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The Association of 4977-Bp Deletion of Mitochondrial Dna with Polycystic Ovary Syndrome in Iraqi Patients

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KEYWORDS
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ABSTRACT:

Polycystic ovarian syndrome (PCOS) is increasingly recognized as a significant health concern among women of reproductive age, exerting its influence on the reproductive system and overall female physiology. Mitochondria plays a pivotal role in generating adenosine triphosphate within human cells while also being a primary source of cellular oxidative stress. Mutation in mtDNA could influence the oxidative stress, thus the defect of PCOS. This study aims to investigate the presence of 4977-bp deletion of mitochondrial DNA in the Iraqi population and its role in PCOS.

A case-control study of 80 women with an age range (of 20- 35) years included in this study; these study populations are subdivided into two groups. Group 1: includes 40 women with PCOS. Group 2: includes 40 age with BMI-matched women with regular menstrual cycles, no signs of hyperandrogenism, and no history of PCOS.

PCOS women demonstrated significantly higher median serum levels of FSH, LH, and Testosterone than healthy women. The results of gel electrophoresis in this study show that there were no deletions in the 4977bp of the DNA of the mitochondrial neither healthy nor PCOS women.

INTRODUCTION

Polycystic Ovary Syndrome (PCOS) is a common hormonal disorder affecting 3–10% of women in their reproductive years. It is characterized by a combination of symptoms related to excess androgens and ovarian dysfunction, including irregular ovulation and the presence of multiple ovarian cysts [1,2].

Mitochondria are organelles enclosed by a double membrane and play crucial roles in various cellular functions, particularly in generating energy through a process known as oxidative phosphorylation [3]. Within human cells, mitochondria produce adenosine triphosphate (ATP) through oxidative phosphorylation, which takes place in the inner membrane and matrix of the mitochondria [3]. Reactive oxygen species (ROS) are produced within the mitochondria as a byproduct of oxidative phosphorylation [4].

Mitochondrial DNA (mtDNA) has a circular, double-stranded genetic structure and contains 13 essential proteins required for oxidative phosphorylation, along with two ribosomal RNAs and 22 transfer RNAs [5]. Unlike the nuclear genome, mtDNA is present in multiple copies within each cell, with numbers ranging from 10 to 10,000, depending on the cell type. Recent research has identified abnormal deletions in mtDNA in individuals with PCOS, suggesting a potential role for these deletions in the development of PCOS [6]. Notably, the 4977 base pair mtDNA deletion is the most common deletion within the mitochondrial genome and results in the loss of approximately one-third of the mtDNA content. This deletion has attracted attention as a potential biomarker indicating the relative health and damage of mtDNA [7]. Given the established link between the mtDNA4977 deletion and oxidative stress, several studies have proposed a connection between this

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deletion and PCOS, suggesting it as a potential biomarker for diagnosing PCOS [8,9].

MATERIALS AND METHODS

A case-control study of 80 females with an age range (20- 35) years was coducted; the study populations were subdivided into two groups:

Group 1: case includes 40 women with PCOS; those patients were diagnosed according to Rotterdam criteria (2003). [10].

Group 2: control include 40 age and BMI-matched women with regular menstrual cycles, no signs of hyperandrogenism, and no history of PCOS.

The calculation of body mass index (BMI) involved dividing the weight in kilograms by the square of the height in meters (kg/m2) [11].

Commercially ready ELISA kits, (AFIAS Elisa kits, Boditech, South Korea) were used to measure Serum Level of FSH, LH, Progesterone and Testosterone and ready ELISA kit (Mindary kit, China) was used to measure estrogen level in serum. The companies' instruction was followed precisely.

mtDNA was extracted from whole blood using a commercially ready kit for DNA extraction; for 4977-bp deletion detection, two pairs of primers were used in a conventional polymerase chain reaction (PCR) (Table 1). The first pair is for confirming mtDNA in the samples, while the other pair is specific for 4977-bp deletion. The 25 µl reaction mixture consisted of 4 µl mtDNA, 1-µl of each of the four oligonucleotides, 13-µl from mastermix and 4-µl deRNAase water (Pioneer, Korea). The reaction conditions in a conventional PCR apparatus (MWG Biotech AG, Ebersberg, Germany) shown in Table 2.

Table 1 The sequences of the primers and Product size for 4479-bp mitochondrial DNA

Primer Sequence 5'→3'	Product size (bp)	Annealing
5-TATACCGCCATCTTCAGCAAAC-3	179	58
5-TACTGCTAAATCCACCTTCGAC-3,	179	
5 CCTTACACTATTCCTCATCACC-3	127	
5-TGTGGTCTTTGGAGTAGAAACC-3	127	

Table 2 showed program of amplification for 4977-bp mitochondrial DNA deletion

Stage	Steps	Temp	Time	No. of cycle
One	Initial denaturation	94	5 min	1
Two	Denaturation	94	60 sec	35
	Annealing	58	30 sec	
	Extension	72	30 sec	
Three	Final extension	72	5 min	1

STATISTICAL ANALYSIS

Mean ± standard deviation (SD) was used to express the results, and student's t-test to compare between groups in Age, BMI, with a P value of 0.05 limits; non- normally distribution data were expressed as median and non-parametric Mann Whitney U test was used to compare hormones between patients and controls.

RESULTS

Table 3 shows the demographic characteristics of the study population. The mean age of women with PCOS was 29.7±15.49 years, which was very close to that of controls (30.18±5.25 years) with no significant difference. Although patients demonstrated higher BMI than controls (29.34±6.06 kg/m2 vs. 28.22±6.78 kg/m2), the difference was insignificant. There were very few cases of T2DM and hypertension in the two groups, with no significant differences.

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Table 3 Demographic characteristics of the study population

Variables	Patients (n=40)	Controls (n=40)	*p*-value
Age, years			
Mean ±SD	29.7±15.49	30.18±5.25	0.487
Range	19-41	20-41	
Body Mass Index, kg/m^2			
Mean ±SD	29.34±6.06	28.22±6.78	0.152
Range	19.7-44.9	20.3-57.26	
Comorbidities			
Diabetes	2(5%)	2(7.5%)	1.0
Hypertension	3(7.5%)	1(2.5%)	0.615

Data regarding hormonal profiles were found to be non-normally distributed. Therefore, these data were expressed as median, and a non-parametric Mann-Whitney U test was used to compare these marker groups between patients and controls (Table 4). The median serum level of FSH and LH in women with PCOS was 8.0 mIU/ml and 7.25 mIU/L, respectively, which was higher than that of controls (6.4 mIU/ml and 5.4 mIU/ml, respectively) with significant differences. Although the median serum level of E2 and testosterone was higher in patients than in controls, the differences were significant in testosterone and insignificant in E2. In contrast, healthy women demonstrated insignificantly higher median serum levels of progesterone than PCOS women (35.1 nmol/L vs. 26.75 nmol/L).

Table 4 Hormonal profile in PCOS patients and controls

Variables	Patients (n=40)	Controls (n=40)	*p*-value
FSH, mIU/ml			
Mean ±SD	8.37±2.27	6.89±2.35	**0.015**
Median	8.0	6.4	
Range	1.13-18.2	3.18-13.0	
LH, mIU/ml			
Mean ±SD	7.93±3.65	5.46±1.66	**0.001**
Median	7.25	5.4	
Range	3.1-20	2.4-9.0	
E2, pg/ml			
Mean ±SD	55.64±32.26	45.39±26.19	0.110
Median	48.0	41.9	
Range	11.7-163	11.9-120	
Testosterone, ng/ml			
Mean ±SD	0.61±0.27	0.47±0.31	**0.031**
Median	0.6	0.38	
Range	0.16-1.27	0.16-1.55	
Progesterone, nmol/L			
Mean ±SD	31.41±15.0	36.28±15.0	
Median	26.75	35.1	0.157
Range	10.51-65.2	13.1-69.5	

FSH: follicular stimulating hormone, LH: luteinizing hormone, E2: estrogen

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Mitochondrial DNA 4977bp- deletion

Gel electrophoresis of the mitochondria DNA amplified with specific primers using conventional PCR (Figure 1).

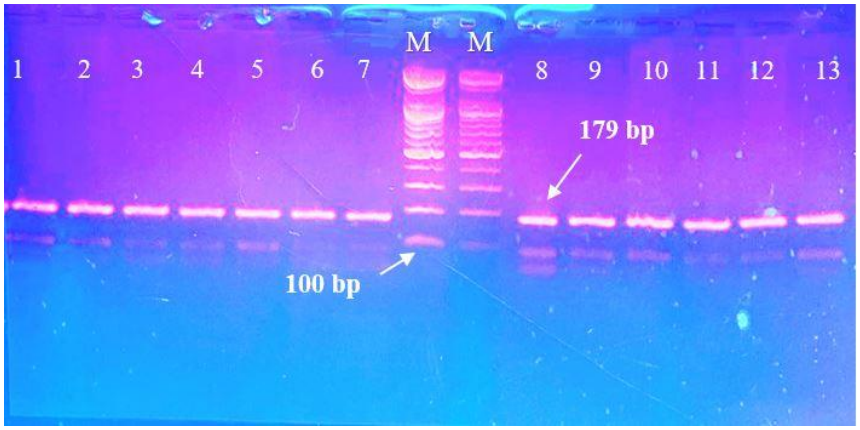

Figure 1 Gel electrophoresis for 4977-bp deletion mtDNA PCR, product stained with ethidium bromide and visualized using Uv- light, Lanes 1-13: intact mtDNA, M: molecular marker.

DISCUSSION

The present study showed no significant difference between patients and control in age, BMI, and comorbidities. This is because individuals in the control group were selected to match patients. On the other hand, some studies show a significant difference in BMI between patients with PCOS and healthy women. In the context of PCOS, BMI plays an important role because there is a strong association between PCOS and weight gain or obesity [12].

Many women with PCOS have a higher BMI than those without the condition. Excess weight and obesity can worsen the symptoms of PCOS and contribute to the development of other health issues, such as insulin resistance, type 2 diabetes, cardiovascular disease, and infertility [12].

Increased body fat levels can lead to insulin resistance, a common underlying PCOS factor. This can disrupt normal hormone production and contribute to the characteristic symptoms of PCOS [13].

In this study, the level of FSH was significantly higher in PCOS compared with the control, with an increase in FSH levels of women with PCOS (8.37±2.27 mIμ/ml) when compared with that of the control group (6.89±2.35 mIμ/ml). This agrees with some studies that found that the level of FSH in PCOS women was higher than in healthy women [14,15].

FSH is a hormone synthesized by the pituitary gland, that plays an essential role in regulating the menstrual cycle and stimulating the growth and development of ovarian follicles. In a normal menstrual cycle, FSH levels rise during the early phase to help stimulate the growth of multiple ovarian follicles. As the follicles mature, they produce increasing levels of estrogen. This rise in estrogen triggers a negative feedback loop, suppressing FSH secretion and allowing for the dominant follicle to develop and ovulation to occur [16].

The result of this study disagrees with Rasool et al., who found that the level of FSH decreases in women with PCOS compared to that of the healthy group [17].

This study showed a statistically significant difference in the LH level among PCOS (7.93±3.65 mIμ/ml) patients compared to the control group (5.46±1.66 mIμ/ml); this agreed with many studies that showed a high level of LH among PCOS patients compared to healthy women [18, 19].

In the present study, serum level of follicular phase estradiol increased in PCOS patients (55.64±32.26 pg/ml) as compared to control women (45.39±26.19pg/ml) but insignificantly, which agrees with the study result that demonstrated that estrogen level like androgens is higher in PCOS women [20]. On the other hand, its disagree with other study that found the serum level of estrogen in PCOS patients was significantly lower than in normal women [21].

This study's results show that the progesterone level was insignificantly decreased in PCOS patients (31.41±15.0 nmol/L) compared to control women

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(36.28±15.0nmol/L). A study of Weir CB et al., agreed with these results and found that the progesterone level was lower among PCOS women than among healthy women [22]. Progesterone is a hormone produced by the ovaries after ovulation, and it plays a crucial role in the menstrual cycle and in preparing the uterus for pregnancy [23].

The progesterone levels in PCOS may be lower than those in healthy women due to anovulation (lack of regular ovulation). In a typical menstrual cycle, the progesterone levels rise after ovulation and decline if pregnancy does not occur, leading to menstruation. However, in PCOS, ovulation might not occur regularly, and as a result, there may be insufficient progesterone production. Lower progesterone levels in PCOS can lead to irregular or absent menstrual periods and difficulty conceiving [24,25].

This study shows that the testosterone in PCOS women is significantly higher (0.61±0.27ng/ml) than the healthy control group (0.47±0.31ng/ml). Serum testosterone is therefore considered to be a sensitive biochemical marker supporting the diagnosis of PCOS. When testosterone hormones are elevated, as in PCOS, symptoms of androgen excess appear in those patients. For some women, this affects their menstrual function and fertility. Others continue to menstruate but have other symptoms like unwanted hair growth, acne, and weight gain [26].

A study conducted by Escobar-Morreale et al. presented findings that differ from the results of this study. They reported that serum levels of total testosterone were less frequently elevated in women with PCOS [27].

Mitochondria, the small cellular structures responsible for energy production, contain a unique type of genetic material called mitochondrial DNA (mtDNA). Deletions in mtDNA can occur as a result of oxidative damage and are associated with aging and various diseases. One of the most common deletions in mtDNA is the 4977-base pair deletion, which spans approximately one-third of the entire mitochondrial genome [28].

Contrary to previous study that found a significant association between the 4977-base pair mtDNA deletion and PCOS, this study's results indicated the absence of this deletion in both PCOS and healthy women. Specifically, the findings did not support the notion that the 4977-base pair deletion was more prevalent in PCOS patients and significantly associated with the condition [29]. This deletion has been established as a pathogenic mutation in humans, leading to the removal of five tRNAs and seven genes responsible for encoding respiratory chain complexes essential for normal oxidative phosphorylation functions. Consequently, the 4977-base pair deletion impairs protein synthesis, reduces ATP production, and decreases mtDNA copy numbers, thus causing mitochondrial dysfunction [29].

Elevated oxidative stress has been reported to induce the accumulation of deletions in mtDNA, contributing to mitochondrial dysfunction [30, 31]. In line with evidence linking oxidative stress to mitochondrial damage, a study by Ye M et al. in 2021 suggested that the mtDNA4977 deletion is a risk factor for PCOS, independent of metabolic parameters. Given that PCOS patients exhibit increased oxidative stress and reduced antioxidant capacity [32], it is reasonable to hypothesize that high levels of oxidative stress may trigger the occurrence of the mtDNA4977 deletion. When the deletion rate surpasses a certain threshold, it can lead to mitochondrial dysfunction and potentially play a role in the development of PCOS [32].

Conclusions:

The present study found no association of the mtDNA4977 with PCOS; Additional research is necessary to clarify the molecular mechanisms that underlie PCOS.

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Authors Contributions

All the authors have contributed equally.

Conflict of Interests

Declared none

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