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The Association between Human Mitochondrial DNA Deletion in Sperm and Infertile Jordanian Men with Asthenozoospermia

العلاقة بين حدوث طفرة في الحمض النووي لميتوكندريا الحيوانات المنوية لدى الانسان والعقم الذكوري الناتج عن ضعف حركة الحيوانات المنوية في الاردن

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Dedications

This thesis is dedicated to my parents, whom without their supplication and prayers I would not be here. I extend my gratitude to my wife and daughters for their patience and putting up with me during my study. I would like to express my deepest gratitude for my brothers and sisters for supporting me.

Mohammad Al Smadi

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Table of contents

Subject

Chapter One

Chapter Two

Chapter Three

Chapter Four

Chapter Five

List of Tables

LIST OF FIGURES

List of Abbreviations

Abbreviation	Description
ATP	Adenosine triphosphate
BLAST	Basic Local Alignment Search Tool
CI	Confidence interval
CO I	Cytochrome Oxidase Subunit I
CO II	Cytochrome Oxidase Subunit II
CO III	Cytochrome Oxidase Subunit III
Cytb	Cytochrome b
DDT	Dichloro-Diphenyl-Trichloroethane
DF	Deleted Forward
DR	Deleted Reverse
EDs	Endocrine Disrupters
HPG axis	hypothalamic–pituitary–gonadal axis
ICSI	Intracytoplasmic sperm injection
IVF	In*vitro* Fertilization
MtDNA	Mitochondrial Deoxyribonucleic Acid

Abbreviation	Description
NADH	Nicotinamide Adenine Dinucleotide Hydride
NCBI	National Center for Biotechnology Information
ND	NADH Dehydrogenase
NO	Nitrogen Oxide
OAT	oligo-astheno-teratozoospermia
OR	Odd Ratio
OS	Oxidative Stress
PAHs	Polycyclic aromatic hydrocarbons
PCR	Polymerase chain reaction
PUFA	Polyunsaturated Fatty Acids
ROS	Reactive Oxygen Species
rRNA	Ribosomal Ribonucleic Acid
tRNA	Transfer Ribonucleic Acid
TUNEL	Terminal deoxynucleotidyl transferase dUTP nick end labeling
WF	Wild type Forward
WHO	World Health Organization
WR	Wild type Reverse

Abstract

The Association between Human Mitochondrial DNA Deletion in Sperm and Infertile Jordanian Men with Asthenozoospermia

Submitted By

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Infertility, a trouble in conceiving a child after 1 year of unprotected intercourse, occurs because of either a female or male factor. Approximately 15% of human couples suffer from infertility. One of the most factors that cause male infertility is reduced sperm motility (Asthenozoospermia). Asthenozoospermia is defined as the proportion of motile sperms under the lower limit for total motility, which is 40 % of total sperm motility. Asthenozoospermia is the second important cause of male infertility after oligozoospermia (reduced sperm count) as sperms with weak motility cannot go through mucus-filled cervices and cannot reach at the site of fertilization. Human mitochondria carry their own genomic DNA. It is circular, double-stranded , and 16,569 bp long consists of 37 genes encoding 13 polypeptides forming a component of respiratory chain oxidative phosphorylation (OXPHOS), in addition to 22 tRNAs and 2 rRNAs (12S and 16S) essential in protein synthesis. The 4977-bp mtDNA deletion is the most widespread and common mtDNA mutation. The molecular approaches have discovered that mitochondrial genes play a significant role in the construction of mature sperm and progressive flagellar movement after ejaculation. Sperms with mtDNA deletion generate insufficient amounts of ATP; this will lead to reduction of the sperm motility.

This study aims to investigate the association between 4977-bp human mitochondrial DNA deletion and Asthenozoospermia in Jordanian men. In this study semen samples of 120 asthenozoospermic infertile men and 80 controls were collected from *Invitro* fertilization IVF units in Jordan. After extraction of spermatozoa DNA, polymerase chain reaction was performed. The deletion of 4977-bp mtDNA was observed in 79.2% of patients with asthenozoospermia compared with 10% in controls, OR = 34.2000, 95% confidence interval = 14.5736, 80.2574, p- value < 0 .001]. It is concluded that there is a significant correlation between sperm 4,977-bp mtDNA deletion and asthenozoospermia in Jordanian population.

Keywords: sperm, asthenozoospermia, mitochondrial DNA, motility, male infertility, Jordan

Chapter One

1. Introduction

Infertility is defined as a trouble in conceiving a child after 1 year of unprotected intercourse. It occurs because of either a female or male factor (Kumar and Sangeetha, 2009). Approximately 15% of human couples suffering from infertility, and the male factor accounts for about 50% of these cases of infertility (Moore and Reijo-pera, 2000).

The diagnosis of male infertility can be achieved by inexpensive semen fluid analysis, as reported by the World Health Organization criteria (WHO 2010). True evaluation for the semen fluid involves the assessment of semen liquefaction, semen volume, antisperm antibodies, sperm count, morphology and motility (Agarwal *et al*., 2008).

Reduced sperm motility (Asthenozoospermia) is the proportion of motile sperms under the lower reference limit for total motility, which is about 40% of total sperm motility. (Eberhard Nieschlag, *et al*., 2010). Asthenozoospermia is the second important cause of male infertility after oligozoospermia (reduced sperm count) (Gavella and Lipovac, 1992). Sperms travel to the oocyte by forward progressive movement; mitochondria in the sperm provide it with the required energy by oxidative phosphorylation (Rajender *et al*., 2010).

Human mitochondrial deoxyribonucleic acid (mtDNA) has a unique and distinctive structure; it does not contain histones and other DNA-binding proteins, so it is replicated quickly without DNA repair system apparatus (Shamsi *et al*., 2008). The lack of protection by histones and the lack of DNA repair mechanisms increase the opportunity of attacks on the unprotected mtDNA by free radicals or by reactive oxygen species. Also mtDNA has a large number of direct repeats of size from 4 to 17 base pair (bp), and then some large-scale deletions may be resulted (Shoffner *et al*., 1989).

The 4977-bp mtDNA deletion is the most widespread mutation among mtDNA deletions (Guo *et al*., 2017). About 40% of individuals with mitochondrial myopathy showed this deletion (Shoffner *et al*., 1989). The molecular approaches have discovered that mitochondrial genes play a significant role in the construction of mature sperm and progressive flagellar movement after ejaculation (Nakada *et al.*, 2006).

The role of 4977-bp mtDNA deletion in asthenozoospermia has been studied in many populations such as Chinese, Italian, British, Greek and Indian. However their results were contradictory. Some researchers found a negative correlation between the mtDNA "common" deletion and fertilization efficiency of spermatozoa (St John *et al*., 2001), while others found that there is an elevated incidence of 4977-bp mtDNA deletion in Asthenozoospermic patients (Kao *et al*., 1995). Therefore, the current study aims to investigate the association between 4979-bp mtDNA deletion and Asthenozoospermia in Jordanian population.

Chapter Two

2. Literature review

2.1 Spermatogenesis

Spermatogenesis is the process of germ cell development in the somniferous tubules of the testes throughout life from teens to elderly men. Spermatogenesis result in production the mature male gametes, which called the spermatozoa. It depends on hormonal control such as follicle-stimulating hormone (FSH) and testosterone, which their sites located in the sertoli cells (Davidoff et al., 1990).

Spermatogenesis can be divided into three distinctive stages: spermatocytogenesis (generating the spermatocytes), spermatidogenesis (production of spermatids) and spermiogenesis where the spermatids form their tail, the upper part of the of the tail is known as midpiece, it is thick due to the arrangement of mitochondria inside, spermatids DNA is packaged and become more condensed (Holstein *et al*., 1987) .After that the acrosome is generated by Golgi-derived vesicles incorporation with many acrosomal proteins (Moreno *et al*., 2000) .

There are two types of spermatocytes divisions, the primary spermatocytes and the secondary spermatocytes. The cells in the primary spermatocytes undergo the stages of prophase: the zygotene, the pachytene and the diplotene stage where the DNA replication, crossing over and reduction of chromosomes to haploid form occurred. While in the secondary spermatocytes produced by the division the germ cells, but unlike primary spermatocytes their division does not include reduction of chromosomes number. DNA-replication and divide quickly to the spermatids. The result of the primary and secondary spermatocytes divisions are 4 haploid spermatids, which they

become mature by spermiogenesis and now they are known as spermatozoa (Griswold, 1998).

Leydig cells are prominent cells consist of groups adjacent capillaries. They secrete the male sex hormone like testosterone, which plays a role in the maintenance of spermatogenesis and stimulation of the sexual behavior (Payne *et al.*, 1996).

2.2 Structure of human sperm

Mature sperm has a simple and well-defined structure, including the head and the tail (Fig. 1). The head contains the acrosome, which is rich in digestive enzymes including hyaluronidase. These enzymes are necessary to breakdown the zona pellucida layer which surrounds the ovum. The tail or flagellum includes the midpiece packed with mitochondria (Baccetti *et al*., 1984).

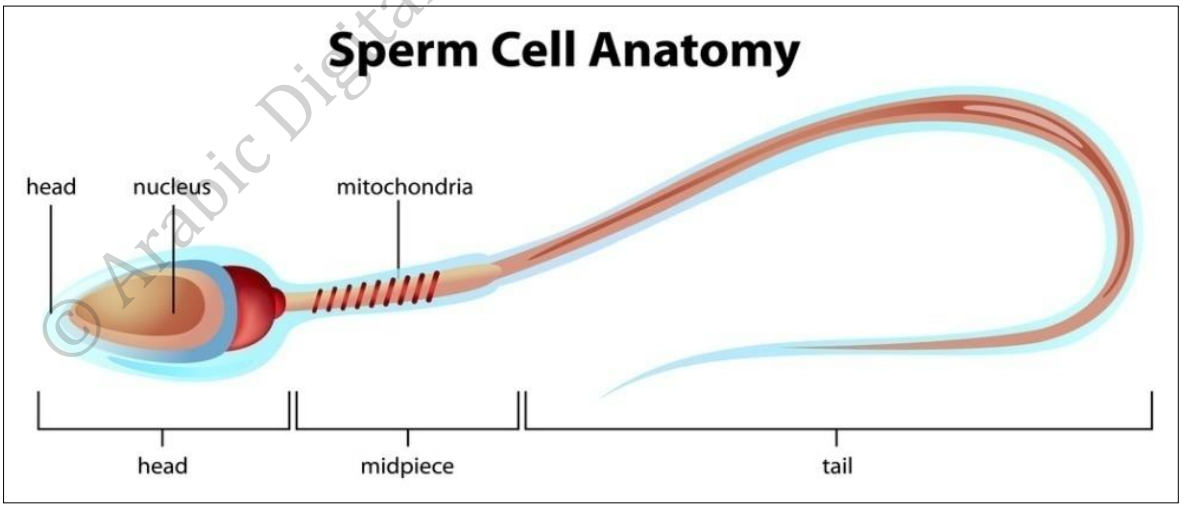


Figure 1: A mature human sperm cell has snake like structure. It has following parts head, neck, middle piece and tail (Mausse *et al*., 2014)

Spermatozoa with their distinctive shape are appropriate to travel for the female gamete. This explain why the nuclear genome of the sperm is highly condensed, and protected

by an acrosome for making contact with oocyte and joined with a flagellum to create a fast forward motility. Sperm head has a diameter ranges between 4–5 μm, while in the flagellum it ranges between 1–2 μm ,furthermore sperm length may reaches about 60 μm (Holstein *et al*., 1987).

Each mature human sperm includes in its midpiece about 50 to 75 mitochondria and one copy of mitochondrial deoxyribonucleic acid (mtDNA) (Michaels *et al*., 1982). The mitochondria in midpiece are considered as energy bank for the sperm tail. Sperm requires a large amount of adenosine triphosphate (ATP) from mitochondrial oxidative phosphorylation to support their rapid movement after ejaculation and active functioning (De Lamirande and Gagnon C, 1992).

2.3 Sperm abnormalities

Male infertility results from decreased number of spermatozoa (oligozoospermia), reduced sperm motility (asthenozoospermia), and many abnormal forms of sperm morphology assessment (teratozoospermia). These abnormalities may come together and are known as the oligo-astheno-teratozoospermia (OAT) syndrome (Jungwirth *et al*., 2012).

The lower reference values according to WHO for evaluation the semen parameters included a semen volume of 1.5 mL, sperm concentration of 15 million/mL, sperm total motility of 40%, and sperm with a normal morphology of 4% (Murray *et al*., 2012).

Idiopathic OAT is usually found to be accompanied by decreased testosterone level, also increase levels of Estradiol (E2), follicle-stimulating hormone (FSH), and luteinizing hormone (LH). Sex hormones (testosterone and FSH) control male germ cell development, and act as survival factors by inhibiting apoptosis. Prolactin regulates

testosterone secretion by testis. It was reported that patients with OAT syndrome have higher levels of prolactin (Wei *et al*., 2013)

2.3.1 Oligozoospermia

According to WHO criteria the male is diagnosed to have oligozoospermia if he has sperm count less than 15 million sperm / ml. There is low pregnancy rate obtained in cases of severe oligozoospermia (Francavilla *et al.,* 1990). Men diagnosed with infertility are establishing to have idiopathic OAT or idiopathic Azoospermia (absence of the sperms in whole semen samples). Idiopathic types of male infertility are mainly caused by genetic and environmental factors like elevation of scrotal temperature, exposure to pesticide and radiation. It is predicted that >1000 genes are implicated in spermatogenesis, so male infertility is considered as a complex disease (Jungwirth *et al*., 2012).

2.3.2 Asthenozoospermia

Asthenozoospermia, or low sperm motility, is a common cause of human male infertility. Sperm motility is a significant factor for transport to the oocyte and for the events leading to penetration of the egg. The presence of leukocytes in the semen considered as a marker for genital tract disease. High levels of round cells in the semen affect sperm motility. Antisperm antibodies decrease sperm motility by formation agglutinated and aggregated sperms (Fraczek *et al*., 2012).

The sperm motility is categorized into four types: Type 1: (fast progressive) sperms are those which move forward quickly in a in a straight line like guided missiles, their speed is >25 μm /sec at 37 °C, type 2: (slow progressive) sperms travel forward, but in curved line, or slow straight line movement, type 3: local motility only, sperms move their flagella without forward motility and type 4 (immotile): sperms without any

movement. The minor reference border for total motility is 40%, and for forward motility is 32% (WHO, 2010).Only motile sperm with progressive forward motility is useful in normal fertilization (Tredway *et al.*, 1975).

2.3.3 Teratozoospermia

The normal sperm has a smooth and oval head. The length of sperm head is approximately 5 µm, with 3 µm in diameter. Furthermore it should be has no defects in the nick, midpiece, tail and without cytoplasmic droplets. One hundred sperms should be analyzed, but it is recommended to asses 200 sperms for true evaluation (Kruger *et al*., 1993).

Each abnormal spermatozoon can have one to four abnormalities, a head abnormality, a neck/midpiece abnormality, a tail abnormality, or the presence of cytoplasmic residues (Fig.2). Head defects involve large or small, tapered, pyriform, round, amorphous, vacuolated. Midpiece defects involve asymmetrical insertion of the midpiece into the head, thick or irregular, sharply bent, abnormally thin. Tail defects involve short, multiple, broken, smooth hairpin bends, sharply angulated bends, of irregular width, and coiled tail. The lower reference limit for sperm morphology is 4 %, if the male has sperm defect less than 4%; male considered to suffer teratozoospermia (Cooper, 2005).

These abnormalities can occur as a single defect or in a combination of two, three or all four abnormalities simultaneously. Sperm morphology affects oocyte penetration, and plays a major role during the early stages of egg fertilization (Wassarman, 1988).

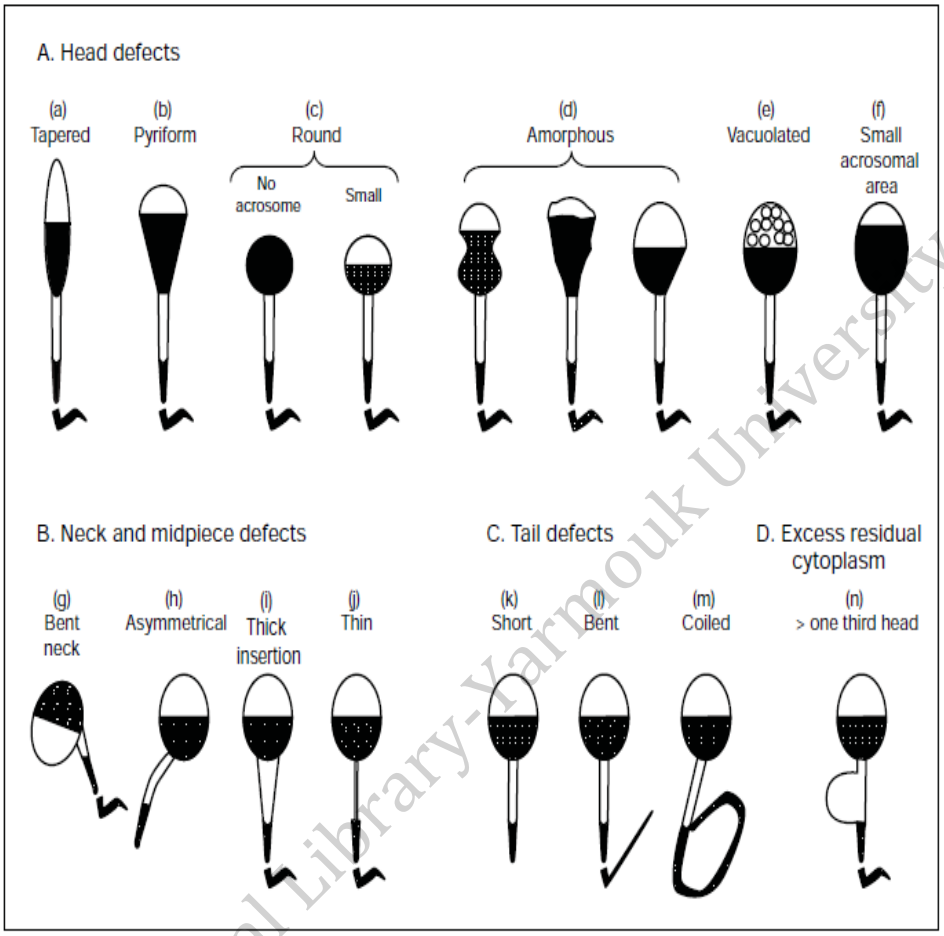


Figure 2: Schematic drawings represent abnormal forms of human spermatozoa. Adapted from (Kruger *et al*., 1993).

These stages can be notable as spermatozoan-egg penetration. It has been suggested that sperm morphology can be strongly associated to the failure in sperm linking with the zuna pellucida (Menkveld *et al*., 1991).

2.4 The human mitochondrial genome

MtDNA was discovered by electron microscope in 1963, and it was isolated from yeast mitochondria in 1964. It was reported that human cells include a second genome in the mitochondria; the human mitochondrial genome was wholly sequenced by Anderson *et al.*, 1981.Human mitochondria carry their own genomic DNA (Fig. 3). It is circular,

double-stranded , and 16,569 bp long consists of 37 genes encoding 13 polypeptides forming a component of respiratory chain oxidative phosphorylation(OXPHOS), in addition to 22 tRNAs and 2 rRNAs (12S and 16S) essential in protein synthesis (Kumar *et al*., 2009).

All 13 proteins encoded by mtDNA are components of the mitochondrial respiratory chain. Seven (Nicotinamide Adenine Dinucleotide Hydride (NADH) Dehydrogenase (ND) 1–ND5, ND4L, ND6) are subunits of complex I, one (cytochrome b) is part of complex III, three cytochrome oxidase subunit I(COX I), cytochrome oxidase subunit II (COXII), and cytochrome oxidase subunit III (COX III) are subunits of complex IV, and two (ATPase 6 and ATPase 8) are subunits of complex V(Smeitink *et al*., 2001).

OXPHOS is an essential biochemical pathway which provides the cells by energy required for metabolism. ATP 6 gene is a mitochondrial gene codes for ATP synthase enzyme, which is responsible for the final step in OXPHOS, while ND genes code for proteins of complex 1, which are responsible for the first step in the electron transport chain. Defective OXPHOS lead to abnormal mitochondrial biosynthesis due to mutated mtDNA, the presence of mutation in mtDNA reduces ATP generation. Defective electron transport chain enzymes lead to insufficient mitochondrial inner membrane potential for ATP production. Mutations in mtDNA stimulate mitochondrial dysfunction, also lead to more damage and mtDNA deletions (Fosslien, 2001).

MtDNA has no introns unlike nuclear DNA, the replication system of mtDNA and the highly oxidative environment increase the rate of mutation 10 to 20 times higher than its occurrence in nuclear DNA (Merriwether *et al*., 1991).

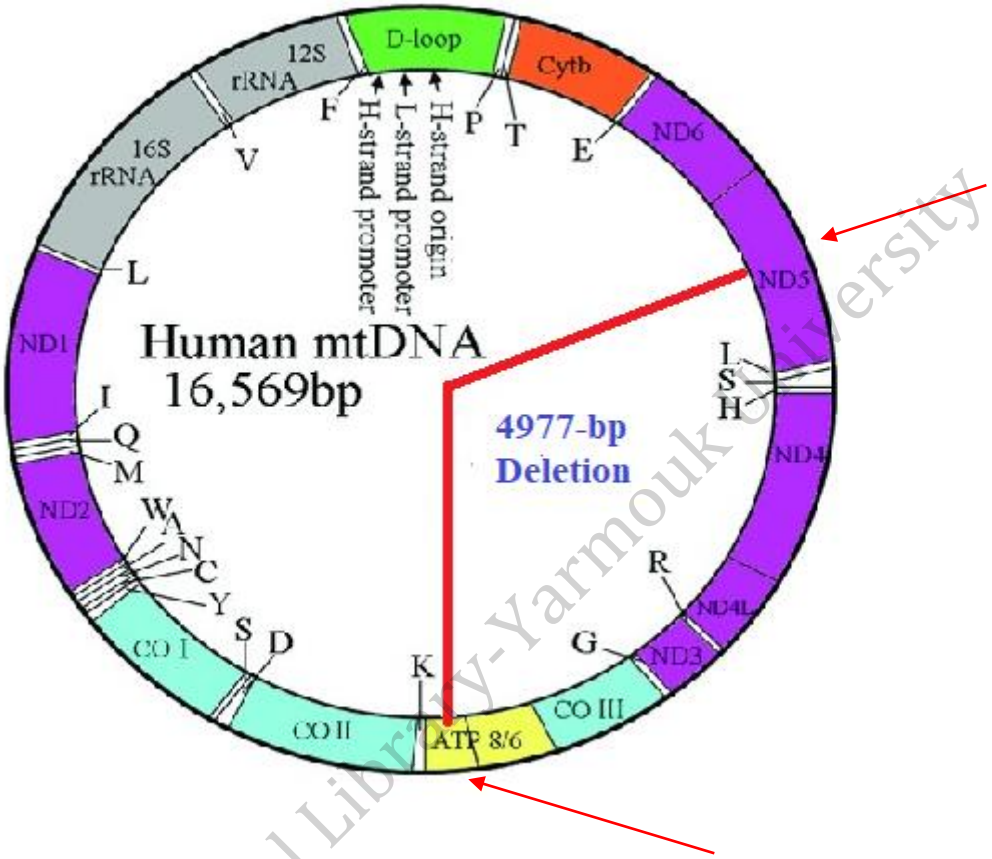


Figure 3: The human mitochondrial genome. This genome encodes 13polypeptides, 22 tRNA, and 2 rRNA. The D-loop includes the H-strand starting point of replication. It is the only region of this genome that is not transcribed but has two hyper variable regions that are used for molecular fingerprinting to determine patterns of maternal inheritance. The 4,977-bp common deletion is indicated by arrows. Adapted from (Shen *et al.*, 2010).

2.5 Impact of Oxidative Stress on Male Infertility

Abnormal spermatozoa consider as a major cause of endogenous reactive oxygen species (ROS) in the seminal fluid (Aitken and Sawyer, 2003), and uncontrolled mitochondrial genesis of reactive oxygen species from defective sperms is related to sperm disorders, particularly sperm motility. ROS may result due to the contaminating

leucocytes, mainly neutrophils and macrophages. The stimulated neutrophils are the sources of toxic reactive oxygen species (Koppers *et al*., 2008).

Sperms with defected mitochondria generate a reduced amount of ATP and produce more ROS and free radicals, this leads to further damage to mitochondria and mtDNA, the result will be an energy problem and reduction of sperm motility (John Sakkas and Barratt, 2000). Sperms usually produce reactive oxygen species in a natural physiological process, and ROS are necessary to sperm function in a small amounts. ROS such as nitric oxide (NO) and the superoxide anion play a role in the capacitation and the acrosome reaction,also ROS have been concerned in sperm oocyte communication, but the disparity in the production of ROS is damaging to the sperms and related with male infertility (Garrido, *et al*., 2004).

Higher ROS levels in the semen decreased sperm variables in infertile men.ROS are usually produced by a diversity of metabolic and physiologic processes.Oxidative stress (OS) occurs as a result of imbalance between the production of ROS and the antioxidant (Wang *et al*., 2004).

Spermatozoa are mainly susceptible to ROS as their plasma membrane contain 5 polyunsaturated fatty acids (PUFA) that contain more than two carbon–carbon double bonds. These lipids in the Sperm membrane play a significant role in regulating the movement of sperm surface antigens during developmental pathways like maturation and capacitation. ROS attacks PUFA in the cell membrane wich will cause lipid peroxidation.Furthermore lipid peroxidation is stimulated by high levels of hydrogen peroxide which may cause cell death (Sanocka and Kurpisz, 2004).

Studies have shown that some environmental factors increase the concentration of reactive oxygen species, like elevated temperatures, electromagnetic radiation, pesticides, pollution, life style, old age, stress, cigarette smoking, fatness and uncontrolled diet (Wong and Cheng, 2011).

Antioxidants protect spermatozoa from ROS , they exist in human semen to scavenge free radicals as self-protection mechanisms. Superoxide dismutase (SOD) is an impotrant antioxidant which conserves spermatozoa by catalysing the dismutation of superoxide anions to hydrogen peroxide and oxygen (Kowalowka *et al*., 2008).

2.6 Effect of male age on fertility

Previous studies reported that there is an increasment in sperm DNA damage with age in healthy men (Spano *et al*.,1998). Sperm DNA-strand damage measured by terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay significantly increases with old- aged men ≥40 years in undergoing infertility treatment.The age-related increase in sperm DNA damage predicts that men with old age may faced dificulties normal pregnancies as a consequence of fertilization with damaged spermatozoa(Vagnini *et al*., 2007).

The increasing age is related with a higher frequency of aneuploidies and point mutations, loss of apoptosis, genetic imprinting and other chromosomal abnormalities (Singh *et al*., 2003). Male germ cells always divide and undergo many mitotic duplications than oocytes . Men older than 50 years would have carried out 840 mitotic divisions , this increase the possibility of occurrence a DNA copy error (Crow, 2000).

Reducing numbers of Sertoli cells, Leydig cells and germ cells is related with old age ,furthermore it affects the thickening of the basal membrane of the seminiferious tubules, also reduct the epithelium tissues in the testis and defective vascularization of the testicular parenchyma (Ku¨hnert and Nieschlag, 2004).The male age affect the level

of the the hypothalamic–pituitary–gonadal axis (HPG axis), which cause adecreasing in the androgen levels in the blood and effects the androgen target cells (Juul and Skakkebaek, 2002).

2.7 Environmental factors and male infertility

Elevation of scrotal temperature to normal core body temperature results in complete failure of spermatogenesis in man and most mammals. Sperm counts from the same men collected in summer and winter were compared and showed an average 30% reduction in sperm counts in summer versus winter (Jørgensen *et al*., 2001).

Toxic substances affect the cells involved in spermatogenesis. Long-term exposure for toxinc compounds becomes significant as the human age increases. Also occupation affects semen parameters (Magnusdottir *et al*., 2005). Polychlorinated biphenyls (PCBs) are organic pollutants The accumulation of PCBs with time in men is associated with sperm abnormalities, mainly asthenozoospermia (Meeker and Hauser 2010).A previous study reported that the percentage of sperm motility in exposed men to PCBs was 35% compared to 57% in unexposed men to PCBs (Guo *et al*., 2000).

Environmental substances and pesticides affect male germ cells like some glycol ethers and benzimidazole fungicides. Endocrine disrupters (EDs) composed of: vinclozolin, lindane, procymidone, and methoxychlor. EDs are usually used in pesticide, and they are considered toxic compounds for reproductive system. Furthermore other compounds used as insecticides such as DDT and react with sex steroid receptors in target cells, leading to inhibit the biochemical processes of steroid action (Neubert D, 1997).Agricultural workers with the highest exposure to pesticides undergo reduction in fertility, also the abortion increase among wives of workers in floriculture (De Cock *et al*., 1994).

The effect of radiation on spermatogenesis was reported by (Clifton and Bremner, 1983). According to their results the repression of sperm counts was remarkable with doses of 15 rad or greater. Sperm counts in all 11 volunteer receiving 40 rad dropped below one million between 15 and 47 weeks after exposure. Furthermore they found that the exposure to 400 rad dose causing a complete inhibition of sperm counts in four of five individuals for at least 40 weeks (Clifton and Bremner, 1983).

2.8 Smoking and male infertility

Cigarette smoke is composed of about 4,000 compounds, released by many chemical processes of hydrogenation, oxidation, decarboxylation and dehydration. The most common released compounds are carbon monoxide, nicotine and tar (Colagar *et al*., 2007). Smoking increases the concentration of ROS and free radicals, also it decreases the antioxidants levels and enhances the lipid peroxidation of sperm membrane, leading to DNA damage and inhibit the sperm production in Leydig cells (Harlev *et al*., 2015).

Reduced sperm motility was related positively to the number of cigarettes smoked per day, also in cigarette smokers the sperm penetration assays have revealed an association with weak sperm function (Sofikitis *et al*., 1995).Exposure to ten cigarettes every day decrease sperm count about 15% (Vine *et al*., 1996). Smoking inhibits maturation of sperm by affecting the epididymis, which is the cellular space where sperms generate their tails; pieces of spermatids especially cilia have been extracted from the semen of heavy smokers isolated in the semen of smokers (Bornman *et al*., 1998).The main active ingredients of smoke that affect sperm parameters are the heavy metals cadmium and lead, it was reported that high levels of cadmium and lead in the semen of smoker are associated with decline in sperm count and motility (Pant *et al*., 2015),

Spermatozoa need large amounts of energy, and for this purpose creatine kinase enzyme plays an important function in adenosine triphosphate (ATP) and adenosine diphosphate processes , furthermore it provides an ATP optimal conditions, in smokers creatine kinase action in spermatozoa was decreased leading to asthenozoospermia (Ghaffari and Rostami, 2013). Smoking affect testicular functions by reducing oxygen supplement, this Oxygen insufficiency lead to hypoxia, and impair the spermatogenesis which needs elevated energy supply (Jensen *et al*., 1991).

Smoking increases DNA methylation, studies revealed that there are variations in the methylation profile of 95 locations in smokers, also suggested a possible relationship between methylation and infertility. Yu reported that heavy smokers with normozoospermia have histone abnormality with high rate, whereas it was lower among non smoker normozoospermia (Yu *et al*., 2014). Furthermore there is a remarkable reduction in protamine concentrations in smokers, which play a major role in facilitating the compaction of the nucleus, protecting the sperm DNA from harmful conditions in the reproductive tract during fertilization (Fortes *et al*., 2014).

2.9 Mitochondrial DNA deletions

The molecular approaches have discovered that mitochondrial genes ATPase 6, ATPase 8, COX 3,COX 2, CytB, ND3, ND4, ND5, and ND6 (Fig.4) play a significant role in the construction of mature sperm and progressive flagellar movement after ejaculation (Nakada *et al.*, 2006). The 4977-bp human sperm mtDNA deletion is the most recognized deletion among mitochondrial DNA deletions. Also it involves the deletion of some structural genes (ATPase 6/8, COIII, ND3, ND4L, ND4 and ND5) and 5 tRNA genes of mitochondrial genome (Fig.5) (Guo *et al*., 2017).

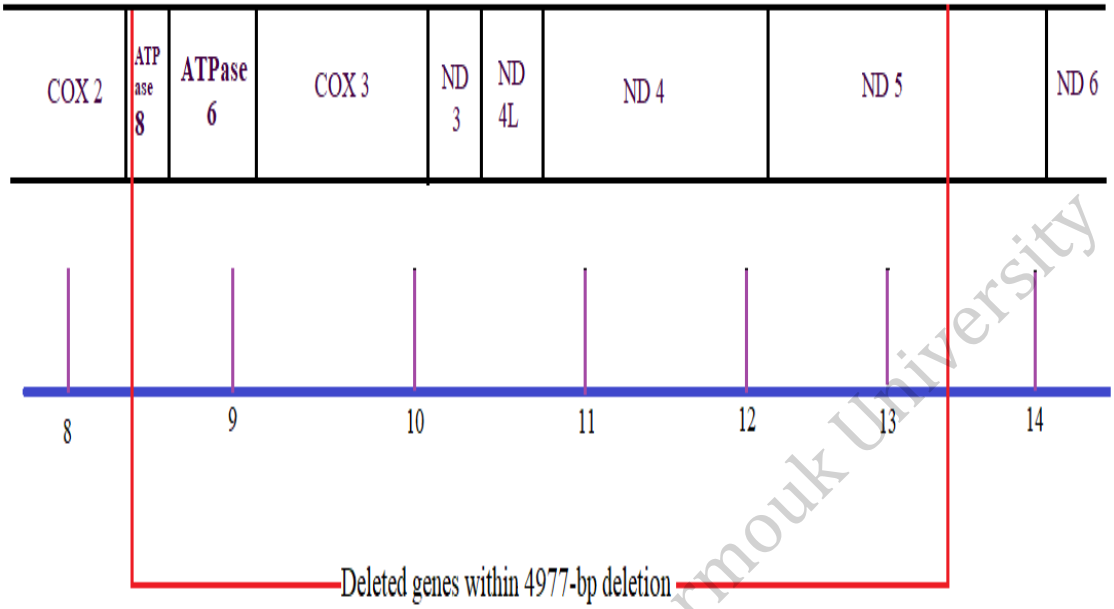


Figure 4: Deleted genes in 4977-bp mtDNA deletion. Adapted from (Ambulkar *et al*., 2016).

Another deletion of 7599 bp deletion lies between 8643bp and 16243bp is also found in human-sperm mtDNA (figure 5), this mtDNA deletion has seven-nucleotide repeated sequences (5'-CATCAAC-3'), also it has ATPase 6 gene within this region. this deletion involve removal of some structural genes (ATPase6/8, COIII, ND 3, ND4L, ND4, ND5, ND6, and Cytb) and 8 tRNA genes, the removal of those genes leading to the production of incomplete respiratory enzymes, causing a significant failure in bioenergetical function of mitochondria and sperm motility. Kao *et al*. (1995) reported that 7599 bp-deleted mtDNA in human spermatozoa are related with asthenozoospermia (Kao *et al.* 1998).

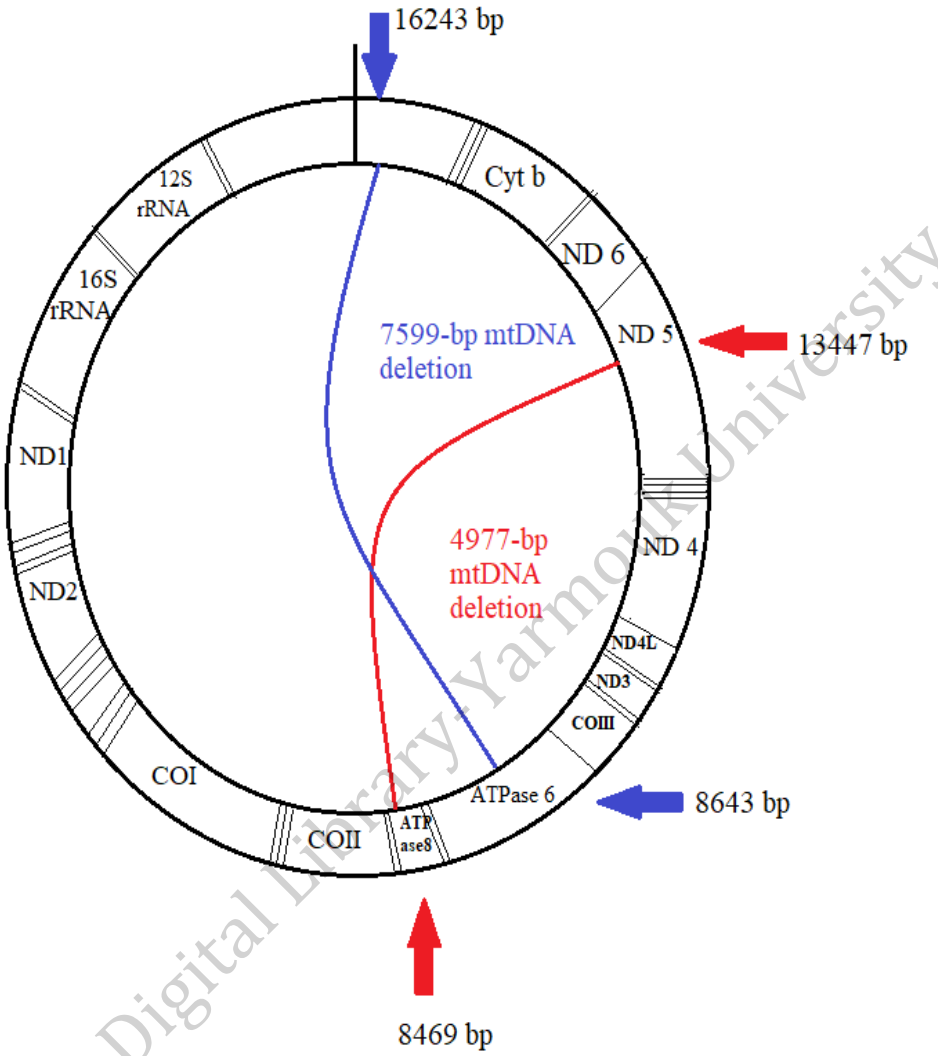


Figure 5: Human mtDNA with the sites of 4977-bp and 7599-bp deletions. Red arrows indicate for the site of 4977-bp mtDNA deletion which located between nucleotides 8,469 and 13,447 bp. While blue arrows indicate for the site of 7599-bp mtDNA deletion which located between nucleotides 8643 and 16243 bp

Adapted from (Rajender et al., 2010).

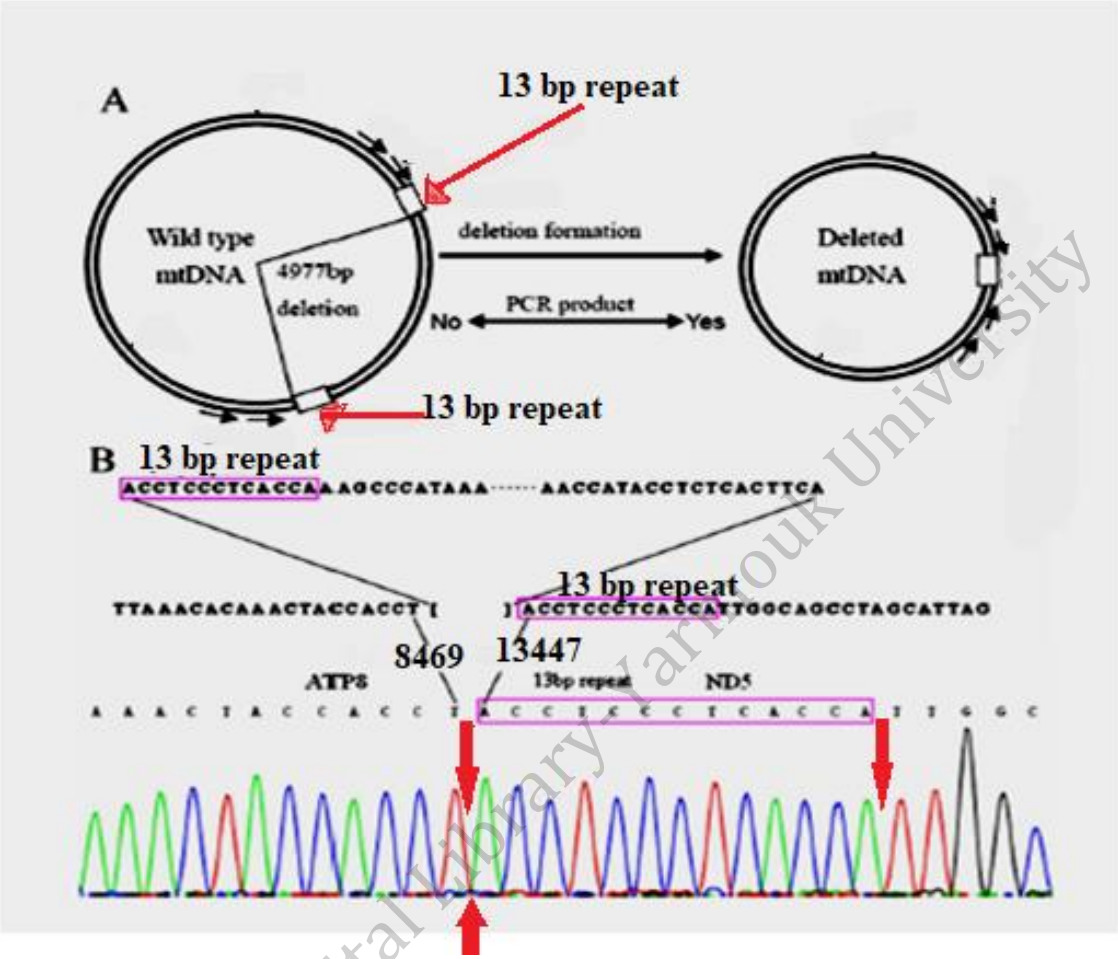


Figure 6: Human mtDNA with or without the 4977-bp deletion (A), verification of the 4977- bp deletion by sequencing profile of the sperm mtDNA amplicon from the patients have the deletion, arrows indicate for the site of deletion (B) Adapted from (Chen *et al*., 2011).

The 4977-bp mtDNA deletion results from the breakpoints within the 13-bp direct repeat of 5'-ACCTCCCTCACCA-3' (Fig. 6) (Wang and Lü, 2009).A previous research in Italy indicated that the mtDNA deletions play a significant role in decline the sperm motility. Only the non-motile sperms were found to have mitochondrial DNA deletions. They observed that the reduced sperm motility coincided with the increase of mtDNA defects. In addition they observed that there is a negative association between mtDNA deletions and the time after ejaculation, their research also showed that normal sperms

were successful in oxygen utilization in their specific organelles, clearly investigate that the mitochondrial enzymatic action is necessary to the sperm motility (Carra *et al*., 2004).

Kao *et al*. 1995 found that there is an elevated prevalence of mtDNA 4977- bp deletion in sperms of Asthenozoospermic patients. They examined the accumulation of the 4977- bp mtDNA deletion in sperm obtained from infertile men, then they scan the presence of mtDNA deletions in normozoospermic men, applying polymerase chain reaction (PCR) techniques, they determined the frequency of incidence of the common mtDNA deletion in sperms with various motilities. Percoll gradients are applied for sperm separation. The maximal occurrence of incidence of the common mtDNA deletion was detected in sperm with the weak motility, their study showed that the proportion of the deleted mtDNA in the sperms with weak motility were notably higher than the proportion of deleted mtDNA in the sperms with normal motility, furthermore their results proofed that the mtDNA deletion is found in germ cells which are correlated to the change in environment of germ cell such as hormones and ROS (Kao *et al*., 1995).

While another study in the United Kingdom did not find a significant relationship between the common deletion and asthenozoospermia, St John *et al.,* (2001) they found that there is unclear relationship between the different groups of Asthenozoospermic patients and fertile men for the 4977- bp deletion, in their experimental procedure they applied the magnetic bead antibody isolation technique to reduce contaminating cells like white blood cells. In addition to that they justify the previous studies which supported the role of mtDNA deletion in asthenozoospermia may be they selected samples of uncontaminated sperm, as it is probable that their samples were impure with other cells like leukocytes (St John *et al*., 2001).

Also another study by Cummins *et al.*, 1998 indicated that the identification of 4977-bp mtDNA deletion in spermatozoa is not correlated with male infertility .Also in their study they found that even normozoospermic males can be confirmed to have considerable levels of the 4977 bp ` deletion, so they concluded that the estimation of mtDNA deletions in the sperm is unsuitable for identifying male infertility (Cummins *et al*., 1998).

Ieremiadou and Rodakis, 2009 reported that there is no association between the occurrence of the 4979-bp mtDNA deletion and asthenozoospermia. In their study in Greece, they found that the incidence of the common deletion did not change obviously between fertile and infertile men, but their results showed that the detection of mtDNA deletion depends on using Percoll density gradient, where the detection of mtDNA deletion in defective sperms by using 45% density gradient was higher than using 90% Percoll fraction.

Moreover, several studies proved that widespread deletions of 7436-bp of mtDNA are related with asthenozoospermia. The deletions of 7436-bp DNA fragment with genes accountable for oxidative phosphorylation, while sperms with normal motility did not have those deletions. The 7436-bp deletion of mtDNA in sperm is considered to be an important cause of reduced sperm motility (Ambulkar *et al*., 2016).

Furthermore point mutations (A73G and A3243G) are positioned in D-Loop region of mitochondrial DNA. The mutations in D-Loop are caused by changing in the binding affinities of the factors that play an important role in the replication and transcription of mtDNA, leading to decrease the content of mtDNA (Parisi and Clayton, 1991).Theses point mutations affects mRNA constitution and protein role depending on their location in the mitochondrial genome (Karimian *et al*., 2015).

A previous study reported that there is a significant relationship between A3243G and sperm motility (Spiropoulos *et al*., 2002), while another study found that A3243G mutation does not correlated with asthenozoospermia (Huang *et al*. 1994).

2.10 Rationale

Male infertility is considered as serious global socio-medical predicament that affects many couples in Jordan. Reduced sperm motility (Asthenozoospermia) is a main cause for male infertility and it may result because of genetic abnormalities. Screening human mitochondrial DNA for deletions can help in the early diagnosis of male infertility, and further refer couples for genetic counseling to decide if they can attend a program of assisted reproductive techniques like intracytoplasmic sperm injection (ICSI). While few studies have been done in the Middle East, no previous similar studies have been carried out in Jordan.

2.11 Objectives

1. To investigate the association between 4977-bp human mitochondrial DNA deletion and Asthenozoospermia in Jordanian population.
2. To evaluate the use of this association as a pre-diagnostic tool in male infertility.

Chapter three

3. Materials and Methods

3.1. Patients and Sample collection

One hundred and twenty asthenozoospermic Patients aged 18- 40 years were divided into six groups according to percentage of sperm motility (Carra *et al*., 2004). group one includes patients with percentage of sperm motility 0% (100% immotile), group two includes patients with percentage of sperm motility from 0% to 5%, group three represents patients with sperm motility from 5% motility to 10%, group four includes patients with sperm motility from 10% to 15%, group five includes patients with percentage of sperm motility from 15% to 20% and group six includes patients with percentage of sperm motility from 20% to 35%,. The patients with varicocele, alcoholism, cigarette smoking and over 40 years were excluded from this study (Ambulkar *et al*., 2016).

Samples from eighty normozoospermic men with high percentage of sperm motility were collected as a control. The samples from normozoospermic men also were divided according to the percentage of sperm motility into three groups, group one includes samples with percentage of sperm motility from 50% to 55%, group two; samples with percentage of sperm motility from 55% to 60% and group three; samples with percentage of sperm motility from 60% to 75% (Bahrehmand and Vaziri 2017).

Semen samples were collected from IVF unit, where infertile patients provide their seminal fluid for evaluation. Semen fluid from all subjects was obtained by masturbation after three to five days of sexual restraint. The semen were collected in a sterile, wide-mouthed, non-toxic, and special container , then proceed in the lab within

an half hour of ejaculation, Seminal fluid samples were incubated at 37° to allow liquefaction to occur for 30 min. Then semen samples were evaluated by a senior clinical embryologist according to the WHO laboratory manual for the examination and processing of human seminal fluid WHO (2010).

Then semen sample were fractionated by Percoll media (45% and 90% gradient) by centrifugation at 1000g for 22 minutes to avoid contamination from other cells like leucocytes and epithelial cells. After that the pellet were collected and washed two times with sperm washing medium to remove particles of density gradient, then sperms were collected by using microcentrifuge at speed equals 664 g for 10 minutes. Finally the sperm pellet was kept at −20°C for DNA extraction (Ieremiadou and Rodakis, 2009).

3.2 Sperm mtDNA extraction

Mitochondrial DNA was extracted from the spermatozoa using The REPLI-g Mitochondrial DNA Kit (by QIAGEN), as recommended by the kit instruction manual. Isolated DNA specimens with an optimal density ratio 260/280 of 1.8 or more only were chosen and stored at − 20°C.

3.3 Polymerase chain reaction

To examine the occurrence of mtDNA in whole genomic DNA extract and the 4,977-bp deletion, two primer pairs were used: Primers WF WR for wild type with amplicon size equals 177 bp . For the 4,977 bp deletion DF and DR pairs of primers were used with amplicon size equals 127 bp (Table 1).

Table 1: Oligonucleotides primers used for PCR amplification of the 4977-bp deleted mtDNA and wild type mtDNA (Thayer *et al*., 2003)

Primer	Nucleotide location	Sequences (5′ → 3′)	The length of amplified product (bp)
Primer WF	(1257–1279)	TATACCGCCATCTTCAGCAAAC	177 bp
Primer WR (wild type)	(1411–1433)	TACTGCTAAATCCACCTTCGAC	
Primer DF	(8416–8437)	CCTTACACTATTCCTCATCACC	
Primer DR (Deleted)	(13498–13519)	TGTGGTCTTTGGAGTAGAAACC	127 bp

The mtDNA was predictable by amplification of a 177-bp sequence from the preserved its 12S region, and deleted regions of mtDNA was recognized by primers closest the deleted area which produced 127 bp product if the mutation was there. PCR amplifications were done in 25 µl reaction volume including 4µl diluted amplified mtDNA, 12.5µl 2X master mix, 1×1µl (10 pmol) of every pair of primers (WF WR or DF DR) and 6.5µl free nuclease water.

The first cycle of PCR involve heating at 94°C for 5 min, then 33 cycles of a denaturation step at 94°C for 30 second, followed by annealing at 60°C for 30 second and the last extension was at 72°C for 5 min. The resulting mixture containing 10 µL of the PCR products was investigated on a 2% agarose gel at 75 V for 1 hour in 1×Tris-borate-EDTA buffer then visualized by UV box and using a 50 bp DNA ladder as a reference.

3.4. Statistical analysis

Statistical analysis was performed using the chi-square test and Minitab 18. The association between human mitochondrial DNA with 4977-bp deletion in Sperm and Asthenozoospermia was measured by OR with 95% CI. P-value is considered significant if ≤ 0.05.

Chapter Four

Results

Mean ages were 34.3 years (range: 19-40) for asthenozoospermic patients, and 32.6 (range: 20-40) years for normozoospermic men. Seminal fluid analyses were carried out by clinical embryologist according to WHO (2010) criteria. The percentage of sperm motility for patients and control groups were listed in Table 2 and 3 respectively.

For the patient groups, DNA analysis revealed that the presence of mtDNA deletion among all groups as follows: 18 patients out of 20 with the deletion in group one and group two; 17 patients out of 20 in group three; 15 patients out of 20 in group four; 14 patients out of 20 in group five, and 13 patients out of 20 in group six (Table 2).

On the other hand the presence of mtDNA deletion in the three sets of healthy volunteers as control groups as follows: among control group one 4 healthy volunteers out of 26 with the deletion, while among control group two 3 healthy volunteers out of 26 with the deletion, and in control group three 1 healthy volunteer out of 28 with the deletion (Table 3).

Table 2: Frequency of 4977-bp mtDNA deletion in patient groups

Percentage of sperm motility	Number of males	Presence of 4997-bp mtDNA deletion	Percentage of MtDNA deletion
0%	20	18	90%
0-5 %	20	18	90%
5-10%	20	17	85%
10-15 %	20	15	75%
15-20 %	20	14	70%
20-35 %	20	13	65%
total	**120 patients**	**95**	**79.2%**

Table 3.Frequency of 4977-bp mtDNA deletion in control group

Percentage of sperm motility	Number of males	Presence of mutation	Percentage of mtDNA deletion
50-55	26	4	15.4 %
55-60	26	3	11.5 %
60-75	28	1	3.6 %
total	**80 control normal males**	**8**	**10%**

The results of sperm mtDNA amplicon revealed two major bands (with lengths of 127and177-bp) on 2% agarose gel, the 177-bp band indicates for wild type or the presence of mitochondrial DNA, while the 127-bp band is generated by the 4977-bp deletion (Fig.7).

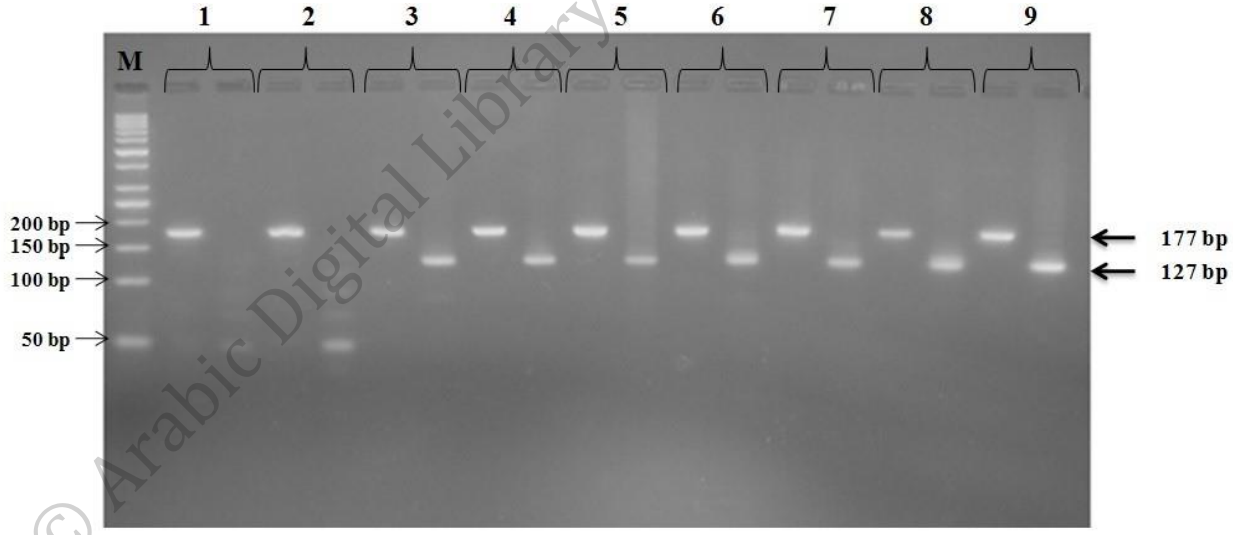


Figure 7: Agarose electrophoretogram for the detection of 4977-bp sperm mtDNA deletion. The 177- bp band is produced from the presence of mtDNA, while 127-bp band is produced from the 4977-bp mtDNA deletion. Lane M, 50bp DNA ladder .The first two patients in lane 1 and lane 2 without the deletion, while the other 7 patients in lane 3, 4, 5,6,7,8, and 9 have the 4977-bp mtDNA deletion.

We confirm also the 4997-bp mtDNA deletion by Sanger sequencing the sperm mtDNA amplicon (Fig.8).

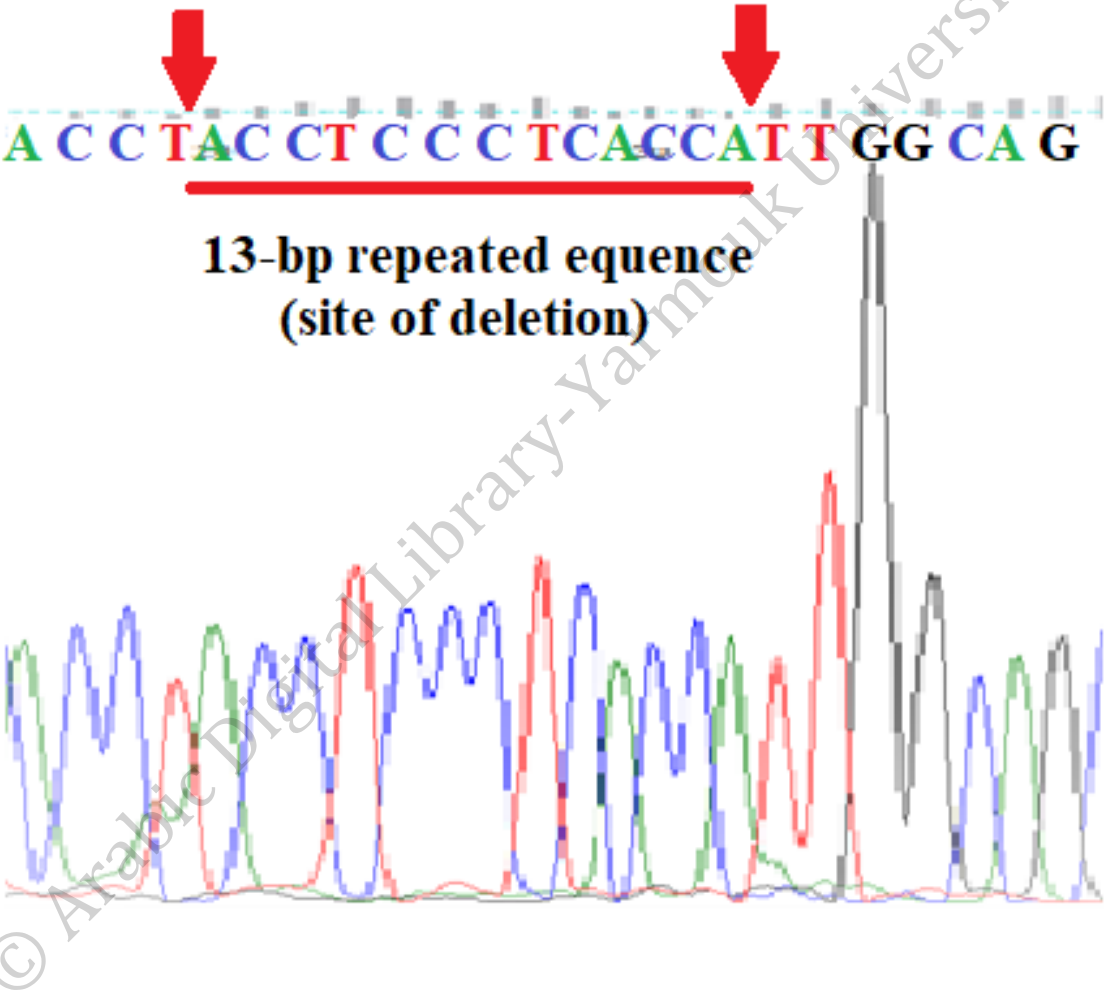


Figure 8: A representative chromatogram shows the sequence of sperm mtDNA amplicon, arrows indicate 13 bp repeated sequence which represents the site of deletion of 4977-bp breakpoint.

The sequencing analysis showed that the 4977-bp mtDNA deletion is founded in the patient's sample (BLAST Fig. 9).

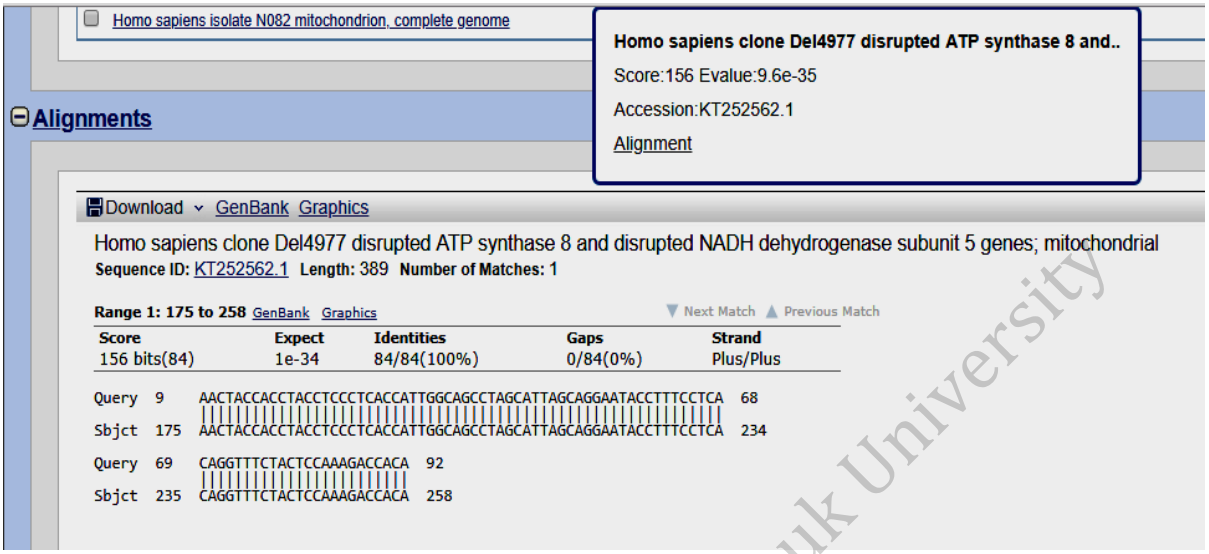


Figure 9: Sequencing analysis by Basic Local Alignment Search Tool (BLAST) from The National Center for Biotechnology Information (NCBI), reported that the mtDNA deletion is present in our sample.

The deleted genes within the 4977-bp mtDNA deletion are 7 genes and 5 tRNAs (Fig. 10).

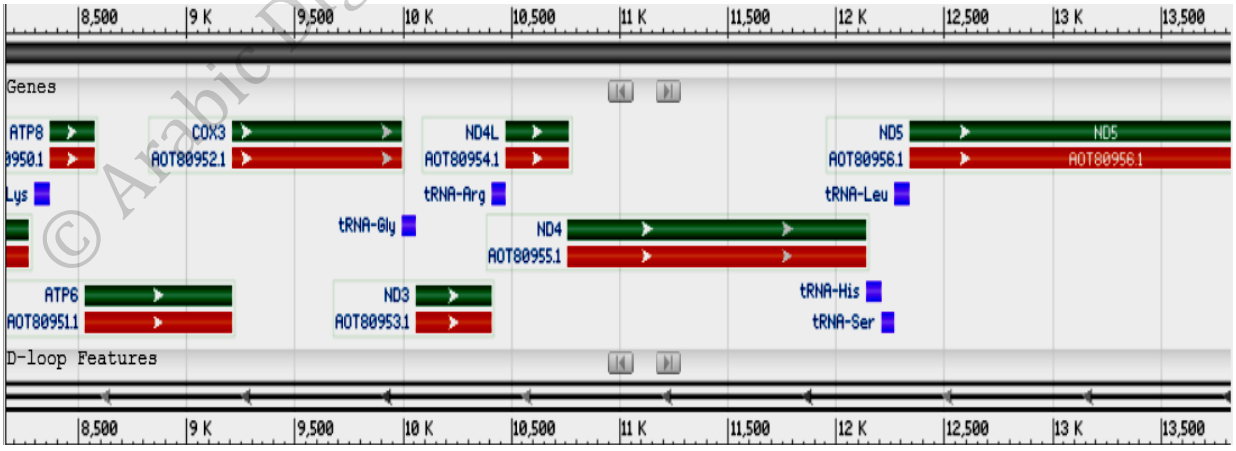
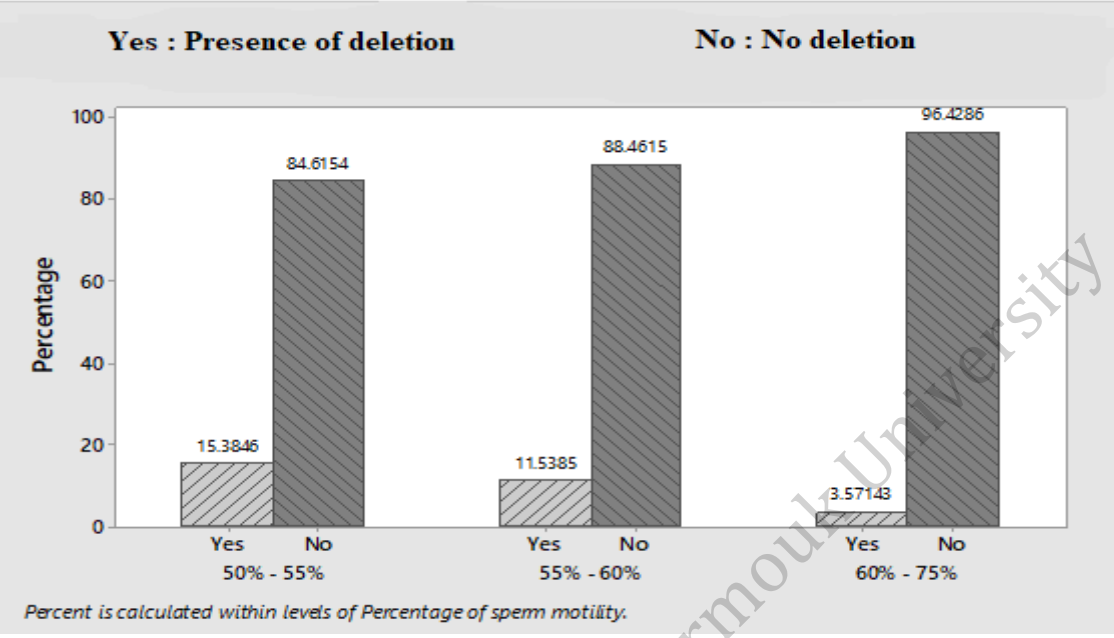
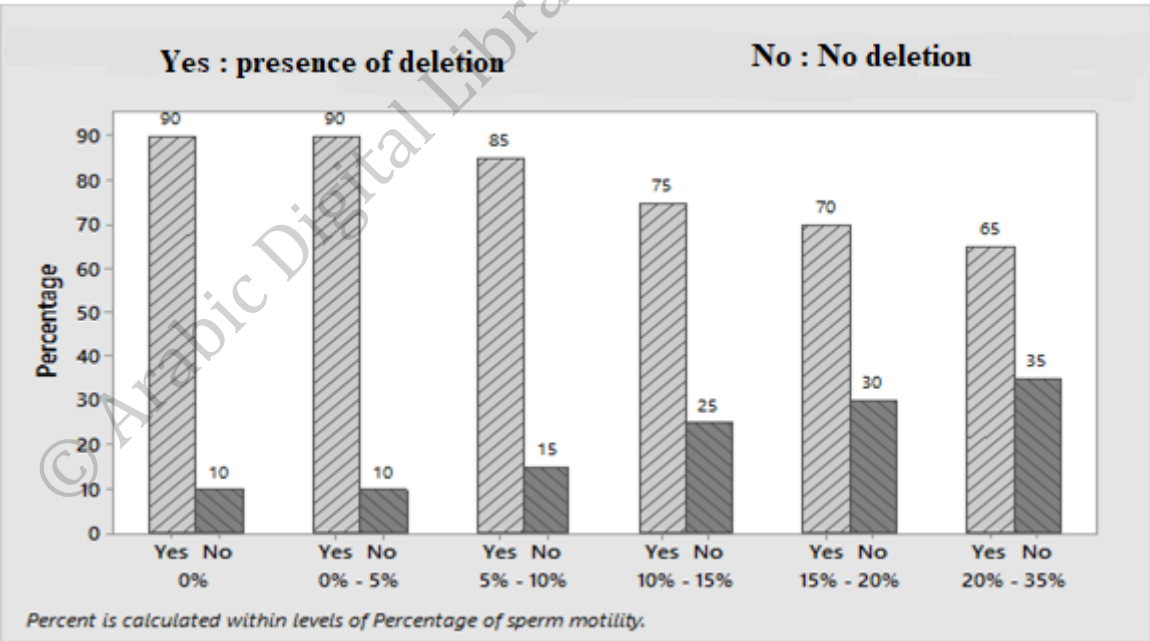


Figure 10: The deleted genes within 4997-bp mtDNA deletion (NCBI).



Control group (A)



Patients group (B)

Figure 11: Profile of the control subdivided into three groups (A) with Profile of the patients subdivided into six groups according to percentage of sperm motility (B). The percentage of mtDNA deletion in each group is indicated.

The percentage of mtDNA deletion in the patients ranged between 65-90 %, while in control groups the prevalence of deletion was (3.5, 11.5, 15) % in groups 1, 2 and 3 respectively (Fig. 11).

It is obvious that the prevalence of mtDNA deletion is increased as the percentage of sperm motility decreases (Fig. 12).The percentage of mtDNA deletion ranged between 85-90 % for groups 1, 2 and 3(0-10 % sperm motility). Whereas , it ranged between 65-75 % for groups 4,5 and 6 (10-35 % sperm motility).the percentage of mtDNA deletion in normozoospermic was significantly lower , where it ranged between 3.5-15 % for the different groups (50-75 % sperm motility).

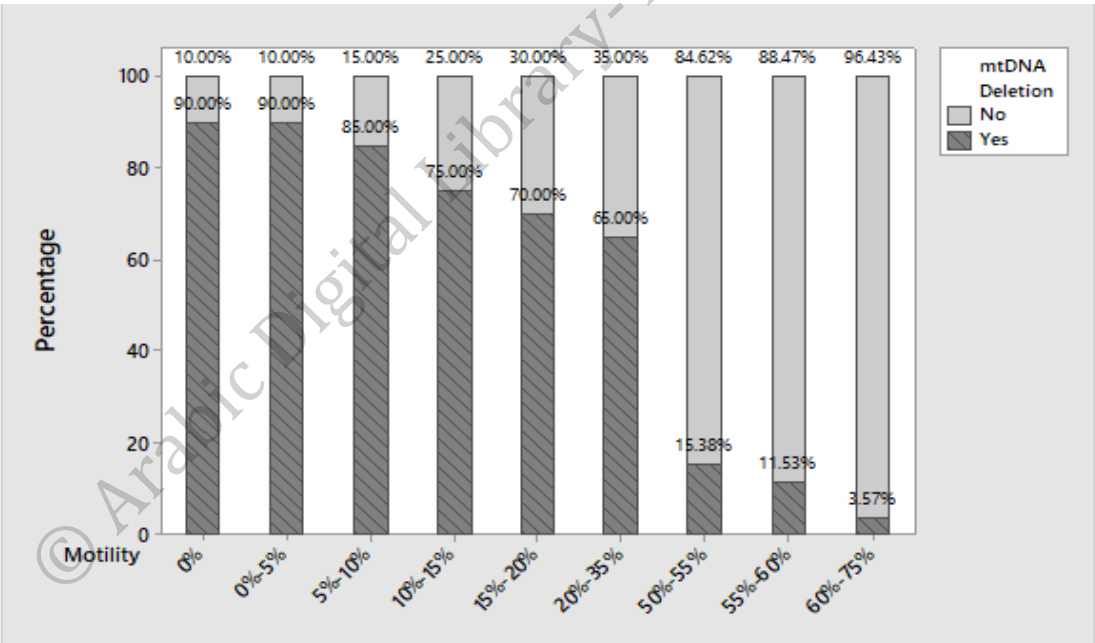


Figure 12: Percentage of sperm motility with the prevalence of 4997-bp mtDNA deletion in all groups

In the present study , there are 95 patients out of 120 have 4997-bp mtDNA deletion. This represents a percentage equals to 79.2 %, while in control group the the 4997-bp mtDNA deletion was found in 8 normozoospermic men out of 80. This represents a percentage equals to 10 % (Fig. 13).

This study reported a significant association between 4997-bp mtDNA deletion and reduced sperm motility. Also, the Odds Ratio and its 95% confidence interval were calculated and found to be 34.2, (14.5736, 80.2574), With a p-value less than 0.001.

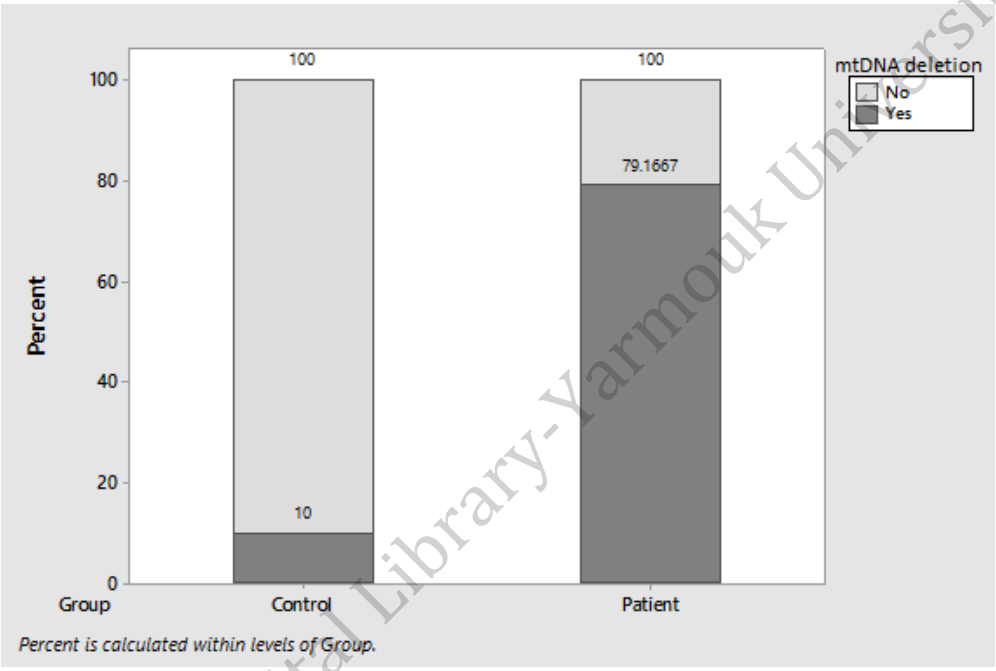


Figure 13: Percentage of 4997-bp mtDNA deletion in Control and Patient's groups.

Chapter Five

Discussion

The mtDNA is more susceptible to mutations than nuclear DNA, due to lack of protection by histones and lack of DNA repair mechanism (Shoffner *et al*., 1989). Moreover the highly oxidative environment due to the free radicles formation increases the rate of mutation 10 to 20 times higher than its occurrence in nuclear DNA (Merriwether *et al*., 1991). Therefore, mtDNA coding sequences are at high risk for mutations. Mitochondrial respiratory chain function depends on the synchronized gene expression of both the mitochondrial and nuclear genomes (Merriwether et al., 1991).

The mitochondria in human are maternally inherited, due to the elimination of paternal mtDNA in early embryogenesis by mechanism involves the destruction of sperm mtDNA after fertilization. Mitochondrial DNA plays a key role in etiopathology of the infertility. It generates more than 90% of our cellular energy that provides the oocyte with energy required for the continuation of meiosis II, fertilization, and development (Stumpf *et al*., 2011). ATP deficiency is associated with the failure in oocyte fertilization. The prevalence of 4977- bp mtDNA deletion in unfertilized metaphase II (MII) oocytes was 34.6 %. The transmission of abnormal paternal DNA is harmful for embryonic growth, which may result by selecting a sperm with mutant mtDNA during intracytoplasmic sperm injection (Chan *et al*., 2005).

Successful birth of a child by IVF technique was recorded, after anucleate donor oocyte cytoplasm was transferred into recipient egg (Cohen et al., 1997). Mitochondrial replacement technique (MRT) was applied for a Jordanian woman with an mtDNA mutation which lead to mitochondrial disease(Leigh syndrome) in her children. Two of

her children had died because of this disease. After MRT, an embryo was chosen and transferred to her uterus, resulting in the birth of a normal child (Palacios et al., 2017).

The 4977- bp mtDNA deletion has been reported as the most common mutation in human sperm mtDNA. It was associated with a decline in sperm motility (Kao *et al*., 1995). Nakada et al., 2006 reported that the genes within 4977-bp mtDNA deletion (ATPase 6/8, COIII,ND3, ND4L, and ND4) play an important role in the construction of mature sperm and progressive flagellar movement, which is required for the sperm to reach the site of fertilization. Aitken *et al*., 2013 proposed a mechanism of 4977-bp mtDNA deletion occurrence by breaking DNA strands through topoisomerase, or by oxidative stress generated by free radicals that attack naked mtDNA easier than nulclear genome. Furthermore the resistance to oxidative stress in sperm is less efficient than somatic cells due to the absence of antioxidant defence enzymes, making sperm more susceptible to oxidative DNA damage.

In this study we identified the presence of the 4977-bp mtDNA deletion in Jordanian patients with asthenozoospermia. Furthermore we investigate the association between this deletion and asthenozoospermia. According to our results, in Jordanian population the prevalence of 4977-bp mtDNA was 79.1 % in asthenozoospermic, while it was 10 % in normozoospermic. Our results agree with a previous research in Italy (Carra et al., 2004), which found a positive correlation between asthenozoospermia and 4977-bp deletion. Also they found that only the non- motile sperm (neither the motile nor the control) has specific mitochondrial DNA defects.

However our results are not consistent with a study in Greece by Ieremiadou and Rodakis (2009), which found that there is no association between 4997-bp mtDNA deletion and asthenozoospermia. Also they observed that the incidence of the common

deletion did not change between fertile and infertile men. Another study in the United Kingdom by St John, *et al.*, 2001 disagree with our findings. They found that there is unclear relationship between the different groups of Asthenozoospermic patients and fertile men for the 4977- bp deletion. According to their results the mtDNA deletions were detected in 33 % of fertile men, while 36 % of oligoasthenoteratozoospermic patients showed the mtDNA deletions. So they reported that there is no correlation between the mtDNA deletions and sperm abnormalities among British population. In their study they have used magnetic beads antibody technique to reduce sperm contamination with leukocytes and they suggest that the researchers who found an association between mtDNA and asthenozoospermia like Kao *et al.*(1995) may have contaminated sperms with other cells like white blood cells because they did not use the appropriate technique to avoid the contaminating cells. However, in our study we used a density gradient technique to prevent the continuations of sperms with other cells in the semen.

The contradictory results may refer to the different technique in methodology, and to the enrichment of mtDNA before DNA analysis. This was a critical step during our study. Without the amplification of mtDNA and using genomic DNA directly in PCR, we did not manage to see the expected band in any sample, but after we amplified the mtDNA our target band appeared in the same samples and the same PCR conditions. The small size of mtDNA which is around 16.5 kb versus 3200 mega bases for nuclear genome (Morton, 1991) explain why we need to amplify mtDNA if it is combined with nuclear genome.

In our study, the presence of 4977-bp mtDNA deletion in some control samples, may refers to environmental factors such as elevated scrotal temprature , long-term exposure to toxic substances and pesticides, radiation and smoking, these environmental factors

increase the concentration of ROS, which they attacks PUFA in these sperm cell membrane leading to lipid peroxidation and the damaging of the sperm (Wong and Cheng, 2011).

Conclusion

In this study which is the first research in Jordan about correlation between asthenozoospermia and 4997-bp mtDNA deletion , we reported that there is a strong association between human mtDNA 4997-bp deletion and asthenozoospermia, and it can be used as prediagnosic tool for male infertility.

Recommendations

May be the choises for the patients with the mtDNA detetion are limited.for those patients it is highly recommended to attend assisted reproductive techniques programs, like intracytoplasmic sperm injection(ICSI), where is the motility in this case is not important as the sperm injected by microscopic needle inside the cytoplasm of the egg. Sperm freezing also is useful in such cases, to save the sperms and prevent any decline in the motility by avoiding environmental factors such as smoking, life style and radiation which affect sperm motility. Further research are needed to study other mitochondral DNA deletions, and their impact on male infertility.

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9. Appendix

استبانة

نموذج موافقة على المشاركة في اجراء بحث علمي

يهدف هذا البحث الى الكشف عن وجود علاقة بين حدوث طفرة في الحمض النووي للميتوكندريا وانخفاض نسبة الحركة المسببة للعقم الذكوري في الأردن.

سوف يتم التعامل مع المعلومات بسرية تامة واحترام خصوصية المريض ، علما بانه يحق له الانسحاب من الدراسة متى ما شاء.لذلك يرجى تحلي الدقة عند الاجابه على الاسئلة لما يعود من فائدة على نتائج البحث.

الاسم :

اوافق على المشاركة في البحث المذكور اعلاه وعلى الاجابة عن الاسئلة بشكل صحيح

التوقيع

رقم العينة المتسلسل :

التاريخ / /

الرقم الطبي:

رقم الهاتف:

العمر:

الوزن:

الطول:

طبيعة العمل:

فترة الامتناع عن الجماع :

هل انت متزوج ؟

كم هي فترة الزواج؟

هل اتبعت وسائل تمنع الحمل؟

هل لديك اطفال؟

هل حصل الحمل بشكل طبيعي ام باستخدام وسائل المساعدة على الانجاب؟

كم عمر اصغر طفل لديك؟

هل يعاني احد افراد العائلة من تاخرالانجاب؟

هل انت مدخن؟

هل تخضع لحمية غذائية عن طريق اخصائي تغذية؟

هل قمت باجراء الفحوصات المخبرية التالية؟ ادخل النتائج المتوفرة بجانب كل فحص

FSH	**MIU/ML**	**Testosterone**	**ng/dl**
B 12	**pg/ml**	**VIT D**	**ng/ml**
Prolactin	**ng/dl**	**Estradiol (E2)**	**pg/ml**

10. Abstract in Arabic

الملخص

العلاقة بين حدوث طفرة في الحمض النووي لميتوكندريا الحيوانات المنوية لدى الانسان والعقم الذكوري الناتج عن ضعف حركة الحيوانات المنوية في الاردن

إعداد: محمد أحمد الصمادي

المشرف الرئيس: د. خالد البطاينة

المشرف المشارك: د.بهاء طراد

يعرف العقم بانه عدم القدرة على انجاب اطفال بعد مرور سنة من الزواج دون استخدام وسائل منع الحمل. ويتشارك كل من الرجل والمرأه مناصفة في اسباب العقم. يعتبر العقم الذكوري من العوامل المهمة في تأخر الانجاب ؛ ويساهم بما نسبته 50% من حالات العقم لدى الأزواج. إن ضعف حركة الحيوانات المنوية من أهم أسباب العقم عند الذكور، حيث أنها لا تتمكن من الوصول الى البويضة في موقع الإخصاب. في هذا البحث تم دراسة احتمالية وجود علاقة بين حدوث طفرات حذف في الحمض النووي للميتوكوندريا وانخفاض حركة الحيوان المنوي للرجال الذين يعانون من عقم في وحدات الاخصاب الخارجي واطفال الانابيب في الاردن.

تمت هذه الدراسة على 120 رجل يعاني من ضعف الحركة في الحيوانات المنوية وأيضا تمت الدراسة على 80 رجل طبيعي لديهم حركة عالية في الحيوانات المنوية. تم الكشف عن طفرات الحذف باستخدام تفاعلات السلسلة المتبلمرة ومن ثم الكشف عن مدى ارتباط هذة الطفرات بالعقم عند الرجال . وجدنا من خلال هذه الدراسة أن 95 رجل عقيم من أصل 120 يحملون طفرة الحذف في الحمض النووي لميتوكندريا الحيوانات المنوية وبما نسبته 79.2%. بينما كانت نسبة حدوث هذه الطفرة في عينات الرجال الذين لديهم حركة حيوانات منوية طبيعية 10% وبعد إجراء التحليل الإحصائي كانت النتائج كالتالي

،[OR = 34.2000, 95% confidence interval = 14.5736, 80.2574, p <0 .001].

وبالتالي خلصت هذه الدراسة –وهي الاولى في الاردن - الى وجود ارتباط وثيق بين طفرة الحذف في الحمض النووي لميتوكندريا الحيوانات المنوية وانخفاض سرعة الحيوانات المنوية المؤدية للعقم الذكوري. هذه الدراسة تساهم في التشخيص المبكر للعقم الذكوري، وتوفر الجهد والمال الذي يبذله المريض عبثا محاولا ايجاد حل لمشكلته وبالتالي تقديم النصيحة لهؤلاء المرضى من خلال توجيههم إلى الإستشارة الوراثية، وإلى وسائل المساعدة على

الإنجاب خاصة الحقن المجهري للبويضة بالحيوان المنوي والى ارشاد بعضهم خاصة الذين يعانون من انحدارمستمر في مستوى حركة الحيوانات المنوية الى الاستفادة من تقنية التجميد بواسطة النيتروجين السائل في بنوك الحيوانات المنوية، وبالتالي منع تاثير العوامل البيئية التي تؤثر على عملية انتاج الحيوانات المنوية مثل التدخين وارتفاع درجة الحرارة والتعرض للمركبات الكيميائية الضارة مما يزيد من فرص حصولهم على أطفال.هناك حاجة الى المزيد من الدراسات في هذا الموضوع والى الكشف عن انواع اخرى من الطفرات في الحمض النووي لميتوكندريا الحيوان المنوي والتي قد يكون لها دور في حدوث العقم الذكوري.

العلاقة بين حدوث طفرة في الحمض النووي لميتوكندريا الحيوانات المنوية لدى الانسان والعقم الذكوري الناتج عن ضعف حركة الحيوانات المنوية في الاردن

إعداد

محمد احمد الصمادي

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2018

العلاقة بين حدوث طفرة في الحمض النووي لميتوكندريا الحيوانات المنوية لدى الانسان والعقم الذكوري الناتج عن ضعف حركة الحيوانات المنوية في الاردن

ماجستير

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جامعة اليرموك

2018