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Genetic studying of Mitochondrial DNA fingerprint using specific Primers to determine the differences in Saudi society

Salman S. Aloufi[1], Waleed A. Almalki[2], Naif N. Alshareef[3], Murad M. Alsaedi[4], Rakan M. Alqahtani[5], Siraj B. Alharthi[6], Abdullah S. Alsulaiman[7], Amani A Alharthi[8], Wafaa M. Almalki[9]

[1]Department of biotechnology, Faculty of science, Taif University, Taif, **kingdom of Saudi Arabia**
[2]Department of Medical Laboratory, Taif University, Taif, **Kingdom of Saudi Arabia**
[3]Medical Diagnostic Laboratory, king Faisal Hospital, Makkah, **kingdom of Saudi Arabia**
[4]Nursing management, king Salman medical city, medina, **kingdom of Saudi Arabia**
[5]Teacher at sons of Riyadh school, Riyadh, **Kingdom of Saudi Arabia**
[6]Department of Biological Sciences, King Abdelaziz University, Jeddah, **Kingdom of Saudi Arabia**
[7]Molecular Diagnostic Unit, Alhada Armed Forces Hospital, Taif, **Kingdom of Saudi Arabia**
[8]Taif University, **Kingdom of Saudi Arabia**
[9]Department of Medical Laboratory, Prince Sultan Military Hospital, Taif, **Kingdom of Saudi Arabia**

***Correspondence:** sirajj1411@gmail.com **Received: 26-02-2023, Revised: 25-03-2023, Accepted: 27-03-2023 e-Published: 31-03-2023**

Mitochondria are organelles within a mammalian cell that consist of inner and outer membranes in which important molecules pass through the outer membrane. Mitochondria are considered to be the energy centers within the cell because they produce energy for the body, producing nearly 90 percent of the energy in the form of adenosine triphosphate (ATP), through a process called oxidative phosphorylation. Mitochondria also have a special genome that is different from the nuclear genome, as it is double stranded and consists of thirteen polypeptides. The mitochondrial genome is circular and consists of 16,569 base pairs. Mitochondria are inherited through the mother, as diseases caused by mitochondrial abnormalities are passed down through the maternal line, whereas the mitochondrial genome has a higher rate of mutation than the nuclear genome. Saliva samples were used in this study, and deoxyribonucleic acid (DNA) was purified by a kit (Dnabler). Polymerase chain reaction (PCR) was used to amplify target sites of `seven specific locus, and samples were electrophoresed on an agarose gel. The results of this study indicate that there are both similarities and slight differences between ten samples from the Saudi community. This study concludes that with regards to the seven specific mitochondrial DNA locus, there were significant differences among people from Saudi, Pakistani, and British societies.

Keywords: Mitochondrial DNA, Primer, Fingerprinting, Forensic evidence.

INTRODUCTION

Mitochondria are cellular organelles with an extra chromosomal genome that is distinct from the nuclear genome (Sharma et al. 2019). The mitochondrial genome is made up of double-stranded deoxyribonucleic acid (DNA), exactly like the nuclear genome, which encodes genes. However, it varies from the nuclear genome in crucial ways (Falkenberg , 2018). The mitochondrial inner membrane is dispersed throughout the mitochondrial network (Yan et al. 2019). Furthermore, mitochondria are responsible for redox homeostasis, signaling, metabolism, immunity, survival, and apoptosis. They are the cell's powerhouse that converts food chemical energy into adenosine triphosphate (ATP), which is used for a variety of cellular processes (Chial and Craig 2008). Mammalian mitochondria are double-membrane organelles that produce around 90 percent of cell energy in the form of ATP through the oxidative phosphorylation (OXPHOS) process. The nuclear genome is linear, whereas the mitochondrial genome is circular and is made up of 16,569 DNA base pairs. While the nuclear genome has three billion, there are 37 genes in the mitochondrial genome that code for 13 proteins, 22 transfer ribonucleic acids (tRNAs), and 2 ribosomal ribonucleic acids (rRNAs). The 13 mitochondrial gene-encoded proteins all direct cells to make protein subunits of the OXPHOS system's enzyme complexes, which allow mitochondria to function as our cells' powerhouses.

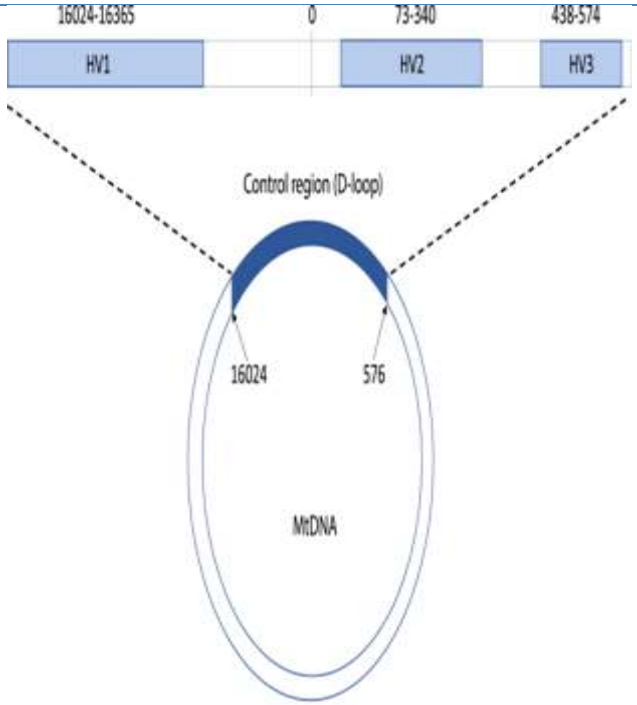


Figure 1: This figure shows the depiction of the mitochondrial genome's basic structure, highlighting the hypervariable areas (HV1, HV2, and HV3) that sit within the genome's control region, or D-loop section, which are of great interest to forensic scientists (adapted from Daeid et al. 2021).

Human mitochondrial DNA (mtDNA) encodes several important proteins; mtDNA is packed by multiple proteins to create a nucleoid that distributes uniformly throughout the mitochondrial matrix, which is necessary for mitochondrial function. A variety of disorders are caused by DNA defects or mutations. Mitophagy can destroy damaged mtDNA, and endonuclease G or mitophagy degrade all paternal mtDNA during fertilization. Mitochondria also have a little quantity of their DNA. This is sometimes referred to as mitochondrial DNA. Moreover, each mitochondria includes one or more copies of mitochondrial DNA, which are found in the mitochondrial matrix. Mitochondrial DNA is distributed throughout the mitochondria network and is required for the maintenance of mitochondrial function. In addition, the maternal line passes along mtDNA, whereas paternal mtDNA is destroyed during conception. Still, the removal of paternal mtDNA during fertilization has always been a controversial scientific topic. Mitophagy and endonuclease G have been implicated in paternal mtDNA clearance during fertilization. mtDNA is found in the mitochondrial matrix, which is coupled with the mitochondrial inner membrane and is dispersed throughout the mitochondrial network. Also, mitochondrial fusion is a two-step process that requires outer-membrane fusion followed by inner-membrane fusion. Mitofusin 1 and Mitofusin 2 regulate mitochondrial outer membrane fusion, while optic atrophy type 1 is involved in mitochondrial inner membrane fusion. Some human disorders induced by mtDNA mutations are passed down through the maternal line.

1.2 Mitochondrial genome

In eukaryotes, maternal inheritance is practically universal (Camus et al. 2022). The mtDNA sequence refers to a group of people from the same maternal lineage whose DNA is passed down from mother to child, as seen in Figure 2 (Daeid and Hackman 2021).

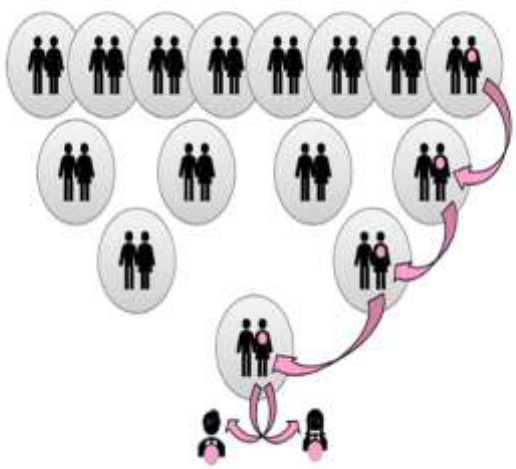


Figure 2: This figure illustrates mitochondrial DNA inheritance through four generations, showing ancestral mothers with the same mtDNA haplotype as the children at the bottom (adapted from Daeid *et al*., 2021).

The mitochondrial genome has a greater mutation rate of 100 to 1,000 times higher than the nuclear genome. The lack of mtDNA repair systems has been proposed as an explanation for the relatively high mutation rate in contrast to nuclear DNA. Normally, the mtDNA of all cells have the same sequence (homoplasmy). Mutations in mtDNA can affect bioenergetic genes, and those that are passed down to the next generation can cause severe hereditary diseases (Daeid and Hackman , 2021). Heteroplasmy shows itself in a variety of forms. In a single tissue, an individual could have many mtDNA types. In one tissue sample, a person may be heteroplasmic, whereas in another they are homoplasmic. Finally, an individual's mtDNA may be one type in one tissue, but a different type in another. The last scenario is the least likely to happen out of the three possibilities. When heteroplasmy is discovered in an individual's mtDNA, it usually consists of a single base difference in HV1 or HV2 (Wonnapinij et al. 2008).

1.3 Application of Mitochondrial DNA in the Forensic Field

In recent years, multiple parallel sequencing (MPS) technology has ushered in dramatic improvements in a variety of sectors. With this innovative technology, gigabytes of sequencing data may be gathered at a reasonable cost. As a result, forensic geneticists may be able to get more information from standard forensic identification markers and evaluate numerous markers at the same time, thereby reducing the amount of sample DNA used while also increasing the statistical weight of evidence. mtDNA typing is a valuable addition to forensic analysis, especially when nuclear DNA is limited or severely damaged. Due to technological limitations and prohibitive costs, forensic mtDNA analysis has traditionally relied on Sanger sequencing, which is the equivalent of 600 bp hyper-variable portions of mtDNA. The MPS platform has recently been utilized in research to sequence the entire genome of mtDNA, which includes updating the population structure of mtDNA. In forensic research, mtDNA profiles are used to identify human remains and compare profiles of missing people (Yao et al. 2018).

Objective

This study is a graduation project for a master's degree in genetic fingerprinting and forensic diagnosis. The study relies on the use of mtDNA to study the genetic differences between people of Saudi descent on specific mitochondrial genetic sites, which have also been studied in previous research in people of other nationalities.

MATERIALS AND METHODS

sample preparation and collection:

This research adopted the reliable method of simple random sampling. In this method, everyone from the population is chosen at random and has the same probability of being selected to be part of the tested samples. Consideration is given to their descent from different regional ,familial environments , and different ages , to enhance the chances that some of the differences will be studied.

The volunteers who used their samples for this research included a group of master's students and teaching staff from the Department of Biotechnology at Taif University, who came from a variety of familial and regional backgrounds. Written consent was gathered from the participants after explaining the study and its purpose. Saliva samples were then collected in a sample container, with a volume of two ml for each sample.

Subsequently, the DNA was extracted on the same day as the samples were collected, and the remaining samples were preserved, as research suggests it is possible to use a saliva sample when it is kept at room temperature for several days without influencing the accuracy and stability of the samples.

DNA Extraction

As mentioned previously, the samples were extracted on the same day as they were collected. Samples were used within hours to ensure accuracy, despite the existence of the study that concluded that samples could be kept for several days or more.

The DNA extraction kit from HAVEN SCIENTIFIC (Dnabler), was used for extraction. The protocol is compatible with a wide range of liquid clinical samples, including viral transport media and saliva.

Protocol work began by switching on a water bath and setting it at 50–60 degrees Celsius. We then transferred 200 µL of the sample to a separate, clean sterile tube, which was a standard 1.5 ml. After that, we added 20 µL of proteinase K before incubating for 60 minutes. We then performed the following protocol steps after the samples were completely homogenized:

We spun down the tube to collect the droplets that had condensed on the inside of the lid, then added 400 µL of 66–100% ethanol, and vortexed from thirty–sixty seconds. We then spun down the tube for 1–2 seconds.

We applied the whole lysate-ethanol mixture to a spin column embedded in a collection tube, centrifuged, discarded the flow-through, and dabbed the drop on the edge of the collection tube onto a tissue until dry. We replaced the spin column in the collection tube and centrifuged the spin column empty for 10 seconds before proceeding to the next step without discarding.

We added 500 µL of wash buffer one to the spin column, centrifuged and then discarded by dabbing the drop on the edge of the collection tube onto a tissue until dry. We then replaced the spin column in the collection tube and centrifuged the spin column empty for 10 seconds before proceeding to the next step without discarding.

We began to add 500 µL of wash buffer two to the spin column, centrifuged at 12,000 g for two minutes, then discarded the collection tube and did the spin column in a new, DNase/RNase-free 1.5 ml tube (elution tube).

We then added 30–50 µL of elution buffer directly to the center of the spin column filter without touching the filter with the tip.

The sample was then incubated at room temperature for two minutes before storing it in the freezer at –20 degrees.

The kit was purchased from www.havensci.com

Polymerase Chain Reaction Principle

Mullis founded and patented the polymerase chain reaction (PCR) in 1983. Its concept is based on the usage of DNA polymerase, which is a DNA polymerase that replicates specified DNA sequences in vitro. From a DNA extract, this approach may make tens of billions of copies of a Specific DNA fragments (the fragments of interest, DNA of importance, or target DNA) can be amplified using the polymerase chain reaction (PCR) technique (Mullis, 1990).

PCR Preparation

The sample was prepared in a PCR tube, and approximately 10 µl of master mix, 1 µl of forward primer, 1 µL of reverse primer, 1 µL of a saliva-extracted DNA sample, and 7 µL of distilled water were added. Furthermore, seven PCR tubes were prepared for each sample, and seven forward and reverse primers were added. Before adding the primers to the PCR tube, about 250 µl of distilled water was added to the primer in the primer tube. A dilution for the primer was then made in an Eppendorf tube, by adding 80 µl of distilled water and 20 µl of the primer (Kadri, 2019).

Targeted Mitochondrial DNA Primer

The first primer (MT1), has been used in a previous study to detect mtDNA in humans (Taylor et al. 2001). The second primer, consisting of MT2, MT3, and MT4, which are located on the mtDNA, has also been used in a previous study (Daud et al. 2014). For example, MT2 targeted the control region HV1, MT3 targeted the control region HV2, and MT4 targeted the control region HV3. Primers from the fifth to the seventh were used in a previous study; their purpose was to detect mitochondrial mutations that cause leukemia (Hutter et al. 2004). The fifth primer encoded MT5 in the HRV1 region of the mitochondria, while the sixth primer that encoded MT6, was used for the same purpose but in another mitochondrial region—the HRV2 region. The seventh primer encoded MT7 in the HRV3 region of the mitochondria.

PCR Primer

Primer Name	Sequence primer	TM	Molecular Weight g/mol
MT1F	(5- TGTAAAACGACGGCCAGT-3)	62.3	5532.7
MT1R	(5- CAGGAAACAGCTATGACC-3)	57.9	5501.7
MT2F	(5- TGTAAAACGACGGCCAGT-3)	62.3	5532.7
MT2R	(5- CAGGAAACAGCTATGACC-3)	57.9	5501.7
MT3F	(5- CAGGAAACAGCTATGACC-3)	57.9	5501.7
MT3R	(5- TGTAAAACGACGGCCAGT-3)	62.3	5532.7
MT4F	(5- CAGGAAACAGCTATGACC-3)	57.9	5501.7
MT4R	(5- TGTAAAACGACGGCCAGT-3)	62.3	5532.7
MT5F	(5- TCCACCATTAGCACCCAAAGC-3)	66.0	6304.1
MT5R	(5- TCGGATACAGTTCACTTTAGC-3)	60.6	6396.2
MT6F	(5- GGTCTATCACCCTATTAACCAC-3)	60.6	6614.4
MT6R	(5- CTGTTAAAAGTGCATACCGCC-3)	62.7	6390.2
MT7F	(5- CGCACCTACGTTCAATATTAC-3)	59.8	6325.2
MT7R	(5- GGGTGATGTAGCCCGTCTAA-3)	64.2	6173.1

PCR Cycle Steps

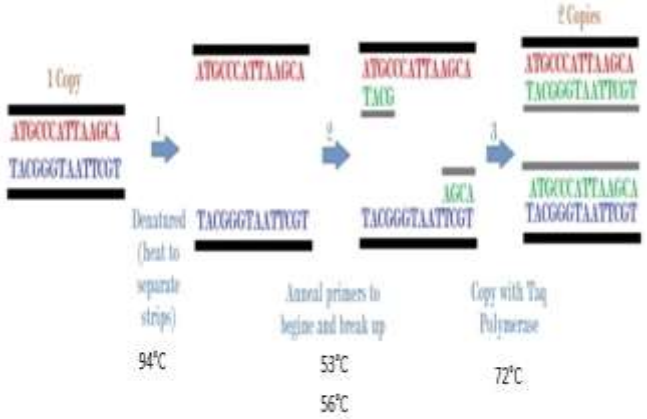


Figure 3: An illustration of how the PCR steps work.

The sample was processed in the PCR tube.

The total sample (n= 70) was warming up at 94 degrees Celsius for five minutes. This process included 42 cycles and began with the following steps:

Initial denaturation of DNA template by heat at 94 degrees Celsius for 45 seconds.

Primer annealing in two groups at different melting temperatures (TM) that aimed the denatured targeted DNA at a specific temperature of 53 degrees Celsius for 1 minute 40 seconds (n= 39) and the other group of the sample, the primer annealing to denatured targeted DNA at a specific temperature of 56 degrees Celsius for 1 minute 40 seconds (n= 31).

DNA synthesis by thermostable DNA polymerase (TAQ polymerase) at 72 degrees Celsius for 1 minute.

The final extension step occurred at 72 degrees Celsius for 10 minutes.

Gel Electrophoresis Principle

The most successful approach to separating DNA fragments of diverse sizes ranging from 100 bp to 25 kb is to use an agarose gel electrophoresis. Agarose is made up of repeating agarobiose (L- and D-galactose) subunits and is found in the seaweed species Gelidium and Gracilaria. During gelation, agarose polymers create a network of bundles whose pore widths control the molecular sieving capabilities of the gel. The separation of DNA was revolutionized by the use of agarose gel electrophoresis. Before the use of agarose gels, DNA was predominantly separated using sucrose density gradient centrifugation, which only provided a rough estimation of size. With agarose gels, DNA is placed into pre-cast wells in the gel and a current is supplied to separate it using agarose gel electrophoresis. Because the (DNA and RNA) molecule's phosphate backbone is DNA fragments that are negatively charged, it will start moving toward the positively charged anode whenever it is contacted by an electric field. Furthermore, DNA molecules have a constant mass/charge ratio, so they are segregated in a pattern based on their size on an agarose gel: the distance traveled is inversely proportional to the log of their molecular weight (M.W). The following factors influence the rate at which a DNA molecule migrates through a gel: the size of the DNA molecule, concentration of agarose, shape of the DNA, applied voltage, presence of ethidium bromide, agarose type, and the electrophoresis buffer after separation. Finally, DNA molecules can be seen under ultraviolet light after staining with a suitable dye (Lee et al. 2012).

Gel Electrophoresis Steps

The gels were then optioned by dissolving 1.5 g of agarose (ABM) in 100 ml of 0.5% of an electrophoresis buffer -Tris/Borate/EDTA (TBE, 50 ml 10 X TBE in 950 ml striatal distilled water). Then, it was heated with the help of a microwave for two minutes. After the agarose was dissolved and cooled down for five minutes, a green safe stain was added (10 µl / in 100 agarose). After that, an electrophoresis buffer (0.5% TBE) was added to fill the electrophoresis tank, and approximately 10 µl of each sample was loaded per well in the gel. GeneRuler 100 bp DNA ladder (Gendirex) was utilized to mark the different M.Ws 10 µl was added to. Electrophoresis was carried out at 120 V for approximately 60 minutes. Lastly, the DNA bands were imaged using a Gel Documentation Systems from UVP imager and software.

RESULTS

Molecular analysis

In this study, mitochondrial genomes molecularly to study genetic differences between some Saudi people on specific genes. The first gene was selected to identify the human mitochondrial mutation related to age through the use of primer MT1 (Taylor et al. 2001). The second, third, and fourth genes were selected from previous studies for three hypervariable regions (HV1, HV2 and HV3), that are encoding to 2 rRNAs, 22 tRNAs and 13 polypeptides mitochondrial genomes in humans by using primers MT2, MT3, and MT4 respectively (Hutter et al. 2004).The fifth, sixth, and seventh primers have targeted three hypervariable regions (HRV1, HRV2, and HRV3) to identify contamination of human hematopoietic cell lines that could be used for studying the differences between people in forensic fields (Hutter et al. 2004).

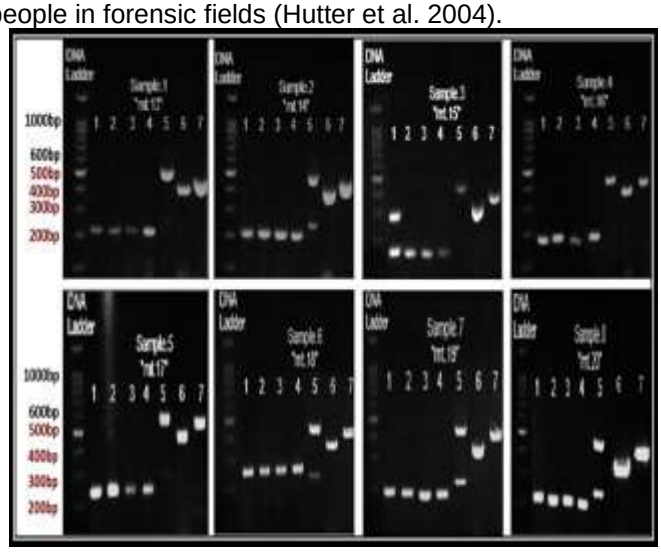


Figure 4: PCR gel electrophoresis for some mitochondria genes in humans after being photographed by UV rays.

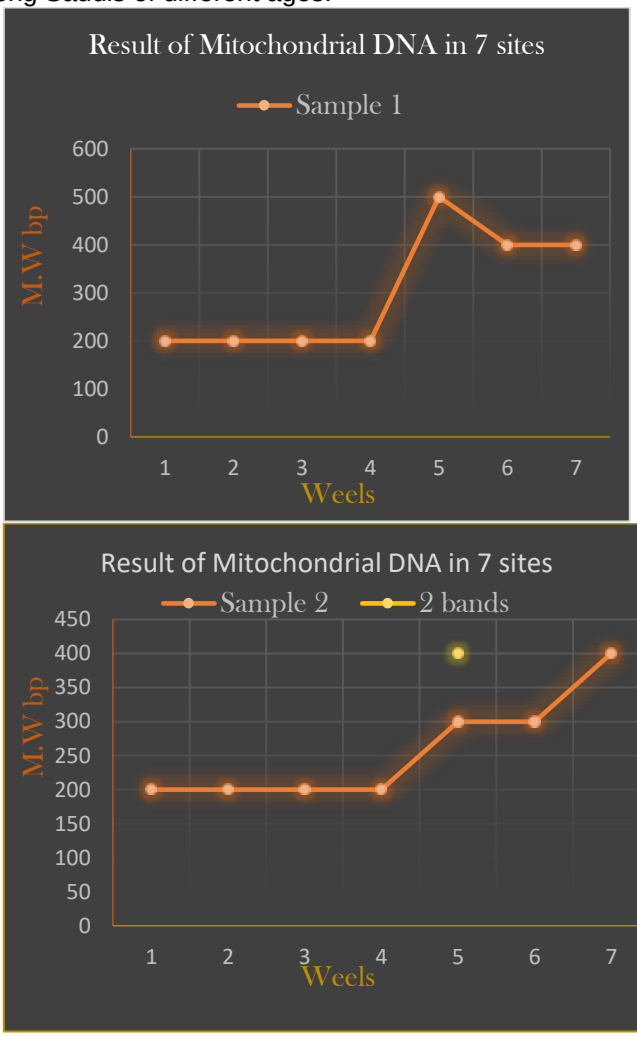
Agarose gel electrophoresis was used to detect seven specific genes in the mitochondrial genome for eight Saudi nationals. Lane 1 contained (MT1) amplification to detect the mitochondrial DNA in the samples that showed 100 bp DNA ladder from Gendirex. Lane 2 contained (MT2) amplification to detect the gene of HV1, which showed a band of around 200 bp. Lane 3 contained the PCR product using primers (MT3) to detect the gene of HV2, with a band of around 300 bp. Lane 4 showed amplification of (MT4) primer to detect the gene of HV3, which presented one band with around 400 bp. Lane 5 contained the amplification of the mitochondrial mutations

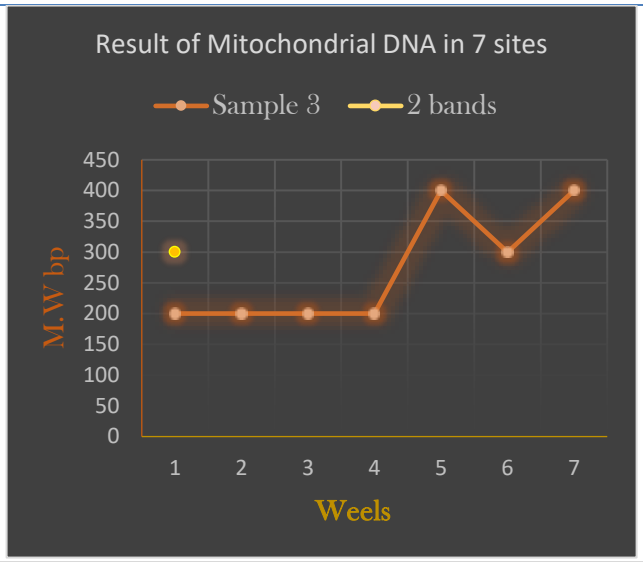
gene HRV1, and used primers (MT5) to show a band of around 500 bp. Lane 6 contained the amplification of the mitochondrial mutations gene HRV2, using primers (MT6) that showed a band around 600 bp. Lane 7 contained the amplification of the mitochondrial mutations gene HRV3, and used primers (MT7) that showed a band of around 700 bp. As shown in figure 4 above, the first well in the gel had primer 1, which detects human mtDNA heteroplasmy in single cells; both forward and reverse, depending on the sequence of MT1 that was used in a previous study (Taylor et al. 2001). In addition, samples 1, 2, 3, 4, 7, and 8 had similar M.Ws of approximately 200 bp. However, the weight of samples 5, 6, and 7 were higher than 200 bp. Apart from sample 3, there are 2 bands. One has a M.W of about 200 bp, while the other has a weight of around 300 bp. Moreover, primers were also observed in the three wells (2, 3, and 4) next to the previous well. These primers (MT2, MT3, and MT4) are responsible for three hypervariable regions, respectively HV1, HV2, and HV3 in human mtDNA. This enables them to analyze highly polymorphism sequences of degraded forensic samples and thereby to identify people who have lost a parent, as mentioned in the scientific article (Hameed et al. 2015). In addition, samples 4 and 7 are evenly sized—close to 500 bp. Furthermore, samples 5 and 8 had a M.W of around 500 bp.As can be seen in the agarose gel, well number 2 contained the position HV1 of mitochondrial DNA where there is a closeness in the M.W between samples 1, 2, 3, 7, and 8 at nearly 200 bp. On the other hand, in the remaining samples, such as samples 5 and 6, their M.W went up to more than 200 bp. Nevertheless, one band in this primer 2 (MT2) at sample 4 seemed to be close to 200 bp. However, it was not clear.

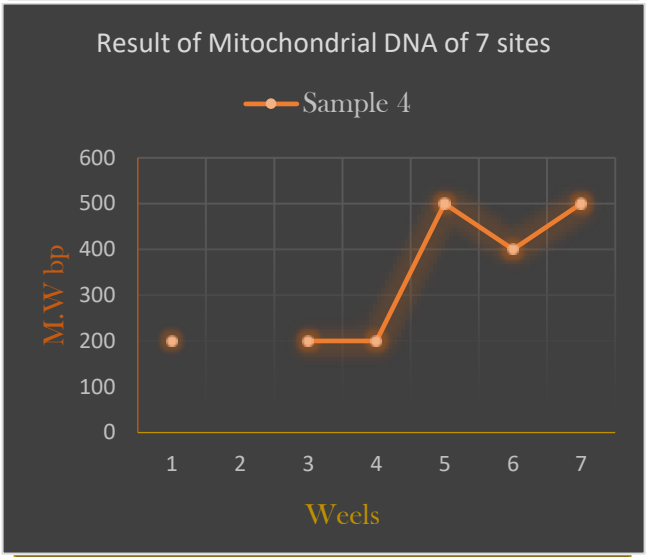
In addition, primer 3 (MT3) was found in well number 3 to know the position of mtDNA (HV2). This primer has the same M.W (200 bp) in samples 1, 2, 3, 4, 6, 7, and 8, while in sample 5 the M.W was higher than 200 bp. As shown in the image of the gel, well number 4 consisted of primer 4 (MT4) for the sit HV3 of mtDNA. There was a similarity between samples 1, 2, 3, 7, and 8, which had the same M.W of about 200 bp. Also, samples 4, 5, and 6 had the same M.W, which ranged higher than 200 bp.The last three samples in each gel were detacted with MT5 , MT6, and MT7 primers . These primers were used to know polymorphism in the noncoding region of the human's MT genome to understand the scientific meaning of human infection with leukemia and lymphoma on private sites like HRV1, HRV2, and HVR3. In well number five, the frequent appearance of two bands was evident for most of the samples for primer 5 (MT5), such as samples 2, 6, 7, and 8. To illustrate, there were two samples, 6 and 8, that had the same M.W; one band of them measured around 300 bp and the other around 400 bp. Furthermore, sample 7 had the same M.W equal to approximately 300 bp in one band and 500 bp in the other. The last sample had two bands in this well: sample 2 had a M.W between 200 bp and 300 bp in one of the bands, and 400 bp in the other. Otherwise, samples 1, 4, and 5 had a similar M.W of around 500 bp, while the M.W of sample 3 was around 400 bp. Primer 6 (MT6) was found in well number 6 for position HRV2 of mtDNA. As a result, samples 1, 4, 5, and 8 had the same M.W, almost equal to 400 bp, and sample 7 is also close to this M.W. Samples 2, 3 and 6 had a M.W upward of 300 bp. Finally, the last position, HRV3 for primer 7 (MT7), could be observed in well 7. Samples 1, 2, 3, and 6 had the same M.W of around 400 bp.

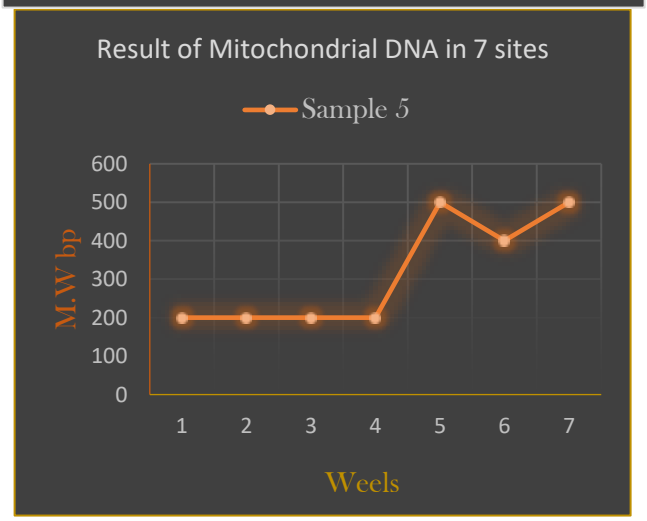
Statistical analysis:

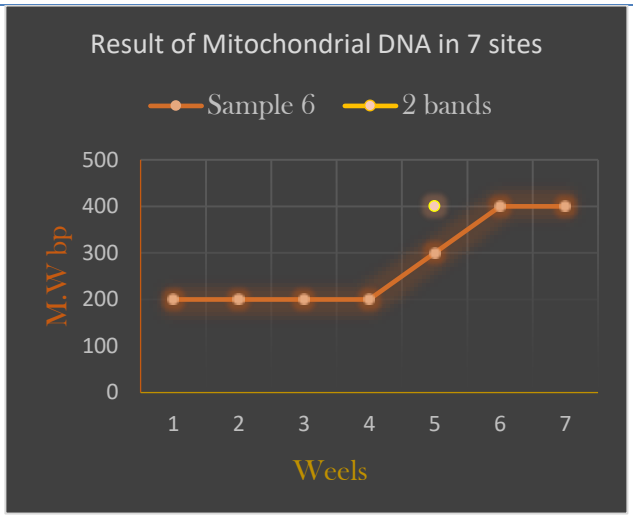
Based on the molecular results after the PCR, a graph was created to explain the similarities and differences in the seven target genes in the mitochondrial genome among Saudis of different ages.

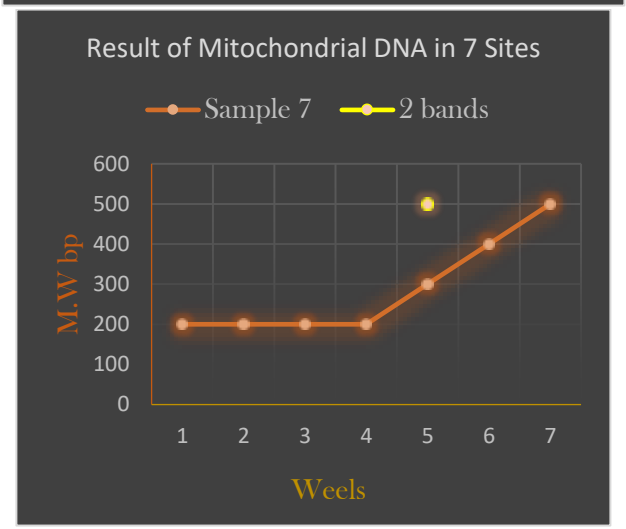












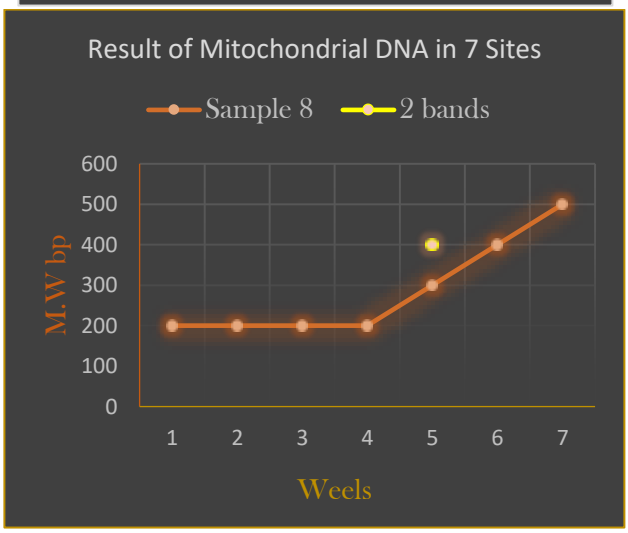


Figure 5: Statistical analysis to facilitate the interpretation of the results of the study for samples.

The above graphs simplify the interpretation and explanation of the molecular results of our research, as it became clear to us that the M.W of MT1, MT2, MT3, and MT4 are similar for all samples, except one more band in

sample number 3 with MT1. The result of MT5, MT6, and MT7, showed a significant difference in the M.W of these sites, with the presence of one more band for samples 2, 6, 7 and 8 with MT5.

DISCUSSION

This research aimed to study the differentiation between people depending on mtDNA in specific genetic sites in mitochondria by using a saliva sample and comparing the results to previous studies. This research adds information about mtDNA, which can help in many fields, such as the detection of the genetic mutations that occur in mtDNA and identifying people and distinguishing between them in forensic fields. We used seven primers to detect the target sites in mtDNA, both forward and reverse, and determine the differences between study samples. Compared with previous results, we found there were slight differences between people. The most important result we obtained is that the genetic sites that were targeted showed differences and some similarities between people from Saudi, British, and Pakistani societies. Previous studies used the same genetic sites that were studied in our research, thereby allowing comparison.

Regarding the primer 1 (MT1) in our result, most of the samples have similar M.Ws, ranging from approximately 200 bp and above. Apart from sample 3, two bands are doubled , one of which has a M.W of about 200 bp and the other 300 bp. The result from previous research was a M.W of 1,905 bp based on the different criteria during the procedure. There are three primers responsible for three hypervariable regions respectively: HV1, HV2, and HV3. These analyze sequences of high polymorphism of degraded forensic samples in human mtDNA. In our results, primer MT2 showed the M.W for all samples were around 200 bp or more than 200 bp. However, the result of the previous study is approximately 480 bp band for the HV1 region of mtDNA, which was visualized under UV light. Regarding the position, HV2 showed the M.W in all the samples to be around 200 bp although in the previous study by Hutter*.,et al* (2004), was 420 bp. The HV3 region of mtDNA in our result showed all the samples were 200 bp or higher. On other hand, the M.W in the previous result was 255 bp. Finally, three specific polymorphism regions in mtDNA were used for studying contamination of human leukemia-lymphoma cell lines (HRV1, HRV2, and HRV3) for primers MT5, MT6, and MT7. HRV1 showed two bands in most of the samples, with a M.W of around 300 bp, while others were around 400 bp.A few samples with one band had a M.W of 500 bp, while the previous result showed a M.W of approximately 521 bp. Regarding HRV2, all samples showed a M.W between 400 and 500 bp compared with the previous result that showed a M.W of around 422 bp. HRV3 with primer, showed the M.W for the most samples between 400 and 500 bp in one band, except for one sample with two bands.

This compares to a previous result that showed a M.W of 541 bp (Hutter et al. 2004). We noticed that when comparing the results from our study and previous studies of the Saudi, British, and Pakistani populations, that there is some similarity in some primers and differentiation in others (Liu et al. 2006). Different results in MT1, MT2, and MT3 were found between our study and previous results, while MT4, MT5, MT6, MT7 showed comparable results. The differences between the populations of these nationalities may play a role in the different results. Also, the different types of samples may be another reason, as we used saliva samples while other studies used blood samples. Furthermore, the mitochondrial genome has a 100-fold higher mutation rate than the nuclear genome. As a result, mitochondria are heteroplasmic with variations within the same cell, and even within the same mitochondria.

CONCLUSION

In general, more than one site has been detected for identifying seven specific (target) sites of mitochondrial genomes of Saudi people. According to the readings obtained in this research for the length of bands, and after comparing them with British and Pakistani studies, we recommend the use of other primers that have a design character of another type to identify the similarities and differences between people. We also recommend searching genomic mitochondrial sites other than the ones mentioned in our study, which are HV1, HV2, HV3, HRV1, HRV2, and HRV3. In addition, consideration should be given to setting fixed global standards for the same type of sample, whether it be saliva, blood, or plasma, etc., to ensure that we know the accuracy of the convergence or difference between races. Finally, we recommend increasing research in the field of mitochondrial DNA and its sequences in Saudi society due to the lack of published research. This study was done to prove that the rate of mutations in the mitochondrial genome is high, and that the repair system is very weak. For this reason, we found significant differences between people. Therefore, the mitochondrial genome cannot be relied upon as a tool in the field of genetic fingerprinting to prove or deny crimes and can only be used in paternity cases from the mother's side.

CONFLICT OF INTEREST

The authors declared that the present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

All authors contributed equally and have been involved in the writing of the manuscript at draft, any revision stages, and have read and approved the final version.

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