VARIATION OF Y-CHROMOSOMAL STRS IN YEZIDI AND CHALDEAN POPULATIONS IN IRAQI KURDISTAN

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ABSTRACT

Background: Many ethnic groups live in the northern part of Iraq which represents the Iraqi part of Kurdistan. Short tandem repeats are widely used in population genetics and forensic science. **Objective:** This research aims to analyze the Y-chromosomal STR markers of two ethnic groups living in Iraqi Kurdistan, Yezidi, and Chaldean groups. **Methodology:** Samples of peripheral blood from a total of 44 unrelated males (22 for each ethnic group) were taken. DNA was extracted by using a DNA Extraction kit and analyzed for eight Y-chromosomal short tandem repeats (Y-GATA-H4, Y-GATA-C4, DYS458, DYS456, DYS448, DYS437, DYS392, and DYS19). Then, the PCR products were run on 10% polyacrylamide gel and stained by silver nitrate. The results were analyzed by Power marker V3.25 and the dendrogram was created by Mega X software. **Results:** The highest diversity was observed at Y-GATA-C4 (GD: 0.81) while the lowest diversity was observed at DYS456 (GD: 0.64) in the Yezidi group. In the Chaldean group, DYS458 (GD: 0.88) was the most diverse, while the least diverse marker was Y-GATA-H4 (GD:0.66). The marker Y-GATA-C4 was found to be the most informative marker in both groups with a PIC value of 0.8605. **Conclusions:** The study confirmed the high discrimination ability of the Y-chromosomal STRs analysis and provided a dataset on these two ethnic groups of Iraqi Kurdistan. The dendrogram of Yezidi and Chaldean datasets reveals that the Yezidi individuals are more closely related to each other as compared to the Chaldean group because intermarriage among Yezidi people is more than that among the Chaldean individuals.

Keywords: Forensic science, Iraqi Kurdistan, Y-chromosome, ethnic group, dendrogram.

INTRODUCTION

Kurdistan is a geographic region located in Western Asia and it includes parts of Iran, Turkey, Syria, and Iraq **(Dahlman, 2002; Sadeghi, 2016).** The adjacent Kurdish areas of Iran, Iraq, Turkey, and Syria are located in the Mid East's northern center region **(O'Leary, 2002).** Many ethnic groups have immigrated to, established in, or lived there naturally over centuries. A significant amount of archaeological evidence suggests that this region is the site of the Neolithic transition **(Gkiasta *et al.*, 2003; Dogan *et al.*, 2017).** Yezidis are a minority group that speaks Kurmanji and are indigenous to Kurdistan **(Omarkhali, 2017)**. The majority of Nineveh and Duhok are the two most populous governorates in Iraq where Yazidis still live in the Middle East **(Dulz, 2016)**. Among Yazidi scholars and in Yazidi circles, there is disagreement about whether Yezidi people are a specific ethnoreligious group or a sub-group of the Kurds but have different religion **(Rodziewicz, 2018)**. The religion of the Yezidi people is known as Yazidism which is monotheistic, and it has roots in the pre-Zoroastrian religion of Iran **(Foltz, 2017)**. The Chaldean ethnic group is an Aramaic-speaking, Eastern Rite Catholic. In Mesopotamia, the cradle of civilization, they have a history dating back more than 5,500 years. A separate Church heads the Chaldean Catholic Church, under the auspices of a Patriarch (the Patriarch of Babylon for the Chaldeans), **(Hanoosh, 2008; Sevdeen and Schmidinger, 2019).** Our previous study involved two ethnic groups, Muslim Kurds and Muslim Arabs in Iraqi Kurdistan **(Fattah *et al.*, 2019).** Short Tandem Repeats (STRs) are DNA regions that have repeating units of 2-6 bp and are found throughout the human genome,

including autosomal and both sex chromosomes X and Y. Unlike autosomal STRs, Y chromosome STRs are in a haplotype state due to the lack of an X chromosome counterpart homolog. Y chromosome STRs (Y-STRs) are located outside of the chromosome's pseudo-autosomal region and are thus passed down from father to male offspring without recombination **(Sayyari *et al.*, 2020).** Polymorphisms in Y-STRs are regarded as valuable evolutionary tools that could be used as genetic markers in regional and population studies. **(Ohied and Al-Badran, 2022).** Furthermore, such polymorphisms could be used for forensic purposes, such as paternity determination **(Rustamov *et al.*, 2004).** No previous study has involved the Chaldean population for Y STR analysis. Therefore, this study aims to use eight specific STR loci of the Y chromosome for characterizing the genetics of the Yezidi and Chaldean populations in Iraqi Kurdistan. In addition, to find the genealogical relationship and use cluster analysis to measure the genetic distance between these two different ethnic groups living in the Iraqi Kurdistan region.

MATERIALS AND METHODS

A. Ethical Committee Approval

The approval of ethics was performed by the ethical committee at the Duhok province Ministry of Health (Reference number: 21082022-6-9). Informed consent for each volunteer was made, genealogical information was documented, and each volunteer confirmed that their fathers, grandfathers, and great-grandfathers belong to the Yezidi or Chaldean ethnic group.

B. Methods

A total of 44 blood samples were collected from unrelated males of two ethnic groups who live in Iraqi Kurdistan, the Yezidi, and Chaldean. We also collected genealogical information about the donors. The DNA was extracted from the whole blood samples using DNA Extraction Kit according to the instructions provided by the supplier company (Dongsheng Biotech Company, China, CAT No. NH 1121). Eight primers of the Y chromosomal STRs were used, namely: Y-GATA-H4, DYS437, DYS392, DYS458, DYS448, DYS456, Y-GATA-C4, and DYS19.

The PCR program was; initial denaturation at 94˚C for 5 min (one cycle); then 34 cycles of 94˚C denaturation for 60 sec, specific annealing temperature (Table 1) for 35 sec and 1 min extension at 72˚C; followed by one cycle at 72˚C for 6 min (final extension). The amplified products along with 20 bp ladder DNA markers were run on 10% polyacrylamide gel electrophoresis for band sizing, and the bands were stained by silver nitrate for visualization .

Table, 1: The characteristics of primers used in this study.

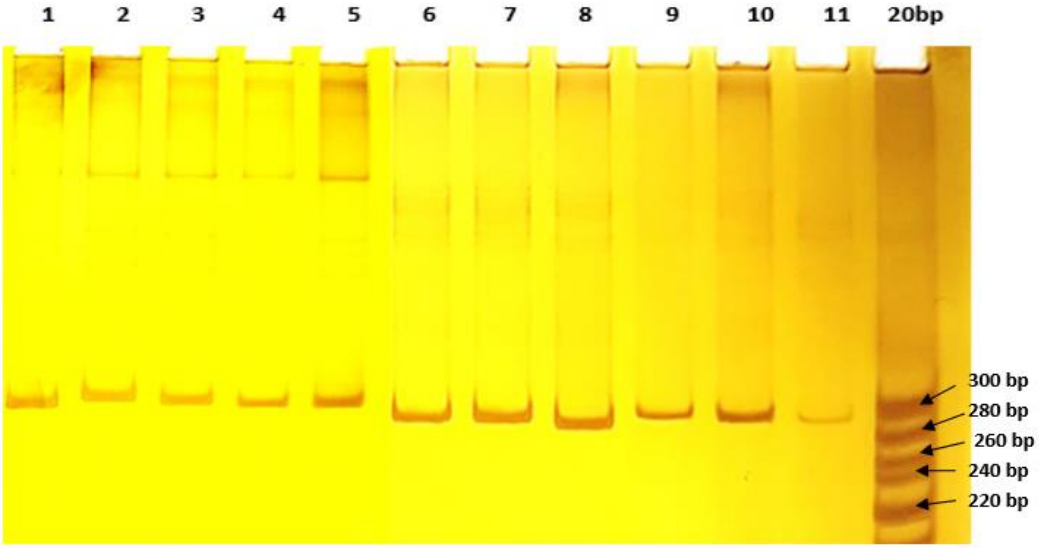
Primer		Primer sequence	Repeat motif	Annealing Tm. °C	Expected Size (bp)	Ref
DYS19	F- R-	5′-CTACTGAGTTTCTGTTATAGT-3′ 5′-ATGGCCATGTAGTGAGGACA-3′	$[TAGA]_3$tagg[TAGA]n	52	176-212	Naji and Al Saadi. 2020
DYS392	F- R-	5′-TCATTAATCTAGCTTTTAAAAACAA-3′ 5′-AGACCCAGTTGATGCAATGT-3′	[TAT]n	52	234-267	Rustamov *et al*., 2004
DYS437	F- R-	5′′-GACTATGGGCGTGAGTGCAT-3′ 5′-AGACCCTGTCATTCACAGATGA-3′	$[TCTA]_n[TCTG]_2$[TCTA]4	59	181-197	Bai *et al*., 2016
DYS448	F- R-	5′-TGTCAAAGAGCTTCAATGGAGA-3′ 5′-TCTTCCTTAACGTGAATTTCCTC-3′	$[AGAGAT]_nN_{42}$[AGAGAT]n	54	279-321	Fattah et al.2019
DYS456	F- R-	5′-GGACCTTGTGATAATGTAAGATA-3′ 5′-CCCATCAACTCAGCCCAAAAC-3′	[AGAT]n	56	137-161	Mizuno, 2008
DYS458	F- R-	5′-AGCAACAGGAATGAAACTCCAAT-3′ 5'-CCACCACGCCCACCCTCC-3′	[GAAA]n	61	111-139	Ohied & Al-Badran, 2022
YGATA-C4	F- R-	5'-GGCTTCTCACTTTGCATAGAATC-3′ 5′-ACCAGCCCAAATATCCATCA-3′	[TAGA]n N_{12} $[gatc]_2$ aa $[taga]_4$	57	151-171	Al-Zubaidi, 2019
Y-GATA-H4	F- R-	5′-ATGCTGAGGAGAATTTCCAA-3′ 5′-CTATTCATCCATCTAATCTATCCATT-3′	[TAGA]n N_{12} $[gatc]_2$ aa $[taga]_4$	52	122-142	Alaqeel, 2020

Tm. = temperature, Ref= reference, bp= base pair, F=Forward, R= Reverse.

Statistical data analysis: The data of the results were analyzed by using the Power Marker V3.25 software and MEGA X was used for constructing the phylogenetic tree. The genetic relationship parameters were calculated according to Reynolds's (1983) statistics **(Reynolds *et al.*, 1983).** The similarity matrix was used to construct the dendrogram using the unweighted pair group method arithmetic averages (UPGMA) procedure **(Sokal, 1958)**. Phylogenetic tree construction was created by using MEGA-X software.

RESULTS

Using power marker V3.25 software analyzed some molecular parameters such as mean allele number, gene diversity, allele frequency, genetic distance, and polymorphic information content. The total number of alleles identified in the two populations was 88 alleles. The allele sizes range was from 111-315 bp (Table 2 and fig. 1).



Figure, 1: 10% Polyacrylamide gel PCR amplified products of primer DYS448, 1 to 5 represent Yezidi individuals and 6-11 represent Chaldean individuals.

Table, 2: Range of allele size of both populations Yezidi and Chaldean.

Primer		Allele Size Range, bp	Primer		Range of allele size, bp
DYS19	Yezidi	185—219	DYS456	Yezidi	153-161
	Chaldean	175—197		Chaldean	137-157
DYS392	Yezidi	258-267	DYS458	Yezidi	119-133
	Chaldean	255-267		Chaldean	111-137
DYS437	Yezidi	185—197	YGATA-C4	Yezidi	153-171
	Chaldean	185—197		Chaldean	145-171
DYS448	Yezidi	285-315	YGATA-H4	Yezidi	122-142
	Chaldean	279-303		Chaldean	130-142

bp=base pair.

In the Yezidi population, the alleles number per locus ranged from 3 at locus DYS456 to 6 alleles at locus DYS448, DYS458, Y-GATA-C4, and Y-GATA-H4, with an average of 4.8750 alleles per locus. Allele frequency ranged from 0.2727 in Y-GATA-C4 to 0.4545 in DYS456 while the mean was 0.3597. The range of gene diversity was from 0.6405 in DYS456 to 0.8140 in Y-GATA-C4 while the mean was 0.7384, indicating a high level of diversity (Table 3).

In the Chaldean group, the number of alleles per locus range was from 4 at DYS437 and Y-GATA-H4 to 11 alleles at the DYS458 locus and the mean was 6.1250. The range of allele frequency ranged from 0.1818 in DYS458 to 0.4545 in Y-GATA-H4 with a mean of 0.3068. The range of gene diversity was from 0.6653 in Y-GATA-H4 to 0.8884 in DYS458 and the mean was 0.7748, this value is higher than the Yezidi population (Table 4).

Table, 3: Summary of the statistic in the Yezidi population.

Marker	Major Allele Frequency	Sample Size	No. of obs.	Number of Alleles per locus	Availability	Gene Diversity	PIC
DYS19	0.4375	22.0	16.0	4.0	0.7273	0.6953	0.6445
DYS392	0.4286	22.0	14.0	4.0	0.6364	0.6837	0.6261
DYS437	0.3158	22.0	19.0	4.0	0.8636	0.7202	0.6668
DYS448	0.3500	22.0	20.0	6.0	0.9091	0.7550	0.7184
DYS456	0.4545	22.0	22.0	3.0	1.0	0.6405	0.5669
DYS458	0.3000	22.0	20.0	6.0	0.9091	0.8050	0.7779
YGATA-C4	0.2727	22.0	22.0	6.0	1.0	0.8140	0.7879
Y-GATA-H4	0.3182	22.0	22.0	6.0	1.0	0.7934	0.7638
Mean	0.3597	22.0	19.3750	4.8750	0.8807	0.7384	0.6940

No. of obs.= Number of observations, PIC= Polymorphic Information Content

Table, 4: Summary of the statistic in the Chaldean population.

Marker	Major Allele Frequency	Sample Size	No. of obs.	Number of Alleles per locus	Availability	Gene Diversity	PIC
DYS19	0.2727	22.0	22.0	6.0	1.0	0.8058	0.7778
DYS392	0.3182	22.0	22.0	5.0	1.0	0.7727	0.7362
DYS437	0.3636	22.0	22.0	4.0	1.0	0.6983	0.6430
DYS448	0.3636	22.0	22.0	6.0	1.0	0.7355	0.6938
DYS456	0.2727	22.0	22.0	6.0	1.0	0.7975	0.7670
DYS458	0.1818	22.0	22.0	11.0	1.0	0.8884	0.8781
DYS635	0.2273	22.0	22.0	7.0	1.0	0.8347	0.8130
YGATA-H4	0.4545	22.0	22.0	4.0	1.0	0.6653	0.6042
Mean	0.3068	22.0	22.0	6.1250		0.7748	0.7391

No. of obs.= Number of observations, PIC= Polymorphic Information Content

The availability value (alleles observed per sampled individuals) was calculated for accurate data analysis and its value was higher in the Chaldean group, with an average of 1.00, but it was 0.8807 in the Yezidi population because of the null alleles of the locus of some Yezidi samples (Tables 3 and 4).

The value of PIC (polymorphic information content) was also calculated for the eight primers in both populations (Tables 3 and 4). The values ranged from 0.5669 in the Yezidi population for the least informative marker, DYS456, to 0.8781 for the most informative marker, DYS458 in the Chaldean population.

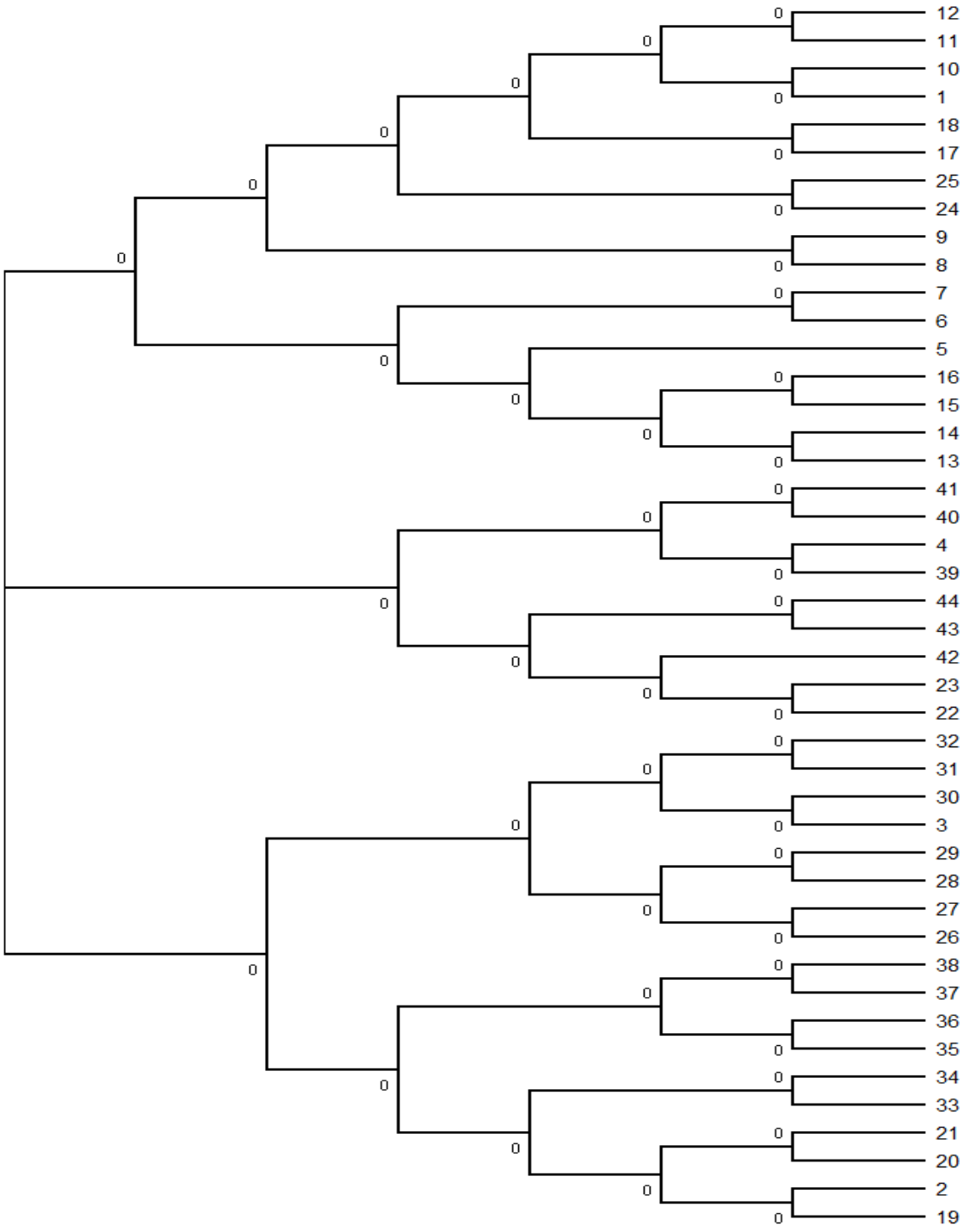
According to Table 5 in both populations together, the range of allele number was from 4 at DYS437 to 11 alleles at DYS458 locus with a mean of 7.2500 alleles per locus. The allele frequency ranged from 0.1818 in Y-GATA-C4 to 0.3659 in DYS448 and the mean was 0.2726. The range of gene diversity was from 0.7139 in DYS437 to 0.8740 in Y-GATA-C4 with a mean of 0.8035. Availability of alleles ranged from 0.7727 in DYS392 to 1.000 in DYS456, Y-GATA-C4, and Y-GATA-H4. The range of PIC values was from 0.7165 in Y-GATA-H4 to 0.8605 in Y-GATA-C4.

The results of Phylogenetic analysis created a dendrogram which resulted in the separation of the populations into three main clusters, Yezidi in one cluster and Chaldean in two other clusters except a few individuals were admixed with another cluster or sub-cluster from both populations, (fig. 2).

Table, 5: Summary of statistics in both Yezidi and Chaldean populations together.

Marker	Major Allele Frequency	Sample Size	No. of obs.	Number of Alleles per locus	Availability	Gene Diversity	PIC
DYS19	0.1842	44.0	38.0	9.0	0.8636	0.8643	0.8493
DYS392	0.3235	44.0	34.0	5.0	0.7727	0.7578	0.7186
DYS437	0.3415	44.0	41.0	4.0	0.9318	0.7139	0.6608
DYS448	0.3659	44.0	41.0	7.0	0.9318	0.7710	0.7397
DYS456	0.2273	44.0	44.0	7.0	1.0	0.8244	0.8003
DYS458	0.2381	44.0	42.0	11.0	0.9545	0.8662	0.8526
YGATA-C4	0.1818	44.0	44.0	9.0	1.0	0.8740	0.8605
Y-GATA-H4	0.3182	44.0	44.0	6.0	1.0	0.7562	0.7165
Mean	0.2726	44.0	41.0	7.2500	0.9318	0.8035	0.7748

No. of obs.= Number of observations, PIC= Polymorphic Information Content



Figure, 2: Phylogenetic relationship of Yezidi and Chaldea population using 8 Y STR- markers. The Yezidi individuals are from no. (1-22). While the Chaldean individuals are from no. (23- 44).

DISCUSSION

In this study, eight loci of the human Y-STRs were used to determine the genetic variation and allele frequency between Yezidi and Chaldean populations in Duhok province. The results showed that within a total of 88 alleles, their sizes range from 111 to 315 bp (Table 2). These results are in agreement with those reported previously for the Iraqi Arab families living in the middle Euphrates and their PCR product size of the DYS392 locus

ranged from 93 to 125 bp, and DYS19 ranged from 176 to 212 bp **(Naji and Al Saadi, 2020**). The mean number of alleles per locus scored in this study (Yezidi 4.8750, Chaldean 6.1250 alleles) was lower than those published in the NIST fact sheet, USA with an average of 9 alleles per locus **(NIST, 2017).**

The high amount of genetic diversity in the population is suggested by the high number of alleles per population. Fattah and his colleagues reported that the average number of alleles in the Kurd population was 5.125 **(Fattah *et al.*, 2019).** The high number of alleles within each population indicates a great level of genetic diversity. The allele frequency in the two groups, Yezidi and Chaldean was not similar to each other. A study by Ohied and Al Badran in the Basrah population with many similar primers used showed high allele frequency in all studied loci **(Ohied and Al Badran, 2022).** In another study by Imad and his colleagues in the middle and south of Iraq population, all eight primers used in this study were also used by them **(Imad *et al.*, 2013).** Allele frequencies in all loci were higher than the results in this study. The data in Tables (3 and 4) indicate that the mean value of gene diversity in the Chaldean population is the highest (0.7748) then followed by the mean gene diversity in the Yezidi population (0.7384). Both **Imad *et al.*, (2013)** and **Naji and Al Saadi, (2020)** reported much lower gene diversity than that reported in this study. In Northern Greece, genetic diversity value of 0.9992 also has been scored in 17 Y STR loci, five of these STRs were similar to those used in this study **(Leda *et al.*, 2008).** The results also revealed that the genetic diversity in the Chaldean population was higher than those in the Yezidi Kurd population (Tables 3 and 4). These variations in genetic diversity values in different populations may be attributed to the gene flow and migration during different times in history. An important factor determining whether a genetic marker is informative is its polymorphism information content (PIC) value. Values of PIC greater than 0.5 (PIC>0.5) are considered a highly informative primer **(Botstein *et al.*, 1980)**. In this study, the value ranged from 0.5669 at the DYS456 locus with 3 alleles in the Yezidi Kurd population to 0.8781 at the DYS458 locus with 11 alleles in the Chaldean population. All these primers used in this study, therefore, can be considered informative due to their high values. These results are in agreement with those of Fattah *and colleagues in* 2019, **(Fattah *et al.*, 2019)** whom they reported high PIC values. **Naji and Al Saadi, (2020)** found that DYS19 and DYS392 primers were the most polymorphic compared to other primers. Primer Y-GATA-C4 was found to be the most informative marker regarding both populations collectively with a PIC value of 0.8605. To evaluate the genetic differentiation and the distance between different populations, a phylogenetic tree was constructed. The phylogenetic tree (fig. 2) separated the populations into two major clusters. The first cluster was subdivided into two other subclusters, one of the Yezidi and the other of the Chaldean subcluster but the other main cluster contained most of the Chaldean. There were few individuals from one clad clustered to another clad in both populations. Compared to Chaldean populations, Yezidi populations have a smaller genetic distance than Chaldean populations do, because of the intermarriage between the Yezidi population individuals. The admixture of a few individuals from one population to another one can be attributed to their long-sharing history of living together for thousands of years. Also, wars, genocides, immigration, and gene flow have their role in admixing some of the individuals from clusters. Another explanation for this is that there is an unknown number of males who have the same Y-STR profile **(de Knijff, 2022).** Tömöry and colleagues through their research study explained that there was not much genetic separation among Hungarian-speaking communities in the Carpathian Basin **(Tömöry *et al.*, 2007).** The Hungarian gene pool was affected by neighboring gene flow and migration. Therefore, the gene flow may be one of the reasons for the admixing among these two populations.

CONCLUSION

All investigated loci have a high power of discriminating values, indicating that a DNA-based database can be created using these loci. The highest gene diversity was seen at Y-GATA-C4 (GD: 0.81) while the lowest diversity was observed at DYS456 (GD: 0.64) in the Yezidi group. In the Chaldean group, DYS458 (GD: 0.88) was the most diverse marker, while the least diverse marker was

Y-GATA-H4 (GD: 0.66). The phylogenetic tree separated the two populations into three major clusters. Most Yezidis were included in one cluster; while the Chaldean group was in the two other clusters. This indicates that the Yezidi individuals are more closely related to each other than the Chaldean because of the intermarriage between relatives in the Yezidi group which is not common in the Chaldean group.

This Study Limitation

This study used the Y-chromosomal short tandem repeats (STRs) loci but did not use autosomal STRs or Mitochondrial DNA. Also, taking more samples and analyzing more primers is needed in future research studies so that further data on the genetic structures of the Yezidi and Chaldean populations of Iraqi Kurdistan be provided

DECLARATION OF CONFLICTING INTERESTS

The authors declare that there is no conflict of interest.

Funding: University of Zakho

ACKNOWLEDGEMENT

The authors are grateful to every volunteer who participated in donating their blood for this research which made this research study succeed.

Abbreviations:

bp: base pair

NIST: National Institute of Standards and Technology.

No. of obs.: Number of observations

PIC: polymorphic information content

STR: Short Tandem Repeat

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الملخص العربي

تنوع المترادفات القصيرة المتكررة في كروموسوم Y في سكان اليزيديين و الكلدانيين في كردستان العراق

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الملخص:

الخلفية: تعيش العديد من المجموعات العرقية في الجزء الشمالي من العراق الذي يمثل الجزء العراقي من كردستان, تستخدم المترادفات القصيرة المتكررة على نطاق واسع في علم الوراثة السكانية وعلم الطب الشرعي. **الهدف:** يهدف هذا البحث إلى تحليل علامات المترادفات القصيره المتكرره في كروموسوم Y للمجموعتين العرقيتين اليزيديين والكلدان. **طرق العمل:** تم أخذ عينات دم من المجموعتين العرقيتين اليزيديين والكلدان و تم استخراج الحمض النووي من الدم باستخدام طقم استخراج الحمض النووي من إجمالي ٤٤ ذكرا غير مرتبطين (٢٢ لكل مجموعة عرقية) تم تحليلها باستخدام ثمانية من المترادفات القصيرة المتكررة في كروموسوم Y(DYS458, DYS456, DYS448, DYS437, DYS392 Y-GATA-H4,Y-GATA-C4, و DYS19) وبعد ذلك, تم تهجير نواتج الـ PCR على هلام بولي أكريلاميد بنسبة ١٠٪ وتم تصويرها عن طريق الصبغ بنترات الفضة. تم تحليل النتائج بواسطة برنامج Power marker v3.25 اما الـ dendrogram فقد تم إنشاؤه بواسطة برنامج Mega x. **النتائج:** لوحظ أعلى تنوع في Y-GATA-C4 (GD: 0.81) بينما لوحظ أدنى تنوع في DYS456 (GD: 0.64) في مجموعة اليزيديين. في المجموعة الكلدانية كانت DYS458 (GD: 0.88) الأكثر تنوعًا ، بينما كانت أقل العلامات تنوعًا في Y-GATA-H4 (GD: 0.66). وجد ان العلامة Y-GATA-C4 الأكثر إفادة في كلا المجموعتين بقيمة PIC تبلغ ٠,٨٦٠٥. **الاستنتاجات:** تؤكد هذه الدراسة القوة التمييزية لـ Y-STR عالية الدقة وتوفر أول مجموعة بيانات أولية عن هاتين المجموعتين العرقيتين في كردستان العراق. يكشف مخطط شجر الإنسان لمجموعات البيانات اليزيدية والكلدانية أن الأفراد اليزيديين أكثر ارتباطًا ببعضهم البعض مقارنة بمجموعة الكلدانية لأن زواج الاقارب يحصل بشكل أكبربين اليزيديين مقارنة مع المجموعة الكلدانية.

الكلمات المفتاحية: علم الأدلة الجنائية، كردستان العراق, كروموسوم Y، خريطة النشوء والتطور.