International Journal of Medical Toxicology & Legal Medicine **Volume 28, No. 1, 2025**
e-ISSN: 0974-4614
p-ISSN: 0972-0448
https://doi.org/10.47059/ijmtlm/V28I5/015

Study the genetic variation of Kirkuk population by using mitochondrial DNA

Najeeb Ibraheem Mohamed[1], Yasser Hamad Hamada[1], Rafea Zaidan Mukhlif[2]

[1]Biology Dept., College of Sciences, University of Kirkuk, Iraq.
[2]Biology Dept., College of Sciences, University of Tikri, Iraq.
Email: Najeeb.biotech@uokirkuk.edu.iq, yasirhamad1974@uokirkuk.edu.iq

Received: 17.11.2024 Revised: 13.12.2024 Accepted: 19.01.2025

ABSTRACT
Geographical barriers have been recognized as significant impediments to population mobility, and these barriers may have varying effects on various genetic markers, including mitochondrial DNA (mtDNA). Thus, the current study used mitochondrial DNA and the Y chromosome to investigate the genetic variety of the Kirkuk population.The current study was conducted in the molecular laboratory in the Department of Life Sciences, College of Science, University of Kirkuk. Samples were collected during November 2022, blood samples were collected from 500 person of Kirkuk governorate include individuals who are not related, and the total number of individuals was 500 individuals, 250 males and 250 females. The results showed that the sample of the central Arab population demonstrated limited genetic diversity with few mutations and differences between individuals.The sample shows moderate to low genetic diversity, based on nucleotide diversity (Pi) and number of mutations.for original Arabs of Kirkuk, the value is very negative and indicates an increase in rare mutations compared to expectations under the genetic equilibrium model. Genetic diversity of Kurds of Erbil and Sulaymaniyah is relatively low in the sample studied, with 16 polymorphic sites and 17 total mutations. The results show relatively low genetic diversity with a significant increase in rare mutations for original Kurds of Kirkuk. The genetic diversity in the Kakai sample is moderate to high, with 92 variant sites and 96 mutations. The Turkmen of Kirkuk sample shows low genetic variation with an excess of rare mutations. Low genetic diversity may be associated with greater genetic isolation in Christian population compared to others in the region, which may lead to less genetic diversity. about the origins of Kirkuk populations, as for the Arabs of Kirkuk, it was noted that there are many origins to which the Arabs return, as cluster U appeared, which is the highest if its percentage reached 22.22%, which originated from the Middle East, followed by cluster H, which reached 12.7%, which originated from North Africa, the Middle East, Central Asia, Northern Asia. Regarding Kakai Kirkuk, it was noted that there are some origins that Kakai belongs to, as cluster U appeared, which is the highest if it reached 37.5% and its origin goes back to the Middle East. Regarding the Kurd of Kirkuk, it was noted that there are some origins that the Kurd goes back to, as cluster H appeared, which is the highest if its percentage reached 24.19%, which originated from Europe. Regarding the Turkmen of Kirkuk, it was noted that there are some origins that belong to the Turkmen, as cluster H appeared, which is the highest if its percentage reached 20%, which originated from Europe, the Middle East, Central Asia, Northern Asia. for the Christian, it was noted that there are many origins to which the Christian return, as cluster U appeared, which is the highest if its percentage reached 42.86%, which originated from the Middle East.

Keywords: mtDNA, SNPs, genetic variation, haplogroup.

INTRODUCTION

Although the majority of people in a given region may identify as belonging to a particular nationality, the population's actual heritage and historical roots can frequently diverge greatly. A genetic isolate is created gradually and is impacted by a number of variables. Geographic separation, sometimes known as isolation by distance, is a typical component of this process, and the distance between locales greatly influences the genetic variations among populations[1,2,3]. Geographical barriers have been found to be significant impediments to population movement [4]. These barriers may have varying effects on genetic markers, including mitochondrial DNA (mtDNA) and the Y chromosome [5]. Other researchers have found that language barriers, in addition to geographic barriers, cause genetic isolates [6]. However, some linguists are still wary of drawing conclusions about the relationship between the two [7]. Furthermore, social and cultural elements, such as religion and

ethnicity, frequently serve as the foundation for demographic divisions, aiding in their creation and maintenance [8].Significant progress has been made in understanding the genetic components underpinning complex human behaviors thanks to genetic investigations. These research have provided information about disorders like Crohn's disease, autoimmune diseases, obesity, and schizophrenia, for example [9,10]. Finding correlations with autosomal chromosomal single nucleotide polymorphisms (SNPs) has been the traditional focus of GWAS. At the same time, new developments are expanding the types of data that are employed in these investigations. This includes adding copy-number variation to the investigative toolkit, using sequencing-based GWAS techniques, and conducting thorough gene-based analysis [11].However, there has been relatively little research done on the genetic relationships between the Y chromosome and mitochondrial DNA (mtDNA), with only a few genetic studies in these areas [12,13,14]. As we learn more about mtDNA correlations, GWAS, EWAS, and PheWAS become more and more significant [15]. Forensic applications, maternal lineage testing, and ancestry inference can all benefit from the adoption of the mtDNA marker system [16,17].Because of its high polymorphism, maternal inheritance, absence of recombination, and fast pace of mutation, mtDNA loci provide a viable marker system for examining maternal genetic links across various ethnic groups. Because of its high copy numbers per cell, which increase the success rate of mtDNA detection [18], mtDNA has significant benefits over nuclear DNA in forensic applications for evaluating extremely degraded, damaged, or trace amounts of biological samples [19].Therefore, the current study aimed to study the genetic variation of Kirkuk population by using mitochondrial DNA.

MATERIALS AND METHODS

Study Design, Sample, and Data Collection Time

The current study was conducted in the molecular laboratory in the Department of Life Sciences, College of Science, University of Kirkuk. Samples were collected during November 2022, blood samples were collected from 500 person of Kirkuk governorate include individuals who are not related, and the total number of individuals was 500 individuals, 250 males and 250 females. Sample and data collection according to ethical approval of environment.

Blood collection

In the study a samples of venous blood in size three milliliters were collected from each person. Into (EDTA) tubes the blood was collected for DNA extraction and stored at - 20˚C (deep freeze) [20,21].

Amplification of mtDNA

Extracted genomic DNA using Geneaid Company's gSYNCTM DNA extraction kit's rapid methodology. Since each cell contains many copies of mt-DNA, HV1 was amplified using a straightforward technique for extracting and measuring DNA. We independently intensified these polymorphic areas using the PCR preliminaries that are included.Tsutsumi et al. [22] amplified 15,997 nt to 16,401 nt of HV1 by synthesizing forward primer 5′-CTCCACCATAGCACCCAAAGC-3′ and reverse primer 5′-CCTGAAGTAGGAACCAGATG-3′. By using Basic search tool with local alignment with 2.2.27 release number, tested these primers in silico against reference human genome. Denaturation at 94 °C for one minute was the PCR amplification condition. A final extension at 72 °C for 10 minutes was performed after 35 cycles of denaturation at 94 °C for 1 minute, annealing at 55 °C for 1 minute, and extension at 72 °C for 1 minute. The South Korean company Macrogen, which can be found at httb://dna.macrogen.com, received the PCR product for sequencing.

Phylogenetic and statistical analysis

Using the SPSS version of the software, significant changes at a probability threshold of 0.05 were expressed using the independent t-test, spearman test, and ANOVA table. The outcomes were shown as a M ± SE [23,24].

Multiple alignment

Using BioEdit 7.1 [25], the electropherograms were read. All sequences were aligned with the updated Cambridge Reference Sequence (rCRS) using Clustal W from Mega 6.0. PhyloTree 16 was used to resolve the mt-DNA haplogroups according to analytic sites [26]. The method was used in HaploGrep 2.0 to reduce the mistake rate during haplogroup organization.

Inference of Haplogroup

MitoTool, an online bioinformatics program designed for analyzing mt-DNA, was used to resolve the Haplogroup assignments. MitoTool obtains the differences in each batch mode sequence by aligning HVS1 sequences with rCRS, which makes use of Clustal W. Taking into account the distinct variation motifs of each haplogroup, the mt-DNA haplogroup was regulated by the best possible accurate coordination as well as fluffy or close coordination. By independently validating the mthap software in MitoTool, we confirmed the induced haplogroups [27]. In the end, we integrated the results of the MitoTool and mthap classifications, and our

phylogenetic tree was created (not shown) for crossing, approving and resolving the differences between the inferred haplogroups.To designate people to a potential haplogroup whose MitoTool characterisation and mthap fizzled or have created ambiguous haplogroups (more than one conceivable haplogroup per individual), phylogenetic defined haplogroups or branches were used.

RESULTS & DISCUSSION

Results of Genetic studies

By using a DNA extraction kit (munual), the genome was extracted, purified from the blood sample of the individuals and families members then it was migrated by using agarose gel as a first step to ensure the presence of DNA samples after extraction. Figure (1).

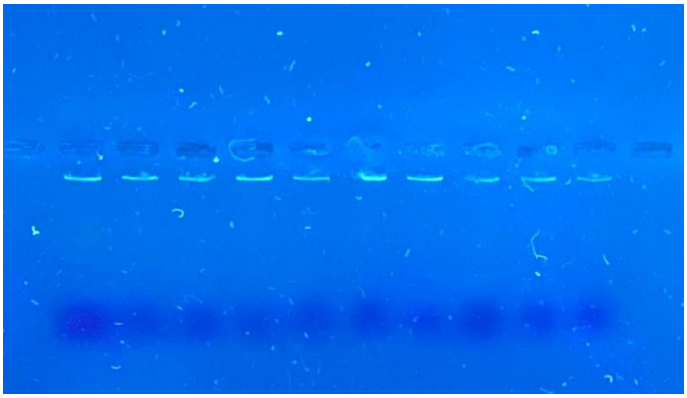


Figure 1: Extraction of whole DNA from blood sample, 1% agarose gel electrophoresis, SB1X, at a voltage 75 volt 20 mA for 1 hour (10 µl in each well).

HVI amplification

PCR technique was used to allow DNA amplification. The HVI region was targeted using specific primers. (Figure 2).

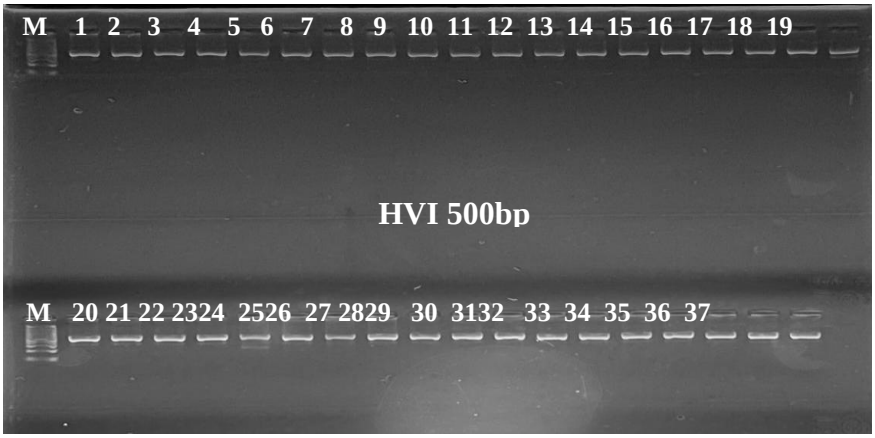


Figure 2: The electrophoreses pattern of PCR product for HVI region. This amplification produced bands about 500 bp under condition 1% agarose, annealing 54 C°, 75 V, 20 mA for 1h. M: DNA ladder, Lane 1-37 PCR product of HVI region.

Genetic variation of Kirkuk population

Middle Arabs

The sample of the central Arab population shows limited genetic diversity with few mutations and differences between individuals. A negative value of Tajima's D indicates that the sample may follow an evolutionary pattern that suggests genetic imbalance or unbalanced evolution, but the result is not statistically significant. A low value of genetic diversity (Pi) could indicate relatively low genetic diversity in the sample, which may be related to the population or environmental history of the region, Table 1.

Southern Arabs

The sample shows moderate to low genetic diversity, based on nucleotide diversity (Pi) and number of mutations. Negative values of Tajima's D may reflect an event such as a historical population contraction or the presence of other evolutionary factors affecting genetic distribution, Table 1.

Original Arabs of Kirkuk
The value is very negative and indicates an increase in rare mutations compared to expectations under the genetic equilibrium model. This deviation could be the result of one of the following scenarios: Recent population collapse: an event that affected the size of the population, leading to a concentration of rare mutations. Removal natural selection: the removal of harmful mutations from the population, while leaving rare mutations without widespread distribution, Table 1.

Kurds of Erbil
Genetic diversity is relatively low in the sample studied, with 16 polymorphic sites and 17 total mutations. A negative Tajima's D result indicates an excess of mutations, which may reflect historical events such as population shrinkage, Table 1.

Kurds of Sulaymaniyah
The genetic diversity in this sample is relatively low as shown by the low values of Pi and k and the limited number of mutations. A negative Tajima's D indicates that there may have been a population collapse in the history of this sample or that natural selection is removing deleterious mutations, Table 1.

Original Kurds of Kirkuk
The results show relatively low genetic diversity with a significant increase in rare mutations.Possibilities include: A recent population collapse. Moderate natural selection may have led to this pattern. The results show relatively low genetic diversity with a significant increase in rare mutations, Table 1.

Kakai
The genetic diversity in the sample is moderate to high, with 92 variant sites and 96 mutations. Tajima's D is positive, indicating the possibility of population expansion in the studied group, Table 1.

Turkmen outside of Kirkuk
The sample shows limited genetic diversity with a slight excess of rare mutations. These results may reflect recent population shrinkage or natural selection, Table 1.

Turkmen of Kirkuk
The sample shows low genetic variation with an excess of rare mutations. Possible causes include population shrinkage or natural selection, Table 1.

Christian
Low genetic diversity: This may be associated with greater genetic isolation in Christian population compared to others in the region, which may lead to less genetic diversity.Negative Tajima's D (-0.98): This may indicate variation in population history, such as population shrinkage or reduced mixing with other groups, which contributes to a greater than expected accumulation of mutations, Table 1.

Table 1: genetic variation of Kirkuk population

Population	S	Eta	K	Pi	Tajima_D
Middle Arabs	21	21	6.35556	0.01681	-0.67812
Southern Arabs	30	31	8.65455	0.01837	-0.84606
Original Arabs of Kirkuk	25	26	2.67828	0.01834	-1.87748
Kurds of Erbil	16	17	4.77273	0.02727	-0.66032
Kurds of Sulaymaniyah	9	9	2.61111	0.02123	-0.97334
Original Kurds of Kirkuk	24	25	2.96585	0.01854	-1.66072
Kakai	92	96	39.14286	0.23868	0.31118
Turkmen outside of Kirkuk	20	20	5.77778	0.01452	-1.05652
Turkmen of Kirkuk	9	9	2.25455	0.02967	-1.11904
Christian	10	10	3.33333	0.01022	-0.98373

S: Number of polymorphic (segregating) sites, Eta: Total number of mutations, k: Average number of nucleotide differences, Pi: Nucleotide diversity.

Origins of Kirkuk populations

As for the Arabs of Kirkuk, it was noted that there are many origins to which the Arabs return, as cluster U appeared, which is the highest if its percentage reached 22.22%, which originated from the Middle East, followed by cluster H, which reached 12.7%, which originated from North Africa, the Middle East, Central Asia, Northern Asia. cluster R, which reached 11.11%, which originated from South Asia. cluster L3, which reached 7.94%, which originated from West Asia and South Asia. cluster W, which reached 1.59%, which originated from Indo-European ancestry. cluster A, which reached 1.59%, which originated from Europe and the Middle East. cluster T, which reached 4.76%, which is spread in the Middle East (especially the South Caucasus, southern Iraq, south-west Iran, Oman and southern Egypt) and in Europe, South Africa. cluster J, which is 3.17%, is associated with the Norse ethnicity and originates in Europe. cluster I, which is 3.17%, is associated with the Druzes of Lebanon, Daghestan, Avars. cluster HV, which is 4.76%, is distributed in Europe and the Near East, figure 1.

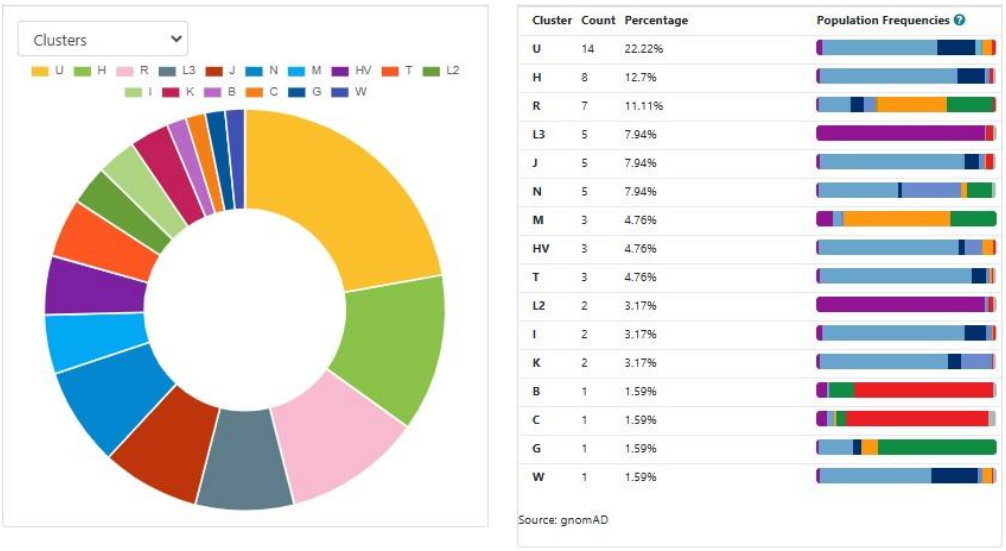


Figure 2: origin of Arab populations in Kirkuk city.

Regarding Kakai Kirkuk, it was noted that there are some origins that Kakai belongs to, as cluster U appeared, which is the highest if it reached 37.5% and its origin goes back to the Middle East. cluster T, which reached 12.5%, which is spread in the Middle East (especially the South Caucasus, southern Iraq, south-west Iran, Oman and southern Egypt) and in Europe, South Africa. cluster J, which is 12.5%, is associated with the Norse ethnicity and originates in Europe. cluster H, which reached 12.5%, which originated from North Africa, the Middle East, Central Asia, Northern Asia. cluster K, which reached 12.5%, which originated from Northwest Europe, figure (3).

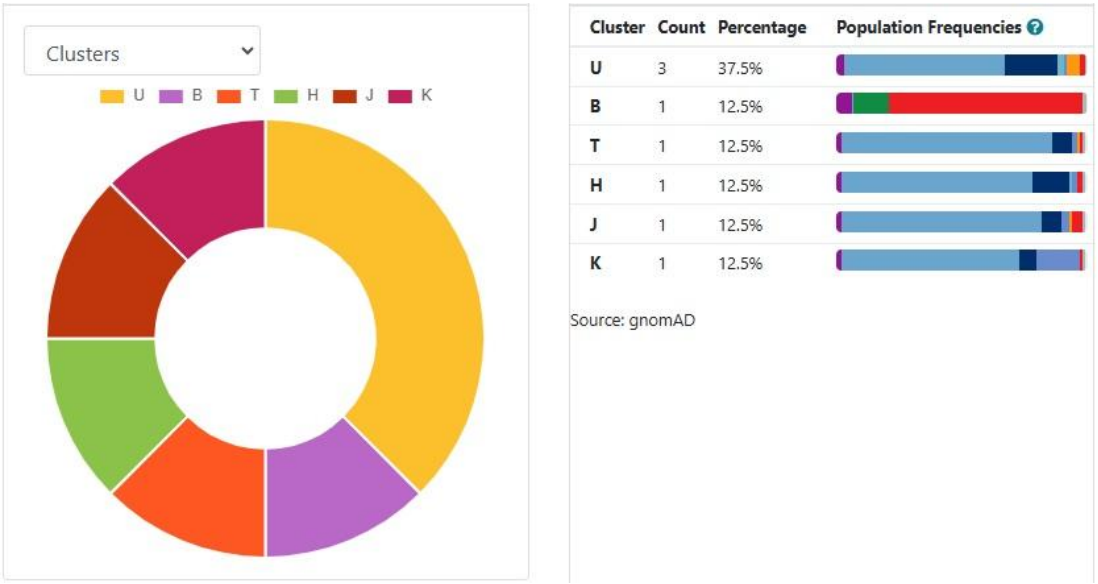
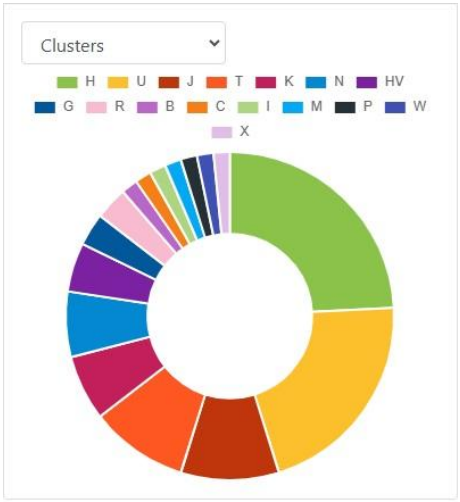


figure 3: origin of Kakai populations in Kirkuk city.

Regarding the Kurd of Kirkuk, it was noted that there are some origins that the Kurd goes back to, as cluster H appeared, which is the highest if its percentage reached 24.19%, which originated from Europe, the Middle East, Central Asia, Northern Asia, followed by cluster U, which reached 20.97%, which is associated with the Norse ethnicity and originates in Europe. cluster J, which is 9.68%, is associated with the Norse ethnicity and originates in Europe. cluster T, which reached 9.68%, which is spread in the Middle East (especially the South Caucasus, southern Iraq, south-west Iran, Oman and southern Egypt) and in Europe, South Africa. cluster K, which reached 6.45%, which originated in Eurasian lineages. cluster HV, which reached 4.84%, which is spread in Europe and the Near East. cluster R, which reached 3.23%, which originated in South Asia. cluster W, which reached 1.61%, which originated from Indo-European ancestry. cluster X, which reached 1.61%, which originated from Assyrians. cluster C, which reached 1.61%, which originated from East Africa, figure (2).

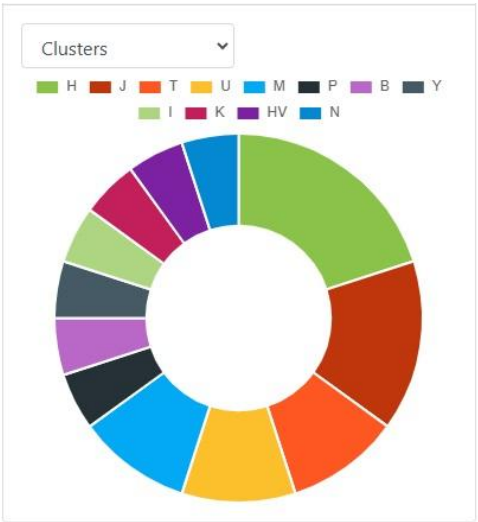


Cluster	Count	Percentage	Population Frequencies
H	15	24.19%	
U	13	20.97%	
J	6	9.68%	
T	6	9.68%	
K	4	6.45%	
N	4	6.45%	
HV	3	4.84%	
G	2	3.23%	
R	2	3.23%	
B	1	1.61%	
C	1	1.61%	
I	1	1.61%	
M	1	1.61%	
P	1	1.61%	
W	1	1.61%	
X	1	1.61%	

Source: gnomAD

Figure 4: origin of Kurd populations in Kirkuk city.

Regarding the Turkmen of Kirkuk, it was noted that there are some origins that belong to the Turkmen, as cluster H appeared, which is the highest if its percentage reached 20%, which originated from Europe, the Middle East, Central Asia, Northern Asia, followed by cluster J, which reached 15%, which is associated with the Norse ethnicity and originates in Europe. cluster T, which reached 10%, which is spread in the Middle East (especially the South Caucasus, southern Iraq, south-west Iran, Oman and southern Egypt) and in Europe, South Africa. cluster U, which reached 10%, which originated in the Middle East. cluster HV, which reached 5%, which is spread in Europe and the Near East. cluster K, which reached 5%, which originated from the Eurasian lineages. by. cluster I, which reached 5%, and originated from the Druzes of Lebanon, Daghestan, Avars.



Cluster	Count	Percentage	Population Frequencies
H	4	20%	
J	3	15%	
T	2	10%	
U	2	10%	
M	2	10%	
P	1	5%	
B	1	5%	
Y	1	5%	
I	1	5%	
K	1	5%	
HV	1	5%	
N	1	5%	

Source: gnomAD

Figure 5: origin of Turkmen populations in Kirkuk city.

As for the Christian of Kirkuk, it was noted that there are many origins to which the Christian return, as cluster U appeared, which is the highest if its percentage reached 42.86%, which originated from the Middle East, followed by cluster H, which reached 28.57%, which originated from North Africa, the Middle East, Central Asia, Northern Asia. cluster HV, which is 14.29%, is distributed in Europe and the Near East, figure 1.

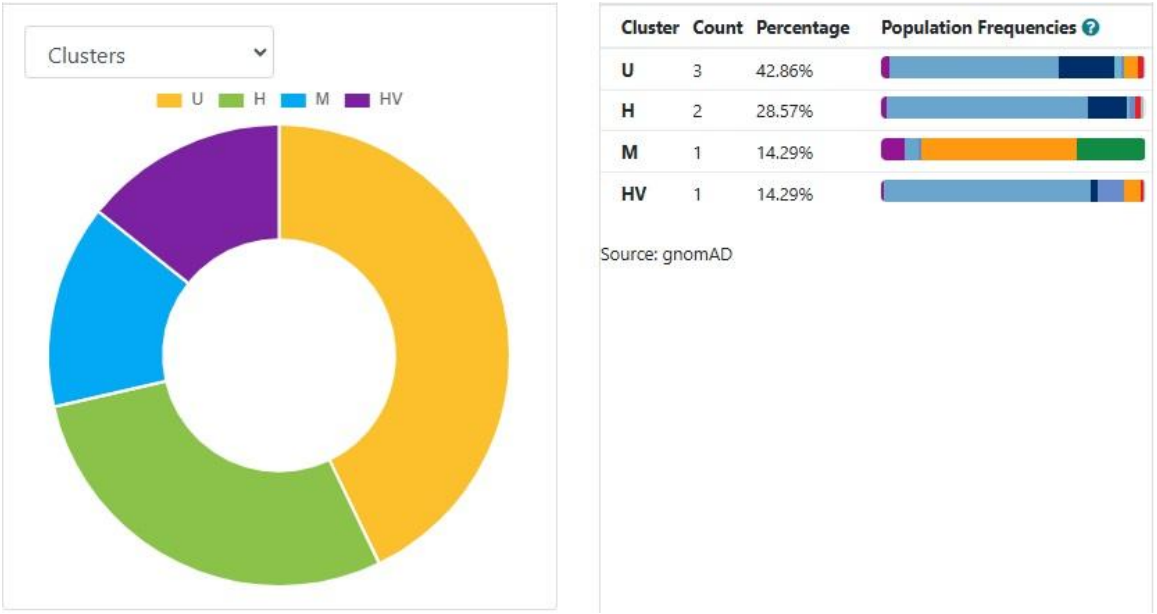


Figure 6: origin of **Christian** populations in Kirkuk city.

The majority of the mtDNAhaplogroups in the 63 Arab people were haplogroup U (22.22%), haplogroup H (12.7%), haplogroup R (11.11%), haplogroup L3, J, and N (7.94%), whereas the lowest haplogroups were B, C, G, and W (1.59%), according to Underhill and Kivisild'shaplogroup geographic associations [28]. Haplogroup U (37.5%) accounted for the majority of the mtDNAhaplogroups in the eight Kakai individuals. Regarding Kurd populations, haplogroup H (24.19%) accounted for the majority of the mtDNAhaplogroups in the 62 Kurd individuals. However, B, C, I, M, P, W, and X had the lowest haplogroup (1.61%). The majority of the 20 Turkmen individuals' mtDNAhaplogroups were haplotype H (20%) for Turkmen populations.The haplogroups with the lowest percentages were P, B, Y, I, K, HV, and N (5%). The majority of the seven Christian individuals' mtDNAhaplogroups were haplotype U (42.86%), which is typical of Christian communities. However, M and HV had the lowest haplogroup (14.29%). L lineages from Sub-Saharan Africa make up 7.94% of the Arab population in Kirkuk. Regarding the most likely geographic origin of the sub-Saharan African lineages in Kirkuk City, 7.94% of them match those in East Africa. The majority of these African lineages are thought to have arrived in the region as a result of the slave trade, but there is also ample evidence of earlier historical contacts with northeast Africa [29,30].According to previously published research, Nasidze et al. [31] found that the Kurdish groups exhibited a similar pattern with differences in maternal and paternal inheritances. Based on mtDNA, they were more closely related to European populations than the other populations of Kirkuk city, but Y-chromosome markers showed the opposite relationship. The region where the Kurdish tribe lived was a key hub for trade between Asia and Europe and bordered both continents. The Kurd group's genetic structure is Eurasian in autosomal inheritance, which may be explained by the possibility for genetic exchanges created by this geographic location.The bulk of the unusual M lineages found in Kurk Kirkuk had Indian ancestry, according to genomic sequencing of one M lineage. Representatives of the majority of the U clades and Central Asian, Indian, and Indonesian M lineages make up an important group of the Krud of Kirkuk that appears to have originated in the East and traveled through Iran to reach the Arabian Peninsula, where, in contrast to the Near East, the H clades (24.19%) have the highest frequency rather than the U (20.97%) group [32]. Accordingly, the Eastern and Southern regions of the Arabian Peninsula were considerably more affected by this Eastern gene flow.However, the bulk of the Krud N and R lineages had a Europe source.The principal European haplogroups were found in the Kurd population, being the haplogroup H the most frequent.

CONCLUSIONS

It is concluded from the current study that there is a great genetic diversity, especially in the Arab and Kurdish populations in the city of Kirkuk, while the Kakai and Christian populations were the purest compared to the rest of the Kirkuk populations.

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