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Research Article

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Prevalence of D3S1358 and D16S539 Loci in the Population of Lorestan and Comparison of Its Prevalence with the Rest of Other Kurdish Regions

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ABSTRACT

Tiny satellites or simple sequences are of DNA molecular markers and has the repeating units of2-6 pair matches. Maintaining genetic diversity in order to modify crops and to improve the levels of genetic resources management has a great importance in maize breeding programs. Also, they have a great importance in forensic identification, genomic mapping and analysis of genetic linkage. It was estimated that around 400 millions of STR markers are exist in the genome which most of them have polymorphic by different. Various lengths in alleles are caused by the differences in the number of times that the original monomer sequence has been repeated. Since the STRs are easily reproducible by PCR, PCR analyzing methods with the help of STR or STR Typing is very useful technique in the field of genomic DNA profiling. This method is more sensitive than alignment techniques such as RFLP. Because of such benefits, these markers are used as selective markers in forensic identification, particularly in destructed cases. 100 blood samples from unrelated-blood-healthy volunteers that three generations of them have been living in the Kurdish regions of Lorestan, i.e. their race were of Kurds, were collected for the present study. Salting out method was then used in order to extract DNA. Monoplex PCR was carried out for each locus of (D3S1358, D16S539) and finally, detection and typing of alleles have been done by agarose gel electrophoresis 5%. The obtained data were analyzed by statistical software of Genepop. Both locus of (D3S1358, D16S539) have polymorphism and heterozygosity which were more than 0.7; were the most polymorphisms (0.7805/0) and heterozygosity (0.8500) related to the locus of D16S539. The applicable results of these two locus have been approved in population identification survey, respectively. In addition, D16S539 Locus showed a more degree of heterozygosity and polymorphism rather than D3S1358 in the population of Lorestan.

Keywords: STR, PCR, genetic diversity, population of Lorestan, STR Typing

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INTRODUCTION

Short Tandem Repeats or (STR) locus with alleles of short repeated sequences of 2 to 7 are pair matched (Ashuri, 1388). These sequences have been scattered throughout the human genome and show considerable differences among human population. Autosomal STR are used as a useful tool in identifying individuals as well as parent-child relationship in criminal and court investigation cases (Salimi, 1389; Butler, 2006). These sequences are highly polymorphic and can easily be reproduced by PCR method (Salimi, 1389). Off-gene duplication areas are divided into two categories of regular and consecutive iterations or dispersed. Scattered repetitive sequences throughout the genome are in decentralized manner which are often areas that have the ability of Retro-transposition or due to Retro-transposition were seen there. Such scattered iterations are divided into two main categories of SINEs and LINEs; two other categories are added to this category which includes Mega-satellite and macro-satellite.

The size of repeating unit in the last two categories is very large, which can be about several Kilo Base (Butler, 2006; Gill, 2002). Tandem repetitive sequences constitute a large part of human genome. Iterations associated with genes, which are called gene family, can be simple iterations within the genes, or the genes itself or parts of them that are repeated. The name of Satellite is taken from their optical spectrum. Regular tandem repetitions are those

repetitions existing in such areas as blocks which were divided into three categories of satellites, small satellites and micro satellites (Butler, 2006; Gill, 2002).

EXPERIMETAL SECTION

Methodology was involved extracting DNA, determine the quantity and quality of DNA on agarose gel 1.5 percent, setting up and regulating Monoplex PCR for each locus separately, electrophoresis on agarose gel 5% in order to scan and gel analyzing, determining the size of bands and related allele, and finally, calculating the frequency of allelic markers and heterozygosity percent and many other statistical parameters using Genepop software and interpretation of the data.

Sampling Method

The population of study were the people of Lorestan that according to official census in 1390 were about 1,754,243 people. In this study, 100 blood samples of 5 ml were randomly collected from people have been living in Lorestan. The blood samples were put in dried blood tubes containing anticoagulant (EDTA) foundry which were stored in the refrigerator to do the next steps and DNA extraction. All of the samples were from healthy people, equally in number of male and female, that they were not relative and up to three generations before them, have been living in Lorestan. In order to comply with current ethical standards in research and meeting the rights of study participants, a letter of satisfaction was designed, which includes characteristics such as demographic, race, geographic location of living place and an introduction about the research, that was signed by the volunteers. The objectives were fully described for subjects; thus, all donors have attempted to donate blood samples with the consent and adequate information about the study. In this study, DNA extraction kit of Kosar Biotech Company based on salting out method was used to extract DNA from the white blood cells.

Determine the quantity and quality of DNA

After extraction, the accurate measurement of DNA quantity and quality is important. Adding the right amount of DNA to PCR reactions will improve the results in lesser time. Adding too much or insufficient amounts of DNA would lead to form a profile that its interpretation is difficult and impossible. Such issues would be outstanding and important in determining forensic samples' profile, in cases where the biological material storage conditions are unknown, and also in some cases that is difficult to estimate the amount of collected cellular material. The extracted DNA was placed under PCR reactions in order to amplify examining locus.

- Data Analysis

The obtained results of typing 2 locus allele autosomal STR (D3S1358 - D16S539) were as follows:

Table 1: 2 locus allele frequencies among the studied population

Allele \ Locus	D16S539	D3S1358
Allele 1		
Allele 2		
Allele 3	0.0500	
Allele 4	0.1400	
Allele 5		
Allele 6	0.0950	
Allele 7	0.3000	
Allele 8	0.2350	
Allele 9		
Allele 10	0.1150	0.0100
Allele 11		
Allele 12	0.0650	0.0400
Allele 13		
Allele 14		0.2200
Allele 15		
Allele 16		0.2200
Allele 17		
Allele 18		0.3600
Allele 19		0.1400
Allele 20		0.0100

Table 2: general 2 locus allele information

Locus	Sample Size	na*	ne*	I *	****Exact p-value
D16S539	200	7.0000	5.1600	1.7766	0.6047
D3S1358	200	7.0000	4.0355	1.5301	0.0974
Mean	200	7.0000	4.5977	1.6533	
St. Dev		0.0000	0.7951	0.1743	

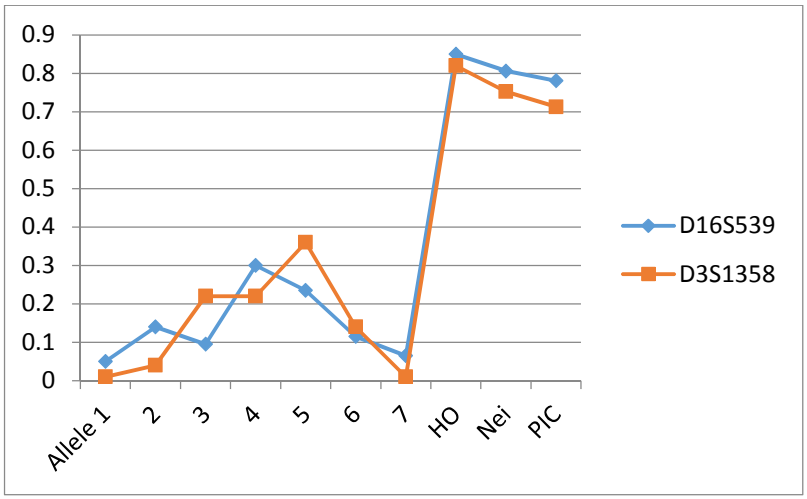
*na = Observed number of alleles
*ne = Effective number of alleles
*I = Shannon's Information index
****Exact p-value for Hardy-weinberg equilibrium

Table 3: heterozygosity and genetic diversity indices and polymorphisms 2 locus STR

Locus	Sample Size	Obs_Hom	Obs_Het	Exp_Hom*	Exp_Het*	Nei**	Ave_Het	PIC***
D16S539	200	0.1500	0.8500	0.1897	0.8103	0.8062	0.8062	0/7805
D3S1358	200	0.1800	0.8200	0.2440	0.7560	0.7522	0.7522	0/7127
Mean	200	0.1650	0.8350	0.2169	0.7831	0.7792	0.7792	
St. Dev		0.0212	0.0212	0.0384	0.0384	0.0382	0.0382	

* Expected homozygosty and heterozygosity were computed using Levene
** Nei's .expected heterozygosity
*** Polymorphism information content

Figure 1: changing level of the parameters for different 2 locus alleles



Details of locus allele D16S539

It is a simple tetra-nucleotide repeat which is located in the long arm of chromosome 16. More than 21 alleles in different populations has been already reported for it which in the population of present study 7 alleles were identified for this locus; they have a size between 129-177 bp, it is illustrated in below gel (Figure 1).

Figure 1: locus D16S539 which is used from Ladder 50 bp, the first column on the left side is the size of the marker alleles.

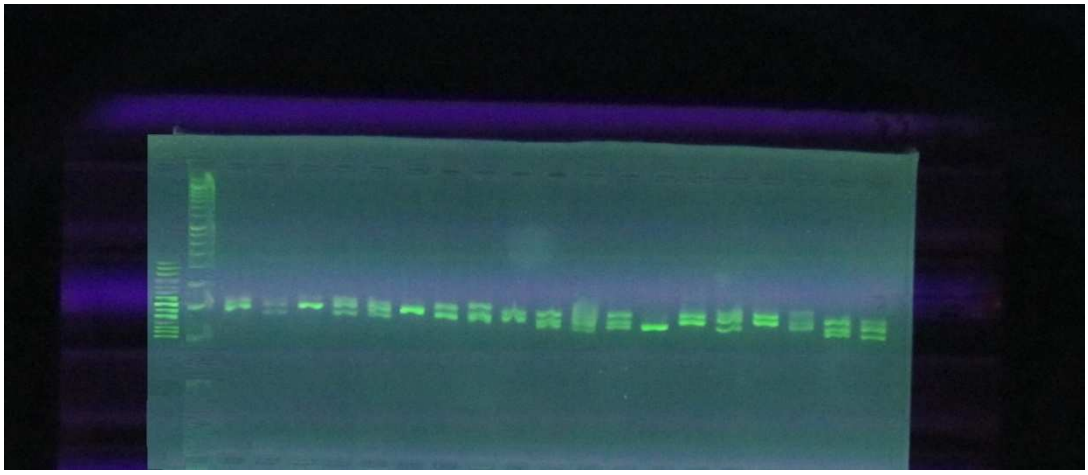
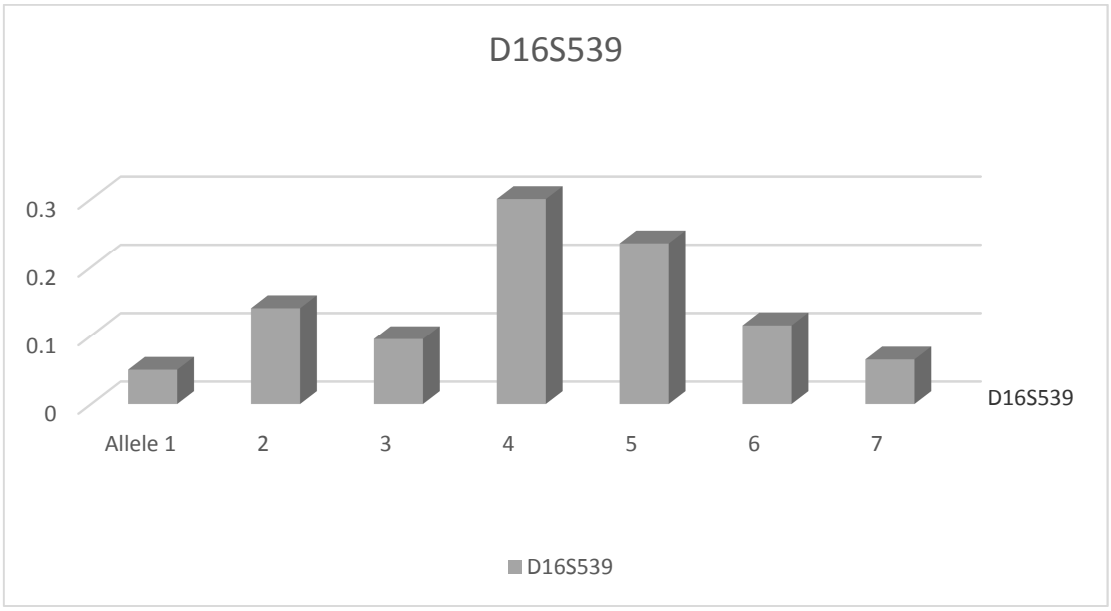


Chart 2: changing frequencies in allele of locus D16S539



Details of allelic loci 3DS 1358

It is a combined tetra-nucleotide repeat which is located in the short arm of chromosome 2. More than 27 alleles in different populations has been already reported for it which in the population of present study 7 alleles were identified for this locus; they have a size between 99-147 bp, it is illustrated in below gel (Figure 2).

Figure 2: locus D3S1358 which is used from Ladder 50 bp, the first column on the left side is the size of the marker alleles.

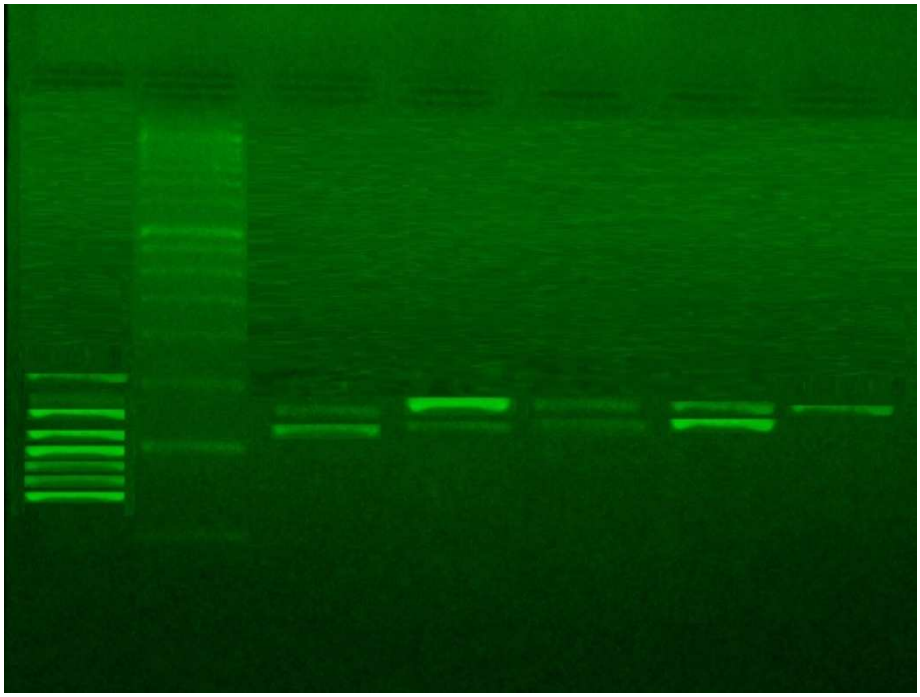
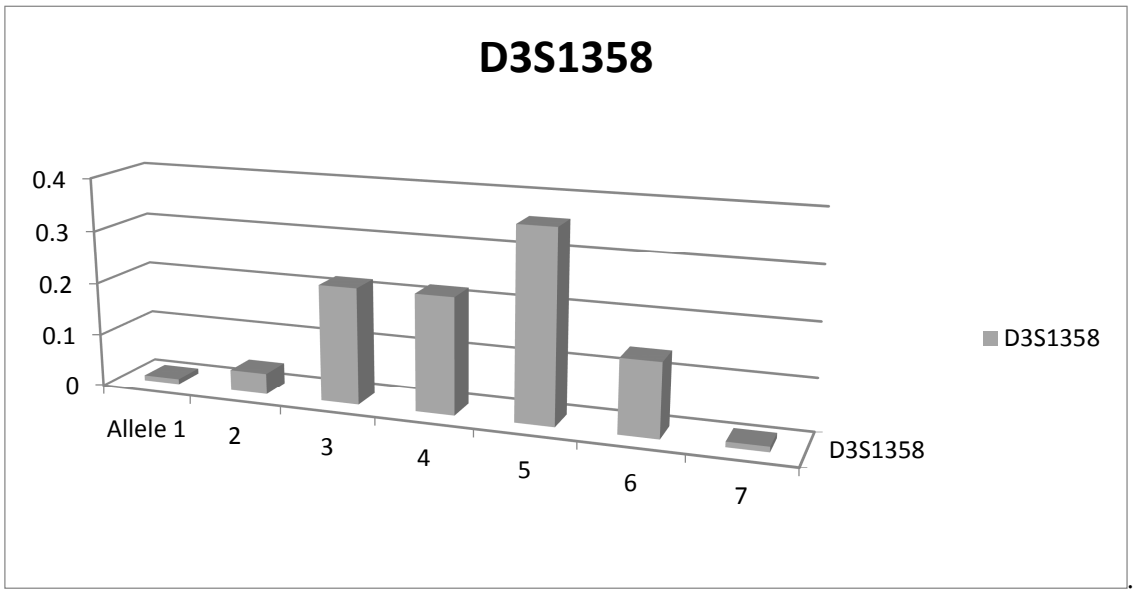


Chart 3: changing frequencies in allele of locus D3S1358



CONCLUSION

In this study, 100 people of Lorestan province were analyzed that were not relative. The allelic frequency, allele diversity and heterozygosity for 2 autosomal STR locus were studied. All locus were in Hardy - Weinberg equilibrium, have had polymorphism and a high variation. Diversity and high polymorphism have proved the applicability of such locus in forensic investigations, identifying and many other issues among this population. 7 different alleles were detected for locus D16S539, which has heterozygosity, diversity and Polymorphism (0.7805 – 0.8062 – 0.8500), diversity and high polymorphism have proved the applicability of such locus in forensic investigations, identifying and many other issues among this population. The most frequent alleles have 11 tetra-nucleotides repeat with the frequency of 0.3000 which in compare with the populations of other regions were as follows: In the population of Azerbaijan, allele with the highest frequency was allele with 11 repeat and allele frequency of 0.259. The data relating to the Philippines, highest allele frequency was allele with 11 repeat with allele frequency of 0.2799 and heterozygosity of 0.7948. Among Palestinians live in Iraq were allele with 11 repeat with allele frequency of 0.292 and heterozygosity of 77.36, respectively. Among Iranian population the highest allele

frequency were related to alleles 11 with the frequency of 31/10 %. In another comparison with Iraqi Kurds allele with the 11 repeat with the frequency of 0.3544 have the highest allele frequencies and heterozygosity of 0.806.

7 different alleles for locus D3S1358 were detected. Which have a diversity and heterozygosity and polymorphism (0.7127 – 0.7522 -0.8200), diversity and high polymorphism have proved the applicability of such locus in forensic investigations, identifying and many other issues among this population.

The most frequent alleles have 17 tetra-nucleotides repeat with the frequency of 0.3600 which in compare with the populations of other regions were as follows: In the population of Azerbaijan, allele with the highest frequency was allele with 15.2 repeat and allele frequency of 0.199. The data relating to the Philippines, highest allele frequency was related to allele 17 with allele frequency of 0.3289 and heterozygosity of 0.7171. Among Palestinians live in Iraq were allele 17 with allele frequency of 0.349 had the highest allele frequency and heterozygosity of 77.36, respectively. Among Iranian population the highest allele frequency were related to alleles 16 with the frequency of 26/30 %. In another comparison with Iraqi Kurds allele with the 17 repeat with the frequency of 0.3641 have the highest allele frequencies and heterozygosity of 0.806.

The most amount of heterozygosity in the study population was for locus D16S539 and D3S1358 locus has the lowest. The most heterozygosity was D16S539 and D3S1358 was the lowest among Philippine population. For Azerbaijan, the highest heterozygosity was D16S539 and D3S1358 was the lowest. For Palestinians live in Iraq, the heterozygosity was equal in both locus. The most heterozygosity was for D16S539 and D3S1358 has the lowest among Iran's population. In the Kurdish population of Iraq, the most heterozygosity was D16S539 and D3S1358 was the lowest. It can relate the proximity and similarity of the results from the comparison of the populations, and the differences could be attributed to geographic proximity, continuity, genetic origin, race and immigration.

5.2 Suggestions

The results of present study have been approved the diversity and polymorphism in 2 locus; it is also showed the applicability of these locus in a population of Lorestan in forensics, identification and immigration cases and so on. In this study, only 2 locus were investigated due to lack of facilities, equipment and automation devices and also have no financial support.

In most forensic, genetics, and identification laboratories around the world, the 15 similar locus STR is used; which would lead shared profiles between populations and laboratories. It is also provide the possibility of comparing the similarity, convergence and divergence between populations and individuals which will be facilitated and reliable, respectively. Therefore, it is suggested that in future investigations, researchers try to analyze 15 common locus in the population of Lorestan in order to provide the ability to compare and make smarter and reliable decisions about genetic relationships between individuals and populations; thus, to be used as a genetic profile for the Kurdish population of Iran.

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