The high variable DNA sequences discrimination efficiency (HVI and HVII) among some Iraqi families

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Abstract
The Iraqi population consists of different categories and different genders in addition to diverted nationalities ,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
Keywords: HVI and HVII ; DNA sequences; Iraqi families

Introduction

There is now a controversy about a handful of entirely distinct mitochondrial inheritance patterns that entail paternal transfer through a process known as "paternal leakage," which is hypothesized to occur during conception. Even though paternal discharge was previously supposed to be impossible, there are several research that support this development. The investigation of mitochondrial polymer in sheep has revealed that paternal inheritance is present. They proposed that each parent's mtDNA be present at an first stage of development because just one kind of mtDNA survived to adulthood (Luo et al. 2018).
Whereas the study involved an instance of mitochondrial disease induced by a 2-bp loss in the mtDNA. Through this investigation, a paternal mt haplotype was discovered inside the patient's extensor muscle in a very quantitative relationship of 10: 1 paternal to maternal kind. which was concerned with the examination of the patient's parents and sister's mt DNA. Yet, since 2002, this was the only example that demonstrated confirmation of paternal mtDNA inheritance in humans. Furthermore, paternal mtDNA was detected in a specific muscle tissue rather than in the blood, which is often used in mt DNA study. As a result of this discovery, the preponderance of paternal mtDNA transmission in this situation may be explained in terms of constrained choice factors, and paternal discharge cannot be generally considered as a key route of mitochondrial heredity. As a result, it was not envisaged that this change would have any bearing on the current investigation, which involves the examination of mtDNA in human blood samples. Several UN researchers, on the other hand, examined the evolution of paternal leaking and compiled a large body of evidence to back up their claims. First, regardless of whether there was paternal leaking, the parental mitochondria were diluted by the large diversity of maternal mitochondria, especially when going through the mitochondrial genetic bottleneck (Que et al. 2021).
Second, there is a proposed process for ubiquitination of the outer membrane of sperm cell mitochondria, which makes them identifiable. As a result, the process of chemical change digestion of the recently formed embryo cellular machinery will be used to target and eliminate them. Furthermore, mtDNA molecules are sequestered at intervals in the inner mitochondrial membrane, preventing heterologous recombination (Ricardo Leung et al. 2021) .
MtDNA is especially effective in situations when the extracted DNA sample is extremely tiny. Because of its manner of inheritance, the benefit of mtDNA studying as an exclusionary technique is frequently neglected. Unlike the nuclear genome, which undergoes chromosomal recombination, Mendelian inheritance, and replication repair, only the mother transfers clonal copies of her mtGenome to her children cross the egg. Thus, kids acquire an identical mtDNA signature that is shared amongst maternally related people, barring mutation (Filograna et al. 2021).

Methodology

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

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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=blastn&PAGE_TYPE=BlastSearch&LINK_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 ≤ 0.01 and P ≤ 0.05).

The discrimination individuals HVI with their families

The discrimination individuals with their families were assessed by detect the identity girls with their mother, boy with their mother, girl with their sister, boy with their brother, girl with brother. Results show high identity of girls with their mother in significant differences (p0.001) than other women (figure 1), the variance value was lower in girls with their mother than with non-mother (figure 2).

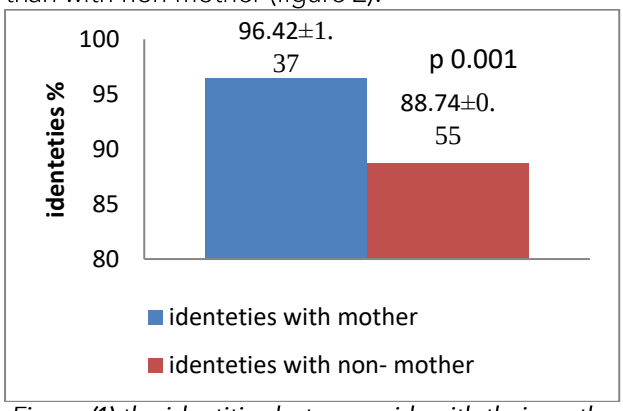


Figure (1) the identities between girls with their mother and other women (independent t test , p <0.05).

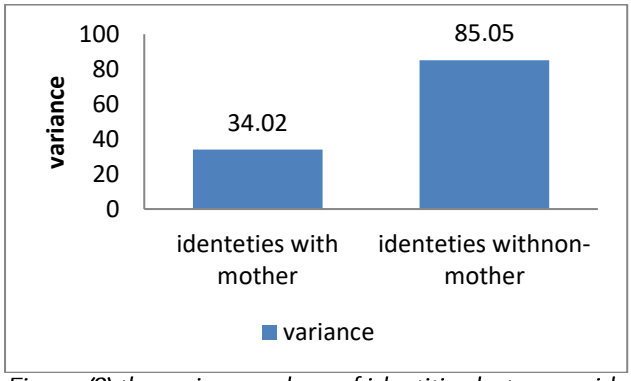


Figure (2) the variance values of identities between girls with their mother and other women

Results show high identity of boy with their mother in non- significant differences (p 0.099) than other women (figure 3), the variance value was higher in boy with their mother than with non-mother (figure 4).

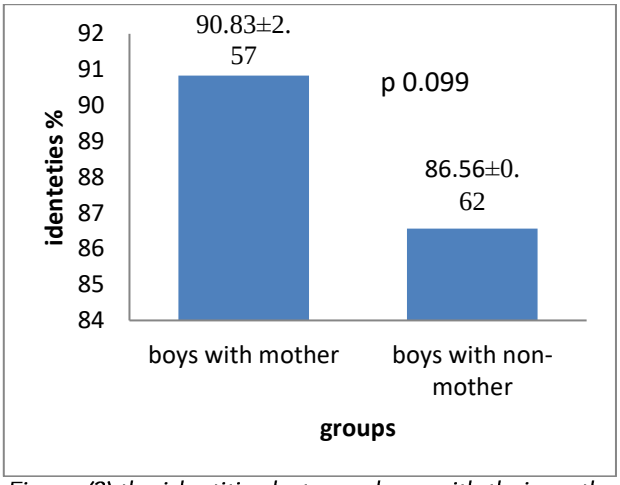


Figure (3) the identities between boys with their mother and other women (independent t test , p <0.05).

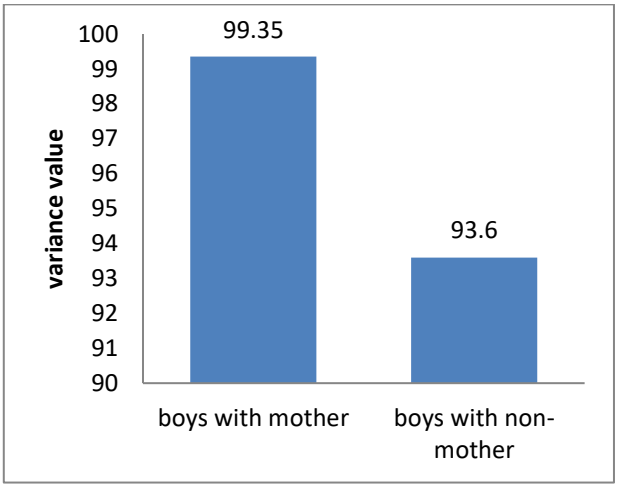


Figure (4) the variance value of identities between boys with their mother and other women

The identity of boys with their sister was higher than non -sister in non-significant differences (p 0.061) (figure 5), and the variance was low of boys with their sister than non-sister (figure 6) .

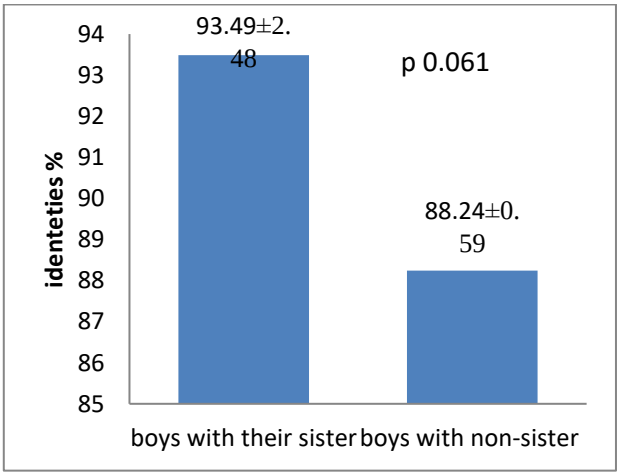


Figure (5) the identities between boys with their sister and non -sister (independent t test , p <0.05).

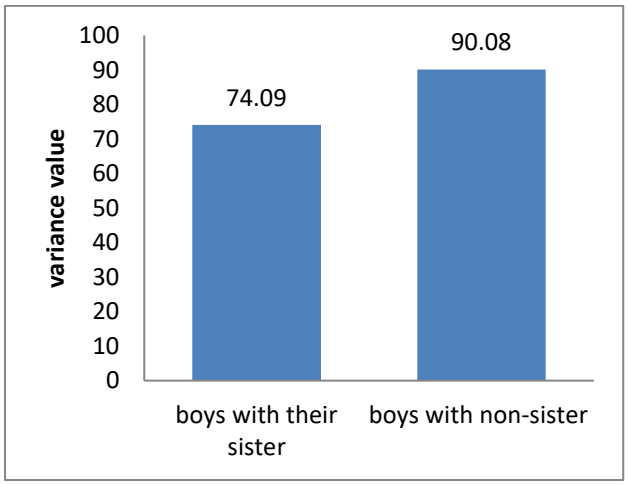


Figure (6) the variance value of identities between boys with their mother and other women

The relation between boys with their brother was lowering than with non-brother in non-significant differences (p 0.152) (figure 7), the variance of boys with their brother was lower than non-brother (figure 8).

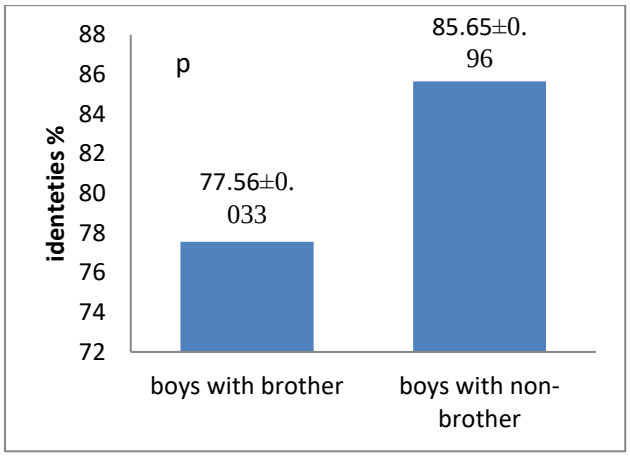


Figure (7) the identities between boys with their brother and non –brother (independent t test, p <0.05).

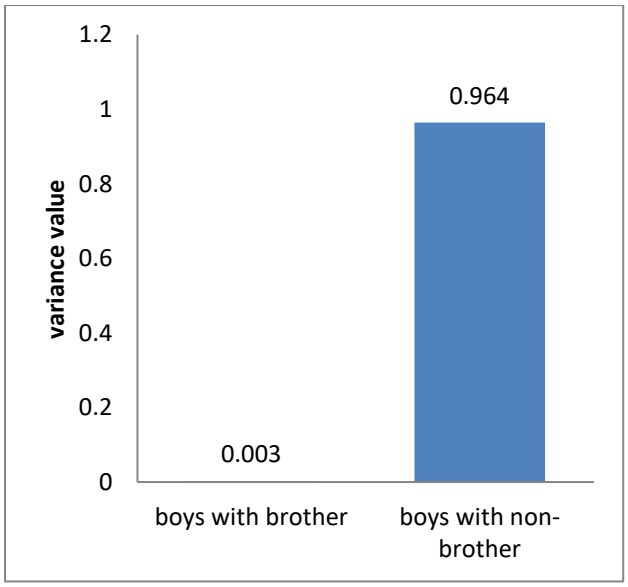


Figure (8) the variance value of identities between boys with their brother and non-brother

Non-significant changes were observed between girls with their sister and non –sister, identity was higher in girls with their sister (figure 9) and the variance was higher also than with non-sister. The variances also higher in girls with their sister than with non-sister (figure 10).

The hetero-plasmy of mtDNA were observed in some cases in both HVI and HVII (This figure for HVI).

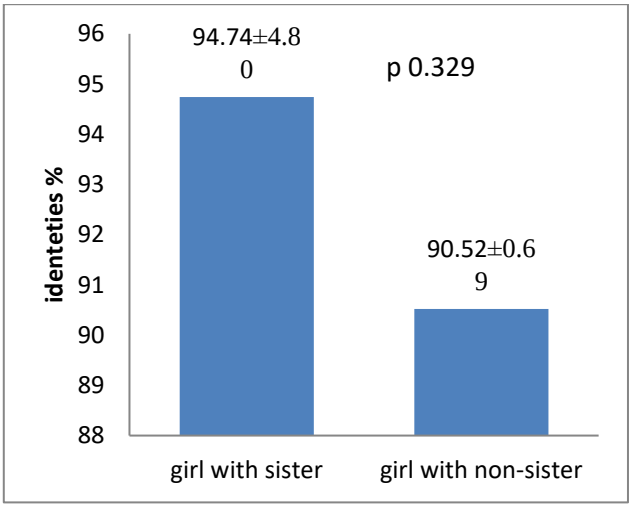


Figure (9) the identities between girls with their sister and non –sister (independent t test, p <0.05).

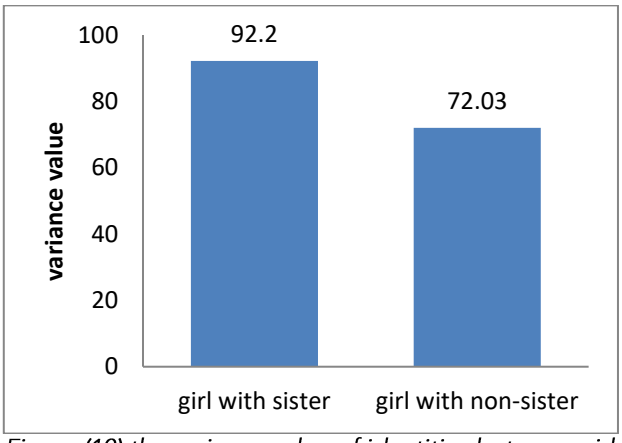


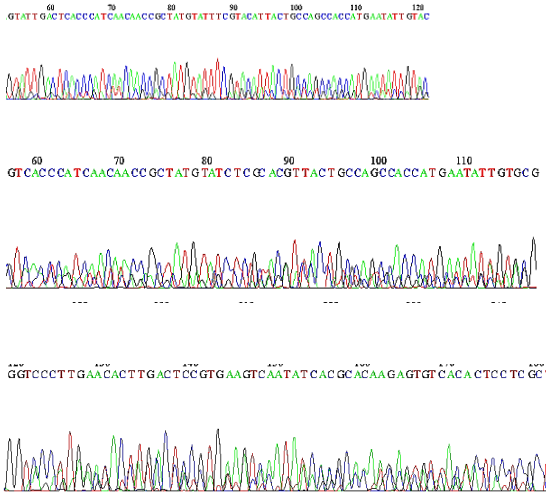
Figure (10) the variance value of identities between girls with their sister and non-sister

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 (Kang et al., 2017).

Chinnery and Hudson (2013) 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 (Wallace and Chalkia 2013, Dowling 2014, Wallace, 2015; Latorre-Pellicer et al., 2016; St John, 2016; Tsai and St John, 2016). Johnston et al., (2015) 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 evidence worldwide. Some investigations buttress the notion that mtDNA

variation associate highly with the ethnic and geographic origin of an individual (Yao et al. 2002; Lee et al. 2011; Catelli et al. 2011). 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 (Hoong and Lek 2005; Stoneking 2000).



This figure (11) the heteroplasmy of mtDNA HVI sequences in some male samples

The discrimination individuals HVII with their families

The discrimination individuals with their families were assessed by detect the identity girls with their mother, boy with their mother, girl with their sister, boy with their brother, girl with brother. Results show high identity of girls with their mother in non-significant differences (p 0.384) than other women (figure 12), the variance value was lower in girls with their mother than with non-mother (figure 13).

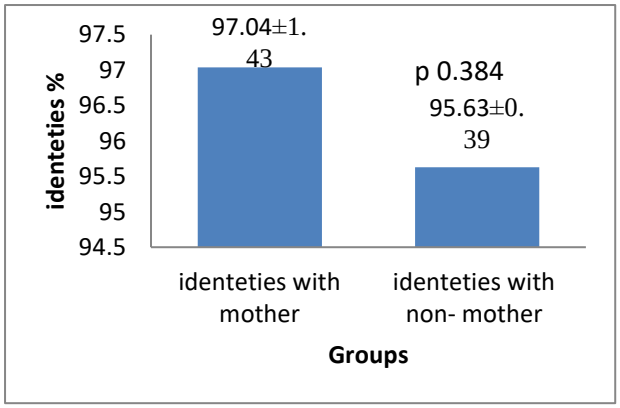


Figure (12) the identities between HV II girls with their mother and other women using HVII (independent t test , p <0.05).

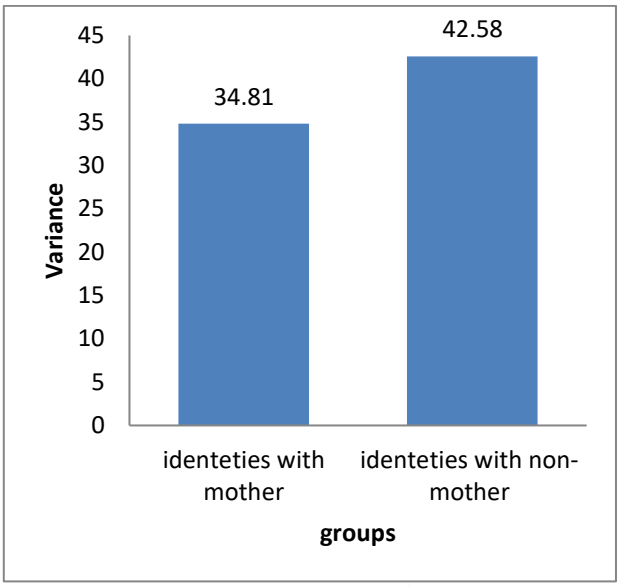


Figure (13) the variance value of HV II identities between girls with their mother and other women

Results show high identity of boy with their mother in significant differences (p 0.035) than other women (figure 14), the variance value was higher in boy with their mother than with non-mother (figure 15).

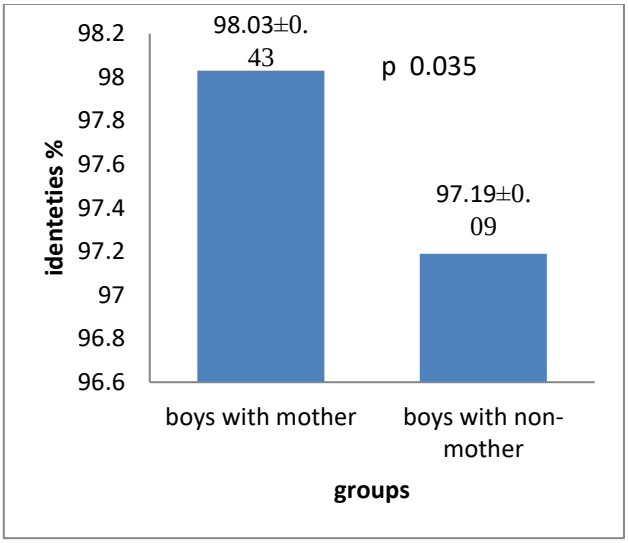


Figure (14) the identities HV II between boys with their mother and other women (independent t test , p <0.05).

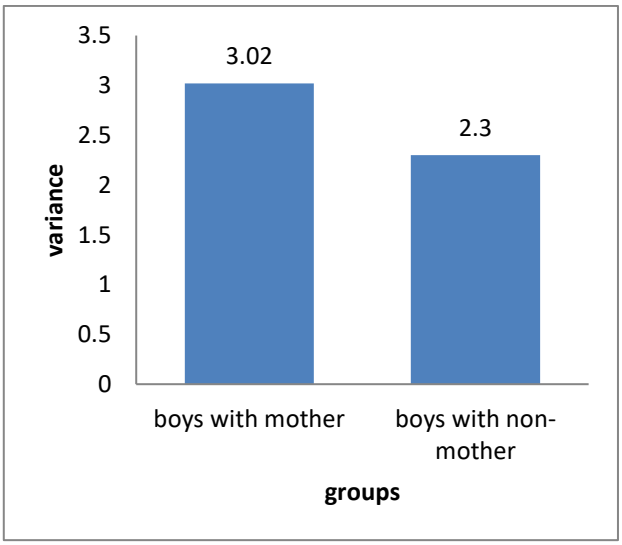


Figure (15) the variance value of HV II identities between boys with their mother and other women

The identity of boys with their sister was higher than non -sister in non-significant differences (p 0.255) (figure 16), and the variance was low of boys with their sister than non-sister (figure 17) .

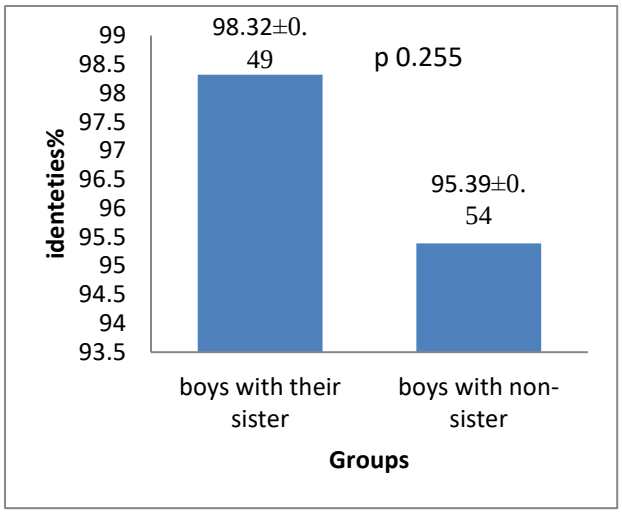


Figure (16) the identities of HV II between boys with their sister and non -sister (independent t test , p <0.05).

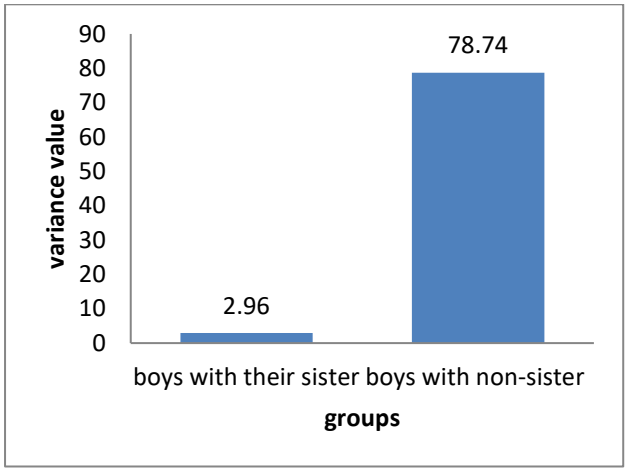


Figure (17) the variance value of HV II identities between boys with their mother and other women

The relation between boys with their brother was higher than with non-brother in non-significant differences (p 0.207) (figure 18), the variance of boys with their brother was lower than non-brother (figure 19).

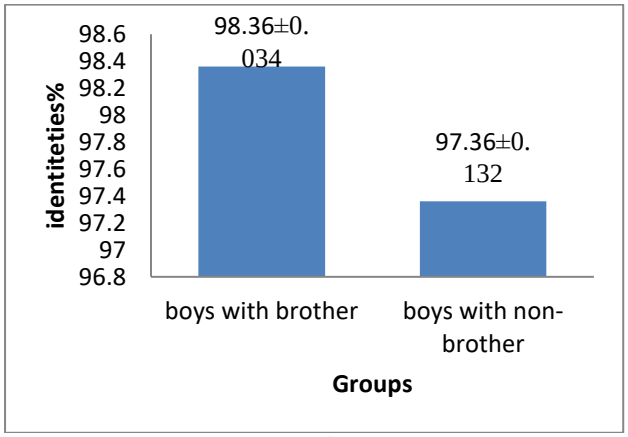


Figure (18) the identities of HV II between boys with their brother and non –brother (independent t test , p <0.05).

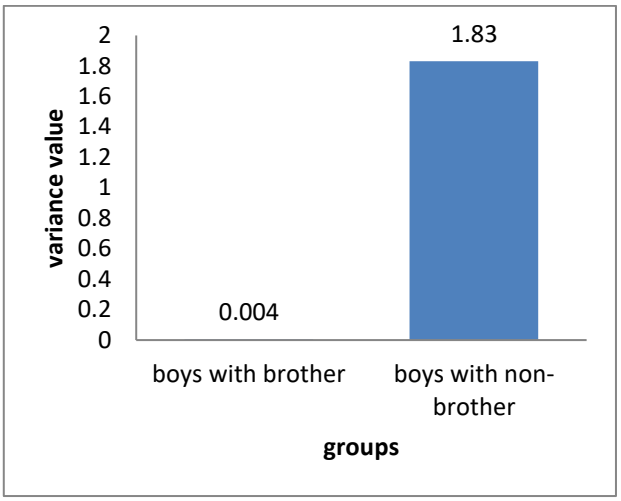


Figure (19) the variance value of HV II identities between boys with their brother and non-brother

Non-significant changes were observed between girls with their sister and non –sister, identity was higher in girls with their sister (figure 20) and the variance was lower in girl with sister than with non-sister (figure 21).

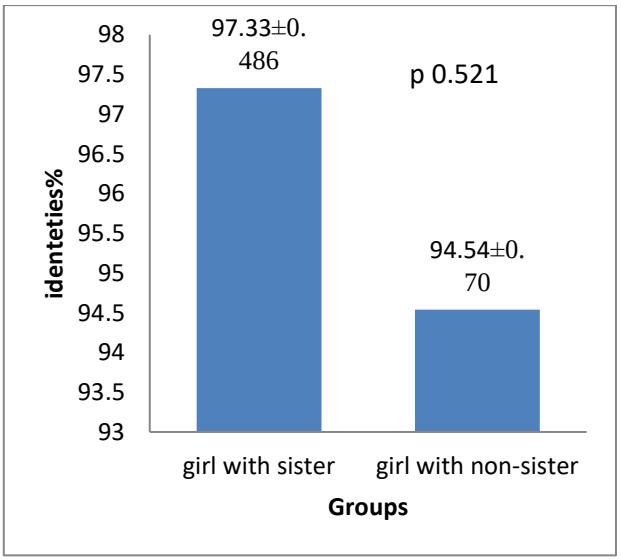


Figure (20) the identities of HV II between girls with their sister and non –sister (independent t test , p <0.05).

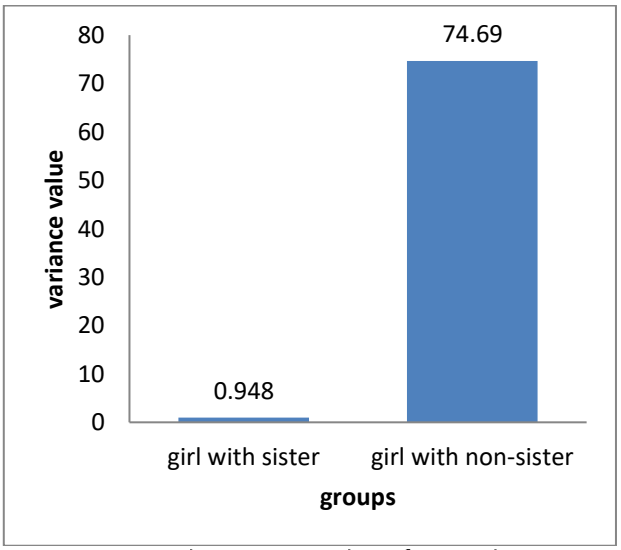


Figure (21) the variance value of HV II identities between girls with their sister and non-sister

The genetic variations of HVI was also depended in the discrimination among individuals and families, results show that there were (13.83) of HVI sequence was mutated (figure 22). According to families and individuals that mutated percentage was lower in families than individuals (figure 23).

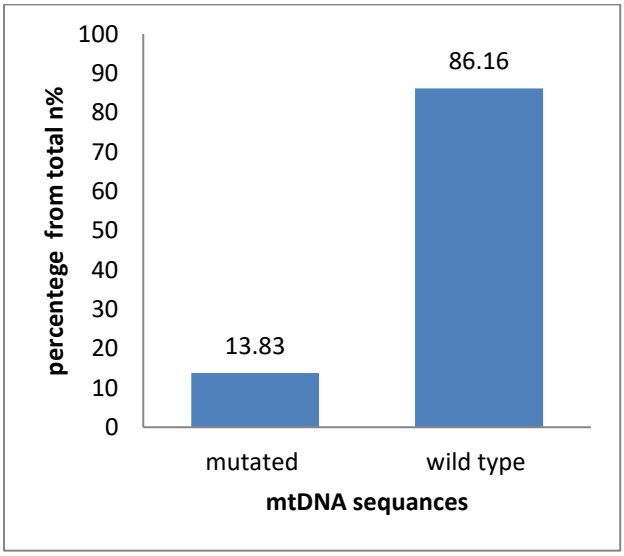


Figure (22) the mutated mtDNA sequences percentage of study population.

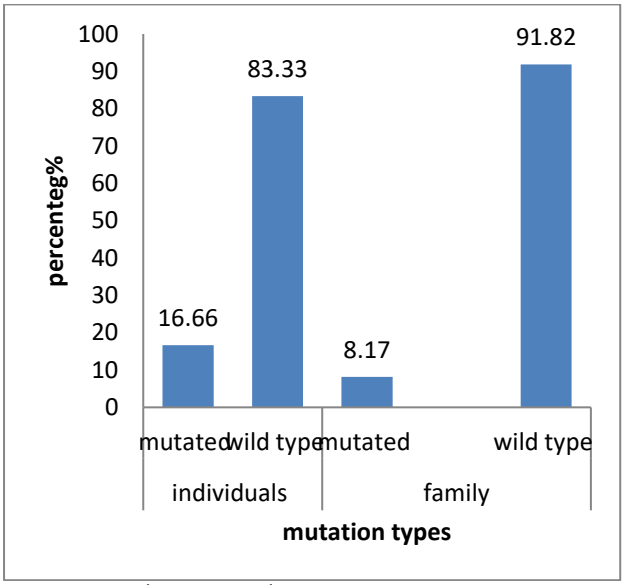


Figure (23) the mutated mtDNA sequences percentage of individuals and families.

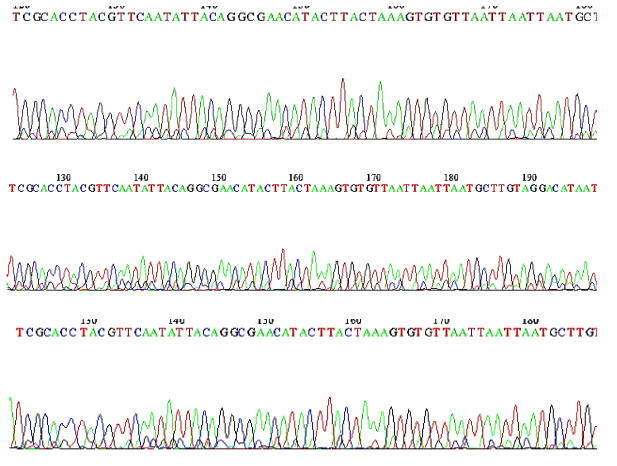


Figure the heteroplasmy of mtDNA HVII sequences in some male sample

3 The discrimination individuals HVI and HVII with their families

the comparison between identities HVI and HV II in family's 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 (Amorim et al., 2019). 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 sequencing mitogenome for more information about forensic applications (Tully et al., 2001; Prinz et al., 2007).

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 (2013) 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.

Low identities was observed between brothers in HVI in current study, studies like Hagelberg, (2003) and Hagstrom et al., (2014) were proved that The mtDNA sequences Differences can elevated in individuals

level in different mitochondria or population variation can be found in the same cell (heteroplasmy), The inherited of mtDNA in human, by mother's egg cell, also there wasn't recombination among mtDNAs . The nature of non-recombining made the diversities exist may be represented in a straightforward phylogenetic tree. The total mtDNA polymorphisms called a haplotype, and any hierarchical clade of haplotypes is a haplogroup, as a results of these features there was robust susceptible to genetic drift, this lead to presence different haplogroup patterns among different geographical areas, particularly on a continental scale (Røyrvik et al., 2016). Heteroplasmy was observed in some family members in current study which explained the lower identities in some relations.

For used myDNA in forensic application there were several concerns in court especially that association with the heteroplasmy document and the possibility of bi-parental inheritage. The important issues that need further investigations to prove the mtDNA robustness as an alternative tool for forensic human identification; are the molecular mechanisms of bi-parental inheritance, the capability of situations that can be occurred , and heteroplasmy characterization with better fidelity, are significant issues that need to be documented (Syndercombe, 2021). the current results found that the heteroplasmy may have role in the some un usual relations that occurred in HVII and HVI.

Syndercombe, (2021) found that the MtDNA sequencing provides an additional useful tool to characterize biological evidence, While its ability to identify an individual is limited because of the lack of recombination, it offers significant advantages when confirmation of maternal lineage is sought or where nuclear DNA is limited such as in the analysis of bones, teeth and hair, because of its high copy number. Understandably it is often used in the analysis of ancient DNA and in the triage of disaster victim identification (DVI). The rarity of a haplotype can be determined by simple counting of how many times the sequence is observed amongst samples of a database. As there may be various databases, both their representativeness and the quality of the sequences then becomes very important (Egeland et al., 2008).

The mtDNA sequence analysis of the remains of the Tsar'sbrother, Grand Duke of Russia Georgij Romanov, also demonstrated heteroplasmyat position 16169 (Ivanov et al., 1996). Comas et al. (1995) detected heteroplasmy at two positions(16293 and 16311) in the mtDNA from an anonymous donor's plucked hair. Wilson et al. (1997) observed a family that carried a mtDNA heteroplasmic state at position16355. In this family (a mother and two children), blood and buccal swab samplesdemonstrated the heteroplasmy, and in some cases individual hairs carried eitheraCoraTatposition 16355. Thus, some hairs from a single individual appearedhomoplasmic, and some hairs differed from one another at only one nucleotideposition. The fact that heteroplasmy occurs more often than originally observedand the mechanism and rate of heteroplasmy are not well defined are often raisedin admissibility challenges in an attempt to exclude mtDNA evidence. But withcareful evaluation, one can avoid erroneous interpretations.In forensic analysis, the mtDNA types between a known exemplar(s) and an ev-idence sample(s) are compared, and established interpretation guidelines are usedto assist in the evaluation. If the mtDNA sequences are the same at all defined sites,the interpretation is a failure to exclude the samples as possibly having the same origin (or under some circumstances the same maternal lineage). When heteroplasmyis observed, guidelines are in place to effect an interpretation. If the compared sam-ples have mtDNA sequences that are heteroplasmic at the same nucleotide sites,the interpretation is a failure to exclude. If one sample is heteroplasmic and theother homoplasmic, and they share the same bases at the defined sites, the interpretation also is a failure to exclude the samples as possibly having the same originbecause these samples share at least one mtDNA species. If both samples yield ahomoplasmic mtDNA profile, yet differ typically, for example, at only one site,additional investigation is warranted. If possible, additional reference samples areobtained and processed. If the evidence and reference samples differ by a singlenucleotide and there is no evidence of heteroplasmy, the comparison is reportedas inconclusive (i.e., there is insufficient information to render an interpretationof inclusion or exclusion). Some have suggested that one-base differences may befurther evaluated based on the rate of mutation or evolution (Alonso et al., 2002; Carracedo et al., 2000; Holland and Parsons, 1999; Tully et al., 2001) . Thisis a reasonable proposition; however, more data may be needed to apply properweight to the interpretation. In cases where two or more nucleotide differencesexist between the two sequences, generally an exclusion is the rendered interpreta-tion, although rarely a false exclusion will occur using this criterion. Heteroplasmyis most often observed in hair samples because genetic drift and bottlenecks are reated due to a hair follicle's semiclonal nature. If an evidentiary hair samplecontains one of the two heteroplasmic lineages observed in a reference sample(or vice versa), then an interpretation of an exclusion is incorrect. Lastly, hairsare the most likely tissue to express homoplasmic profiles that at times may differby one nucleotide within an individual. When the mtDNA sequence from a hairevidence sample differs from a known reference at one nucleotide position, typingadditional known hairs may resolve the issue.The suggestion that the presence of heteroplasmy renders forensic mtDNAanalysis invalid may seem heavy-handed, yet it is routinely raised in admissibil-ity hearings. The following is an example of how the existence of heteroplasmy has

been used to challenge the use of mtDNA evidence in the courtroom. Basedon current knowledge, when heteroplasmy is observed within the HV1 and HV2,sequences typically show evidence of a mixture or differ in sequence at one and,to a much lesser extent, two bases. However, Grzybowski (2000) reported muchhigher levels of mtDNA heteroplasmy in hair samples. Grzybowski sequenced justHV1 in 100 forcibly removed hairs from 35 Polish individuals. Thirteen of the 35people displayed heteroplasmy, and more than half of the heteroplasmic individualscarried multiple heteroplasmic sites. One individual was reported to have six het-eroplasmic positions. This level of heteroplasmy was substantially higher than waspreviously observed. Though inconsistent with typical observations, Grzybowski'sfindings (Grzybowski, 2000) were used to challenge the admissibility of mtDNA analysis of forensic samples (Budowle et l., 2002; D'Eustachio, 2000a and b), though to no avail. A single study may be an outlier and/ormay not be sufficient evidence to change established tenets. Though such a studymight be readily dismissed in the scientific arena, this sort of finding can persistin the courtroom. Regardless, oddities should not be dismissed in forensic DNAanalysis without review and due consideration.Grzybowski (2000) used at least three orders of magnitude more template DNAin the PCR, as well as approximately twice the number of cycles of PCR, thanroutinely used in forensic laboratories. Such analytical conditions are likely tosuccumb to contamination problems, and Tully & Lareu (2001) suggested thiscould be the basis for Grzybowski's findings. Budowle et al. (2002) concurred andcarried out a more in-depth review of the Grzybowski data. Since the same hairsamples were not available for reanalysis, we analyzed the natural human mtDNAHV1 variation within a reference sample population (Allard et al., 2002). Using the phylogeneticanalyses described above, we compared these analyses with that observed in theGrzybowski (2000) study.First, we found the primary sequence data displayed in the tables andfigures of Grzybowski (2000) inconsistent with the reported sites. The sequenceswere misidentified and/or outside the mtDNA regions that were initially ampli-fied. Second, only two of Grzybowski's (2000) four defined hot spots (i.e., rapidlychanging sites) match the most variable sites (16126 and 16311) known for U.S. andEuropean Caucasians (Allard et al., 2000). Using the SWGDAM mtDNA population data set (86)(http://www.fbi.gov/hq/lab/fsc/backissu/april2002/index.htm), many other sites(16093, 16129, 16172, 16183, 16189, 16192, 16261, 16362) change more rapidly n HV1 (Allard et al., 2000) than sites 16294 and 16296, which Grzybowski (2000) described as hotspots. In fact, site 16296 has a relatively slow rate of change (Allard et al., 2000). Sites that changerapidly in the mtDNA HV1 and are not listed as hot spots by Grzybowski (2000)include 16129, 16189, 16192, and 16362. Third, Grzybowski (2000) reported otherheteroplasmic sites that are uncommon in the Caucasian reference data set. Forinstance, sites 16088, 16105, and 16125 showed no variation when compared tothe 1771 Caucasians in the SWGDAM data set (1). Many of the heteroplasmic siteslisted by Grzybowski (52) are rare variants in the SWGDAM data set. These in-clude 16024 (nD6), 16090 (nD2), 16140 (nD4), 16167 (nD6), 16243 (nD7),16288 (nD5), and 16316 (nD11).

Other reason of mtDNA diversity is a Mitochondrial turnover that is stable in post-mitotic cells likes neurons, the damaged and old mitochondria taken away by mitophagy which carried by organelle host. However, the mtDNA sequance are lost continually in intact mitochondria regarding to destroy by chemical processes and ROS. The real rate of mtDNA turnover is ranged from few days to as long as a year in some cells (Poovathingal et al., 2012), it's also affected by requirements energy due to cell type; the excessive ATP generation contributed in the ROS elevation level lead to higher damage rate and turnover in mtDNA. Considering the more conservative evaluate of twelve months for the mtDNA half-life, the mean of non-proliferative cell required duplicate its entire mtDNA complement about 70 times (for instance undergo 70 population doublings) by 70 Y. just to sustained a steady copy number of mtDNA. another published issue estimates about 12 days (Larsson et al., 2010), there were about 2131 times of replication would observed by the age of 70 Y. In fact the picture may be diverted according to tissue thus, Poovathingal et al. (2012) assumed a conservative half-life evaluate of 3 mon., in line with the expected a half-life "in the order of months" proposed by This would imply 280 times of replication from birth to the age of 70 Y.

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