of mtDNA haplotypes within haplogroup U found in the Bosnian population. Circle areas are proportional to haplotype frequencies. Variant bases of the HVS I and HVS II are numbered (in comparison with CRS) and shown along links between haplotypes. *Mv* represents median vectors. Character changes are specified for transversion and transition. The insertion or deletion events at *np* 309 and 315 were not considered.

TABLE 1
DISTRIBUTION (%) OF THE DETERMINED mtDNA HAPLOGROUPS IN THE ANALYZED POPULATION

Haplogroup	N	Frequency
H	129	52.65
I	4	1.63
J	19	7.76
T	12	4.90
W	8	3.27
U5a	6	2.45
U5b3	2	0.82
U5b1b	3	1.22
U4a2	3	1.22
U4c1	1	0.41
0U3	2	0.82
U3a	1	0.41
U2e	3	1.22
U1	1	0.41
U7	1	0.41
K	10	4.08
V	15	6.12
X	14	5.71
Other	11	4.48

but not deviating from the frequencies in nearby populations in Southeastern Europe, with the exception of Macedonian population (Table 3). The recorded frequency of the autochthonous European haplogroup V, noted as a marker of post-glacial expansion of population from the Iberian Peninsula¹² study was 6.12%. According to previous literature data^{3,24}, the frequency of the haplogroup V from our study represents the highest noted frequency in populations of Southeastern Europe. In addition to haplogroup H, supposedly strongly related to late-glacial expansions of populations from ice-age refugia after the LGM (Last Glacial Maximum)^{5,46} other determinants of such process haplogroups W, I, X and K⁵ were also detected in Tuzla region population. Recorded frequency of these haplogroups (with the exception of haplogroup X) is less than 5%, which fits in the range of variations among comparative populations of the Southeastern Europe (Table 3). In European populations, the both subhaplogroups X1 and X2 of haplogroup X are mostly represented with the frequency of up to approximately 5%^{47–50}. In populations of Southeastern Europe the haplogroup X has been represented in a quite small percentage (<2%) (with the exception of Macedonians and Macedonian Romani)^{3,24}. However 5.71% of individuals from our study was revealed to belong to this haplogroup. A rather high incidence of this haplogroup has reported in Macedonians²⁴ and Macedonian Romani²⁴ (6% and 11% respectively), which corresponds to the frequency of haplogroup X in our study.

TABLE 2
FREQUENCIES OF MTDNA HAPLOTYPES WITHIN THE HAPLOGROUP U IN THE POPULATION OF TUZLA REGION

HVSI (–16000)	HVSII	HG	Haplotype (N)
192-256-270	73-263-309.1-315.1	U5a	3
256-270	73-263-309.1-315.1	U5a	1
75-256-270	73-263-309.1-315.1	U5a	1
189-192.1-195-234-256-270-311-330-362-384	73-151-152-263-309.1-315.1	U5a	1
189-192-270	73-150-263-315.1	U5b1b	1
189-192-270-292	73-150-263-309.2-315.1	U5b1b	1
93-189-194-195-197-270	73-150-263-315.1	U5b1b	1
192-270-304	73-150-228-263-315.1	U5b3	2
356	73-195-263-310-	U4a2	2
189-194-195-356	73-195-263-310-	U4a2	1
179-356	73-195-235-263-315.1	U4c1	1
343	73-150-179-195-263-315.1	U3	1
261-343	73-150-263-315.1	U3	1
343-390	73-150-263-309.1-315.1	U3a	1
051-129-182-183-189-	73-150-217-263-310-	U2e	1
050-051-082-091-129-179-183-189	73-152-217-263-309.2-315.1	U2e	1
051-129-182-183-189	73-152-217-263-310-	U2e	1
129-179-182-183-189-249	73-263-285-309.2-315.1-	U1a	1
309-318	73-152-263-309.1-315.1	U7	1

Mutations are shown indicating positions relative to the rCRS³⁴. The nucleotide positions in HVSI and II sequences correspond to transitions and transversions. The presence of insertions or deletions is indicated by .1 respectively, following the nucleotide position.

TABLE 3
FREQUENCY (%) OF WESTERN-EURASIAN MTDNA HAPLOGROUPS IN THE POPULATION OF TUZLA REGION AND COMPARATIVE POPULATIONS OF SOUTHEASTERN EUROPE

Population	N	H	I	J	T	W	U5	U4	U3	U2	U1	U7	K	V	X
Northeastern Bosnia	245	52.65	1.63	7.75	4.89	3.27	4.48	1.63	1.22	1.22	0.41	0.41	4.08	6.12	5.71
Bosnians ³	144	48.00	2.78	6.94	4.86	1.39	6.94	5.56	0.69	0.00	1.39	0.00	4.17	0.00	1.39
Bosnians ²⁴	247	47.00	2.40	7.30	2.80	3.60	8.50	2.40	0.00	0.80	0.00	1.60	5.30	2.40	2.00
Herzegovinians ²⁴	130	43.00	0.80	8.50	6.15	2.31	4.60	4.60	0.00	2.30	1.50	0.00	9.20	3.85	0.80
Croats ²⁴	277	45.00	1.40	11.91	8.66	2.20	11.60	2.20	0.70	1.40	0.40	0.00	3.61	4.00	2.20
Slovenians ³	104	47.00	1.92	9.62	5.77	4.81	2.00	5.77	1.92	0.96	0.00	0.00	3.85	0.00	0.96
Serbs ²⁴	117	41.00	3.40	6.84	5.13	3.40	9.40	6.80	0.90	1.70	0.00	1.70	4.37	3.40	0.00
Macedonians ²⁴	146	41.00	1.40	7.53	11.00	2.70	8.90	3.40	2.10	1.40	0.70	0.00	3.42	1.40	6.20

The values of haplogroup diversity of observed population and comparative populations of Southeastern Europe are given in Table 4. Haplogroup diversity in the analyzed population was slightly lower in relation to the comparative populations of Southeastern Europe. Lower values of this parameter may be due to slightly higher frequencies of specific haplogroups as noted in our study (H, V and X), relative to comparative populations. Analysis of genetic differentiation between pairs of the observed populations (pF_{ST}) indicated that the differentiation between the analyzed population and surrounding populations (populations of former Yugoslavia: Herzegovinians, Croats and Slovenians), including the previously studied population of Bosnians was relatively uniform in the range from 0.0076 to 0.0100 (Table 5). A slightly increased genetic differentiation between the population of Tuzla region and populations of Serbs and Macedonians was noted. Somewhat lower values of genetic differentiation were observed in comparison with the population of Slovenians, and those were in correlation with a previous study by Malyarchuk³. It is indicative that slightly increased intergroup differentiation of the population of Tuzla region and Bosnians (sampled in previous studies^{3,24}) remains within 1%. It should be noted that in our study mtDNA lineages were analyzed within local geographical origin, while the studies by Malyarchuk et al.³ and Cvjetan et al.²⁴ investigated Bos-

TABLE 4
HAPLOGROUP DIVERSITY IN THE ANALYZED POPULATION AND COMPARATIVE POPULATIONS OF SOUTHEASTERN EUROPE

Population	Haplogroup diversity
Northeastern Bosnia	0.6967±0.0298
Bosnians ³	0.7151±0.0315
Bosnians ²⁴	0.7444±0.0229
Herzegovinians ²⁴	0.7665±0.0298
Croats ²⁴	0.7410±0.0214
Slovenians ³	0.7267±0.0369
Serbs ²⁴	0.7657±0.0288
Macedonians ²⁴	0.7766±0.0262

nian population as of heterogeneous origin. Genetic relations of the comparing populations are presented with the values of genetic distance (Table 5). Minimum genetic distance (0.007) is observed between the population of Tuzla region and Slovenian population while Macedonians were the most distant from Tuzla population (0.0131).

In PC analysis (Figure 3), first PCA component shows separation of Northeastern Bosnia from Croatian and

TABLE 5
MATRIX OF GENETIC DIFFERENTIATION (pF_{ST}) (BELOW DIAGONAL) AND RESULTS OF GENETIC DISTANCE (ABOVE DIAGONAL) BETWEEN COMPARATIVE POPULATIONS OF SOUTHEASTERN EUROPE BASED MTDNA HAPLOGROUPS

Population	Northeastern Bosnia	Bosnians ³	Bosnians ²⁴	Herzegovinians ²⁴	Croats ²⁴	Slovenians ³	Serbs ²⁴	Macedonians ²⁴
Northeastern Bosnia		0.0109	0.0101	0.0095	0.0090	0.0077	0.0201	0.0131
Bosnians ³	0.01087		0.0005	0.0037	0.0000	0.0000	0.0016	0.0005
Bosnians ²⁴	0.01006	-0.00313		0.0000	0.0063	0.0000	0.0000	0.0028
Herzegovinians ²⁴	0.00951	-0.00046	-0.00235		0.0050	0.0006	0.0003	0.0012
Croats ²⁴	0.00894	0.00365	0.00630	0.00499		0.0000	0.0029	0.0007
Slovenians ³	0.00768	-0.00549	-0.00255	0.00057	-0.00278		0.0000	0.0000
Serbs ²⁴	0.01994	-0.00273	-0.00083	0.00032	0.00298	-0.00350		0.0000
Macedonians ²⁴	0.01303	0.00160	0.00281	0.00118	0.00067	-0.00110	-0.00043	

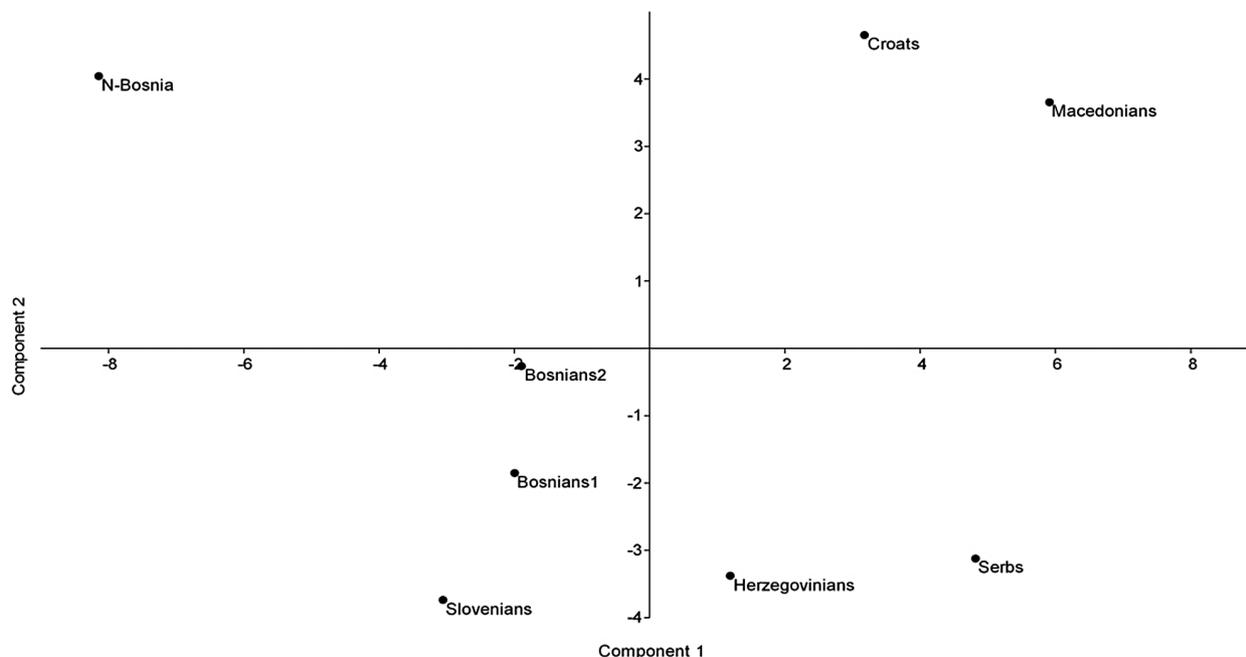


Fig. 3. PC analysis performed using the frequencies of the mt-DNA haplogroups in the population of Tuzla region (N-Bosnia) and comparative populations of Southeastern Europe. On the whole, 62.15% of total variance is represented by first (39.15%) and second (23%) PCA component.

Macedonian population, as well as other observed Bosnian, Herzegovinian and Serb populations. The second PCA component separates Northeastern Bosnia from other two observed Bosnian populations. On the whole, 62.15% of total variance is represented by first (39.15%) and second (23%) PCA component.

For the AMOVA test we stratified five groups: 1) population of Tuzla region; 2) Bosnian-Herzegovinian population (previous studies – Bosnians^{3,24}; Herzegovinians²⁴); 3) populations of the former Yugoslavia, Southeastern European populations (Croats, Serbs and Macedonians²⁴; Slovenians³); 4) populations of Central and Eastern Europe (Poles and Russians²⁹) and 5) populations of Northern Europe (Finns and Swedes¹⁶). From total detected genetic variation, 0.50% belongs to variation between the groups, 0.04% between populations within groups, and 99.55% within populations. The observed F_{ST} value was 0.0045.

Discussion and Conclusions

The position of the investigated Bosnian-Herzegovinian population compared to the observed populations of Southeastern Europe

The nine identified general Western-Eurasian haplogroups in the population of Tuzla region represent nearly all mtDNA variations that are present in Europe today. Our results show that the investigated population of Northeastern Bosnia has haplogroup combination typical for European populations. This conclusion is supported by the analysis of molecular variance in this study, with intergroup variation of 0.50%, which gives a clear

picture of the position of population from the area of Northeastern Bosnia among European populations. Many previous studies on mtDNA variation showed a high degree of homogeneity among European populations. Southeastern European populations, where Bosnia and Herzegovina belongs, contain a large number of common mtDNA haplogroups^{3,24}. However, analysis of the distribution of mtDNA variations and their combinations can be rather informative assessment tool about ethnic history of a given population. Our study suggests that the population of Tuzla region shows more genetic similarities with populations of former Yugoslavia such as Slovenians, Croats and Herzegovinians, than in relation to the populations of Serbs and Macedonians. This fact can be supported by some historical records as well, which suggested that the Illyrians, as the dominant population group in the Balkans and in Bosnia and Herzegovina, mixed with the Slavic tribes and became the common ancestors of modern Slovenians, Croats and Bosnian-Herzegovinians while there is a probability that the Serbs are descendants of the Thracians people who have assimilated with the Slavs³.

The analysis of frequency distribution of observed mtDNA haplogroups showed that the genetic specificity of investigated Bosnian-Herzegovinian population is in relation to the observed population of Southeastern Europe, represented in slightly increased frequency of haplogroup H, characteristic of all Paleolithic European settlers¹⁸ and autochthonous European haplogroup V¹². The most frequent mtDNA haplogroup in the population of Tuzla region is haplogroup H, originated in the Middle East approximately 30 to 25 kya. This haplogroup is presumed to be strongly involved in the late-glacial expan-

sions from ice-age refugia after the LGM and expanded in association with the second wave of Paleolithic migrations, possibly contemporary with the diffusion of Gravettian culture (25 to 20 kya)^{5,12}. According to previous studies about 30–50% of European mtDNA lines (excluding the population of Basques) is classified as the haplogroup H^{4,5,44,46}. The recorded frequency in this study (52.65%) belong to the category of »slightly« increased frequency among the populations of Southeastern Europe and other European populations. A relatively increased frequency of this common European haplogroup may be the result of some influence by »later« population expansions that occurred during the second Paleolithic wave of migrations in Southeastern Europe, and consequently in the territory of Bosnia and Herzegovina. In studies on the presence of Y-haplogroups in the Bosnian-Herzegovinian populations, Marjanovic⁵¹ reported that increased frequency of Y-subhaplogroup I (P-37) is an indicator of re-expansion after the LMG in the Balkan populations. Given the fact that the mtDNA haplogroup H, strongly involved in the late-glacial expansion of human populations after the LGM and expanded in association with the second wave of Paleolithic migrations^{5,12}, is seen as equivalent to Y haplogroup I (P-37), we can stipulate that increased frequency of haplogroup H in this study is following a trend in that direction – in line with the hypothesis of the post-glacial expansions from a LGM refuge area in the Balkan. Of course, to establish this statement more precisely, additional studies are necessary to dissect haplogroup H within sub-haplogroups as a parameter of spatial and temporal frequency for assessing prehistoric and historical migratory events in this region. The noted frequency of haplogroup V, as a marker of post-glacial population expansion from the Iberian Peninsula¹², was slightly increased compared to the frequency in other populations of Southeast Europe. Although the noted frequency does not exceed the upper limit on the presence of this haplogroup in Europe, its slightly increased incidence in the analyzed population is an interesting indicator of specific mtDNA characteristics of this population, minding the fact that the previous studies reported the absence of this haplogroup in European populations of Slovenians, Russians, Poles, Macedonian Roma people and a relatively lower frequency in other populations in Southeastern Europe^{3,29}. Also, an absence of this haplogroup in previously analyzed population of Bosnians was observed³.

The data obtained on the frequency of determined haplogroup, results of exact-test and intergroup analysis all point to mtDNA specificity in the area of Tuzla region and significant geographic-genetic structuring of the analyzed area. Further, PC analysis showed certain distinctions of Northeastern Bosnia population compared to other observed Bosnian, Herzegovinian and other populations in the region. Our suggestion is that in the future work, in an extended study, emphasis should be given to the analysis of mtDNA lines from all regions of Bosnia and Herzegovina and the consideration of increased local share of the aforementioned haplogroups in the area of northeastern Bosnia should be taken into account.

Specific frequencies of the mentioned haplogroups and the results of intergroup analysis suggest that the genetic history of the analyzed Bosnian-Herzegovinian population is very complex and requires more detailed future studies. The region of northeastern Bosnia has attracted immigrants from all over the world due to its mineral resources, so it is not surprising that this region represents the geographical crossroads of historical migratory events in the past. The share of unidentified haplogroups in the analyzed population of close to 5% may indicate that the unidentified mtDNA lines belong to different gene flows, which retained their »genetic traces« until very day.

The presence of mtDNA haplogroups as an influence of the Paleolithic and Neolithic mtDNA gene flow on genetic structure of populations of Northeastern Bosnia

Based on the spatial frequency of mtDNA lines (for European mtDNA^{5,10}; for ancestral mtDNA⁵²) it is suggested that European gene flow contains about 80% Paleolithic and 20% Neolithic markers. The analyzed mtDNA data in our study show that the determined portion of Paleolithic mutations of about 75% is in accordance with the portion of Paleolithic genes in today's European mitochondrial gene pool. Beside the identified typical Western-Eurasian haplogroups of mtDNA flow (i.e. H, K, I, W and X), observed as determinants of later population expansions of the Upper Palaeolithic (14 kya)^{5,9}, we also detected the oldest haplogroup present in Europe (U5), which appeared among the first European settlers in the early Upper Palaeolithic (45 kya)^{5,9} as well as the haplogroups U1, U2 and U4, designated as components of the middle Upper Paleolithic (26 kya)^{5,9}. The results of archaeological researches in the municipalities of Tuzla region also indicate traces of the Paleolithic, which date probably to 20–30 kya. The determined Paleolithic mutations are an important indicator of the portion of Paleolithic migrations in the region of northeastern Bosnia. The determined portion of pre-Neolithic and Neolithic mutations in the analyzed population amounts to approximately 14%, which is relatively consistent with the previous statement about the share of Neolithic markers in the European mitochondrial profile. Neolithic mitochondrial gene flow is also supported by many archeological evidences found at the Neolithic settlement in Gornja Tuzla, as well as in Donja Tuzla (Tuzla city today), where a large part of the Neolithic pottery, stone and bone material has been collected²⁷.

Some of the earlier analyses^{16,22}, have suggested that European haplogroups represented in European populations with a small percentage (as I, W and X) have a mutation in the D-loop, which is specific to the Asian and African haplogroups. Given the slightly increased frequency of haplogroup X in this study, which can be labeled as one of more important haplogroup determiners of the genetic structure of populations in Tuzla region, it is assumed that the demographic events in the mtDNA gene flow of the analyzed populations resulted from population expansions not only at the end of the Paleolithic, but also in some earlier demographic processes.

Our data suggest that the Paleolithic markers are dominant characteristic of gene pool of the analyzed Bosnian-Herzegovinian population, meaning that the present mtDNA lines were brought to the area of northeastern Bosnia by various waves of migrations throughout Paleolithic age. However, a somewhat smaller impact of the pre-Neolithic, Neolithic and some later migrations on the genetic structure of investigated population should not be neglected.

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Acknowledgements

We thank all who voluntarily gave samples of their buccal mucosa and to all institutions that participated in their collection. This study was conducted within the scientific project titled »Possible factors of genetic variation in the population of Bosnia and Herzegovina« (Nr. 11-14-21811) supported by the Ministry of Education and Science of Sarajevo Canton, Bosnia and Herzegovina.

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UČESTALOST OSNOVNIH ZAPADNO-EUROAZIJSKIH MTDNA HAPLOGRUPA I PALEOLITSKE I NEOLITSKE LOZE U GENETIČKOJ STRUKTURI POPULACIJE SJEVEROISTOČNE BOSNE

SAŽETAK

Varijacije mitohondrijske DNA (mtDNA) su analizirane u uzorku od 245 osoba bosansko-hercegovačke populacije iz područja sjeveroistočne Bosne (poznat kao tuzlanska regija). Pripadnost haplogrupni je određena koristeći RFLP metodu (polimorfizam dužine restrikcijskih fragmenata), analizirajući haplogrupno-specifične markere kodirajuće regije mtDNA, karakteristične za osnovne zapadno-euroazijske haplogrupe. Izvršena je dodatna analiza sekvencioniranja dva hipervarijabilna segmenta (HVSI I HVSII) kontrolne regije mtDNA za identifikaciju subhaplogrupa haplogrupe U. Studija je otkrila da 95,51% analiziranih osoba pripada tipičnim zapadno-euroazijskim haplogrupama: H, I, J, K, T, U, V, W i X. Najučestalija haplogrupa u analiziranoj populaciji bila je haplogrupa H (52,65%) koja zbog povećane učestalosti predstavlja markirajuću haplogrupu populacije sjeveroistočne Bosne. Rezultati međugrupne genetičke analize su ukazali na veću genetičku sličnost bosansko-hercegovačke populacije sa predhodno studiranim populacijama Hercegovaca (iz dijela Bosne i Hercegovine), Slovenaca i Hrvata u odnosu na susjedne populacije jugoistočne Europe. Naša studija, također, sugerira da su u genetičkoj strukturi populacije tuzlanske regije dominantne mutacije klasificirane kao »paleolitske«. Ove mutacije su vjerojatno unesene na područje sjeveroistočne Bosne migracijama iz prehistorijskog i historijskog perioda, ali se ne mogu zanemariti ni utjecaji pre-neolitskih, neolitskih ili nekih kasnijih migacija, koje su na genetičku strukturu ove populacije imali manji doprinos.