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ANTI-MULLERIAN HORMONE GENE POLY MORPHISM IN OBESE WOMEN WITH POLYCYSTIC OVARY SYNDROME

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Abstract: Polycystic ovary syndrome (PCOS), a complex condition that affects women of reproductive age, women with PCOS present higher prevalence of obesity. The purpose of the current research is to examine the possible relationship between polycystic ovary syndrome (PCOS) and Anti-mullarian hormone with their gene polymorphism (+430 T/G). The patients and controls women samples were collected during period May 2022 to March 2023. Its 140 participated in this study the ages of patients and control women ranged from 18 to 40 years old. These 140 person were divided into two groups: Group I: 70 obese control group and Group II: 70 obese PCOS group. Using amplification refractory mutation system (ARMS), also termed allele-specific PCR. In addition, serum specimens were collected for the measurement of many biochemical parameters. Showed that increased significantly in serum AMH and the frequencies of the G and T alleles were showed that there were significant differences between the case PCOS group and control. MDA parameter increased significantly while GPX1 decreased non significantly, about T-AOC the result of current study showed decreased significantly as compared to control group. Women with PCOS had a significantly higher mean in serum LH and Prolactin than control women, while FSH increased non significantly in PCOS patient when compared to control women. Women with PCOS had decreased significantly in GRA% and significant higher mean value in LYM%, PLYand WBC, respectively than control women, while RDW% decreased significantly in PCOS patient when compared to control women. The participants with homozygous for the G allele of the AMH (+430 T/G) showed an increased risk of PCOS and the value was statistically significant among obese women with polycystic ovary syndrome in Erbil city and associated with significant increase in serum Anti-mullarian hormone in PCOS group.
Keywords: AMH, AMH gene promoter (+430 T/G) polymorphism, Oxidative stress and antioxidant parameters and Biochemical parameters.

1.Introduction

Polycystic ovary syndrome (PCOS) is a complex condition that affects women of reproductive age and is characterized by ovulatory dysfunction and androgen excess(1). PCOS is a lifelong endocrine dysfunction affecting 10 to 15% of women worldwide (2). In worldwide the prevalence of obesity in women with PCOS is estimated at 49% (3).

Anti-Mullerian hormone (AMH) is a dimeric glycoprotein with a molar mass of 140 kDa (4).The molecule consists of two identical subunits linked by sulfide bridges, and characterized by the N-terminal dimer (pro-region) and C-terminal dimer, AMH binds to its Type 2 receptor AMHR2, which phosphorylates a type I receptor under the transforming growth factor (TGF) beta signaling pathway (5). AMH is produced by the granulosa cells of growing follicles in the ovary, and AMH levels correlate with the number of antral follicles as

observed by transvaginal ultrasound (6). PCOS women display a 2- to 3-fold increase in serum AMH levels, compared with normal ovulatory women, reflecting the increased number of small antral follicles (7, 8).The follicular development beginning at the primary and secondary stages when AMH receptor II protein is expressed (9). Experiments were performed to assess follicular function and oocyte competence, as well as expression of genes that are critical for gonadotropin signaling, and oocyte quality (10). After birth there is an initial increse in AMH, then a gradual continuous rise with a peak at 25 years throughout childhood with some fluctuation around puberty (11). Thereafter, initial studies suggested that AMH decreases with age, and it becomes undetectable with 5 years before menopause (12). Polycystic ovary syndrome and obesity are closely linked both from an epidemiological genetic perspective, obesity affects between 38 and 88% of women with PCOS (13). There is much potential for altered gene expression to play an important role in the pathogenesis of PCOS, including genetic pathways implicated in steroid synthesis, cell communication, reproductive function and carbohydrate metabolism(14). Malondialdehyde (MDA) is a highly reactive compound that occurs as an enol and is one of the final products of polyunsaturated fatty acids (PUFAs) peroxidation in the cells. An increase in free radicals causes the overproduction of MDA. The level of MDA increases both in PCOS and obesity but also in hyperandrogenism and insulin resistance (IR). Lipid peroxidation of polyunsaturated fatty acids generates malondialdehyde (15). Kuscu and Var showed that MDA levels were increased in PCOS patients but were independent of obesity (16). PCOS is associated with decreased antioxidant concentration. It is one of the states with increased oxidative stress, leading to disturbance in the cycle of ovarian follicular and luteal phases (17). Follicular fluid in women with PCOS demonstrated increased levels of reactive oxygen species (ROS) and MDA. It decreased total antioxidant capacity (TAC), which was directly associated with reduced oocyte maturation and fertilization rates, poor embryo quality, and lower pregnancy rates(18).

The current study aimed to investigate the association of AMH with detection of single nucleotide polymorphism of AMH, oxidative stress and antioxidant and some hormones in patient with PCOS.

2. Materials and Methods

Sample and Study design

Seventy women with PCOS patients conducted gynaecology in maternity and teaching hospital in Erbil city were diagnosed by physicians, sonography and the history and clinical laboratory examination. The control group were composed of 70 healthy women free from signs and symptoms of PCOS, with regular menstrual cycles and the absence of obvious acne or hirsutism. None of the subjects PCOS or control group had clinically evident chronic or acute diseases such as infection, tumours, thyroid dysfunction, cardiovascular disease. All subjects with PCOS met the 1990 NIH criteria (19) and thus had clinical hyperandrogenism (i.e. hirsutism) and/or hyperandrogenemia and chronic oligoovulation. The patients and controls women samples were collected during period May 2022 to March 2023, which consists of 140 participate women, the ages of patients and control women ranged from 18 to 40 years old. These were divided into two groups: Group I: 70 obese women without PCOS (control group) and Group II: 70 obese women with PCOS (PCOS group). The recruited women were counseled and written informed consent such as (age, body mass index (BMI) and waist circumference(WHR)) BMI = body mass (kg)/height^2 (m^2). obese: BMI $\geq$ 25 kg/m^2, The waist-to-hip ratio is calculated by dividing waist circumference at its narrowest point by hip circumference at the widest point, was obtained from each woman before her participation in the current study.

Blood Sample Collection

From each participating subject, 10 ml of venous blood was drawn using disposable syringe, and distributed into two aliquots. The first aliquot (7 ml) was dispensed into a plain tube and it was centrifuged 15 minutes at 3000 rpm. The separated serum was distributed into 5 aliquots in Eppendorf tubes, which were frozen at -20˚C until assessment of hormones and biochemical tests. The second aliquot (3 ml) was transferred to EDTA tube for complete blood count (CBC) and for gene polymorphism detected.

Hormonal and Biochemical analysis

1- Determination of Anti-Mullerian hormone

Sera of patients and controls was assessed for the level of AMH using commercially available kits (Elabscieence Biotechnology, USA) by ELISA.

2- Determination of hormones

Serum levels of Luteinizing Hormone (LH), Follicle-Stimulating Hormone (FSH) and

prolactin (PRL) were done by using Cobas 6000 (Roche, Switzerland) immunoassay analyzers.

3- Determination of oxidative stress and antioxidant

Sera of patients and controls were assessed for the levels of) and malondialdehyde (MDA), glutathione peroxidase (GPX) and total antioxidant capacity (T-AOC) using commercially available kits (Elabscieence Biotechnology, USA) by ELISA.

4- Determination of some Hematological variables

Hematological variables were assessed on the day of the collection of blood. Haematological parameters including WBC count, PLT count, LYM%, GRA% and red blood cell distribution width (RDW%) were measured by a fully automated hematology analyzer (Medonic mserries, Sweden).

Molecular analysis
DNA extraction

DNA from whole blood was extracted with PureLink™ Genomic DNA Mini Kit from Thermo Fisher, USA. The following steps showed the DNA extraction steps according to the manufacturer's instructions.

Quantification and Qualification

Quantification and qualification of total DNA concentration was performed by using one Drop TOUCH Nano Drop with absorbance wavelengths of A 260/280 ratio and a ratio of ~1.7 is generally accepted as "pure" for DNA.

ARMS PCR

The amplification refractory mutation system (ARMS), also termed allele-specific PCR, is a simple method for detecting any known mutations involving single base changes which is based on the use of sequence-specific PCR primers that allow amplification of the DNA only when the target allele is contained within the sample. ARMS technique demands only PCR amplification and gel electrophoresis of the amplicons.

The targeted regions of AMH, was amplified. A total of 20 µl volume of reaction mixture was prepared to contain 3 µl DNA template, 10 µl of AddStart Taq Master 1µl of each primer and 4 µl double deionized water (ddH2O) in the thermocycler, Applied Biosystem (AB).

Cycling profile for AMH consisted of an initial denaturation step of 2 min at 94˚C followed by 30 cycles at 94˚C, 30 sec. at 55˚C, 30 sec, 72˚C 30sec., and final extension 2 min at 72˚C.

The PCR for AMH, primers were employed and the protocols are listed in the table below.

Table 1. Thermocycler Condition

Components	**Volume(µL)**
Master Mix	10 µL
Forward Primer(N)	1 µL
Forward Primer(M)	1 µL
Reverse Primer	1 µL
dH2O	4 µL
Template (DNA)	3µL
Total Volume	**20 µL**

Table 2. Thermocycler Condition of AMH

Temperature	**Time**	**Cycles**
94 °C	2 mins	1×
94 °C	30 sec	30 cycles
55 °C	30 sec	
72 °C	30 sec	
72 °C	2 mins	1×
12 °C	∞	

Table 3. Designed primer sequences used in T-ARMS-PCR genotyping detection and interpretation.

No.	Primer Name	Primer Sequence 5'-3'	bp	Amplicon Size
1	AMH-F	CCTTCCACTCGGCTCATTTA	20	
2	AMH-R	CACCAGGATGTGGACCTCCT	20	196bp

Agarose gel electrophoresis (POST-PCR Detection)

Agarose gel electrophoresis (2%) was employed to check the efficiency of PCR reactions and stained with ethidium bromide (EtBr) to make the DNA visible under UV light. The power supply condition was set at 100V for 40 minutes. The expected fragment sizes of the bands are indicated in figure 1 and 2.

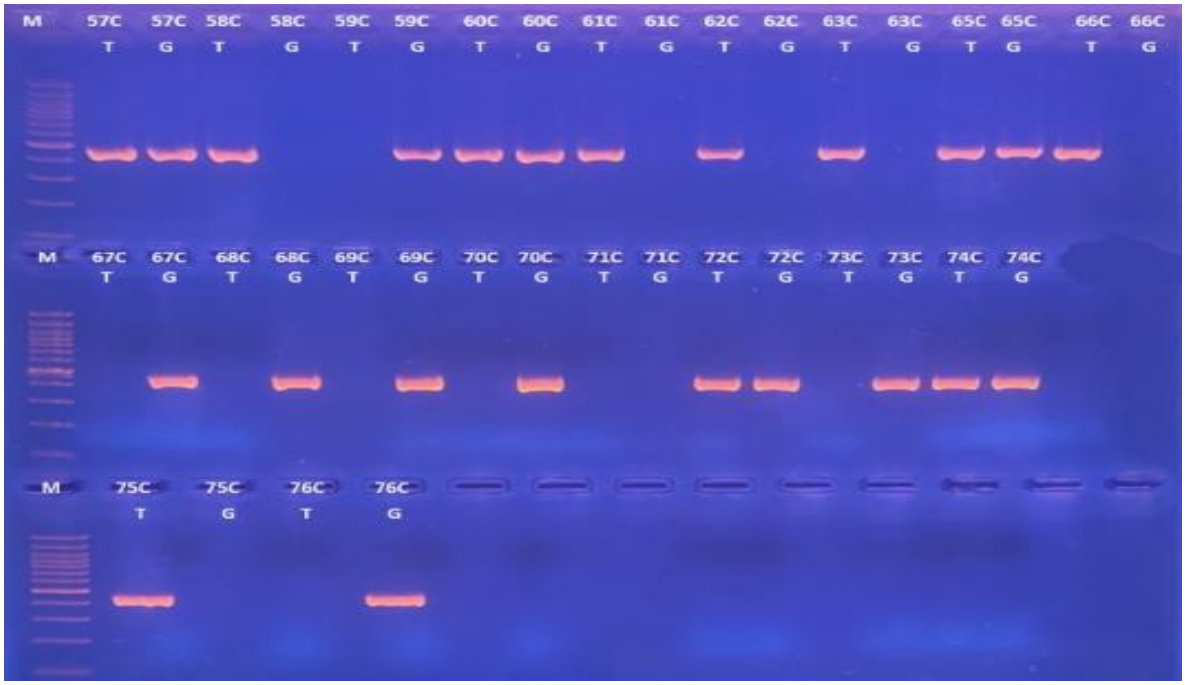


Figure 1. Agarose gel electrophoresis of control sample of the T-ARMS-PCR products for the AMH (+430 T/G) SNPs.

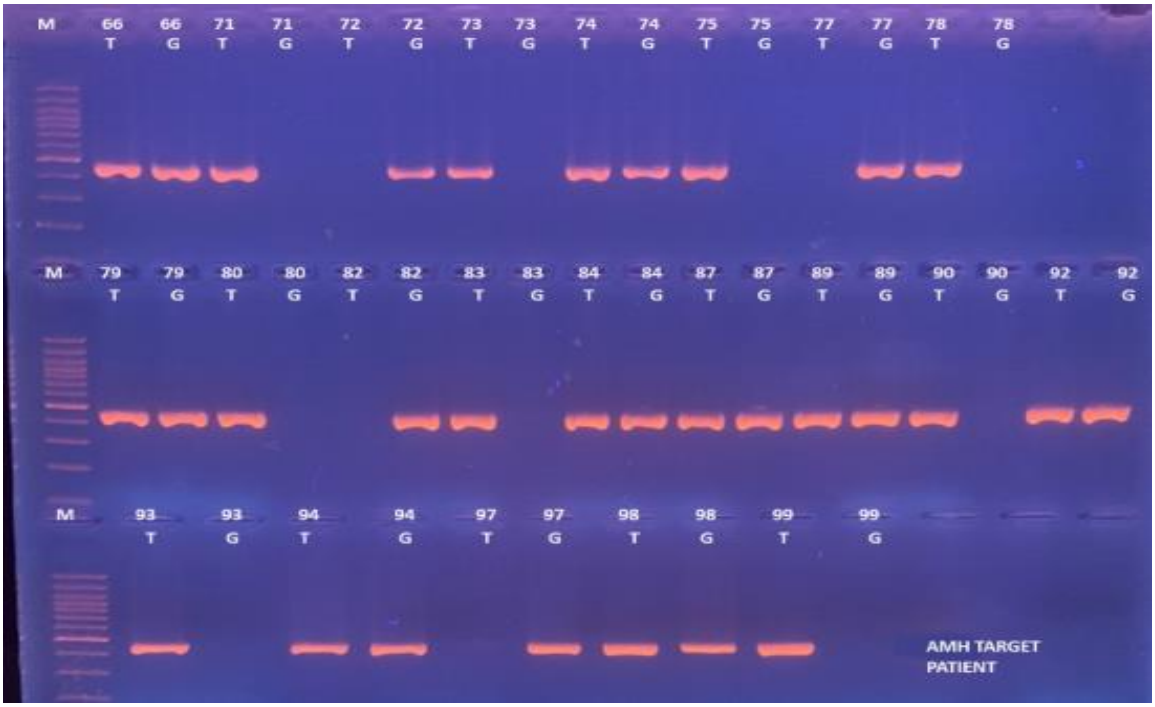


Figure 2. Agarose gel electrophoresis of patient case of the T-ARMS-PCR products for the AMH (+430 T/G) SNPs.

Statistical Analysis

Graph Pad Prism version 9 and MedCalc version 18 were used for the data analysis. The Kruskal Wallis test calculated Mean±SD, p-value≤0.05 was considered as significant, and the predictive significance of the study determined severity via Receiver Operator Characteristic (ROC) Curve analysis. The Hardy-Weinberg equilibrium was estimated using the H-W calculator for two alleles. Using stepwise multiple regression modeling to assess factors affecting serum AMH levels.

3.Results

3.1-Baseline Patient Characteristics

Mean age of the all cases 18-40 years and the mean age of PCOS patients and control group were 28.20±5.755 and 30.71± 6.181 respectively the mean baseline of BMI was 31.82±5.110 and 30.78± 5.843 kg/m2, and WHR 0.84±0.053 and 0.84±0.048 respectively. As in table 4.

Table 4. Baseline patient characteristics

Group of demographic	Patient	Control	p. value
Age	28.20±5.755	30.71± 6.181	NS
BMI	31.82±5.110	30.78± 5.843	NS
WHR	0.84±0.053	0.84±0.048	NS

BMI, body mass index. WHR, waist hip ratio.

3.2-Anti-mullarian hormone

Anti-mullarian hormone in PCOS group increased significantly ($p<0.05$) as value 1680±146.6 when compared with control group as value (433.7±54.51) shown in figure 3.

According to area under the curve(AUC) values, serum AMH exhibit a good marker for PCOS patients. The AUC of serum AMH was 0.914 and $p<0.001$ as showen in figure 4.

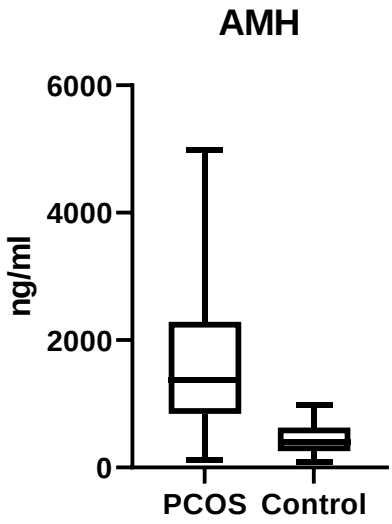


Figure 3. The levels of AMH between PCOS and control

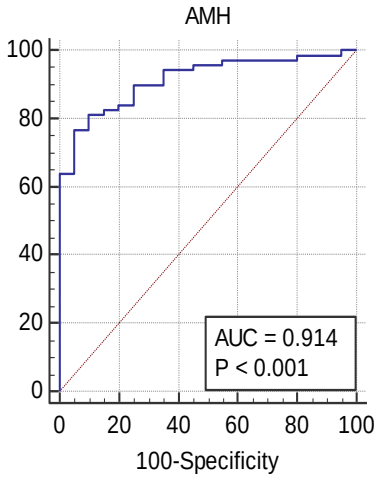
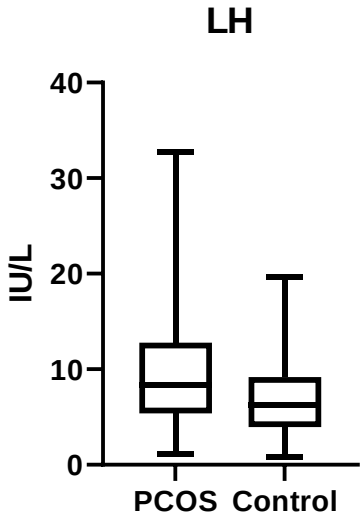


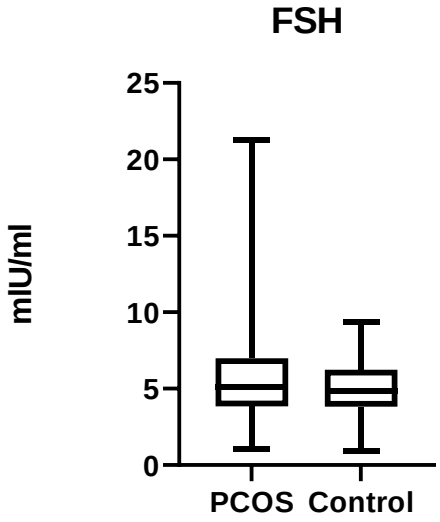
Figure 4. ROC curve analysis of AMH

3.3-Hormonal analysis

Women with PCOS group had a significantly higher mean in serum LH as value (9.734±0.682) and Prolactin (18.36±1.052), than control women as value (9.734±0.682) and (14.28±1.024) respectively with p value p<0.05 while FSH increased non significantly in PCOS patient as value (6.172±0.474) when compared to control women as value (4.952±0.225) as shown in figure 5.

According to area under the curve(AUC) values, serum hormonal levels were exhibit a good markers for PCOS patients. The AUC of serum LH was 0.609, FSH was0.545,Prolactin was 0.642 and pvalue=0.028,0.365 and 0.003 respectively as showen in figure6





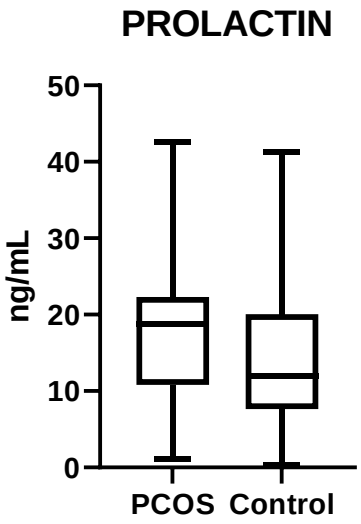
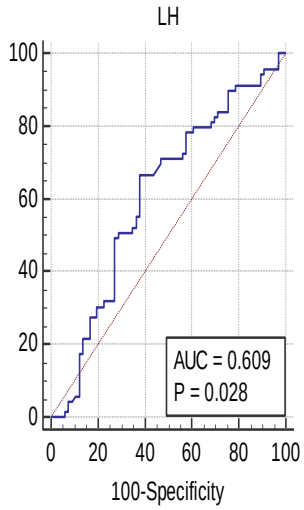
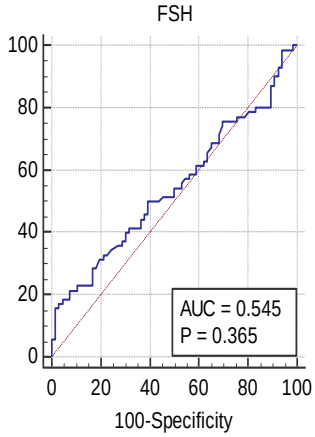


Figure 5. The Biochemical Parameters of PCOS Patients and Control group.





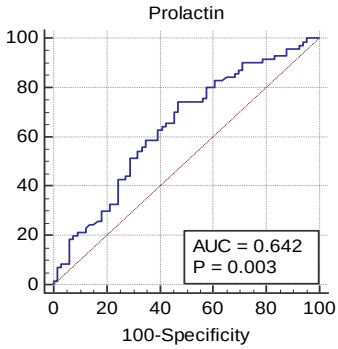


Figure 6. Prediction of hormones in patients with PCOS based of the ROC curve

3.4-Oxidative stress and antioxidant parameters

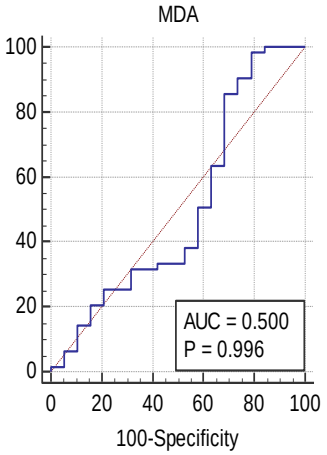
According to the antioxidant parameters, Table 5 demonstrated that MDA parameter increased significantly between PCOS patient and control group (938.7±75.10) and (511.8±115.6), respectively with p value <0.05, GPX1 in female PCOS decreased non significantly and their value was (17.00±1.236) when compared with control group as value (28.55±5.983), about T-AOC the result of current study showed that the PCOS group decreased significantly (0.195±0.009), (0.207±0.011) and (0.207±0.007) respectively as compared to control group with p value <0.05. Table 5.

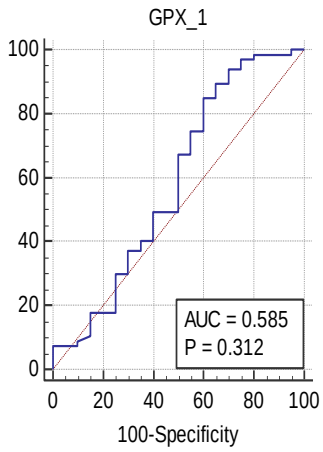
According to area under the curve(AUC) values, serum MDA, GPX1 and T-AOC exhibit a good markers for PCOS patients. The AUC of serum MDA was 0.500, GPX1 0.585, T-AOC was 0.654 and p value<0.996, 0.312 and 0.024 respectively as showen in figure 7.

Table 5. Serum Oxidative stress and antioxidant parameters levels in PCOS and control group.

parameters	PCOS	Control	P- value
MDA	938.7±75.10	511.8±115.6	0.005
GPX1	17.00±1.236	28.55±5.983	NS
T-AOC	0.195±0.009	0.207±0.011	0.045

NS: Non-significant. PCOS: Polycystic ovary syndrome, MDA: malondialdehyde GPX1: Glutathione peroxidase 1, T-AOC: Total antioxidant capacity.





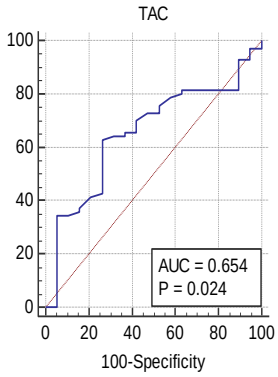


Figure 7. ROC curve analysis of serum oxidative stress and antioxidant level in PCOS and control

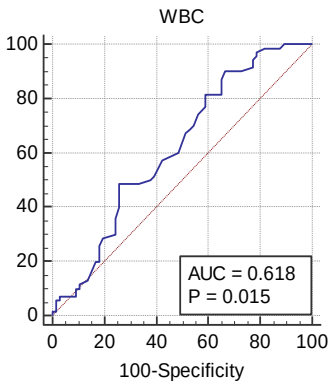
4.5-Hematological parameters

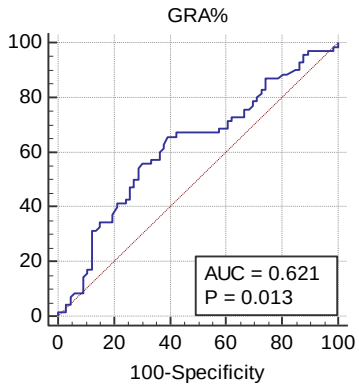
Table 6 showed a significant increase in WBC count in patient group as value 7.739±1.564 compared to control group as value 7.055±1.775 with p value <0.05. the granular percentage was higher significantly in PCOS group as value 61.53±7.687 when compared with control group as value 64.26±7.283 with p value <0.05. A significant increase in LYM% in patient group as value 30.70±6.691 compared to control group as value 28.83±6.776 with p value <0.05. PLT was higher significantly in PCOS group as value 277.1±47.61 when compared with control group as value 252.4±53.21 with p value <0.05. while RDW% decreased significantly in PCOS patient group as value 11.69±0.9450 compared to control group as value 12.23±1.096 with p value <0.05.

According to area under the curve(AUC) values, some blood parameters were exhibit a good markers for PCOS patients. The AUC of WBC was 0.618, GRA% was 0.621, LYM% was 0.604, PLT was 0.615, RDW% was 0.645 and p value=0.015, 0.013, 0.033, 0.020, and 0.0030 respectively as showen in figure 8.

Table 6. The Hematological parameters of PCOS Patients and Control group.

Hematological Parameters	PCOS	Control	P values
WBC	7.739±1.564	7.055±1.775	0.017
GRA%	61.53±7.687	64.26±7.283	0.014
LYM%	30.70±6.691	28.83±6.776	0.035
PLT	277.1±47.61	252.4±53.21	0.021
RDW%	11.69±0.9450	12.23±1.096	0.003





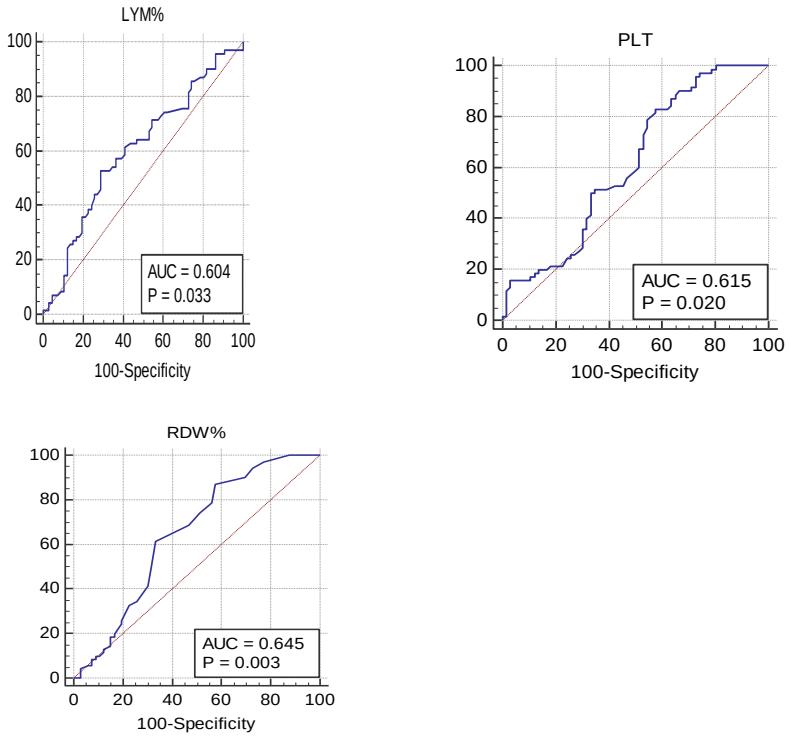


Figure 8. ROC curve analysis of hematological parameters in PCOS and control group.

Associations of AMH (+430 T/G) genotypes distributions and alleles frequencies in PCOS

Our results showed that the numbers of individuals of TT, TG and GG genotypes of AMH (+430 T/G) (rs10407022) were 21(14.7%), 36(25.9%) and 13(9.1%) in the case PCOS group and 45(31.5%), 16(110.2%) and 6(4.2%); in the control group, respectively. Moreover, the frequencies of the T and G alleles were 78(54.9%) and 62(43.4%) in the case PCOS group and 106(74.2%) and 28(19.6%) in the control group, respectively. Statistical analysis showed that there were significant differences between PCOS and control group However, non-statistically significant differences showed between the PCOS and control group, in the distribution of AMH genotypes GG (p=0.139 with P < 0.05). 57(39.9%) the recessive model (TT+TG) vs GG of the AMH showed a decreased risk of PCOS among control group 61(42.7%). 49(34.3%) the only recessive model (GG+TG) vs TT of the AMH showed an increase risk of PCOS among control group 22(15.4%) the value was statistically significant (p=0.000). As shown in table 7.

Table 7. Statistical evaluations of associations between AMH genotypes or alleles and PCOS patients.

AMH (rs10407022)	Patient N%	Control N%	R R	Etiology or Preventive Fraction	p	95% C I
Genotype						
TT	21(14.7%)	45(31.5%)	0.21	0.53	0.000	0.10-0.43
TG	36(25.9%)	16(110.2%)	3.37	0.36	0.001	1.63-6.98
GG	13(9.1%)	6(4.2%)	2.32	0.10	0.139	0.83-6.46
(TT+TG) vs GG	57(39.9%)	61(42.7%)	0.43	0.51	0.139	0.15-1.20
(GG+TG) vs TT	49(34.3%)	22(15.4%)	4.77	0.55	0.000	2.33-9.77
Allele						

T allele	78(54.9%)	106(74.2%)	0.33	0.52	0.000	0.20-0.57
G allele	62(43.4%)	28(19.6%)	3.01	0.29	0.000	1.77-5.12

RR: Relative risk, CI: Confidence Intervals, Exact Fishers Probability (P).

The genotype frequencies of AMH among the categorie of PCOS was assessed by the Hardy-Weinberg equilibrium (HWE) calculation. The differences in frequency of heterozygous genotype (TG) between observed and expected in PCOS group was 36(25.2%), 34.54(24.17%), 16(11.2%), 22.15(15.5%), in control group respectively. The variance between the observed and expected values of genotype frequencies was statistically non-significant, indicating that the distribution of this cohort was under HWE (Table 8).

Table 8. Observed numbers and Hardy-Weinberg (H-W) equilibrium of AMH genotypes and alleles in PCOS patients and control.

Case Categories		AMH gene at position 430 bp (re 10407022)					
		Genotypes			HWE p. value	Alleles	
		TT	TG	GG		T	G
PCOS patients	Observed	21(14.7%)	36(25.2%)	13(9.1%)	0.724	78(54.6%)	62(43.4%)
	Expected	21.73(14.95%)	34.54(24.17%)	13.73(9.61%)		Not estimated	
Control	Observed	45(31.5%)	16(11.2%)	6(4.9%)	0.023	106(74.2%)	28(19.6%)
	Expected	41.93(29.35%)	22.15(15.5%)	2.93(2.05%)		Not estimated	

5-Discussion

Polycystic ovary syndrome still lacks a definitive etiology, and a thorough diagnosis is challenging due to subjective phenotypes. The main findings of the present study the relationship between AMH and PCOS. Women with PCOS had significantly higher mean in serum AMH than control women, as reported by Humburg and Crawford, 2014 study, showed that the AMH level increased 2-3 times more in PCOS than women with normal ovary (20). AMH was secreted by granulose cells from tiny antral and prenatal follicles in the ovary. This hormone inhibited the recrument of primordial follicles and reduced the aromatase induction caused by FSH in the antral follicles. This results in a quicker than usual recruitment of primordial follicles, which accelerates the ovaries' loss of follicular reserves(21). Menstrual abnormalities will develop sooner as a result of the influence, and the likelihood of infertility and trouble becoming pregnant will rise (22). Moreover, another study suggested that the combination of AMH had higher sensitivity to diagnose PCOS when compared with normal group(23). The results of Alhassan et al., 2023 showed a substantial difference in AMH levels between PCOS cases and controls. This difference may have been caused by the higher levels of androgen in PCOS-affected individuals, which can lead to abnormalities in the ovaries and a relative decrease of FSH production (24).

The present study postulated that the antioxidant parameters, demonstrated that GPX1 in female PCOS decreased non significantly when compared with control group, our results align with the study in (2015) As an antioxidant defense against PCOS, women with PCOS demonstrated considerably lower GPx activity as compared to the control group. The mitochondrial antioxidant enzyme GPx1 is mainly an internal antioxidant that detoxifies H2O2 into water and is essential for shielding cells from the harmful effects of H_2O_2 (25). Certain disorders may be associated with an increase in H2O2 both inside and outside of cells as a result of increased H2O2 sources and/or decreased GPxl activitie owing to genetic variance (26).

Oxidative stress is caused by an imbalance between the body's antioxidant defenses, which act as a buffer against oxidative damage, and the generation of free radicals, which are highly reactive molecules with unpaired electrons (27), which could harm cellular and tissue structures at the molecular level (28).The current result showed that the level of MDA increased significantly between PCOS patient and control group. Because oxidative stress has a

negative impact on ovarian tissue, patients with cellular damage can exhibit elevated MDA levels. Consequently, using antioxidants might lessen the damaging impact that free radicals have on reproductive tissues (29). loss of follicular function may cause oxidative stress to may damage the ovarian cell and weaken the antioxidant to defense against this damage (30). As a result, there is an increase in inflammation and oxidative stress, which may exacerbate insulin resistance and hyperandrogenism in PCOS (31), while level of T-AOC showed significant decrease in PCOS group this could be explained by compensation in response to high circulating levels of oxidants(32). However study of (33) showed that patients with Polycystic ovary syndrome, significant difference recorded observed in activity of total antioxidant capacity (TAC) when compared to control group.
Gonadotropin releasing hormone (GnRH) triggers the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the pituitary gland. In PCOS, these two hormones are essential for the two phases of the menstrual cycle; without them, the egg cannot develop or emerge from the follicle. This breaks the cycle and causes either primary or secondary amenorrhea, (34). In the present study, women with PCOS had a higher mean in serum LH and Prolactin, this result is in agree with (35) showed that Compared to controls who are slim, obese PCOS patients had considerably higher mean serum prolactin and LH levels. Additionally, aberrant GnRH release resulting in alterations in LH secretion patterns and higher LH levels with a relative decrease in glucose may be brought on by obesity, metabolic problems, or changed sex steroid synthesis(36).

The current study showed that women with PCOS had a non-significant change in serum FSH than control women, Women with PCOS have disruptions to the normal gonadotrophin axis, which causes a rise in LH and a reduction in FSH (37). The findings contradict the 2017 study by Lal et al., which discovered substantial variations in LH, FSH, and the LH/FSH ratio between non-obese and obese women. In current study women with PCOS had decreased significantly in GRA% in PCOS when compared to control value. In PCOS group significant higher mean in value in LYM%, PLT and WBC, respectively than control women, while RDW% decreased significantly in PCOS patient when compared to control women. PCOS women demonstrated significantly increase levels of white blood cells (WBC) compared to the control group as shown by (36). Regardless of the patient's obesity, PCOS patients have considerably higher WBC and neutrophil counts, indicating that chronic inflammation is a role in PCOS that cannot be disregarded (38). Research indicates that elevated white blood cell counts in PCOS patients are associated with persistent low-grade inflammation, rather than obesity. This suggests a noteworthy relationship between metabolic issues and problems (39). However WBCs did not significantly differ between PCOS-affected women and controls in the research (40). In women with PCOS, a study found that WBCs had a positive prognostic effect and lymphocytes had a negative predictive effect by increase of inflammatory biomarkers in lean and obese women (41). According to Shi et al. (2013), women with PCOS had significantly higher lymphocyte and total WBC counts than controls. Results showed that women in the PCOS group had significantly higher levels of RDW (another inflammatory marker) than women in the control group. This could be because subjects with PCOS have underlying chronic inflammation; hence, an increase in RDW concentration can be linked to low-grade chronic inflammation in PCOS patients. According to a study, patients with PCOS had red blood cell distribution widths (RDWs) that were larger than those of non-PCOS patients. This can lead to oxidative stress by inducing the release of RDW from immature red blood cells (42).

The current result found that AMH (+430 T/G) (rs10407022), of the GG and TG genotypes have a higher risk for PCOS than women with the TT genotype. The G allele is associated with an increased risk for PCOS, which is an T allele. In the case of AMH (+430 T/G), the TG genotype has a greater risk with the T allele. Mutation in study showed that, also known as SNP rs10407022, has been reported to have a clinically significant effect in PCOS (22). Also agreement with (43) discovered that the AMH gene polymorphism at position c.146G>T, p.Ile49Ser (rs10407022) results in a shift in the AMH protein's amino acid composition from serine to isoleucine at position 49 and is known to impact AMH bioactivity. The genotype T/G of AMH is substantially greater in PCOS women. The AMH genotype T/G may be involved in the high serum levels of LH. These elevated levels of LH enhanced the production of androgenic precursors in PCOS patients compared to non-PCOS patients. These precursors can induce oxidative stress by inducing the release of RDW from immature red blood cells (44).

Conclusion

this study was concluded that the levels of AMH and the frequencies of the G and T alleles were showed significant differences between the case PCOS group and control due to increase the number of small antral follicles in PCOS in female patients. The oxidative and anti oxidative stress, hormonal and hematological parameters correlated with poly cystic ovarian syndrome.

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Conflicts of Interest
The authors state that they do not have any conflicts of interest.

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Author Contribution
All authors have contributed significantly to this research. (N.H. O.) took responsibility for collecting samples, laboratory investigations, statistical assessment, and manuscript composition. (K.A. M.) and (S.N. D.) played roles in the conception, and design, with the interpretation of the research's results and offered valuable input and feedback throughout the development of the manuscript. All the authors carefully reviewed and approved the final draught of the manuscript.

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