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GENETIC POLYMORPHISM OF 15 STR LOCI IN THE POPULATION OF TWO BOSNIAN AND HERZEGOVINIAN VILLAGES

Jasmin Mušanović[1]*, Azra Metović[1], Damir Šečić[2], Esad Pepić[2], Svjetlana Loga-Zec[3], Nerman Ramić[4], Senad Šljuka[5] and Ismar Ećo[6]

[1]Department of Biology and Human Genetics, Medical School, University of Sarajevo, Sarajevo, Bosnia and Herzegovina.
[2]Department of Pathophysiology, Medical School, University of Sarajevo, Sarajevo, Bosnia and Herzegovina.
[3]Department of Pharmacology, Medical School, University of Sarajevo, Sarajevo, Bosnia and Herzegovina.
[4]Health House of Vitez, Vitez, Bosnia and Herzegovina.
[5]Department of General Biology, Faculty of Science, University of Sarajevo, Sarajevo, Bosnia and Herzegovina.
[6]Institute for Health Care of workers in Transport-Sarajevo, Sarajevo, Bosnia and Herzegovina.

***Corresponding Author: Jasmin Mušanović**
Department of Biology and Human Genetics, Medical School, University of Sarajevo, Sarajevo, Bosnia and Herzegovina.

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ABSTRACT
In this study, we used 15 STR loci from two Bosnian villages Orahovica and Vukotići located in the central Bosnia and Herzegovina. DNA profiles was perform for 98 individuals from these two villages. Deviations from Hardy Weinberg-equilibrium in similar studies for individual loci were not recorded except for individual loci, which could be related to the size and diversity of the sample itself.

KEYWORDS: STR loci, heterogeneity, Hardy-Weinberg, villages, Orahovica, Vukotići.

INTRODUCTIONS
Bosnia and Herzegovina is located in the heart of Europe and is not a member of the European Union. The population of Bosnia and Herzegovina has about 3.5 million inhabitants of different religious communities. Two villages from wich samplese were taken are Vukotici and Orahovica located in the central part of Bosnia and Herzegovina in the Ze-do canton.[1,2] These are small villages away from the town of Zenica in which the inhabitants have a similar or the same surname, which creates a basis for possible inbreeding. One of the major applications of advanced DNA techniques in forensics is to study. STR markers are wery well known as an effective tool for human identity testing and can be used for forensic identification in forensic investigation, anthropological studies and in studying genetic variation among individuals.[3] Genetic analysis of 15 STR loci (D3S1358, vWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317, D7S820, TH01, TPOX, CSF1PO, and D16S539, Penta D and Penta E present in the Powerplex 16 system (Promega) were analysed.

MATERIALS AND METHODS
The selection of individuals (voluntary donors) whose samples were analyzed was based on the existence of a sibling who was willing to give their samples for analysis. The ethical principles of this research were taken into account, so that the entire research is based on a voluntary basis with the informed consent of all research participants.

60 samples from the village of Orahovica were collected, 46 from male respondents and 14 from female respondents, and 38 samples from the village of Vukotići, 21 from male respondents and 17 from female respondents. The total number of respondents from both villages was 98, and according to the gender representation, 67 were males and 31 were females. All respondents from both villages were of different age structures.

Thirty pairs of relatives from the village of Orahovica and the same number from the village of Vukotići were obtained by pairing relatives of common parents, regardless of the fact that there are a smaller number of respondents.

The control group for both Orahovica and Vukotići was obtained by random pairing of relatives from the same samples, taking into account the elimination of possible kinship, and thus we also got 30 unrelated couples from each village, which we compared with pairs of relatives in a comparative analysis of statistical parameters. A modified Miller[4] protocol was used to extract a total genomic DNA from buccal cells. The DNA profile for every respondent is created by following the same protocol described in our previous study.[5] When it comes to estimation of allelic and genotypic frequencies, allelic frequencies are calculated by the method of *direct counting* according to the formula.

$$\tilde{p}_u = n_u / (2n),$$

where is
n*u* = total number of a particular allelic variant of a given locus in the observed population
n = total number of samples in the observed population,

and genotypic frequencies according to the pattern.

$$\tilde{p}_{uv} = n_{uv} / n,$$

where is
n*uv* = total number of a particular genotype for a given locus in the observed population,
n = total number of samples in the observed population.

By resampling individuals from the data set, a *confidence interval* was formed for allelic and genotypic frequencies.

The deviation of the observed microsatellite loci from the Hardy-Weinberg equilibrium was assessed by the exact p-value test (statistical significance level $p < 0.05$), described by Guo et Thompson (1992).[6] This method is based on the use of the standard Fisher's exact test, namely 2X2 contingency tables extended to a triangular table.

Heterozygosity is one of the indicators of population structure. In the accompanying literature, heterozygosity (Ho) is defined as the proportion of heterozygous individuals in a population.[7] The level of observed heterozygosity per locus was estimated for both villages.

As an indicator of heterogeneity within populations, the measure of estimating the number of genotypes and alleles at each locus as well as for all observed loci (allelic diversity) was used.

In the assessment of the heterogeneity measures of the observed two populations (Orahovica and Vukotići), only non-relatives were taken into account, since if relatives were considered for these analyses, such a sample would be biased.

Genetic diversity is associated with expected heterozygosity (He) and is defined as the probability that two randomly selected alleles from a population are different.

By comparing the levels of observed and expected heterozygosity, it is possible to make a precise change in the structure of populations or taxa, by individual observed loci, as well as in a group.

To determine the influence of genetic drift on genetic differentiation between the observed populations, an analysis of interpopulation Fst (pairwise Fst) was performed.

RESULTS AND DISCUTIONS

The process of collecting traces, extraction, quantification, amplification and detection of STR sequences from the collected samples of buccal mucosa for both villages was completely successful. Profiles were obtained for all 98 collected samples, which can be clearly analyzed for all 16 loci (15 STR loci + amelogenin). The range of peak height variations ranged from 1,200 to 9,000 relative fluorescent RFU units.

The range of peak height variations of the detected profiles for both villages is wider than the range obtained in a previous similar study[8] as well as the suggested range, which is characterized as ideal for buccal mucosal samples, and is 400 to 2000 units, ie RFU[9] However, as in the previous study, these values refer to the AB377 DNA sequencer. According to previous experience in the use of AB310 genetic analyzer, the one in use at the Institute of Genetic Engineering and Biotechnology (because the results may vary within the same model of genetic analyzer), these values move from 1000 to 3000 RFU.

Population heterogeneity analysis

What is a common indicator for Orahovica and Vukotić, as well as for the total population in terms of observed and expected heterozygosity is the fact that the observed heterozygosity is higher than expected indicating possible outbreeding, which was shown by the intrapopulation inbreeding test (Table 1, 2 and 3). This result can be considered expected because only non-relatives are included in the analysis of the genetic structure of the population. A more accurate level of inbreeding can be determined by random sampling which was not the case here, as sampling is designed to compare relatives with non-relatives which has some influence on the results of heterozygosity level analysis as well as deviation from Hardy-Winberg equilibrium.

Table 1: Observed parameters of heterogeneity of the population of the village of Orahovica.

Marker	Maximal allele frequency(MAF)	Number of genotypes	Number of alleles	Expected heterozygosity (He)	Observed heterozygosity(Ho)
D3S1358	0.3250	13.0000	5.0000	0.7685	0.7833
TH01	0.3333	14.0000	6.0000	0.7643	0.7167
D21S11	0.4000	22.0000	9.0000	0.7828	0.8167
D18S51	0.2833	24.0000	9.0000	0.8439	0.8333
PENTAE	0.1917	32.0000	13.0000	0.8861	0.9333
D5S818	0.4083	12.0000	5.0000	0.7268	0.6833
D13S317	0.3417	17.0000	7.0000	0.7451	0.7667
D7S820	0.3250	17.0000	6.0000	0.7811	0.7500
D16S539	0.4583	14.0000	7.0000	0.7132	0.6667
CSF1PO	0.3583	9.0000	5.0000	0.7068	0.7833
PENTAD	0.2583	18.0000	8.0000	0.8060	0.8167
VWA	0.2667	20.0000	6.0000	0.8094	0.8167
D8S1179	0.4250	16.0000	9.0000	0.7197	0.7833
TPOX	0.5000	11.0000	5.0000	0.6532	0.6833
FGA	0.2667	25.0000	8.0000	0.8439	0.8500
Mean value	0.3428	17.6000	7.2000	0.7701	0.7789

Table 2: Observed parameters of heterogeneity of the population of the village Vukotići.

Marker	Maximal allele frequency(MAF)	Number of genotypes	Number of alleles	Expected heterozygosity (He)	Observed heterozygosity (Ho)
D3S1358	0.3167	13.0000	7.0000	0.7758	0.8833
TH01	0.3917	11.0000	6.0000	0.7508	0.8167
D21S11	0.2750	19.0000	10.0000	0.8275	0.8833
D18S51	0.2167	24.0000	10.0000	0.8667	0.9000
PENTAE	0.2000	23.0000	11.0000	0.8832	1.0000
D5S818	0.4417	10.0000	5.0000	0.6836	0.6167
D13S317	0.3750	11.0000	6.0000	0.7733	0.7333
D7S820	0.2667	13.0000	6.0000	0.7949	0.9333
D16S539	0.4167	11.0000	5.0000	0.7128	0.7500
CSF1PO	0.3417	12.0000	6.0000	0.7378	0.6000
PENTAD	0.3167	12.0000	7.0000	0.7682	0.8000
VWA	0.3583	12.0000	6.0000	0.7761	0.9000
D8S1179	0.3583	16.0000	7.0000	0.7761	0.8167
TPOX	0.5750	10.0000	6.0000	0.5953	0.6667
FGA	0.2083	19.0000	9.0000	0.8472	0.6833
Mean value	0.3372	14.4000	7.1333	0.7713	0.7989

Table 3: Observed parameters of total population heterogeneity (all samples).

Marker	Maximal allele frequency(MAF)	Number of genotypes	Number of alleles	Expected heterozygosity (He)	Observed heterozygosity (Ho)
D3S1358	0.3208	15.0000	7.0000	0.7734	0.8333
TH01	0.3625	15.0000	7.0000	0.7659	0.7667
D21S11	0.3375	30.0000	10.0000	0.8135	0.8500
D18S51	0.2042	34.0000	11.0000	0.8687	0.8667
PENTAE	0.1667	49.0000	14.0000	0.8993	0.9667
D5S818	0.4250	15.0000	6.0000	0.7085	0.6500
D13S317	0.3583	20.0000	7.0000	0.7719	0.7500
D7S820	0.2333	18.0000	6.0000	0.8036	0.8417
D16S539	0.4375	18.0000	8.0000	0.7181	0.7083
CSF1PO	0.3042	13.0000	6.0000	0.7308	0.6917
PENTAD	0.2750	22.0000	9.0000	0.8070	0.8083

VWA	0.2958	20.0000	6.0000	0.8031	0.8583
D8S1179	0.3917	22.0000	9.0000	0.7582	0.8000
TPOX	0.5375	13.0000	6.0000	0.6281	0.6750
FGA	0.1708	30.0000	9.0000	0.8653	0.7667
Mean value	0.3214	22.2667	8.0667	0.7810	0.7889

When it comes to the deviation from the Hardy-Weinberg equilibrium (HWE) for individual loci for the villages of Vukotići, Orahovica and for the total population, the results of the analysis for each locus are shown in tables 4, 5 and 6.

Table 4: Deviation from HWE for individual loci for the population of the village of Vukotići.

Marker	p-value
D3S1358	0.1894
TH01	0.0000
D21S11	0.0031
D18S51	0.0030
PENTAE	0.0000
D5S818	0.2646
D13S317	0.0000
D7S820	0.0361
D16S539	0.0001
CSF1PO	0.0021
PENTAD	0.0027
VWA	0.0004
D8S1179	0.0124
TPOX	0.1431
FGA	0.0000

Table 5: Deviation from HWE for individual loci for the population of Orahovica village.

Marker	p-value
D3S1358	0.6242
TH01	0.0373
D21S11	0.0282
D18S51	0.0080
PENTAE	0.1926
D5S818	0.0421
D13S317	0.1546
D7S820	0.5652
D16S539	0.3069
CSF1PO	0.0842
PENTAD	0.0821
VWA	0.0086
D8S1179	0.3786
TPOX	0.1782
FGA	0.0815

Table 6: Deviation from HWE for individual loci for total population.

Marker	p-value
D3S1358	0.1252
TH01	0.0000
D21S11	0.0003
D18S51	0.0000
PENTAE	0.0242
D5S818	0.0305
D13S317	0.0052
D7S820	0.1607
D16S539	0.0573
CSF1PO	0.0003
PENTAD	0.1246
VWA	0.0010
D8S1179	0.5004
TPOX	0.0251
FGA	0.0000

Statistically significant deviations from HWE ($P < 0.05$) were recorded in the Vukotići population for loci TH01, D21S11, D18S51, PENTA E, D13S317, D7S820, D16S539, CSF1PO, PENTA D, VWA, D8S1179, FGA (Table 4). In the case of the village of Orahovica, similar statistically significant deviations from HWE ($P < 0.05$) were recorded for loci TH01, D21S11, D18S51, D5S818 and VWA, and in the total sample for TH01, D21S11, D18S51, PENTA E, D5S818, D13S , CSF1PO, VWA, TPOX and FGA (Table 5) similar to the previous population. Deviations in similar studies for individual loci were not observed, which could be related to the size and diversity of the sample itself. At the $P < 0.05$ level, statistically significant differences in allele frequencies were observed between the Croatian and Serbian populations for D21S11 locus, as well as between the Croatian and Slovenian populations for vWA, D7S820, and D18S51 loci.[10] Namely, studies of a similar nature limit the reliability of the sample size to 100-150, which was not the case in our sample because it had less than 100 respondents.[11-12] It is quite obvious that the number of loci that deviate from the Hardy-Weinberg equilibrium is different in Orahovica and Vukotići. One of the possible reasons is the fact that the number of inhabitants of the village of Vukotići is significantly less than the number of inhabitants of the village of Orahovica. This is not the case with similar studies[13] because the results obtained are probably related to the size of the sample.[14]

CONCLUSION

All of the 15 loci are highly polymorphic among habitants from these two villages and can be used for analysing the genetic polymorphisms of potentially isolated population. To obtain more accurate results, a larger sample should be estimated.

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