

**DIVERSITY OF NUCLEAR SHORT TANDEM REPEAT LOCI IN
REPRESENTATIVE SAMPLE OF NORTH-EASTERN BOSNIAN
AND HERZEGOVINA POPULATION**

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Diversity of nuclear microsatellite markers were analyzed in a
reference sample of the population of northeast Bosnia and Herzegovina.
437 samples taken from unrelated individuals were processed and three
samples of paternity proof were shown. Detection effectiveness profile of
the research, points to a valid choice of method of extraction, amplification

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and genotyping *short tandem repeat* (STR) loci with PowerPlextm16 kit. Genetic analysis of allelic variants of the 15 STR loci PowerPlextm16 kit detected 17 samples determined as rare allelic variants or microvariants. Samples were divided into 15 different allelic variants at 7 different loci, and are: in locus *D7S820*, *D16S539*, *D3S1358*, *D18S51*, *PENTA D*, *PENTA E* and in locus *vWA*. Genetic analysis of mutations in cases of paternity determined three examples of single-step mutations in the loci *FGA*, *Penta D* and *D3S1358*. Genetic analysis of observed STR loci detected three allelic variant of genotype combination 7/10/11.3 in locus *D7S820* Type II. Population genetic analysis of STR loci in a representative sample of the population of northeast Bosnia and Herzegovina included the application of the assessment tests of within-population genetic diversity and interpopulation diversity, as well as genetic differentiation between populations: North-eastern Bosnia and Herzegovina (BH) and BH general reference, then the Croatian population, Macedonian, Serbian and Slovenian. Based on the result analysis of specific forensic parameters, it can be assumed that the most informative marker is *PENTA E* for population genetic analysis and forensic testing in the population of northeast Bosnia and Herzegovina. Research results fit regional STR database of this part of Europe.

Key words: genetic diversity, microvariants, mutations, North-eastern Bosnia and Herzegovina, STRs, three allelic variant

INTRODUCTION

Variation of inherited material (DNA molecules) represents a genetic basis in overall biodiversity in space and time, observing the living world as a whole and especially every species of living beings. Genetic marker is actually a term used in the description of DNA whose characteristics and position (locus) are clearly determined and on the basis on which living systems individually differ (PRIMORAC *et al*, 2008). Analysis of STR (*Short Tandem Repeat*) genetic markers is considered to be a clear population-genetic indicator analysis of the individual, but also of the population biodiversity.

A true value of these markers lies in its simplicity and the speed of the process and their analysis, as well as the possibility of simultaneous processing of larger number of STR markers in so called "*multiplex STR systems*". This enabled extremely high level of individualization at the DNA analysis of trace. Lately these sequences are, in addition to its wide application in forensic DNA analysis, for example: search for missing persons, in mass casualties, paternity testing, police cases (BUTLER, 2005). Application of STR typification is apparent in establishing DNA database, as well as research population diversity (BUDOWLE *et al*, 201). The first molecular studies of nuclear DNA marker diversity of isolated populations of Bosnia and Herzegovina were shown in studies (EINUM 2004) and the research was directed towards autosomal STR markers in BH (Bosnia and Herzegovina)

population (MARJANOVIĆ *et al.*, 2005) and polymorphisms of Y-chromosome molecular markers (MARJANOVIĆ *et al.*, 2006). Hereafter, in parallel with this, there is a continued research on mitochondrial DNA analysis in human populations of B&H. Bosnia and Herzegovina, from demographic genetic perspective, riddled with high number of more or less isolated local population of indigenous population. It represents a very interesting area for population-genetic research of various levels and orientations.

In this regard, within this complex population of molecular-genetic studies there was genetic analysis of rare allelic variants observed of STR loci represented in the commercial PowerPlex™16 (PP16 Promega) kit and assessment of their impact in forensic genetic analysis; then genetic mutation analysis of allelic variants observed at STR locus and assessment of their impact in forensic genetic analysis; and estimated degree of integration achieved results in the Regional and European population-genetic background, and variation in allelic variants of these markers within that environment.

MATERIALS AND METHODS

Collecting blood samples

Collection of blood samples was based on the principles of forming the reference sample for the study population, observation and assessment of genetic structure of human populations of northeast Bosnia and Herzegovina. During sample collection, standards were respected in regards to informing the respondents about the manner and purpose of determination of their genetic profile. Along with this, a form was prepared which specified the objectives of entrepreneurial research, but which required a signature on the voluntary consent of the respondents. The total sample number adds up to 437 respondents. The samples were collected and analyzed by standard methods of forensic DNA laboratory in the University Clinical Centre Tuzla. Besides voluntary respondents involved in the population study, the examples of paternity testing were included, which were considered specific for the analysis in terms of representation of mutation allelic variants.

The collection was done by taking a drop of blood from the fingertip of respondent, and performing bloodstain pattern analysis. The samples were taken directly on Schleisher&Schuell ISOCOD Card.

DNA analysis

Total genomic DNA was isolated by the procedure for Schleisher&Schuell ISOCOD Card, according to the procedure suggested by the producer (Schleisher&Schuell, Dassell, Germany). After collection, samples were dried in a laminar on a room temperature and afterwards put away in the fridge (+4°C) until further processing.

For amplification of 15 STR loci PowerPlex™16 kit (Promega Corp, Madison, WI, USA) was used and these are: *D3S1358*, *TH01*, *D21S11*, *D18S51*, *Penta E*, *D5S818*, *D13S317*, *D7S820*, *D16S539*, *CSF1PO*, *Penta D*, *vWA*, *D8S1179*, *TPOX*, *FGA*, also *Amelogenin* locus used for gender determination. Amplification

was carried out as described previously (MARJANOVIĆ *et al*, 2006). The total volume of PCR reaction was 25 µl. The PCR amplification was carried out in PE GeneAmp PCR System Thermal Cycler 9700 (Applied Biosystems), according to the manufacturer's recommendations.

Electrophoresis of amplification products was carried out on the ABI PRISM 3100 Genetic Analyzer (Applied Biosystems). All electrophoresis data was collected by software program ABI PRISM® *Collection software version 1.0. or 1.1.* For the analysis and interpretation of the observed STR locus, software Genotyper version 3.7 NT (Applied Biosystems) was used.

Confirmation of allelic variants

During the initial detection of microvariants in this study were subjected to re-extraction of DNA and re-amplification with a PowerPlex®16 kit. It asserts that in order to confirm detection microvariants, the condition of electrophoresis on sequencer had to be optimized. Genotyping STR loci showed relative balance at the RFU height (relative fluorescence units). Range of variation in height ranged from 500-3000 RFU, therefore the appearance of stutter bands was minimal. Also, samples containing mutations or three allelic variants have been confirmed by re-extraction and amplification (PROMEGA CORPORATION, 2001).

Date analysis

Regarding analysis of data, allele frequencies, *Hardy–Weinberg* equilibrium (PROMEGA CORPORATION, 2001), observed *heterozygosity* (H_{obs}) and expected *heterozygosity* (H_{exp}), population differentiation test, and also estimation of *genetic differentiation* (F_{st}) and *pairwise* ($pFst$) were calculated using the software package PowerMarker (LIU, 2005). *Matching probability* (PM), *power of discrimination* (PD), *polymorphism information content* (PIC), *power of exclusion* (PE) and *typical paternity index* (TPI) were calculated by Microsoft®Excel workbook template PowerStats program version 1.2 (TERREBA, 2005). Already mentioned population-genetic and biostatistics analysis were implemented at the Institute for Genetic Engineering and Biotechnology in University of Sarajevo.

RESULTS AND DISCUSSION

“Off ladder” allelic variants/microvariants

In this study, the sample of 437 unrelated individuals, 17 observed samples were identified as “off ladder” alleles as shown in Table 1. They were classified into 15 different allelic variants detected in 7 different STR loci PowerPlex®16 kit: D7S820, D16S539, D3S1358, D18S51, PENTA D, PENTA E and vWA.

In this study, from 17 observed “off ladder” allelic variants, 13 samples showed a nominal frequency 0.0588%. In six observed samples, which were classified in four different “off ladder allelic variants” in locus *D3S1358* (allelic variant 11), *D18S51* (allelic variant 7), *Penta D* (allelic variant 18 and 19) represent a complete repeat unit insertions or deletions that were removed out of the size of

allelic ladder. Allelic variants of the remaining 11 were partial repetitions or *microvariants*.

Table 1. Observed alleles variant

Locus	Alleles Variant	Number of Observations	Frequency
D7S820	7.3	1	0.0588
	11.3	1	0.0588
D16S539	15.2	1	0.0588
D3S1358	11	2	0.1176
D18S51	7	2	0.1176
PENTA D	16.2	1	0.0588
	18	1	0.0588
	19	1	0.0588
	14.1	1	0.0588
	12.1	1	0.0588
	11.2	1	0.0588
PENTA E	16.4	1	0.0588
	17.4	1	0.0588
vWA	12.2	1	0.0588
	15.2	1	0.0588
Total		17	

The most observed off-allelic variant in the study was allelic variant 11 in locus *D3S1358*. It was observed 2 times in related individuals (mother and child) with 0.1176% in this study. Allelic variant 7 in locus *D18S51* also appears 2 times with the same frequency (0.1176%). Interestingly, the two alleles were found in locus *vWA* (12.2 and 15.2), one allele 15.2 in *D16S539* and one allele 16.2 in *Penta D* locus. *Penta D* locus had the highest number of unique allelic variants as much as 6. *Penta D* locus was highly polymorphic and most likely; because of it there were a greater number of present *microvariants* loci *Penta D* in this study.

Some authors (HUEL *et al.*, 2007) in their research detected 31 different “*off ladder allele*”, of which nine off-ladder variants represent a full set of repeat insertions and deletions. The remaining 22 were “off ladder” as a partial repetition or *microvariants*.

Sometimes, there is an increase or decrease in length which takes allelic variants within the size rank of neighboring loci, and such examples are to be published in the literature (HEINRICH *et al.*, 2005, GRUBWIESER *et al.*, 2005) or in the STR database (<http://www.cstl.nist.gov/biotech/strbase/>). It is problematic when allele falls within allelic bin of neighboring loci. However, previously described studies and facts, as well as our results, suggest that the official allelic ladder of commercial PowerPlex™16 kit includes all the official allelic variants. But, the publication of new off *ladder variants/microvariants* in STR database or relative

publication helps the availability of information. This way, it will help the interpretation and correct presentation of results in forensic genetic analysis. Otherwise it can lead to wrong conclusions. In this study of detected allelic *microvariants*, they were subjected to re-extraction of DNA and re-amplification with PowerPlex® 16 kit. To confirm the detection of microvariants, condition of electrophoresis on sequencer was optimized. However, for their final confirmation that would be necessary to do the sequencing

Analysis of single-step mutation

In this study 3 examples of single-step mutations were elaborated or in the specific examples of paternity testing. Genetic analysis of the mutations in the cases of paternity determined three types of single-step mutations in the loci FGA (26→27), Penta D (12→13) and D3S1358 (17→18). In this study, three observed mutations *were father's origin*. It is important to state that this single mutation does not affect the proof of paternity as shown in the examples from this study, in which $TPI=99.99998\%$.

Mutations in microsatellites are mainly caused by replication error which results by base change in repetitive unit or by length change within the repetitive unit (CLAYTON *et al*, 2004).

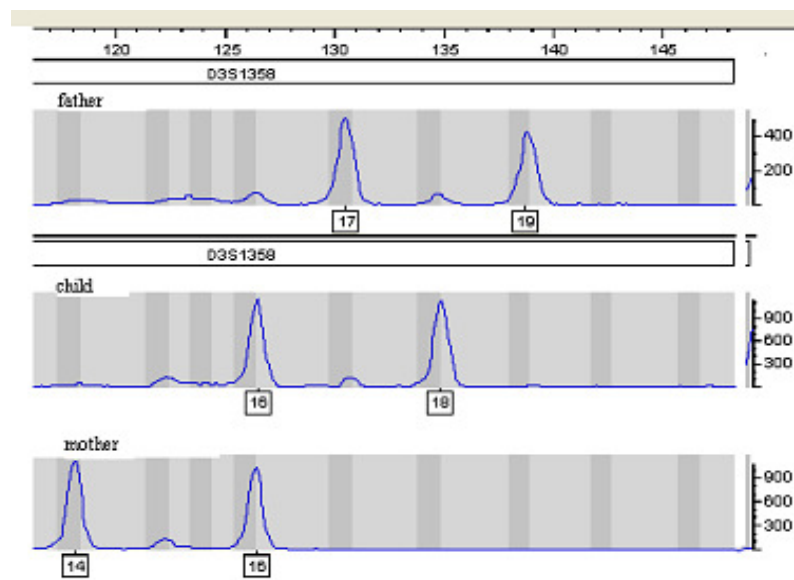


Figure 1. Observed single-step mutation in D3S1358 locus.

In the first diameter, as shown in Figure 1, by genotyping STR loci *D3S1358* allelic profile of the mother was obtained 14/16; father 17/19 and child 16/18. In *D3S1358* locus, the child has inherited the 16 allele from the mother and the 18 allele from the father. Allele 18 is a mutant allele in a child. In other words, the child should have inherited allele 17 or 19 from the father. However, there is an appearance of allelic variation between these two alleles. Most likely, it is a result of changes in allele in adding one total repeating unit to the locus. Allele 17 mutated to allele 18.

In another example, in Figure 2, by genotyping STR locus *FGA* allelic profile of mother was obtained 21/24; father 23/26 and child 24/27. In *FGA* locus, the child has inherited the 24 allele from the mother and allele 27 from the father. The 27 allele is a mutant allele in a child, in other words, the child should have inherited the allele 23 or 26 from the father, but there is an appearance of allelic variant 27. This came as a result of mutation alleles in adding one repetitive unit in locus, meaning allele 26 mutated to allele 27.

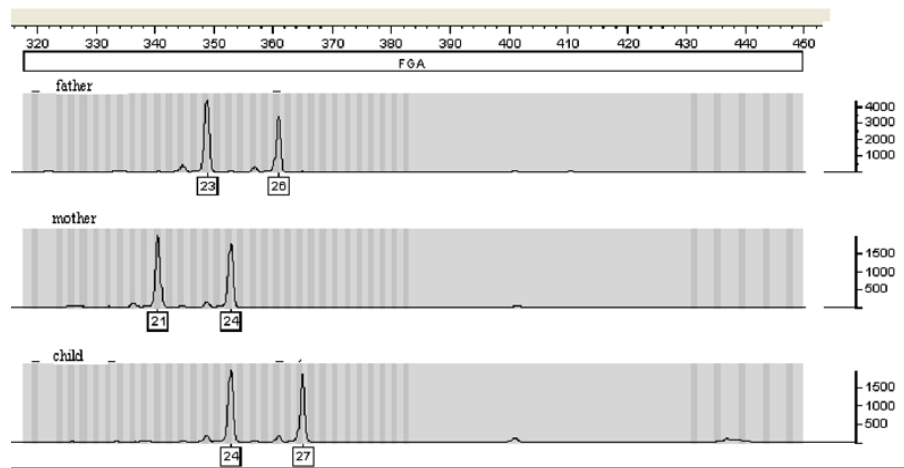


Figure 2. Observed single-step mutation in *FGA* locus.

And third example in Figure 3, by genotyping *STR* locus *Penta D* allelic profile of mother was obtained 10/11; father 12/14 and child 11/13. The child inherited the 11 allele from the mother and the 13 allele from the father. The 13 allele is a mutant allele in a child. The child should have inherited the allele 12 or 14 from the father, but there is an appearance of allelic variant 13 in between these two alleles. This came as a result of mutation, adding one repetitive unit in locus. Allele 12 mutated to allele 13.

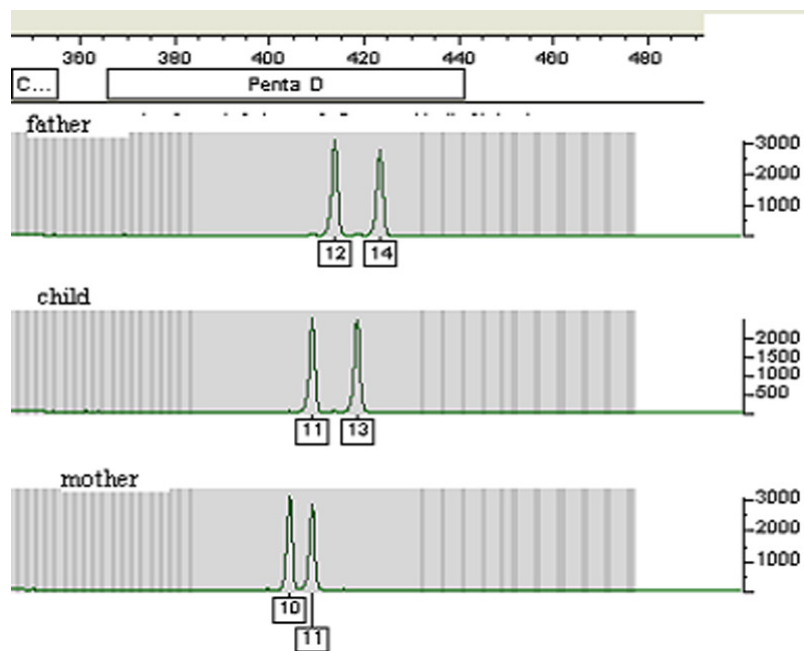


Figure 3. Observed single-step mutation in PENTA D locus.

In situations where there is a direct comparison between the evidence and suspicion, mutation rate is not so important. However, when it comes to relation (relative) comparison in testing the origin and analysis of kinship, and identifying the victims of mass casualties, mutation events play a crucial role (LECLAIR *et al.*, 2004). One classic forensic case of proving the incest, which led to mixing of the traces, describes the importance of detecting mutations that solved this case (MARJANOVIC *et al.*, 2006).

All above mentioned facts lead to the conclusion that mutations that appear in the *STR* loci, are not heavy arguments in proving the identity of the person or question paternity, but on the contrary, in some cases, may be useful.

Three allelic variant at the locus D7S820

Genetic analysis of observed *STR* loci detected three allelic variant genotype combinations 7/10/11.3 at the locus *D7S820* Type II, which suggests chromosome duplication (Figure 4).

In this study of observed locus *D7S820*, there was a detection of three allelic variants of genotype combination 7/10/11.3. The height of allele 7 is 1740 RFU, the height of allele 10 is 1575 RFU and the height of microvariant 11.3 is 1369 RFU. Allele 10 (size 228.25 bp) and allele 11.3 (size 235.00 bp) differ in 6.75 base pairs and are relatively close one to another, unlike allele 7 (size 216.10 bp) which is

significantly further away. Equal intensity and balance of the allelic height suggests that this is about chromosomal duplication Type II. We can only assume that the child inherited a part of chromosomes that contain duplicate region 7/10, while the 11.3 allele was inherited from the other parent. Unfortunately, we could not get confirmation of these results, because we had not samples of their parents.

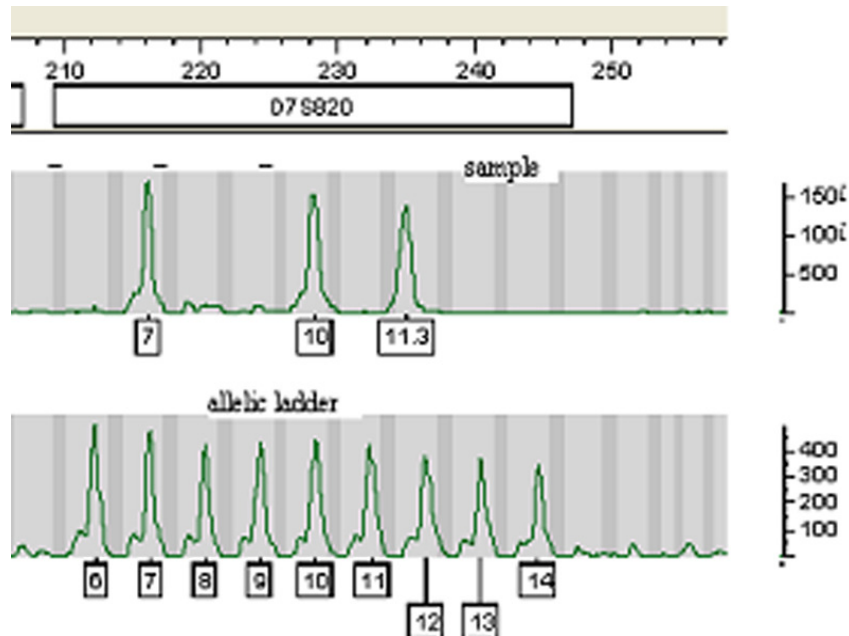


Figure 4. Observed three allele patterns of genotype combination 7/10/11.3 (Type II) in D7S820 loci.

Some authors (CLAYTON *et al.*, 2004) have described the three allelic variants in one locus and classified them into two predominant types. In one form, there appears to be imbalance at the peak height in three allelic variants (unequal intensity peaks) and was described as Type I, and there can also occur an equal intensity at the peak height and was described as Type II.

In several cases (HUEL *et al.*, 2007) the authors have found traces of inherited duplicated alleles responsible for Type II. In the case of duplication, the two alleles that are next to each other transfer to the offspring (CLAYTON *et al.*, 2004).

STR analysis of the PowerPlex™16 kit is extremely precise and accurate in paternity testing, kinship, personal identification, but it also shows the clinical application in detecting diagnosis of possible trisomy.

Genetic characteristics of populations in northeast Bosnia and Herzegovina and its position in relation to European populations from geographically closer regions

Within population analysis of genetic diversity of populations in northeast Bosnia and Herzegovina determined some indication of heterogeneity that represents important characteristics of the assessment of its genetic structure. In order to estimate the population genetic characteristics of northeastern Bosnia and Herzegovina on the basis of nuclear microsatellite markers, an estimation of allele frequencies, variance and standard deviation was carried out. The observed allele frequencies distributions, summarized in Table 2, indicate that each STR loci is moderately to highly polymorphic in a population of northeast Bosnia and Herzegovina. The highest allele frequency among all allelic variants of observed locus has allelic variant 8 in TPOX locus (0.5366).

Analysis of genotypes showed that the highest number of genotypes was recorded in PENTA E locus (92 genotypes), in locus D18S51 (67 genotypes) and the lowest in TPOX locus (18 genotypes). The highest number of alleles was determined in PENTA E locus (17 allelic variants), in locus D18S51 and FGA (16 allelic variants), and the lowest in D3S1358 and THO1 locus (7 each allelic variant). During the analysis of expected (H_{exp}) and observed (H_{obs}) heterozygosity of loci, similar numerical values were noticed, so the average expected value is (0.7930), while the average observed heterozygosity is (0.7951). The highest expected and observed heterozygosity was noted in the locus PENTA E and lowest in TPOX locus. The exact-p value for observed microsatellite loci showed that loci THO1 and TPOX are not within Hardy-Weinberg equilibrium ($p < 0.05$, Table 2).

For each STR locus there is specific forensic index calculation matching the probability (PM), power of discrimination (PD), the power of exclusion (PE), and polymorphism information content (PIC), which are presented in Table 2. Highest values PE (0.757) and PD (0.978) were recorded in a locus Penta E, while TPOX locus had the lowest value PE (0.331) and PD (0.8). *Polymorphism analysis of the contents of polymorphism (PIC)* showed that the highest value was observed in PENTA E and D18S51 locus (0.8595-0.8863), while the lowest value in TPOX locus (0.5712).

Based on heterozygosity values, the polymorphism information content as measures of informativeness, as well as forensic parameters PE and PD, we can consider that the most informative marker is PENTA E, for population genetic analysis and forensic tests in a population of northeast Bosnia and Herzegovina.

Molecular genetics differentiation of populations in northeast Bosnia and Herzegovina shows the characteristics of general reference of Bosnia and Herzegovina population, in other words, it genetically represents a part of it. This was confirmed by the test of interpopulation differentiation based on allele frequencies between the populations of North-eastern Bosnia and Bosnia and Herzegovina reference population⁶, and showed statistically significant difference ($p < 0.05$) just in locus D16S3539.

Table 2. Observed allele frequencies and statistical parameters for forensic testing at the 15 STR loci in North-Eastern Bosnia and Herzegovina population

STR Loci	1	2	3	4	5	6	7	8-->
Allele	D3S1358	THO1	D21S11	D18S51	PENTAE	D5S818	D13S317	D7S820
5	-	0.0011	-	-	0.0606	-	-	-
6	-	0.286	-	-	-	-	-	0.0011
7	-	0.1259	-	-	0.1327	0.0034	0.0023	0.0172
8	-	0.1156	-	-	0.0172	0.0011	0.1316	0.1728
9	-	0.1911	-	0.0023	0.0103	0.0435	0.0847	0.1568
9.3	-	0.2586	-	-	-	-	-	-
10	-	0.0217	-	0.0092	0.1167	0.0755	0.0526	0.2609
11	-	-	-	0.016	0.1098	0.3272	0.3764	0.1968
12	-	-	-	0.1144	0.1693	0.3535	0.2483	0.1545
13	0.0011	-	-	0.0938	0.1339	0.1705	0.0675	0.0332
13.2	-	-	-	0.0011	-	-	-	-
14	0.1087	-	-	0.1705	0.0481	0.0183	0.032	0.0069
15	0.2895	-	-	0.1487	0.0526	0.0069	0.0046	-
16	0.2231	-	-	0.1911	0.0355	-	-	-
17	0.2059	-	-	0.0984	0.0515	-	-	-
18	0.1568	-	-	0.0778	0.0378	-	-	-
19	0.0149	-	-	0.0309	0.0103	-	-	-
20	-	-	-	0.0183	0.0069	-	-	-
21	-	-	-	0.016	0.0046	-	-	-
21.2	-	-	-	-	-	-	-	-
22	-	-	-	0.008	0.0023	-	-	-
22.2	-	-	-	-	-	-	-	-
23	-	-	-	0.0034	-	-	-	-
23.2	-	-	-	-	-	-	-	-
24	-	-	-	-	-	-	-	-
24.2	-	-	-	-	-	-	-	-
25	-	-	0.0011	-	-	-	-	-
26	-	-	-	-	-	-	-	-
27	-	-	0.0172	-	-	-	-	-
28	-	-	0.1808	-	-	-	-	-
28.2	-	-	0.0011	-	-	-	-	-
29	-	-	0.2037	-	-	-	-	-
29.2	-	-	0.0092	-	-	-	-	-
30	-	-	0.222	-	-	-	-	-
30.2	-	-	0.0378	-	-	-	-	-
31	-	-	0.0549	-	-	-	-	-
31.2	-	-	0.0973	-	-	-	-	-
32	-	-	0.0103	-	-	-	-	-
32.2	-	-	0.1156	-	-	-	-	-
33	-	-	0.0023	-	-	-	-	-
33.2	-	-	0.0423	-	-	-	-	-
34.2	-	-	0.0046	-	-	-	-	-
H(obs)	0.7895	0.7437	0.8352	0.8558	0.881	0.762	0.7826	0.8444
H(exp)	0.7874	0.7851	0.847	0.8727	0.8954	0.7309	0.7638	0.8135
Exact	0.5789	0.0236	0.6326	0.2059	0.1901	0.1258	0.8156	0.0967
PIC	0.7542	0.7519	0.8291	0.8595	0.8863	0.6864	0.7323	0.7869
MP	0.079	0.08	0.041	0.031	0.022	0.125	0.088	0.066
PD	0.921	0.92	0.959	0.969	0.978	0.875	0.912	0.934
PE	0.58	0.499	0.666	0.706	0.757	0.531	0.567	0.684

STR Loci	--> 9	10	11	12	13	14	15
Allele	D16S539	CSF1PO	PENTAD	vWA	D8S1179	TPOX	FGA
5	-	-	-	-	-	-	-
6	-	-	-	-	-	0.0023	-
7	-	-	0.0034	-	-	0.0034	-
8	0.0069	0.0011	0.0126	-	0.0126	0.5366	-
9	0.1018	0.0366	0.2426	-	0.016	0.0927	-
9.3	-	-	-	-	-	-	-
10	0.0606	0.3009	0.111	0.0011	0.0675	0.0629	-
11	0.2838	0.2872	0.1831	-	0.0572	0.2735	-
12	0.3249	0.3158	0.1739	-	0.1465	0.0263	-
13	0.1922	0.0458	0.1842	0.008	0.333	0.0023	-
13.2	-	-	-	-	-	-	-
14	0.0286	0.0103	0.0629	0.1133	0.2368	-	-
15	0.0011	0.0023	0.0206	0.1121	0.1087	-	-
16	-	-	0.0023	0.2082	0.0195	-	0.0023
17	-	-	0.0034	0.2757	0.0023	-	-
18	-	-	-	0.2094	-	-	0.0114
19	-	-	-	0.0595	-	-	0.0744
20	-	-	-	0.0103	-	-	0.1133
21	-	-	-	0.0023	-	-	0.1831
21.2	-	-	-	-	-	-	0.0023
22	-	-	-	-	-	-	0.1808
22.2	-	-	-	-	-	-	0.0137
23	-	-	-	-	-	-	0.1625
23.2	-	-	-	-	-	-	0.0057
24	-	-	-	-	-	-	0.1339
24.2	-	-	-	-	-	-	0.0011
25	-	-	-	-	-	-	0.0778
26	-	-	-	-	-	-	0.0263
27	-	-	-	-	-	-	0.0103
28	-	-	-	-	-	-	0.0011
28.2	-	-	-	-	-	-	-
29	-	-	-	-	-	-	-
29.2	-	-	-	-	-	-	-
30	-	-	-	-	-	-	-
30.2	-	-	-	-	-	-	-
31	-	-	-	-	-	-	-
31.2	-	-	-	-	-	-	-
32	-	-	-	-	-	-	-
32.2	-	-	-	-	-	-	-
33	-	-	-	-	-	-	-
33.2	-	-	-	-	-	-	-
34.2	-	-	-	-	-	-	-
H(obs)	0.7551	0.7254	0.8398	0.8307	0.7872	0.6316	0.8627
H(exp)	0.762	0.7237	0.8266	0.8076	0.7912	0.624	0.8639
Exact	0.4979	0.3927	0.575	0.851	0.38	0.0319	0.9912
PIC	0.7245	0.6723	0.8033	0.7806	0.7636	0.5712	0.8488
MP	0.092	0.13	0.055	0.067	0.072	0.2	0.034
PD	0.908	0.87	0.945	0.933	0.928	0.8	0.966
PE	0.519	0.469	0.675	0.657	0.575	0.331	0.72

$H_{(obs)}$ - observed heterozygosity, $H_{(exp)}$ - expected heterozygosity, Exact – p-value of exact test for Hardy-Weinberg equilibrium, PIC- polymorphism information content, MP - matching probability, PD - power of discrimination, PE - power of exclusion

Analysis of genetic differentiation (F_{st}) found extremely small differentiation in observed populations and F_{st} was -0.0003 and proved that the population of northeast Bosnia and Herzegovina belongs to the general B&H population.

Relations between the population of northeast Bosnia and Herzegovina with European populations of the closer region show genetic similarity. This was confirmed by the test of interpopulation differentiation based on the allele frequency between the B&H population (reference population (MARJANOVIĆ *et al.*, 2005) and the population in northeast Bosnia and Herzegovina, Slovenia (DROBNIĆ *et al.*, 2005), Croatian population (PROJIĆ *et al.*, 2007), Macedonian (JAKOVSKI *et al.*, 2006) and Serbian (VESELINOVIĆ *et al.*, 2005) showing a statistically significant difference just in only one locus ($p < 0.05$, Table 3).

Table 3. Test results of interpopulation differentiation between the population of Northeast Bosnia and Herzegovina and B&H reference population with observed European populations

Locus	p
CSF1P0	0.2531
D3S1358	0.1580
D5S818	0.2437
D7S820	0.3471
D8S1179	0.8320
D13S317	0.9674
D16S539	0.3186
D18S51	0.1293
D21S11	0.2631
FGA	0.5014
TH01	0.0448*
TPOX	0.3967
vWA	0.2843

* $p < 0.05$

The estimated F_{st} values across all corresponding loci between those six observed populations indicate slight genetic differentiation, where F_{st} was 0.0002. And also pF_{st} test, as a measure of pairwise population-genetic differentiation shows a very low value. As expected, the population of northeast Bosnia and Herzegovina has the lowest pairwise F_{st} with general reference of Bosnia and Herzegovina population (-0.0002), therefore the highest value is among the population of Slovenia and Macedonia (0.0016, Table 4).

Given the wide application of STR loci in forensic genetics (which is the case with this survey), and because of the high polymorphism loci, we can say that geographic specificity has been partially reduced and subtle differences between the observed populations cannot be detected. On the other hand, nuclear microsatellite

markers (EINUM and SCARPETTA, 2004) have proven to be a good indicator for detecting the degree of isolation of human populations.

Table 4. The value of pairwise f_{st} between pairs of the observed populations

Population	BH	CRO	MAC	SLO	SRB	NE BH
BH	0,0000					
CRO	0,0001	0,0000				
MAC	0,0000	-0,0001	0,0000			
SLO	0,0006	0,0012	0,0016	0,0000		
SRB	-0,0005	0,0000	0,0006	0,0000	0,0000	
NE BH	-0,0002	0,0001	0,0001	0,0007	-0,0001	0,0000

BH – Bosnia and Herzegovina, CRO – Croatia, MAC – Macedonia, SLO - Slovenia, SRB – Serbia, NE BH – North-eastern Bosnia and Herzegovina

CONCLUSION

With regard to research results, we can say that they were expected, and that they fit regional STR database of this part of Europe. Also, it is important to note that the size of the selected sample of 437 unrelated individuals is far greater than almost all so far analyzed for one relatively small population, such as the population of Bosnia and Herzegovina. Of course, that too contributed to a good estimate of its genetic structure and incorporating reference data in Bosnia and Herzegovina STR database.

Based on these studies, which have yielded information on molecular genetic diversity of DNA markers of human population in Bosnia and Herzegovina, we can conclude that in the future, the testing of new DNA markers will continue, detections of mutations within the population of Bosnia and Herzegovina, expansion of a selected sample, and of course incorporating this data into existing regional databases.

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**DIVERZITET NUKLEARNIH KRATKIH TANDEMSKIH
PONAVLJAJUĆIH (STR) LOKUSA U REPREZENTATIVNOM UZORKU
STANOVNIŠTVA SEVEROISTOČNE BOSNE I HERCEGOVINE**

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Bosne i Hercegovine. Obrađeno je 437 uzoraka uzetih od nesrodnih individua, prikazana su tri primjera dokazivanja paterniteta. Uspješnost detekcija profila u istraživanju ukazuje na odgovarajući izbor metode ekstrakcije, amplifikacije i genotipizacije STR lokusa sa PowerPlex[™]16 (PP 16 Promega) na ABI Prism 3100 Genetic Analyser. Genetička analiza alelnih varijanti na 15 STR lokusa PowerPlex[™]16 kita detektirala je 17 uzoraka determiniranih kao rijetke alelne varijante ili *mikrovarijante*. Uzorci su razdijeljeni u 15 različitih alelnih varijanti na 7 različitih lokusa i to: u lokusu *D7S820*, *D16S539*, *D3S1358*, *D18S51*, *PENTA D*, *PENTA E* i u lokusu *vWA*.

Genetička analiza mutacija u slučajevima paterniteta determinirala je tri primjera jednostepenih mutacije u lokusima *FGA*, *Penta D* i *D3S1358*. Genetička analiza observiranih STR lokusa detektovala je i tri alelnu varijantu genotipske kombinacije 7/10/11.3 u lokusu *D7S820* Tipa II. Populacijsko genetička analiza STR lokusa u reprezentativnom uzorku stanovništva sjeveroistočne Bosne i Hercegovine obuhvatila je primjenu testova procjene unutar populacijskog genetičkog diverziteta i interpopulacijskog diverziteta, zatim genetičke različitosti između populacija: sjeveroistočne Bosne i Hercegovine (BH) i BH opšte referentne populacije, te populacija Hrvatske, Makedonije, Srbije i Slovenije.

Na osnovu rezultata analize specifičnih forenzičkih parametara, može se smatrati da je najinformativnijim marker *PENTA E* za populacijsko genetičke analize i forenzička testiranja u populaciji sjeveroistočne Bosne i Hercegovine. Rezultati istraživanja slični su regionalnim «STR bazama podataka» ovog dijela Europe.

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