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

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ABSTRACT

***Purpose**: Because 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. This research aims to analyze the Y-chromosomal STR markers of the two ethnic groups Yezidi and Chaldean.*

***Subjects and Methods:** A DNA from Peripheral blood from a total of 44 unrelated males was extracted (22 for each ethnic group) and analysis for eight STRs of the Y-chromosome (Y-GATA-H4, Y-GATA-C4, DYS458, DYS456, DYS448, DYS437, DYS392 and DYS19). Then, the PCR products were run on 10% gel of polyacrylamide and stained by silver nitrate. The results were analyzed by Power marker V3.25 and dendogram created by Mega X software.*

***Results:** The highest diversity observed at Y-GATA-C4 (GD: 0.81) while lowest diversity was observed at DYS456 (GD: 0.64) at Yezidi group. At Chaldean group DYS458 (GD: 0.88) was the most diverse, while the least diverse marker was in Y-GATA-H4 0.66 in Chaldea and in Chaldean samples loci DYS458. The marker Y-GATA-C4 was found to be the most informative marker in both groups with PIC value of 0.8605.*

***Conclusions:** The study confirmed the high discrimination ability of the Y-STRs analysis and providing dataset on these two ethnic groups of Iraqi Kurdistan. The dendogram of Yezidi and Chaldean datasets reveals that the Yezidi individuals are more closely related to each other as compared to Chaldean group because Yezidi people because of intermarriage among them more than the Chaldean.*

## INTRODUCTION

Kurdistan is a geographical region located in Western Asia and it includes parts of Iran, Turkey, Syria and Iraq (Dahlman, 2002 and 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 and Dogan et al., 2017). Yezidis are a minority group who speak 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 Yezidi people is known as Yazidism which is monotheistic in nature, and it has roots in the pre-Zoroastrian

religion of Iran Foltz, 2017. Chaldea ethnic group is an Aramaic-speaking, Eastern Rite Catholics. 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), and (Sevdeen and Schmidinger, 2019)]. Our previous study involved the two ethnic groups, Muslim Kurds and Muslim Arabs in Iraqi Kurdistan Fattah et al., 2019).

No previous study has involved Chaldean population for Y STR analysis. Therefore, this study aims to use a number of specific STR loci of the Y chromosome for characterization the genetic of Yezidi and Chaldean population in Iraqi Kurdistan Secondly, to find the genealogical relationship and using cluster analysis to measure the genetic distance between these two different ethnic groups living in Iraqi Kurdistan region.

## METHODOLOGY

### Approval of Ethical Committee

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

### Methods & Materials

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

Statistical data analysis: The data of the results were analysed by using the Power Marker V3.25 software and MEGA X was used for the constructing the phylogenetic tree. The genetic relationship parameters calculated according to Reynolds (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.

**Table 1: The characteristics of primers used in this study**

| Primer | | Primer Sequence | Repeat Motif | Annealing Tm. °C | Expected Size( bp) | Ref |
|---|---|---|---|---|---|---|
| DYS19 | F-<br>R- | 5′-CTACTGAGTTTCTGTTATAGT-3′<br>5′-ATGGCCATGTAGTGAGGACA-3′ | $[TAGA]_3$tagg [TAGA]n | 52 | 176-212 | Naji and Al Saadi. 2020 |
| DYS392 | F-<br>R- | 5′-TCATTAATCTAGCTTTTAAAAACAA-3′<br>5′-AGACCCAGTTGATGCAATGT-3′ | [TAT]n | 52 | 234-267 | Rustamov *et al*., 2004 |

| | | | | | | |
|---|---|---|---|---|---|---|
| DYS437 | F-<br>R- | 5″-GACTATGGGCGTGAGTGCAT-3′<br>5′-AGACCCTGTCATTCACAGATGA-3′ | $[TCTA]_n[TCTG]_2[TCTA]_4$ | 59 | 181-197 | Bai *et al.*, 2016 |
| DYS448 | F-<br>R- | 5′-TGTCAAAGAGCTTCAATGGAGA-3′<br>5′-TCTTCCTTAACGTGAATTTCCTC-3′ | $[AGAGAT]_n N_{42}[AGAGAT]n$ | 54 | 279-321 | Fattah et al.2019 |
| DYS456 | F-<br>R- | 5′-GGACCTTGTGATAATGTAAGATA-3′<br>5′-CCCATCAACTCAGCCCAAAAC-3′ | [AGAT]n | 56 | 137-161 | Mizuno, 2008 |
| DYS458 | F-<br>R- | 5′-AGCAACAGGAATGAAACTCCAAT-3′<br>5'-CCACCACGCCCACCCTCC-3′ | [GAAA]n | 61 | 111-139 | Ohied & Al-Badran, 2022 |
| YGATA-C4 | F-<br>R- | 5'-GGCTTCTCACTTTGCATAGAATC-3′<br>5′-ACCAGCCCAAATATCCATCA-3′ | $[TAGA]n\ N_{1\ 2}\ [gatc]_2$ aa $[taga]_4$ | 57 | 151-171 | Al-Zubaidi, 2019 |
| Y-GATA-H4 | F-<br>R- | 5′-ATGCTGAGGAGAATTTCCAA-3′<br>5′-CTATTCATCCATCTAATCTATCCATT-3′ | $[TAGA]n\ N_{1\ 2}\ [gatc]_2$ aa $[taga]_4$ | 52 | 122-142 | Alaqeel, 2020 |

*Source: Authors*

## RESULTS AND DISCUSSION

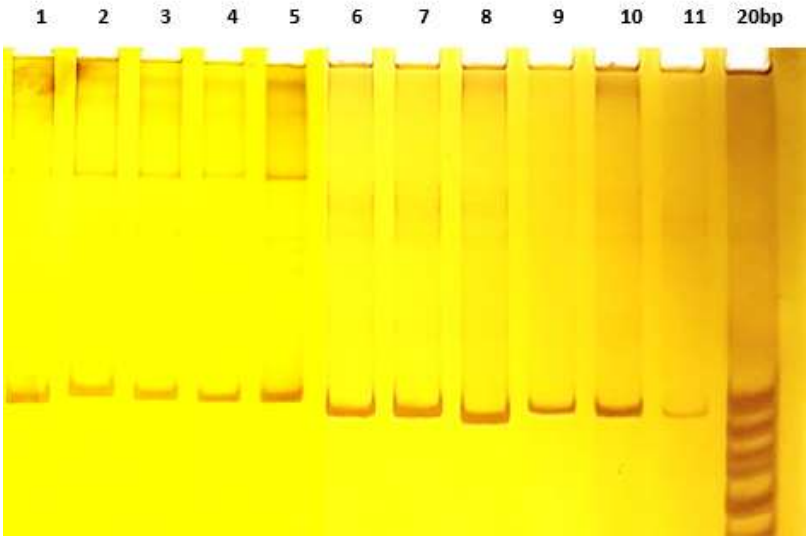
Using power marker V3.25 software analysed some molecular parameters such as mean allele number, gene diversity, allele frequency, genetic distance and polymorphic information content. The total number of alleles identified in the two populations was 88 alleles. The allele sizes range was from 111-315bp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

In the Yezidi population, the alleles number per locus ranged from 3 at locus DYS456 to 6 alleles at locus DYS448, DYS 458, Y GATA-C4 and YGATA-H4, with an average of 4.8750 alleles per locus. Allele frequency ranged from 0.2727 in YGATA-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 YGATA-C4 while the mean was 0.7384, indicating a high level of diversity (Table III).

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

**Table 2: Range of allele size of two 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 |




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

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 in Chaldean group, but it was 0.8807 in Yezidi population because of the null alleles of the locus of some Yezidi samples (Tables III and IV). The value of PIC (polymorphic information content) was also calculated for the eight primers in both populations (Tables III and IV). The values ranged from 0.5669 for the least informative marker, DYS456, to 0.8781 for the most informative marker, DYS458.

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

| Marker | Major Allele. Frequency | Sample Size | No. of obs. | Allele No | 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 |

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

| Marker | Major Allele Frequency | Sample Size | No. of obs. | Allele No | 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 |

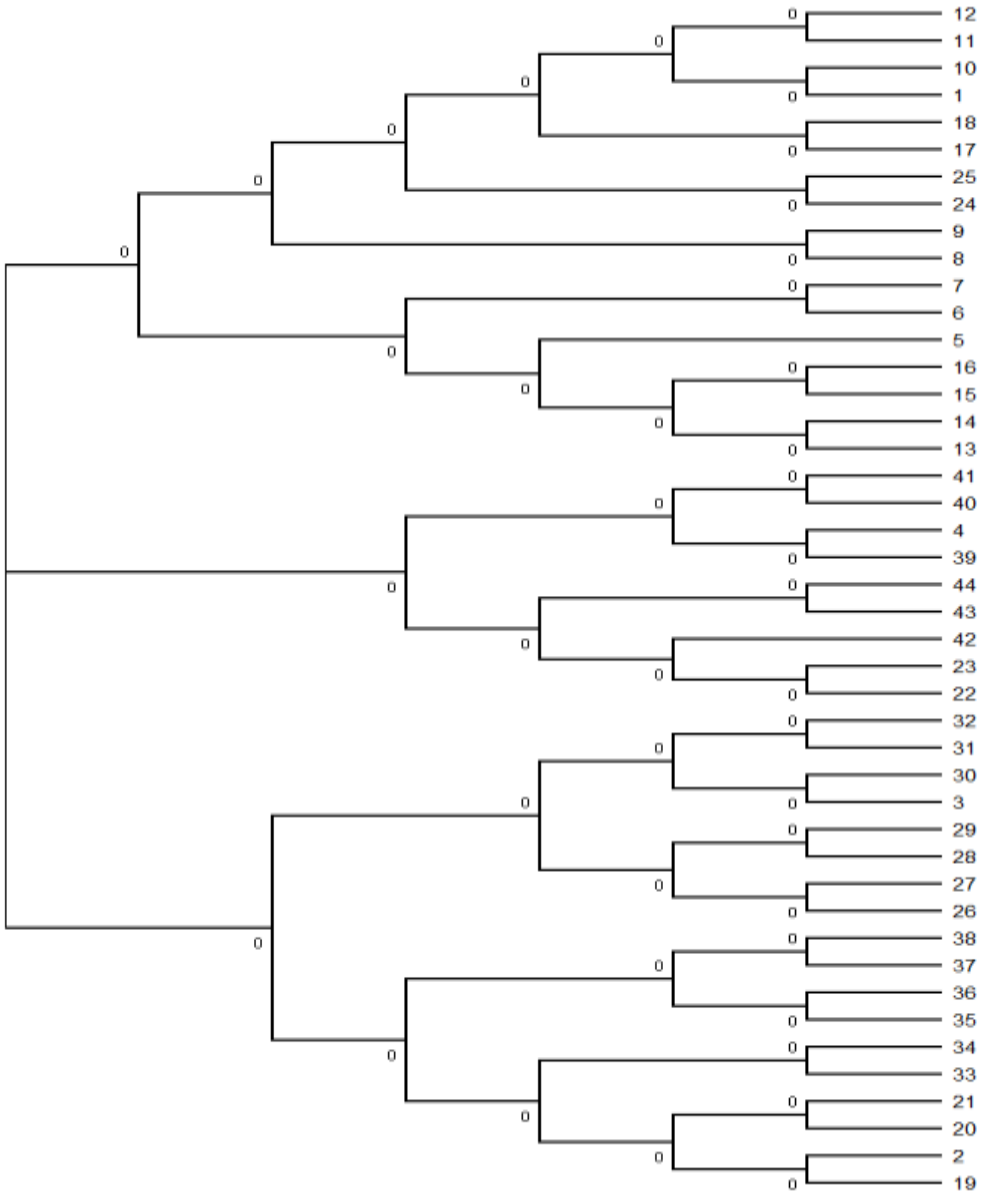
| | | | | | | | |
|---|---|---|---|---|---|---|---|
| YGATA-H4 | 0.4545 | 22.0 | 22.0 | 4.0 | 1.0 | 0.6653 | 0.6042 |
| Mean | 0.3068 | 22.000 | 22.0 | 6.1250 | 1.0 | 0.7748 | 0.7391 |

According to Table V in both populations together, the range of allele number was from 4 at DYS437 and YGATA-H4 to 11 alleles at DYS458 locus with mean of 7.2500 alleles per locus. The allele frequency ranged from 0.1818 in DYS635 to 0.3659 in DYS 448 and mean was 0.2726. The range of gene diversity was from 0.7139 in DYS 437 to 0.8740 in DYS635 with mean of 0.8035. Availability of alleles ranged from0.7727 in DYS 392 to 1.000 in DYS456, DYS635 and YGATA-H4. The range of PIC value was from 0.7165 in YGATA-H4 to 0.8605 in DYS635.

The results of Phylogenetic analysis created a dendogram which resulted in separation of the populations into two main clusters, Yezidi cluster and Chaldean cluster. The former, in turn, is divided into two sub-clusters: The Yezidi individuals in one subcluster and Chaldean in the other subcluster except 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 population together.**

| Marker | Major Allele Frequency | Sample Size | No. of obs. | Allele No | 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 |




*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 the present study, the Y-STRs were used to determine the allele frequency and genetic variation in 8 loci among Yezidi and Chaldean populations in Duhok province. The results revealed that within a total of 88 alleles their sizes range 111-315 bp (Table II). These results are similar to those previously reported for the Iraqi Arab families lives in middle Euphrates that their PCR product size of the DYS392 locus ranged 93 to125 bp, DYS19 ranged from176 to 212 bp [Naji and Al Saadi,2020]. Mean number of alleles per locus scored in this study (Yezidi 4.8750, Chaldean 6.1250 alleles) showed to be lower than those published in fact sheet of National Institute of Standards and Technology (NIST), USA with an average of 9 alleles per locus (NIST, 2017).

High amount of genetic diversity in the population is suggested by high number of alleles per each population. Fattah and his colleagues in 2019 reported that the average number of alleles in Kurd population was 5.125 (Fattah et al., 2019). 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 (2022) in Basrah population with many similar primers used, showed high allele frequency in all

studied loci (Ohied and Al Badran, 2022). Another study by Imad and his colleagues (2013) in middle and south of Iraq population, all the 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 III and IV indicate that the mean value of gene diversity in Chaldean population is the highest (0.7748) then followed by the mean gene diversity in 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 Chaldean population was higher than those in Yezidi Kurd population (Tables III and IV). These variations in genetic diversity values in different populations may be attributed to the gene flow and migration during different time of the history. An important factor determining whether a genetic marker is informative is its polymorphism information content (PIC) value. According to Botstein et al. (1980), values of PIC greater than 0.5 (PIC>0.5) are considered as highly informative primer. In this study the value ranged from 0.5669 at DYS456 locus with 3 alleles in Yezidi Kurd population to 0.8781 at DYS458 locus with 11 alleles in Chaldean population.

All these primers used in this study therefore can be consider as informative due to their high values. These results are in agree with those of 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 YGATA C4 was found to be the most informative marker regarding both populations collectively with 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 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.

Admixing of some individuals from one population to another population can be attribute to the long sharing history of living with other for thousands of years. Also, wars, genocides, immigrations and gene flow have its role in admixing some of the individuals from clusters. Another explanation of this, that there are unknown number of males which have the same Y-STR profile (de Knijff, 2022). Tomory et al, (2007) in their study demonstrated that there has not been much genetic separation among Hungarian-speaking communities in the Carpathian Basin. The Hungarian gene pool has been impacted by migration and neighboring gene flow. Therefore, the gene flow may be one of the reasons of the admixture pattern results among these three populations.

## CONCLUSION

All investigated loci have high power of discriminating values, indicating that a DNA-based database can be created using these loci. Highest gene diversity was seen at YGATA-C4 (GD: 0.81) while lowest diversity was observed at DYS 45456 (GD: 0.64) at Yezidi group. At Chaldean group DYS458 (GD: 0.88) was the most diverse, while the least diverse marker was in YGATA-H4 0.66 in Chaldea and in Chaldean samples loci DYS458. The phylogenetic tree separated the two populations into three major clusters. Most Yezidis were included in one cluster while the Chaldean group were in the two other clusters which indicate that the Yezidi individuals were 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 used the Y Chromosomal short tandem repeat (STR) loci and

did not use autosomal STRs or Mitochondrial DNA. And using large number of samples and more primers to be done in future research studies, so that further data on the genetic structures of Yezidi and Chaldean of Iraqi Kurdistan be provided.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