Analysis of allele frequencies of the selected 15 autosomal STR markers in Tikrit population – Iraq with comparison to Middle Eastern, African, and Europeans

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Abstract. Short tandem repeats (STRs) have been recommended as the highest polymorphic loci among the humana DNA regions. Therefore, STRs are agreeable to many genetic fields like forensic, population genetics and anthropological studies. The main aim of this research is to evaluate the autosomal STRs in Tikrit city-Iraq, to expand the human genetics database and forensic genetics analysis. The DNA database was obtained from 306 unrelated volunteers from native Tikrit population-Iraq, using 15 autosomal STR loci. The current study determined the allele frequencies in the Tikrit population and then compared them with other national Iraqi populations as well as with populations in the Middle East, Africa, and Europe. The highest level of heterozygosity was observed in D8S1179 and TH01 loci (0.797), while the less level was shown by CSF1PO (0.48). The departure from HWE Equilibrium was recorded in only 3 STR loci from a total of 15 loci analyzed ($p < 0.003$). The Combined Match Probability (CMP) for 15 autosomal STR was 1 in 7.89208×10^{-19} and the Combined Discrimination Power (CDP) was 0.9999999997. The discrimination power (DP) was especially high in D2S1338, D18S51, D19S433 and D21S11. Based on the results observed in a Dendrogram, Tikrit population was clustered with other populations, likely reflecting the historical and geographical factors. D2S1338, D18S51, D19S433 and D21S11 markers were recognized as suitable for forensic genetics analysis in Tikrit population. Also, the 15 STRs markers provide information for the studies of genetic distances between the current study and other included populations to be compared with this study.

Keywords: Allele frequencies, Forensic, Iraq, STR, Tikrit

INTRODUCTION

Ancient Mesopotamia (Iraq) is the area between the Tigris and the Euphrates rivers. Mesopotamia is indeed the home of the first appearing civilizations, including those of Sumer, Akkad, Babylon, and Assyria (Al-Quraishi and Negm, 2019). Tikrit is one of the oldest small cities in Iraq, located as a fort on the edge of the Tigris. Historically, the first mentioning of Tikrit is in 615

BC. During the Fall of Assyria Chronicle, it was a shield for Nabopolassar (the Babylonian king) at the time of his campaign on the Assur city (Bradford, 2001). Today, Tikrit city is the center of Salah al-Din Governorate, north-central Iraq. It stands on the west bank of the Tigris River approximately 100 miles northwest of Baghdad as shown in Figure 1. Notably, Tikrit's population is

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now mainly Arabs. It has three main groups: Al-Tikriti (containing some subpopulation), Alhadithi and Al-Bu Nasir. In Tikrit's population, the social organization, historical and geographical factors are expected to have a profound impact on the patterns of the population's genetic structure.

An individual's genetic profile is made up of several markers that are inherited from their parents (Delgado and Neyra, 2019). Studies in human molecular anthropology are interested with a large range of molecular markers (DNA markers), each marker having a different advantage but complementary to other markers (Garrigan and Hammer, 2006). Short tandem repeats (STRs) loci, or so-called microsatellites loci, occur as tandem repetitive sequences ranging of length from one to six bp and representing around ~0.03% of the whole nuclear DNA (Gymrek, 2017). Short tandem repeat (STR) loci are the most informative genetic markers used worldwide for human identification (HID) purposes (Quiroz-Mercado *et al.*, 2017). STRs are robust molecular markers that are used due to their high rate of hypervariability and vast distribution across the nuclear DNA (Yoo *et al.*, 2011). Likewise, data analyses of STR loci have become important tools in common evolutionary researches (Bentayebi *et al.*, 2014; and Reinar *et al.*,

2018). Because of the high mutation rate, autosomal STRs loci have the possibility to yield genetic evidence around recent evolutionary events in the history of human population, which make them the ideal option in population genetics studies and the forensic field (Chbel *et al.*, 2003; El Amri *et al.*, 2011; Pilav *et al.*, 2020). The allele frequency of STR-loci varies within/between populations and geographic region (Silva *et al.*, 2012; Buadu, 2018). Therefore, several prior studies have investigated allele frequencies in the national Iraqi populations by using STR markers, such as; allele Frequency in Al Anbar population among 132 unrelated healthy individuals (Al-Janabi *et al.*, 2016), 302 unrelated individuals from the Kurdistan Population region (Murad and Amin, 2017), 120 samples of unrelated individuals from Wasit population, (Al-Rubai *et al.*, 2015), and 100 individuals from Mesan and Basrah population (Hameed *et al.*, 2015). This study is the first genetics characterization of Tikrit population, using 15 autosomal STRs in AmpFLSTR Identifier Plus kit from the Applied Biosystems company (Foster City, USA). The current study consists of unrelated individuals from Tikrit / Iraq population who were selected to characterize the allele frequencies, forensic descriptive parameters and anthropological studies.

Figure 1. Geographic location of Tikrit city / Iraq.

MATERIALS AND METHODS

Samples collection

In the current study, three hundred and six blood samples from healthy unrelated persons were spotted on FTA Card (GE Healthcare, UK). All samples originate from the native Tikrit population (Al-Tikriti, Alhadithi, and Al-Bu Nasir human tribes). All were volunteers who were chosen to achieve a maximum representation of native tribal groups who have lived in Tikrit city for at least two generations. Also, general information about the participants, their parents and grandparents are self-reported. The age of the individuals involved ranged from 18 to 60 years old (170 males and 136 females).

DNA extraction

Based on the manufacturer's instructions, a 1.2 mm punch from FTA card contains around 5-20 ng of genomic DNA (Alaeddini, 2012). DNA is isolated using the Chelex 100 resin methods with some modification (GE Healthcare, 2010). Then, DNA concentration and quality were measured

by NanoDrop-1000 spectrophotometry (Thermo Scientific) based on the manufacturer's protocol.

DNA amplification and data analysis

From each sample, 1ng/μl of genomic extracted DNA was amplified using 15 + 1 STR markers (D8S1179, D21S11, D7S820, CSF1PO, D3S1358, THO1, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818 and FGA) as well as amelogenin (gender-determining locus) by using the Human Identification Kit, AmpFLSTR Identifier Plus Kit from Applied Biosystems (Foster City, USA) and the GeneAmp PCR system 9700 from Applied Biosystems (Foster City, CA, USA) according to standards recommended by the manufacturer. The PCR program includes three stages: Stage one: 95°C/11 min., Stage two: 94°C/20 sec, 59°C/3 min., and Stage three: 60°C/10 min. final hold: 4°C up to 24 hours as shown in Table 1. An Electrophoresis for PCR product was performed on the ABI 3500xl Genetic Analyzer with 8-capillary from Thermo Fisher Scientific Company (Carlsbad, USA). The GeneMapper1 ID software (version 3.1) was used for the allele's identification and data analysis.

Table 1. PCR program.

Initial incubation step	Cycle (28 cycles)		Final extension	Final hold
HOLD	Denature	Anneal / Extend	HOLD	HOLD
	Cycle			
95°C	94°C	59°C	60°C	4°C
11 min	20 sec	3 min	10 min	Up to 24 hours

Table 2. Comparative populations with Tikrit population

Region	Population	Population size
Iraq	Mesan and Basrah (Hameed et al., 2015)	100
	Wasit (Al-Rubai et al., 2015)	120
	Kurdistan (Murad and Amin, 2017)	302
	Al-Anbar (Al-Janabi et al., 2016)	132
Middle East	Riyadh / KSA (Osman et al., 2015)	190
	Jordan (Al-Eitan and Tubaishat, 2018)	500
Africa	Moroccan (Bentayebi et al., 2014)	320
	Sudan (Babiker et al., 2011)	498
Europe	Swedish population (Montelius et al., 2008)	416
Asia	Eastern Turkey (Tokdemir et al., 2016)	802

Comparative populations

Tikrit population data was used for comparison with the four national Iraqi populations. Two population were from the Arab Middle East, two populations from Arab Africa, one population from Europe and one population from Asia. See Table 2 for more details.

Statistical analysis

The calculation of Allele frequency, test Hardy-Weinberg Equilibrium (HWE) and p-value were performed with Genepop 4.7.5 software, using the Bonferroni corrected test (Glaubitz, 2004; Rousset, 2016). Linkage Disequilibrium (LD) observed and expected heterozygosity, and F-statistics were evaluated using the Arlequin software v3.01 (Rousset, 2016). Forensic parameters including Polymorphic Information Contents (PIC), the Power of Discrimination (PD), Random Matching Probability (RMP) and the Probability of Exclusion (PE) calculated all these parameters manually by using Microsoft Office Excel (Version 2010) and GenAlix (v.6.5) (Excoffier *et al.*, 2009). Additionally, Dendrogram analysis was performed by using XLSTAT (Version 2020.1).

RESULTS AND DISCUSSION

The 306 samples from the Tikrit population-Iraq were subjected to 15 autosomal STR analysis. Figure 2 represents an example of DNA profiling for one sample (male) with STRs markers. Data of allele frequencies are listed in Table 3. The Observed and Expected heterozygosity, P-value, standard deviations and inbreeding coefficient for the STR loci are listed in Table 4. The total number of recorded STRs alleles was 153 and the allele frequency in a population ranged from 0.002 - 0.525. The highest allele number observed at D2S1338 and D19S433 loci was ($n = 15$), while the TPOX locus recorded the lowest number of alleles ($n = 6$). Allele 8 in the TPOX locus appear as the most polymorphic alleles. Basically, increasing size of sample translates to increase the accuracy of the allele frequency estimates in population (Hale *et al.*, 2012). The highest heterozygosity is 0.797 for both D8S1179 and TH01 loci, while the lowest level was identified in

CSF1PO (0.48). Among the autosomal STR makers, F-statistical analyses detected a FIS value ranging from D5S818 (-0.048445) to CSF1PO (0.327640). If the inbreeding coefficient is zero (i.e., random mating), the genotype frequencies are those expected for Hardy-Weinberg equilibrium. On the other hand, if there is a complete inbreeding ($F = 1$), the frequency of heterozygotes is zero, while the negative value of FIS means more heterozygotes than expected under HWE (Pyreritz, 2018). No linkage disequilibrium (LD) was detected among 15 STRs loci in population samples. Only 3 out of 15 STR loci showed significant deviation from expectations of HWE (Bonferroni corrected threshold $p < 0.003$). Human populations do not exactly meet all the conditions of HWE (Padmanabhan, 2014). This deviation was imputed to the popular of consanguineous marriage, the marriage between relatives among Tikrit population increasing homozygosity. In findings of previous studies, some loci did not conform to HWE such as; Al-Anbar study which observed 4 out of 15 STR loci (Al-janabi *et al.*, 2016), Wasit study with 3 of 15 observed and 4 of 15 STR loci (Al-Rubai *et al.*, 2015), and a Saudi study which recorded 3 out of 15 STR loci (Osman and Alsafar, 2015) which is consistent with our data. Population Genetic structure can be affected broadly by cultural histories and genetic evolution which can change the frequencies of different variants at given genetic markers (Adnan *et al.*, 2020).

Markers that are used for human identification may have weaker discrimination power in populations with genetic structure than in unstructured populations, due to the impact that the presence of subpopulation groups has on the random match probability (Haidar *et al.*, 2021). The Polymorphic Information Content (PIC) was greater than 0.6 through all autosomal STR loci. Therefore, these results indicated that STR markers are highly polymorphic DNA markers and would be suitable as valuable markers for discriminating descent of persons in Tikrit population. Any markers of loci containing larger numbers of alleles are expected to have higher PIC values and thus are more instructive (Hildebrand *et al.*, 1994). The analyses of the results are set out in Table 5. It can be seen that the power of discrimination (PD) index for

marker D2S1338 is 0.977 is the highest, followed by D18S51 at 0.970, D19S433 at 0.952 and D21S11 at 0.950. In contrast, the marker with the lowest PD index is D2S1338 at 0.827. This indicates that a DNA-based database for Tikrit population can be safely used by using STRs loci. Within each STR marker, the discrimination power of an allele depends on its frequency in a certain human population (El Andari *et al.*, 2018). The higher PD of a genetic locus led to the increased efficiency in power discrimination between individuals of the human population (Al-Snan *et al.*, 2019). The Combined Discrimination Power (CDP) of the examined 15 loci was 0.999999997 which should be sufficient enough

for the particular individual identification even if the census size of the population is extremely large. The highest and lowest Matching probability values were measured for the D3S1358 (0.166) and D2S1338 (0.023) loci, respectively. In the current study, the combined match probability was 7.89208×10^{-19} . This means that the probability of finding two persons with an identical DNA profile in Tikrit population is impossible. The observed values of the power of exclusion (PE) ranged from 0.171 at CSF1PO to 0.668 at D2S1338. The combined exclusion probability (CEP) was 0.92092 for all autosomal STR loci used.

Figure 2. Electropherogram showing results of STR typing.

Table 3(a). Allele frequency results for 8 of 15 autosomal STR markers.

[illegible]

Table 3(b). Allele frequency results for 7 of 15 autosomal STR markers.

[illegible]

Table 4. Observed and Expected heterozygosity, P-value, standard deviations and inbreeding coefficient.

Fis or f	s.d.	P-value	HE	HO	Locus
D8S1179	0.797	0.821	0.92746	0.00022	0.030362
D21S11	0.739	0.835	0.00194	0.00004	0.122633
D7S820	0.758	0.782	0.00718	0.00009	0.030745
CSF1PO	0.48	0.714	0.00000	0.00000	0.327640
D3S1358	0.742	0.752	0.00576	0.00006	0.012593
TH01	0.797	0.787	0.35013	0.00052	-0.016360
D13S317	0.706	0.774	0.00713	0.00008	0.089464
D16S539	0.742	0.8	0.05642	0.00016	0.071792
D2S1338	0.836	0.891	0.50872	0.00040	0.061160
D19S433	0.784	0.841	0.00069	0.00003	0.071621
VwA	0.748	0.773	0.13287	0.00027	0.032658
TPOX	0.644	0.646	0.69231	0.00034	0.007180
D18S51	0.833	0.875	0.82387	0.00023	0.048181
D5S818	0.81	0.772	0.02232	0.00012	-0.048445
FGA	0.791	0.846	0.00441	0.00007	0.063759

HO = Observed Heterozygosity; HE = Expected Heterozygosity; P-value = the p values of the exact test for Hardy-Weinberg equilibrium; s.d. = standard deviations; Fis= F-Statistics test at $P < 0.05$

Table 5. Summary of forensic statistics parameters.

Locus	MP	PD	PIC	PE
D8S1179	0.056	0.944	0.80	0.594
D21S11	0.05	0.950	0.81	0.490
D7S820	0.083	0.917	0.75	0.524
CSF1PO	0.136	0.864	0.66	0.171
D3S1358	0.166	0.844	0.71	0.496
TH01	0.08	0.920	0.76	0.594
D13S317	0.087	0.913	0.74	0.437
D16S539	0.069	0.931	0.77	0.496
D2S1338	0.023	0.977	0.88	0.668
D19S433	0.048	0.952	0.82	0.570
VwA	0.089	0.911	0.74	0.507
TPOX	0.023	0.827	0.6	0.347
D18S51	0.030	0.970	0.86	0.662
D5S818	0.105	0.895	0.74	0.619
FGA	0.044	0.956	0.83	0.582

PIC = Polymorphism information content; PD = Power of Discrimination; MP = Matching probability; PE = Power of Exclusion

To estimate a differentiation between Tikrit populations and other populations that include national, Middle east, Afro-Arab, Asia and Europe populations, a Dendrogram was conducted based on the data of allele frequencies obtained from the 15 autosomal STRs. Thus, a Dendrogram was used in our study to exemplify the genetic distances between Tikrit populations and four national populations as in Figure 3, while Figure 4 showed genetics distance between Tikrit

population- Iraq and other population listed in Table 2.

As shown in Figure 3, Tikrit, the Tikrit population shared the most genetic relatedness with the Al-Anbar, Kurdistan, and Wasit populations. On the other, Mesan and Basrah population showed relatedness with each other and in distant genetic structure from Tikrit population. The Tikrit population occupy an intermediate location between Al-Anbar and Kurdistan in one hand and Wasit population in

other hand. Also, Figure 4 compares the genetic distance between Tikrit population/Iraq and some other international populations. Thus, Tikrit and Jordan populations share the most genetic relatedness among the other populations, followed by Sudan Morocco, Eastern Turkey and Riyadh KSA in the same cluster. On the other hand, Swedish population showed a distant genetic structure from Tikrit population.

At present, comprising investigation of human genetics population can illustrate the migration, gene flow, mutation and introduction history. However, due to the complicated effects of geography and history factors, the genetic distance varies among human populations. Also, results from previous researches of large STRs marker panels showed wide geographic clustering, which leads to a discussion about whether a human fine-scale genetic variation between human population is better characterized by separate clusters or continuous clines (Rosenberg

et al., 2005; Perez *et al.*, 2018). Besides, studies involving molecular archaeology can give extra information which is useful to interpret important global phenomena such as early migration, arising civilizations and human biology (Ariffin *et al.*, 2007; Hunter *et al.*, 2007).

Figure 3. Dendrogram represent the level of similarity between Tikrit population and other Iraqi national populations.

Figure 4. Dendrogram represent the level of similarity between Tikrit population- Iraqi and some international populations; including Jordan, Riyadh KSA, Eastern Turkey, Moroccan, Sudan, and Swedish.

CONCLUSION

D2S1338, D18S51, D19S433 and D21S11 markers showed to be convenient for forensic genetics analysis in Tikrit population. Also, the data of STRs markers produced information used in the evaluation of genetic distances between genetics data of Tikrit population and some chosen population groups (Iraqi national and foreign populations). These data could be useful to enrich Iraqi STRs database and provide reference data for future studies in the population genetics field.

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