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Estimation of Mitochondrial DNA Sequences (HVI and HV II) Variations in Iraqi Population

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Abstract: The current study aims to estimate the efficiency of mitochondrial DNA sequences (HVI and HV II) variations in the Iraqi population, The study population classified to two groups (individuals and families), the classification according to gender shows the women in families was higher than individuals in significant differences (P 0.007). The identities of HVI and HVII with data base of NCBI were detected in present study, the results showed non-significant changes between HVI and HV II in study population with NCBI data, and there were significant association between study groups (individuals HVI v HVII) and (families HVI v HVII) and also in HVII between individuals and families, significant highly identities of families female (HVII) and of individuals male, significant highly identities in HVI individuals and families with genetic disease and in healthy individuals, high identity observed in HVII in families than individuals. The variance in HVII was higher than HVI while in HVI the family's variance was a higher than individuals, in families' groups about five families have higher variance than others, in HVII the variance in individuals was higher than families and one family has higher and one family has higher variance. The results concluded that current output show fluctuation between HVI and HVII identities and variance

Keywords: mitochondrial MtDNA, Hypervariable region 1, Hypervariable region 1, sequences (scnp's), massive parallel sequencing

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Introduction

Even though the mitochondrion only comprises "0.25" percent of a cell's total DNA, 100 mitochondria live in the cytoplasm, making mtDNA the most abundant genetic molecules in forensic samples. MtDNA analysis is possible due to these properties of known population-specific variation and copy amount. Applications in forensic investigations (1).

The Displacement loop, which is made up of around 1100bp of the noncoding DNA, is known as the hypervariable area because of its higher mutation rate than the rest of the mtGenome. Three segments make up the hypervariable area. Hypervariable region 1 (HV1) encompasses nucleotide sites 16024-16365, hypervariable region 11 (HV2) encompasses nucleotide sites73-340, and hypervariable region III (HV3) encompasses nucleotide sites 438-574. HVI and HV2 are the most commonly tested in forensic settings, but HV3 is rarely examined. Other regions of the nuclear genome, such as one -copy nuclear polymorphic sequences (scnp's), have been discovered to mutate 10 to 17 times faster than the hypervariable area (2).

mtDNA is particularly beneficial in forensic examinations involving calcified tissues and hair in ancient

samples, according to current studies, missing individuals, and mass disasters, where DNA materials are typically damaged or severely deteriorated. Because each nucleated cell has 100 to 1000 mt genomes, the chances of mtDNA forensic indicators alive cellular damage are higher than those of the nuclear genome (3).

Methods

The current study was conducted in the molecular laboratory in the Department of Life Sciences, College of Science, University of Babylon. Samples were collected in the January 2021; they included males and females from sample of Iraqi population Collecting of blood samples from 150 persons consisting of; 100 males and 50 females. Samples and data collection according to ethical approval of environment.

Table (1): HVI and HVII primers Sequences

Primer Name	Sequences
HVI F	5- CTC-CAC-CA-TAG-CAC-CCA-AAG-C-3
HVI R	5- CCT-GAA-GTA-GGA-ACC-AGA-TG-3
HVII F	5-GGT-CTA-TCA-CCC-TAT-TAA-CCA-C-3
HVII R	5-CTG-TTA-AAA-GTA-ACC-GCC-A-3

Sequencing: Sequencing is done by Macrogen company ,then data analysis were implemented using NCBI, blast tool https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastnandPAGE_TYPE=BlastSearchandLINK_LOC=blasthome, and multiple alignment using https://mafft.cbrc.jp/alignment/software/.

Statistically analysis: All statistical analysis was performed via using SPSS version 23. all data were excited as (mean±SE) by using the one-way ANOVA test, Chi-square test, Duncan's, and Pearson correlation analysis used to determine significant variances among groups ($P \leq 0.05$).

Results

The socio-demographic analysis of study population, and study groups (individuals and families)

The socio-demographic distribution of study population according to gender shows 59>3% men and 40.7% women (table 2). Regarding to employment, high percentage was no employments (28%), then employments was (25.3%), house wife was (20.7%) and students was (20%) and finally retired was (7%).

The endogamy shows that (48.7%) was in closed marriage and (51.3%) was hasn't. the output of genetic disease find (5.3%) has genetic disease and (94.7%) was healthy. Other disease including diabetes mellitus type 2 and hypertension were (10.7%) and healthy individuals were (89.3%) (table 2). The study population classified to two groups (individuals and families), the classification according to gender shows the women in families was higher than individuals in significant differences (OR 0.0943, CI95% 0.0491 to 0.1814, P 0.007). The employment shows significant differences (p 0.000) among employments types, a higher percentage of students (40%) and house wife (28%) in families and no employment in individuals (39%). Significant changes (p 0.000) in endogamy were observed, about (60%) of individuals in closed marriage while in families was (74%). The healthy percentages were higher in both groups in non-significant differences (p 0.519). the other disease shows non-significant differences (p

0.817) in both groups that have high percentages of healthy people.

Table (2): The socio-demographic analysis of study population, and study groups (individuals and families).

Subjects	Population	Individuals	Families	Test	P
Gender					
Men	59.3	77	76	OR 0.0943 CI 95%(0.0491 - 0.1814)	0.007
Women	40.7	23	24		
Employment					
No employment	28	39	6	X^2 48.5689	0.000
Student	20	10	40		
Employment	25.3	26	24		
House wife	20.7	17	28		
Retired	6	8	2		
Endogamy					
Yes	48.7	60	26	OR 0.2342, CI% 0.1286 to 0.4267	0.0001
No	51.3	40	64		
Genetic disease					
Yes	5.3	6	4	OR 0.6528, CI% 0.1785 to 2.3875	0.5191
No	94.7	94	96		
Other disease					
Yes	10.7	11	10	OR 0.8990, CI% 0.3636 to 2.2225, P	0.8176
No	89.3	89	90		

The identities of HV1 with NCBI data

The match with NCBI data was implemented for each study sample of population, individuals and families, results show non-significant (p0.160) between individuals and families (Figure 1). Regarding to gender the identities percentages individuals and families HIV I with NCBI show non-significant changes (p 0.922) between men, and significant between (0.015) between women (figure 2).

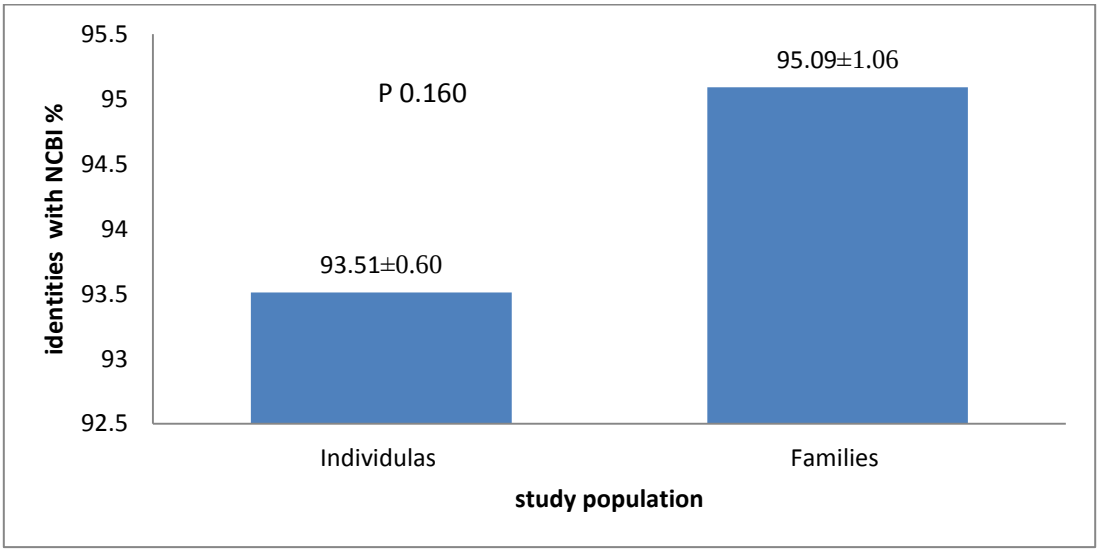

Figure (1): The identities percentages of study population (individuals and families) with NCBI (HV I).

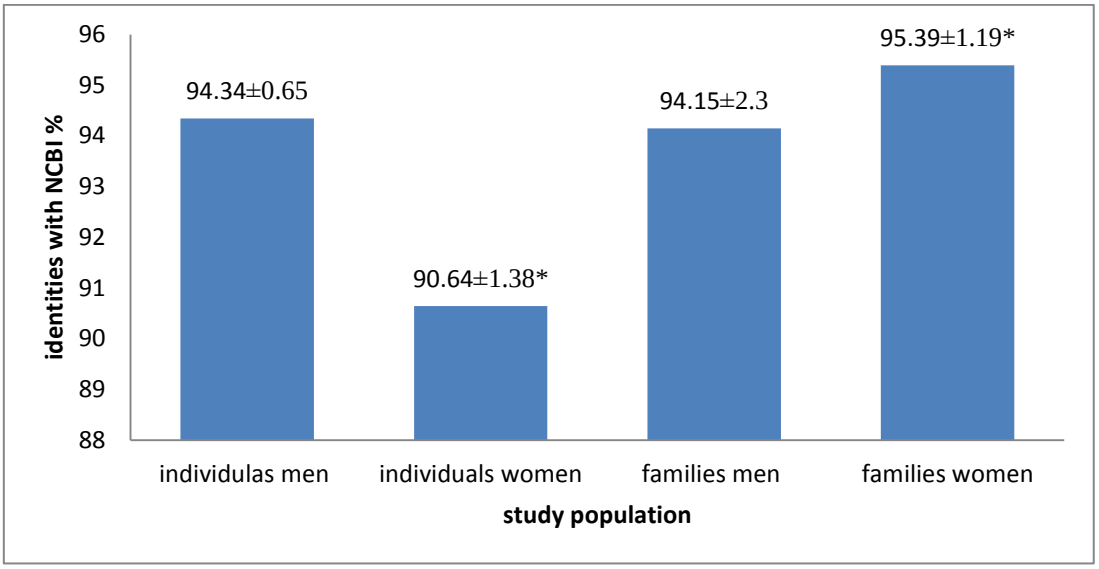

Figure (2): The identities percentages of study population (individuals and families) with NCBI (HIV I) according to gender (mean ±SE, independent t test at p<0.5).

The comparisons among employment types in study groups show non-significant changes for individuals (p0.072) and for families (p 0.700). the higher identities percentages were observed in no employee, students of individuals than families and in employee, house wife and retired of families (figure 3).

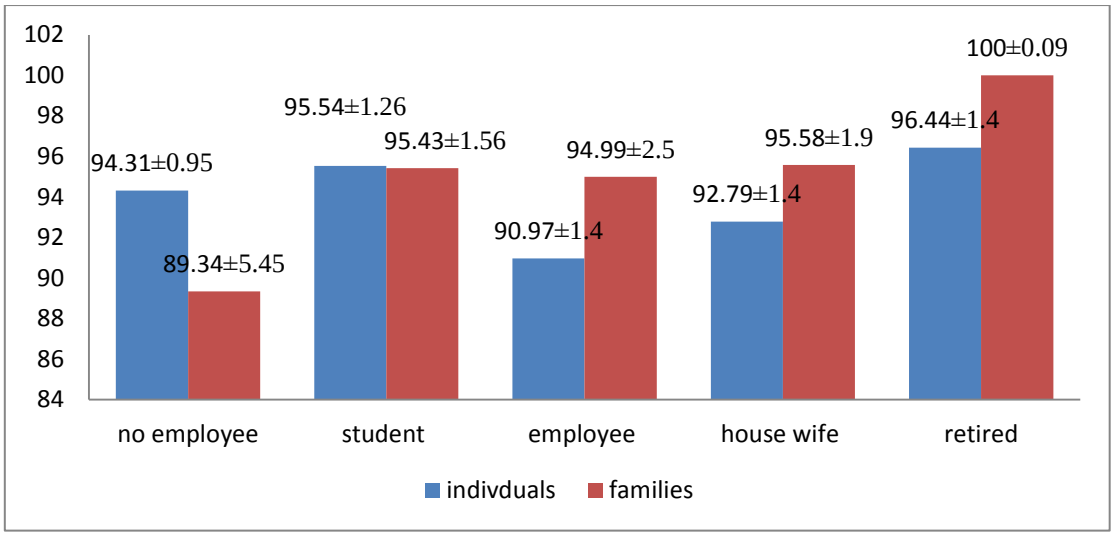

Figure (3): The identities percentages of study population (individuals and families) with NCBI (HV I) according to employment.

The samples with genetic disease show non-significant differences between individuals and families (p0.094) while the healthy recorded less identities (p 0.174) in both individuals and families in non-significant differences (p 0.124, 0.362) respectively (table 3). Other disease also recorded non-significant differences for all comparisons in slightly differences (Table 3).

Table (3): The socio-demographic analysis of study population, and study groups (individuals and families).

Subjects	Individuals	Families	Sig
Genetic disease			
Yes	97.18±0.74	99.90±0.100	0.094
No	93.27±0.63	94.89±1.09	0.174
Sig	0.124	0.362	
Other disease			
Yes	95.16±1.60	93.72±3.7	0.683
No	93.32±0.65	95.24±1.17	0.121
Sig	0.115	0.672	

The identities mean of individuals and families show that some families have high identities percentages (B, C, D, E, G, F, I, L, Q, P, O) while other have low percentages (A, H, J, K, M, N) (figure 6), these differences were significantly (p 0.000) (Table 4).

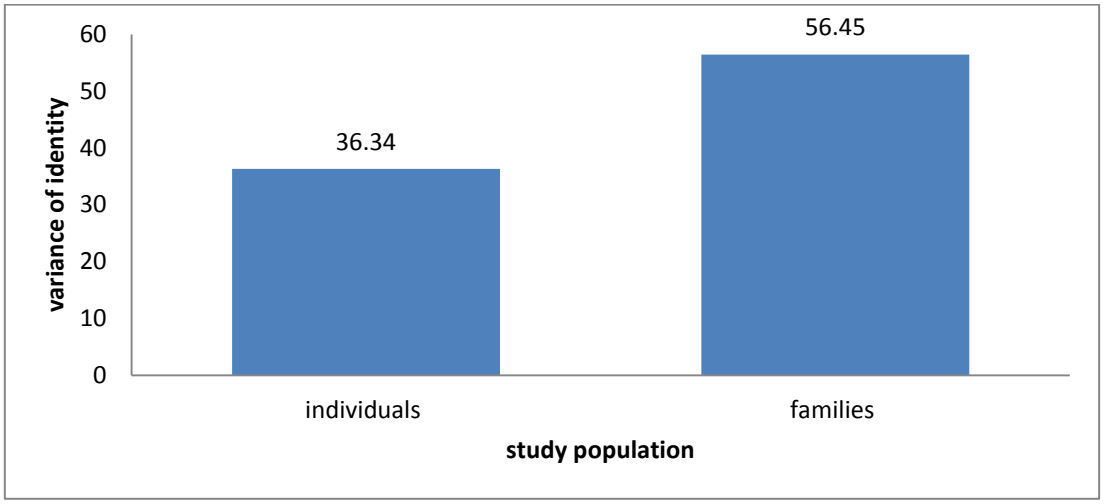

Figure (4): The variance value of identity percentage of individuals and families in study population.

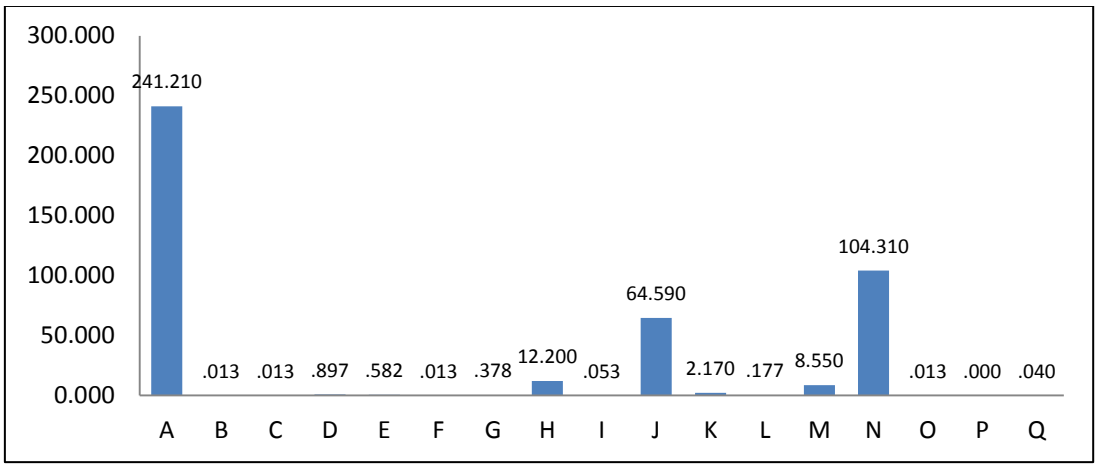

Figure (5): The variance value of identity percentage of families in study population(A-Q).

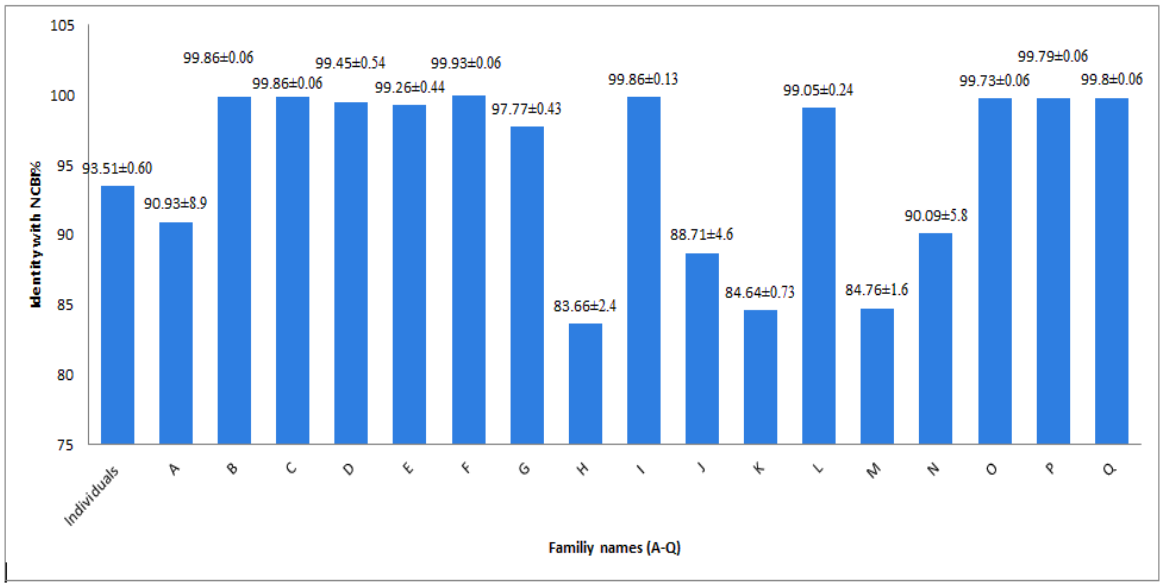

Figure (6): The identity percentages of HV I among individuals and families (A-Q) (mean ±SD).

Table (4): The statistical analysis of the identity percentages of HV I among individuals and families (A-Q) (p 0.000). (ANOVA one way, p<0.05, NS non-significant, S significant).

	Individuals	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
Individuals		NS	NS	NS	NS	NS	NS	NS	S	NS	NS	S	NS	S	NS	NS	NS	NS
A			NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
B				NS	NS	NS	NS	NS	S	NS	S	S	NS	S	S	NS	NS	NS
C					NS	NS	NS	NS	S	NS	S	S	NS	S	S	NS	NS	NS
D						NS	NS	NS	S	NS	S	S	NS	S	NS	NS	NS	NS
E							NS	NS	S	NS	S	S	NS	S	NS	NS	NS	NS
F								NS	S	NS	S	S	NS	S	S	NS	NS	NS
G									S	NS	NS	S	NS	S	NS	NS	NS	NS
H										S	NS	NS	S	NS	NS	S	S	S
I											S	S	NS	S	S	NS	NS	NS
J												NS	S	NS	NS	S	S	S
K													S	NS	NS	S	S	S
L														S	NS	NS	NS	NS
M															NS	S	S	S
N																S	S	S
O																	NS	NS
P																		NS
Q																		

The phylogenic tree of HVI was constructed; it's shown multiple rot with different branches and genetic distances (figure 7).

The identities of HVII with NCBI data

The identity with NCBI data was implemented for each study sample of population, individuals and families, results show significant (p0.000) between individuals and families High percentage of identity was observed in families than individuals (figure 8). According to gender the identities percentages of individuals and families with NCBI show high percentage of identities were higher in men of both groups, significant differences (p 0.02) between men and (p 0.000) between women between individuals and families (Figure 9).

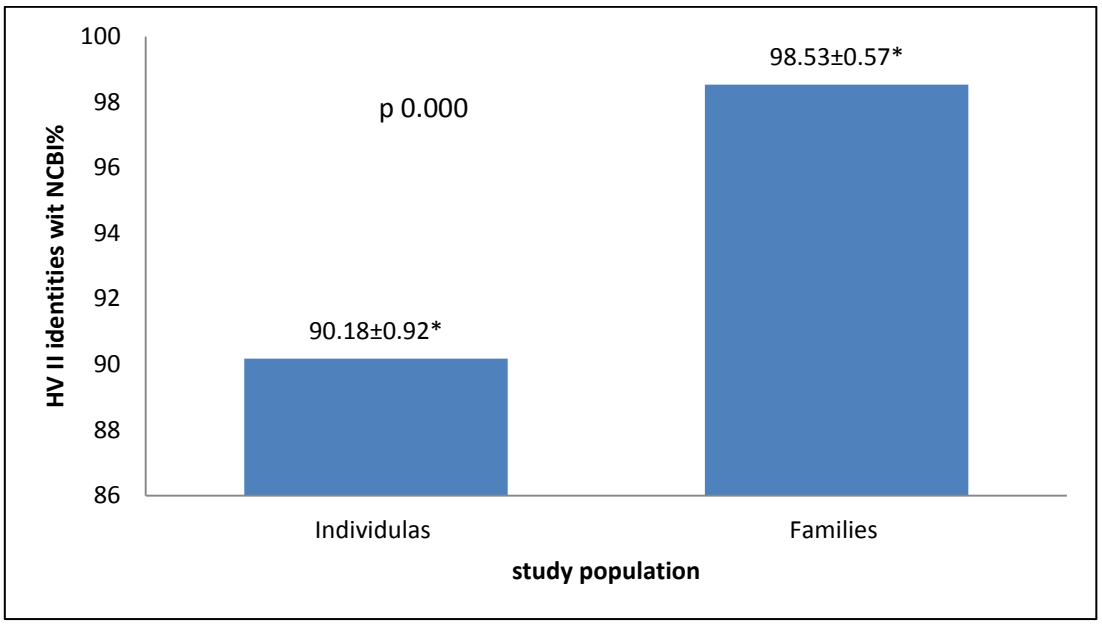

Figure (8): The identities percentages of study population (individuals and families) with NCBI (HV II) (mean ±SE, independent t test at p<0.5, * p 0.000).

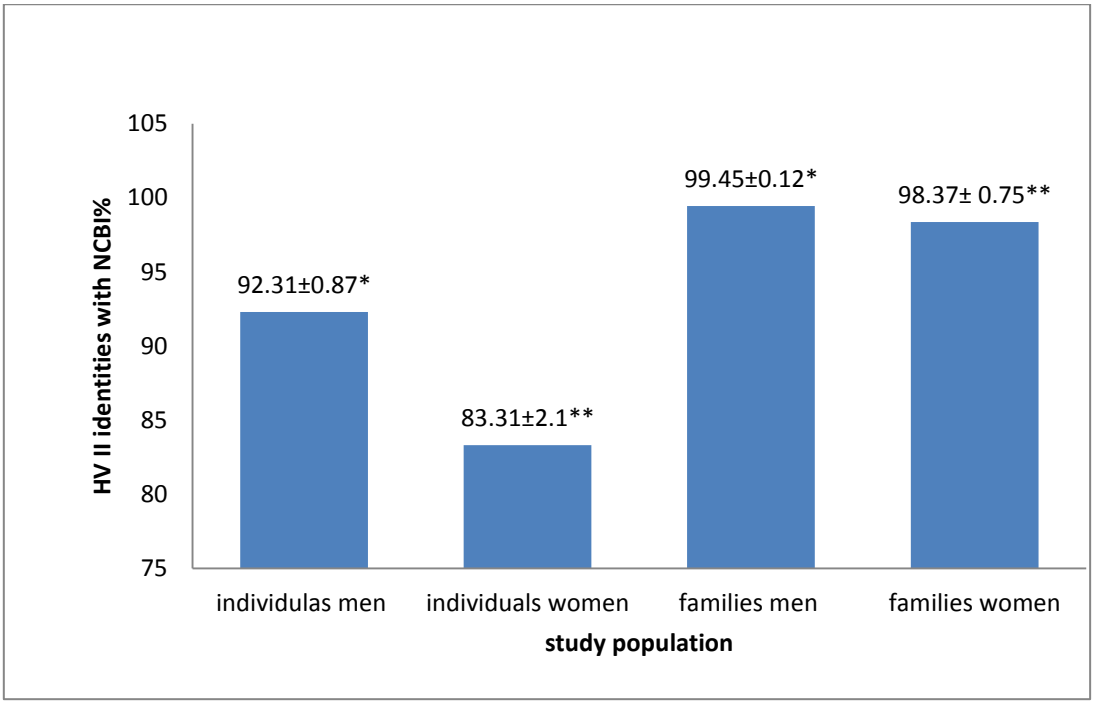

Figure (9): The identities percentages of study population (individuals and families) with NCBI (HIV II) according to gender (* p 0.02 between men, ** p 0.000 between women) mean ±SE, independent t test at p<0.5.

The comparisons among employment types in study groups show significant changes for individuals (p 0.004) and non-significant for families (p0.829). the higher identities percentages were observed in all types of employments of families than individuals (figure 10).

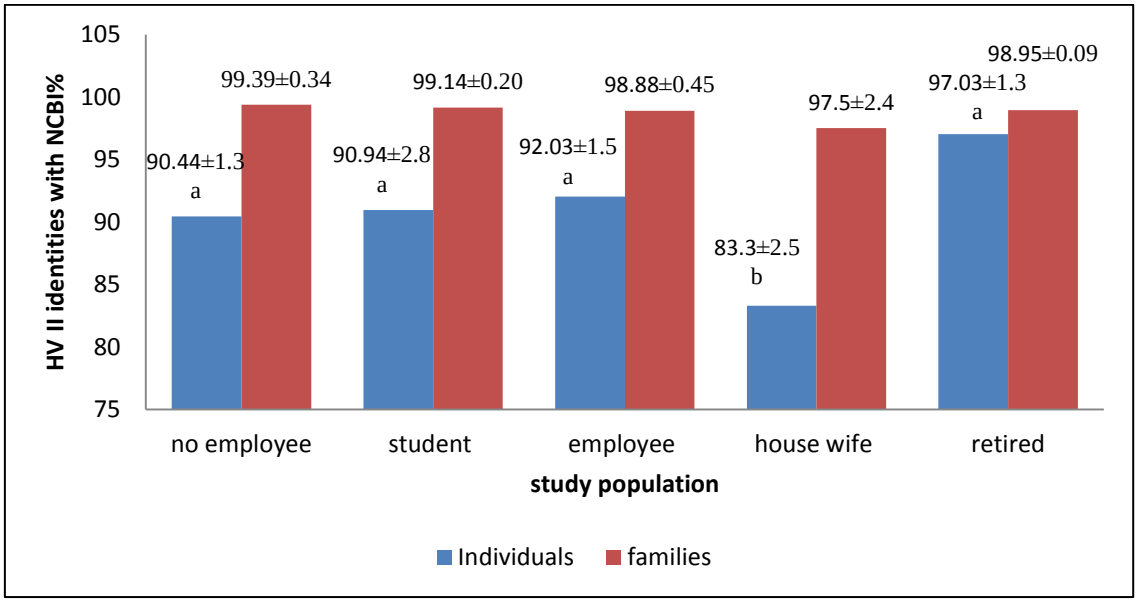

Figure (10): The identities percentages of study population (individuals and families) with NCBI (HV II) according to employment (p for individuals 0.004, Different letters refer to significant differences, p for families 0.829).

The samples with genetic disease show non-significant differences between individuals and families (p0.094) while the healthy recorded less identities individuals in significant differences (p 0.000) (table 5).

Other disease also recorded non-significant differences (p 0.176) between individuals and families that suffered from other disease and significant differences in healthy population (p 0.000) table (5).

Table (5): The identities percentages of study population (individuals and families) with NCBI (HIV II) according to disease infected (mean ±SE, independent t test at p<0.5).

Subjects	Individuals	Families	Sig
Genetic disease			
Yes	90.93±3.50	99.47±0.52	0.231
No	90.17±0.94	98.59±0.59	0.000
Sig	0.843	0.768	
Other disease			
Yes	93.89±2.56	99.42±0.192	0.176
No	89.76±0.96	98.54±0.63	0.000
Sig	0.156	0.560	

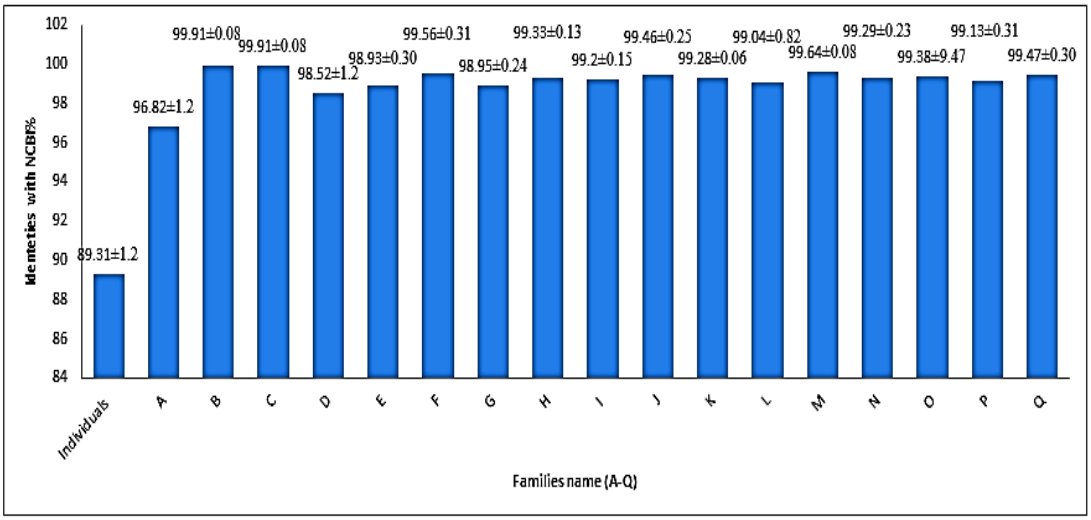

Figure (10): The identity percentages of HV II among individuals and families (A-Q) (mean ±SE, p 0.068).

The variance values of study groups were detected; in families the variance value was lower than individuals (figure 11). Families variance were varied (90.38-99.95) (figure 12).

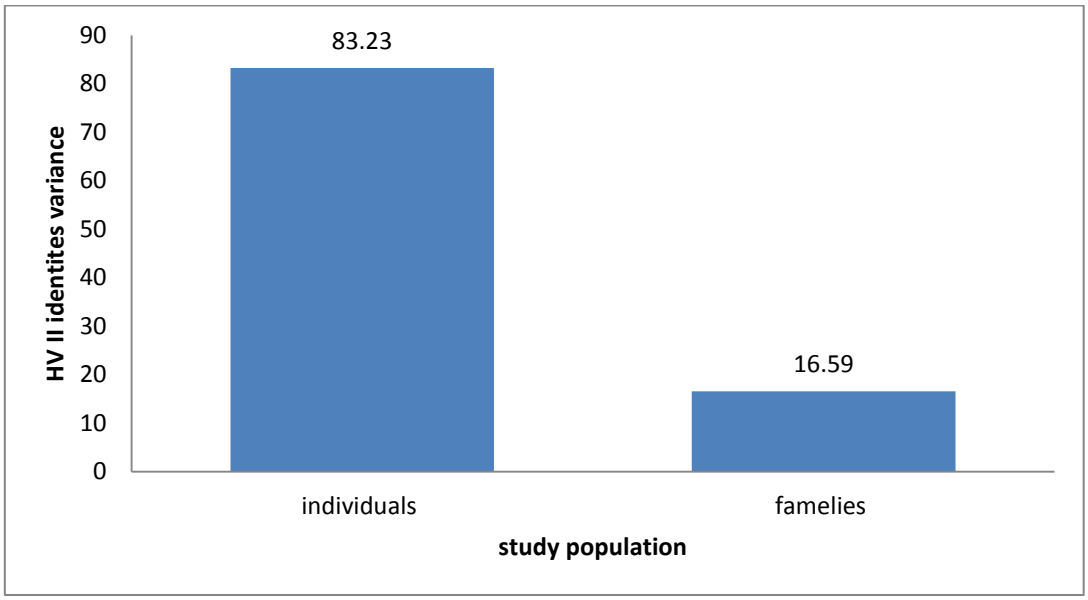

Figure (11): The variance value of identity percentage of individuals and families in study population.

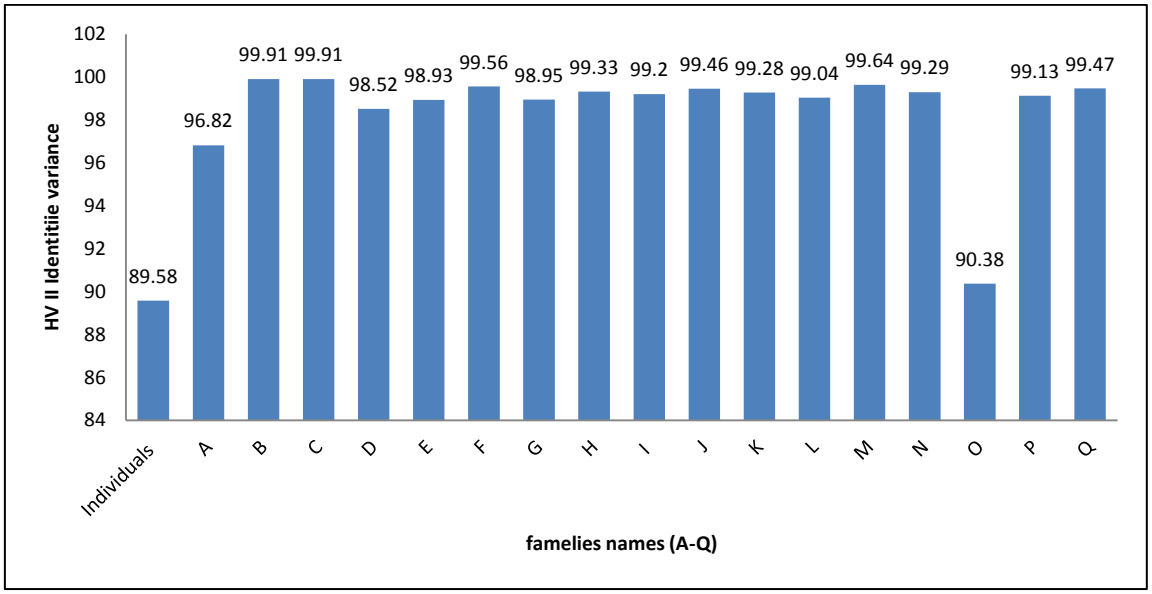

Figure (12): The variances value of identity percentage of families in study population(A-Q).

Discussion

The socio-demographic analysis of study population, and study groups (individuals and families)

The Iraqi population consists of different categories and different genders in addition to diverted nationalities, thus the genetic elements may be affected by other factors like lifestyle, pollution exposures and efficiency of DNA repair mechanisms. Although of absence the genetic data base of Iraqi population and poor information about the genetic diversity, some studies were suggested in last years, Hayder *et al*., (4) used Twenty-three Y-STR loci included in the PowerPlex Y23 (Promega, Madison, WI, USA) were typed in 254 males from the Iraqi Arab population. Ohied and Al-Badran(5) found that the phylogenetic analysis showed a large variability of the communities of Basrah; they didn't cluster on the phylogenetic tree.. Current study was suggested to estimate the genetic variation in 150 individuals in Babylon province utilized HVI and HVII region, The presence D- loop in the mtDNA control region is the most rapidly evolution due to the variations in sequances. There are 3 regions of hypervariable (HVI, HVII and HVIII) which tended to mutate 5–10 round quickly than DNA in nucleus, The mutation rate elevation of these hypervariable regions made utlized in population genetic investigations and for individuals discrimination analyzing (6).

Some investigations characterized the variations in nucleotide in contemporary populations and observed in modern Europeans typical variations of dozens nucleotides, more varied in Africa than in Europe(7, 8), and proved findings expand these output in a wide geographical range(10) suggested that the computational approaches power to process the questions about mtDNA population structure and its establishment by several studies that represented by an automated workflow. Furthermore, several of these statistics' interpretation about the possibility of mtDNA segregation and its contribution in disease still under investigation, like the characterize and expected variation

in different modern populations with social and biological data. In Iraq there were poor data about mtDNA variations, haplogroups and diversity in Iraqi populations in spite of the diverted in gender, nationalities and geographical differences.

The analysis of Mitochondrial DNA (mtDNA) genome has been a robust approach in forensic practice as well as in the understanding of human phylogeny in the maternal lineage. The control region is the traditional mtDNA analysis targeting, but the introduction of massive parallel sequencing (MPS) has made the typing of the entire mtDNA genome (mtGenome) more accessible for routine analysis. The complete mtDNA information can provide large amounts of novel genetic data for diverse populations as well as improved discrimination power for identification (11). The genetic diversity of the mtDNA sequence in different ethnic populations has been revealed through MPS analysis, but the Korean population not only has limited MPS data for the entire mtGenome, the existing data is mainly focused on the control region(12).

The identities of HVI and HVII with NCBI data

The results of current output show fluctuation between HVI and HVII identities and variance, the identities of HVI and HVII with data base of NCBI were detected in present study, the results showed non-significant changes between HVI and HV II in study population with NCBI data, and there were significant association between study groups (individuals HVI v HVII) and (families HVI v HVII) and also in HVII between individuals and families, significant highly identities of families female (HVII) and of individuals male, significant highly identities in HVI individuals and families with genetic disease and in healthy individuals, high identity observed in HVII in families than individuals. The variance in HVII was higher than HVI while in HVI the families variance was a higher than individuals, in families groups about five families have higher variance than others, in HVII the variance in individuals was higher than families and one family has higher and one family has higher variance, The diversity, similarities and difference in mtDNA are generated from different factors, first the high variable region in mtDNA have ability to mutate more times faster than nuclear DNA, second, absence of DNA repair system, third exposure to pollution through the life that made it more mutated(6).

Also, the rate of mtDNA mutation depends on other factors, like long exposure to oxidative stress value (OS) and the accuracy of the mtDNA polymerase, the ROS production is an inescapable of oxidative phosphorylation mechanisms generation in the mitochondrion, and these have harmful effect lead to DNA damage. Thus, the mtDNA experiences a raise mutation induction rate regarding to its proximity with the ROS production source, ROS levels also elevation by excess calories, pre-existing mtDNA damage, genetic variation in the regional mtDNA, and nDNA generation changes of stress response genes (13) where increased ROS production that lead to more ROS production over time. There was an exceptionally elevate mutation rate in mtDNA regarding to several unique characterizations of the mitochondrion, like heteroplasmy, its somatic mtDNA damage, meaning that wild-type and mutant mtDNA is coexisted, which segregated randomly through cell

division that may be caused heteroplasmy shifts. Since human cells have (hundreds – thousands) mtDNA copies (14). The ROS have been proved that Iraqi individuals have higher level of ROS and low level of total antioxidants molecules that may be impacted in the mtDNA.

In current study the comparison between identities with NCBI between HVI and HV II in study groups According to gender shows significant changes, the HVII of families was higher than HVI. The effect of sex in the mtDNA diversity were established to determine its impacted in some sex-based phenotyping (15). Frank and Hurst (16) suggested the theory of Population genetic predict that mtDNA development that male-harming mutations must be accumulated mtDNA when they have simple impact on women fitness. This evolutionary men-harming mutations build-up capable of potentially inflate the responsible of the mitochondrial encoded sequence to the phenotypic and fitness diversity of men, and encourage to asymmetries in mtDNA diversity in men relative to women. Gemmell *et al*. (17) introduced models determined the specific genetic adverbs of population that made big versus tiny asymmetries, in fitness diverted between the men and women. These theory proposed that the structure of population, inbreeding and kin selection perhaps restrict the men-harming mutations accumulation in the mtDNA(18, 19, 20).

The differences between men and women may be because the variation in effective population size of men and women. HEYER *et al*., (21) suggested some anthropological mechanisms that may clarified sex-specific diverted in population size impacted therefore, the human genomic variation: (i) reproductive success differences resulted from polygyny; (ii) rules of descent; and (iii) reproductive success transmission.

The genetic disease and other disease showed high identities in patients than healthy individuals in both HVI and HVII, Investigations clarified that Any mutation in coding sequence of mtDNA molecules combined with mutation in a D-loop construct a strong proliferative feature, would have the potential harmful impacts in old age, especially the neoplastic properties acquired, that lead to load of accumulate mutation in the population of mtDNA will create general problems in function and mitochondrial health and age-related senescence contributing (22). Other studies found there was no association between D- loop region and some disease (23) later other studies found an association between disease and high variable variation (24, 25).

Moreover, the variation in d-loop that including HVI and HVII may be undergo random genetic drift in somatic cells, it has been proved that the variation of D-loop may be impact in the mtDNA proliferation in embryo-derived stem cell lines (26).

Chinnery and Hudson (27) suggested that the mtDNA diversity is actually neutral or nearneutral, despite of the high number of research that deal with humans and animal models, there were non-pathogenic mtDNA sequences correlated with some phenotypic impacts, like longevity, disease susceptibility and livestock fertility (13, 14, 28, 29, 30). Johnston *et al*., (31) noted that there were evidences about the phenotypic impacts, other analyses have non-significant statistically about claims of mtDNA related to different diseases.

The exploitation of mtDNA diversity has increasingly become a source of recent forensic and anthropological evidences worldwide. Some investigations buttress the notion that mtDNA variation associate highly with the ethnic and geographic origin of an individual (32, 33, 34). Hypervariable region typing is powerful because it boasts a higher mutation rate and does not undergo.

Mendelian inheritance (only the mother passes clonal copies of her mtgenome to her progeny) or recombination. Thus, barring mutation, progeny maternally inherit an identical mtDNA haplotype. Although the hypervariable regions 1 and 2 (HV1, HV2) comprise forensic mitotypes, the HV1 region is primarily used to assign haplogroups (hg) in population genetics and anthropological investigations as the HV2 region has been demonstrated to show less genetic variability in various populations (35, 36).

The correlation coefficient of HVI identities with NCBI and individuals age shows non-significant weak positive correlation, in families' identities and age shows non-significant weak inverse correlation the correlation coefficient of HVII identities with NCBI and individuals age shows significant positive correlation. While the correlation of families' identities and age shows non-significant weak positive correlation. The association of age with genetic diversity depended on other factors like stress exposure, lifestyle and inherited patterns, Li *et al*., (22) found that more than 90% of non-proliferating cells may have at least one hindered mutation in each cell at the age of 70, and there wasn't cells would have less than ten mutations, proved in mtDNA the mutation perhaps implicate significantly with adult onset diseases.

The discrimination individuals HVI and HVII with their families

The comparison between identities HVI and HV II in families' relations shows non-significant in Girl identities with mother (p 0.757) and girl with sister (p 0.610), while other relation were significantly**,** these identities were performed among families members with them and with others families, results indicated that HVII was the best in these relations.

The mtDNA typing was used from 25 years cross the world to overcome the many human identification mass disasters, violent crimes, terrorism acts, simple crimes, in addition of missing persons problems. The typing of mtDNA still developing with the technologies progressing from tiny fragments examination to multiple mtDNA genomes sequencing in a short time. Forming a lineage genetic typing, the mtDNA genome can predicted the ancestors state, like health and disease expected (37). In spite of many peoples have acceptable reasons of information found about an unknown suspect's potential ancestral information, others have been found a potential genetic dispositions to disorders as being unacceptable. Moreover, de-nova approaches can sequence mitogenome for more information about forensic applications (38, 39).

DNA typing was initially used to bolster a case against a suspect previously identified through traditional means of evidence gathering. However, the evolution of DNA typing has far surpassed the limited confirmatory role of DNA evidence and led to the increase of DNA's probative value. Reid (40) supports this role by demonstrating that mtDNA HV1-based sequence analysis has a higher power of

discrimination for self- identified U.S. African Americans than for U.S. European Americans. With the Reid findings, he challenged the preconception of limited mtDNA utility and make three observations concerning mtDNA analysis for forensic typing: First, the discriminatory power of mtDNA analysis will vary based on the maternal lineage. There is greater mtgenome variability amongst populations with maternal African ancestry, Second, in cases of weak or alternative suspects, mtDNA HV1-based sequence analysis and haplogroup discrimination may provide new avenues for consideration and offer investigative leads, such as, inferring ethnicity from an unknown evidentiary sample. He also demonstrated greater than 90% concordance of self-identified ethnicity and haplogroup assignment, which supports the idea of inferring ethnicity based on mtDNA haplotype. Third, an increase in mtDNA HV1 population data will help to increase the power of discrimination for mtDNA typing by providing stronger statistical support.

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