DIVISION OF ALLELES IN THE DETERMINATION OF KINSHIP IN SMALL LOCAL POPULATIONS

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ABSTRACT

In this study the efficacy of short tandem repetitive sequences - STR was examined to prove kinship among the descendants of one parent pairs within the small local population by analyzing sharing of alleles per locus at siblings and non-siblings of the two villages: Vukotići and Orahovica and comparing these results with the results of a heterogeneous Bosnian-Herzegovinian population of our previous research. There was a significant difference in this division in the group of siblings and a group of non-siblings as well and the phenomenon of polarization when it comes to the representation of sharing zero and two alleles in the samples for both villages, with significantly overrepresented sharing of zero alleles at non-siblings and significantly overrepresented sharing of two alleles at siblings. By comparing sharing of alleles between sample from the small local population and the sample from mixed population from our earlier research, there is no statistically significant difference in the distribution of 2, 1 and 0 alleles between samples of these two population. This obtained ratio of dividing 2, 1 and 0 alleles in these two samples is about that both samples belongs to the same Bosnian-Herzegovinian population. Number of loci that differ from the Hardy-Weinberg equilibrium is different in the village of Orahovica and in the village of Vukotići. One of the possible reasons is the fact that Vukotići is less than the Orahovica. Using STR system in determining the kinship, or lack of the kinship in a small local populations within the Bosnian-Herzegovinian population, is proved to be a successful method based on the results of this research.

KEYWORDS: short tandem repetitive sequences, siblings, non-siblings, allele sharing.

INTRODUCTION

It is known as a postulate that for informative estimating of kinship the method of sharing two alleles at a higher number of loci can be used.[1, 2] Sometimes it is very difficult to discriminate siblings from non-siblings especially because of sharing possibilities. Siblings can share 0, 1 or 2 alleles at any recognizable locus.[3] According to the literature the expected probability of sharing of alleles at siblings when do not share a single allele would be 0.25 (25%) and the same probability when shared two alleles, and when they share one allele 0.5 (50%). How many alleles will be shared is a matter of chance as well. What is important to emphasize is that neither the division of one nor a zero allele does not have full informative value in determining the full kinship because the same value in assessment of the kinship show half-siblings while some authors in their papers report sharing of one allele in approximately the same proportion (about 50%) and at siblings.[2-7] The literature states that the sharing of 2 alleles on a multiple number of loci may be informative in demonstrating of biological kinship[1, 2] but not as the exclusive method, but with combination with different limit values of CSI (Cumulative or combined sibship index) what can increased the probability of kinship. Also in the case of various forms of disasters in which people suffer, for the preliminary screening of kinship is recommended sharing of 2 alleles at least in one locus.[5] In this research we tried to examine the possibility of using of sharing alleles in determining of kinship in a small local populations, often isolated populations according to their demographic status.

Description of the village Orahovica

Orahovica is a housewarming in Orahovica field in the north of the city of Zenica in the the Federation of Bosnia and Herzegovina. It is located at the mouth of the Orahovica creek into the river Bosna.

Orahovica is composed of the following villages: Rajčevići, Babići, Hasanovići, Gornja Mahala, Kovačevići, Mala Rijeka, Spahići and Imamovići. According to the 1991 census in Orahovica were 2,535 citizens.[8]

Description of the village Vukotići

Vukotići is located 30 kilometers from the town of Zenica in the central Bosnia between the villages Šerići and Jastrepac on a hill facing south-east. This village with 115 housing units has 958 inhabitants. The villagers are mostly farmers and herders. Wealthier population migrated to urban and suburban areas. There are no visible remains of any previous civilization.[9]

MATERIALS AND METHODS

Selection of individuals whose samples were analyzed are based on the existence of a brother or sister who were willing to give their samples for analysis. While sampling the ethical principles of this project is taken into account, so that taking the material was performed only on a voluntary basis by the well-known donors and collected samples and the results are not used for other purposes. Informed consent was obtained on the basis of filling in forms for taking a buccal swab cells. All donors were signed up that voluntarily participate in the study as a donor of its genetic material.

Pairs of analysis are created on the basis of genealogical connections, because it was the people who are known to be determined by the same parental pair.

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

By pairing siblings of common parents is obtained the thirty pairs of relatives from the village of Orahovica, and the same from the village of Vukotići.

The possibility of obtaining the same number of pairs of siblings and from the village Vukotići is enabled because some families included in the study had three children.

The control group and for Orahovica and for Vukotići was obtained by random pairing of unrelated individuals from the same samples. It has been created the 30 unrelated pairs from each village by using that method. Those pairs were included in the comparative analysis of statistics parameters compared with couples of siblings.

Combining the results obtained from both samples it is obtained a new sample of pooled population that has been compared with a sample of mixed population of our previous research.

As a source of DNA from respondents buccal swabs were used. The DNA profile for every respondent is created by following the same protocol described in our previous study.[4]

Method used in this research to determine the biological kinship is the sharing of alleles per locus at siblings and non-siblings, already described in the literature as well as in our previous work.[2-4] The kit used for the PCR amplification was the Power Plex 16.[14]

Probability of kinship is determined by calculating the Likelihood Ratio (LR) parameter for each of the 15 STR loci observed for each of the pairs of siblings and non-sibling. The CSI is calculated for each of the pairs by multiplying the value of LR of all 15 loci.[1,4,5,7,10,11] As a form for calculating LR the form proposed by Brenner has been used.[12]

To prove statistical significance Student's t-test, χ^2-test and ANOVA has been used. Statistical analysis was done in the home made Microsoft Excel sample sheet.

RESULTS AND DISCUSSION

Report, analysis and discussion of the sharing of alleles per locus for the siblings and for the non-siblings from both observed villages - separately

For the village Orahovica at siblings there were 450 separate analysis, ie. 15 loci x 30 pairs = 450 and for the non-siblings there were also 450 observations, ie. 15 loci x 30 pairs = 450.

Distribution of sharing of alleles (2, 1 and 0) for the siblings and for the non-siblings obtained in this study is shown in figure 1. On the basis of the calculated χ^2-test, it was observed statistically significant difference in the distribution of sharing of alleles for the siblings and for the non-siblings from the village Orahovica (p = 1.38532E-22) as well as a statistically significant difference between expected and obtained values (p = 0.005946984) (Figure 2).
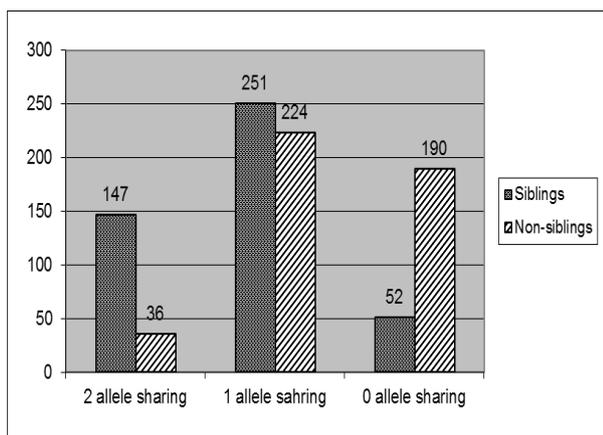
Figure 1. Distribution of sharing of alleles at siblings and non-siblings from the village of Orahovica
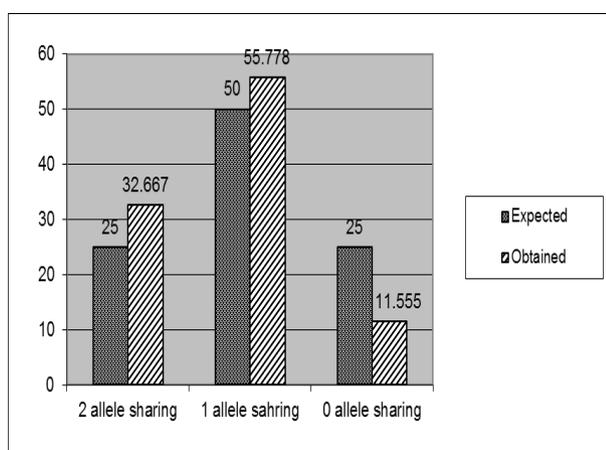
Figure 2: Obtained and expected distribution of allele sharing for the siblings from the village of Orahovica expressed in percentages

For the village of Orahovica it is noted that at the division of 1 allele for the siblings and non-siblings, the difference is very small (55,778% and 49,778%), but also for the siblings in relation to the expected value (55,778% and 50%). This result in the sharing of one allele for the siblings and for the non-siblings with very little difference can be, but not necessarily, a consequence of the homogeneity of the tested sample, as indicated in the literature[7], and some authors report of this way of sharing of one allele among siblings and non-siblings.[6,7] According to the Wenk-in (1995), a division of one allele does not have an informative importance in assessing the kinship.[2]

In contrast, the difference in the representation of the sharing of zero alleles or two alleles is higher among groups of siblings and non-siblings, and this has already been reported in the literature as a phenomenon of polarization.[1,4,7]

In the first case, the sharing of zero alleles, a significantly higher number of those cases was noticed in the group of non-sibling 190 (42.222%) versus 52 (11.555%) for the siblings what is expected, and in another case the sharing of 2 alleles, significantly higher

number of cases was noticed for the siblings 147 (32.667%), versus 36 (8%) for the non-siblings, which was also expected.
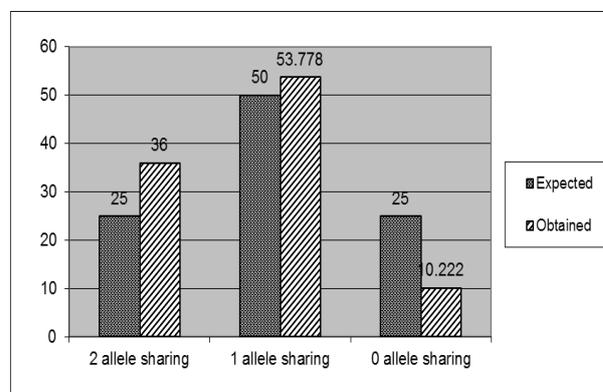
For the sisblings from the village Orahovica the ratio obtained of sharing 2, 1 and 0 alleles was about 33%: 56%: 11% while the other authors report their results 40%: 50%: 10%[6] and 28.5%: 59.2%: 12.3%.[7] It can be said that the achieved results do not significantly differ from the results of similar studies.

For the village Vukotići at siblings there were 450 separate analysis, ie. 15 loci x 30 pairs = 450 and for the non-siblings there were also 450 observations, ie. 15 loci x 30 pairs = 450.

Distribution of sharing of alleles (2, 1 and 0) for siblings and non-siblings obtained in this study for the village Vukotići is shown in figures 3 and 4. On the basis of the calculated $\chi 2$-test, it was observed statistically significant difference in the distribution of sharing of alleles at siblings and non-siblings from the village Vukotići p = 9.97284E-34 as well as a statistically significant difference between expected and obtained values p = 0.000977494.
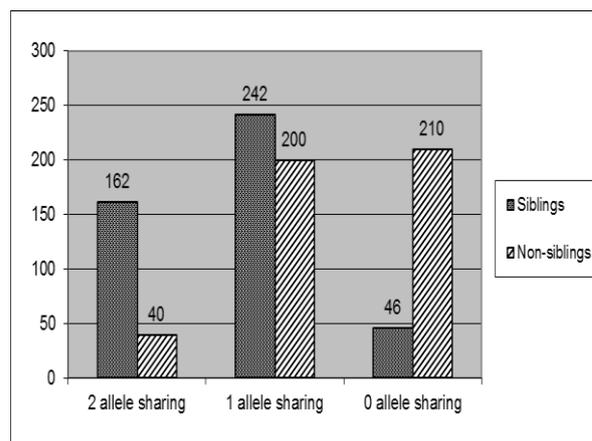
Figure 3. Distribution of sharing of alleles at siblings and non-siblings from the village of Vukotići

Figure 4. Obtained and expected distribution of allele sharing for the siblings from the village of Vukotici expressed in percentages-phenomenon of polarisation

For the village of Vukotići it is also noted that at the division of 1 allele at siblings and non-siblings, the difference is very small (53,778% and 44, 4444%), but also at siblings in relation to the expected value (53,778% and 50%). This result in the sharing of one allele at siblings and non-siblings has been previously described.[2,6,7]

In contrast, the difference in the representation of the sharing of zero alleles or two alleles is higher among groups of siblings and non-siblings, and this is already known as a phenomenon of polarization.[1,7]

In the first case, the sharing of zero alleles, a significantly higher number of those cases was noticed in the group of non-siblings 210 (46.667%) versus 46 (10.222%) at siblings what is expected, and in the second case the sharing of 2 alleles, a significantly higher number of cases was noticed at siblings 162 (36%), versus 40 (8.889%) at non-siblings, which was also expected.

For this village the obtained ratio of sharing 2, 1 and 0 alleles was about 36%: 54%: 10% in relation to the village of Orahovica where the ratio was 33%: 56%: 11% with no statistically significant difference p = 0.756997755 and in accordance with results of similar studies.[1,6]

Comparison and discussion of the sharing of alleles per locus at siblings and non-siblings from a common sample of small local population and the sample of mixed population

For comparison of results from small local population with the results obtained from previous studies[4] from the sample of mixed population the results of the two villages are connected on the basis of the above mentioned criteria and are obtained the following results (Graphs 5 and 6).

There were a total of 900 observations, 60 pairs x15 = 900 in the common sample of small local populations.

By using χ2-test here was observed statistically significant difference in the distribution of sharing alleles at siblings and non-siblings p = 1.18817E-27 and statistically highly significant difference between expected and obtained values p = 0.002598562. The phenomenon of polarization previously described here is very expressed. The approximate ratio of sharing 2, 1 and 0 alleles at siblings is 34%: 55%: 11%, which also doesn't deviates from the results of similar studies.[1,6]

By comparing siblings from small local population described above and mixed populations of earlier research the following results are obtained.

Comparing non-siblings from small local populations and mixed populations of earlier research the following results are obtained.

And in both cases is not detected a statistically significant difference in the distribution of sharing 2, 1 and 0 alleles between the samples of these two populations (p> 0.05) with an approximate relations of sharing of alleles at siblings 34%: 55%: 11% in the sample is small local population and 34%: 54%: 12% in the mixed population sample.

The literature states that such obtained relationship in sharing of alleles at siblings could be the result of heterozygosity of STR system more than the distribution of allelic frequencies in certain populations.[6] To this can be added the fact that the same STR system is being used in all research in this field in Bosnia and Herzegovina.[4]

However, looking at the sharing of alleles per formulas that separate sharing of alleles in homozygote and heterozygote between siblings and non-sibling of both villages and sample of a small local population and mixed population, in the first case was detected„ statistical significance borderline" in the sharing of alleles between non-siblings of both villages (p = 0, 059517555), and a statistically significant difference between groups of non-siblings originally from a small local population and mixed population (p = 0.004465626).

In the first case, it can be noticed higher sharing of 2 alleles in heterozygous and higher sharing of 0 alleles among non-siblings of the village Vukotići, while the sharing of 2 alleles in homozygote is much more expressed at non-siblings in the village of Orahovica.

In the second case, between non-siblings originating from small local and from a mixed population, notes the higher sharing of 2 alleles in homozygote and higher sharing of 0 alleles at non-siblings originally from a small local population.

Looking at the distribution of sharing alleles per locus, it may be noted that few siblings with the lowest CSI value in the sample of small local population has CSI 5.4853 which is a much higher value than the value of the same pair in the sample of mixed population (CSI = 0.0536) and they do not share one allele at even 5 locus which is the highest number of loci in which siblings do not share a single allele.

In this pair of mixed population there were not shared alleles at 3 loci. These couples share 2 alleles at 4 loci in a pair of the local population in the homozygote at 1 locus and in the heterozygous at 3 loci and at the pair of mixed population at 1 locus in the homozygote. This division of 2 alleles may be associated with the value of the CSI in these pairs.[13]

Analyzing of siblings couples with the highest CSI value in both populations at pair with the highest value of CSI 2969454702.4902 of combined population was detected sharing of two alleles at eight loci and it was 6 loci in

heterozygotes and 2 loci in homozygote. At the local population the pair with CSI 534,211,727.2030 share two alleles at even 11 loci which is the highest number of loci with the sharing of 2 alleles in the sample of siblings of this population and with a much smaller CSI value to what can be influenced by the fact that this couple shared 2 alleles in homozygote only in one locus, and in the 10 in heterozygote. The number of loci in which siblings shared two alleles is also higher than the largest found in the literature, with 10 loci.

A pair of non-siblings with the lowest CSI = 0.0000001 in a sample of small local population does not share even a single allele at 12 loci, which is reported in the literature while in the sample of mixed population couple with the lowest CSI = 0.0000005, which is higher CSI than in the previous pair, does not share one allele at 9 loci, which can be linked to the value of CSI.

These pairs are shared two alleles at one locus in the local population in heterozygotes, and not a single locus in a mixed population.[1]

A pair of non-siblings with the highest CSI = 0.5261434 in the local population had a sharing of 2 alleles at two loci, at one in the homozygote, the one in heterozygotes, and ain a mixed population pair with the highest CSI = 62.3941, sharing of two alleles at three loci and on 1 locus in the homozygote, and the 2 in heterozygotes. These couples do not share a single allele at 3 loci in the local population and 2 loci in a mixed population.

At the sample of non-siblings of small local population was observed that as many as 12 pairs of non-siblings have not registered division of two alleles at any locus, as described in the literature[1], and is more than is found in a sample of the mixed population, at 9 non-siblings pairs.[13]

Even in this small sample of the local population have not been found even one pair of siblings in whom there is no sharing of two alleles in any of the observed loci, as well as in the sample of mixed population, which is described in the literature.[1,13]

CONCLUSION

Based on the above, and the results obtained in a previous study[13] and in the information given in the literature[5] there is enough place to be said about the impact of the sharing of two alleles with the increasing significance of the sharing of two alleles in homozygote in order to increase the value of CSI and thereby facilitate the determination of biological kinship and the impact of the sharing of 0 allele what can reduce CSI and reduce the probability of kinship but not as independent indicators of kinship but in combination with various CSI limit values.

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