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Relationship between Anti-Müllerian Hormone, Antral Follicle Count, Thyroid Hormone, Age and BMI in Patients with Polycystic Ovary Syndrome

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Abstract:-

➢ ***Background:***

One of the most prevalent endocrine disorders affecting women of reproductive age is Polycystic ovary syndrome (PCOS). The long-term health issues it can create in women, like obesity, diabetes, metabolic syndrome, and most significantly, infertility, are what make it so problematic.

➢ ***Methods:***

Between July 2022 to the end of October 2022, Dr. Youssef Al-Hussein Center for Fertility and Infertility Treatment in Tartous conducted this study with two groups: patients (83) and healthy women (67). Blood samples were collected from patients and healthy subjects, and laboratory analyses were performed, including Anti-Mullerian Hormone (AMH) using Cobas e 411 devices, TSH analysis using an I chroma device, and AFC measuring using a transvaginal ultrasound probe.

➢ ***Results:***

The levels of AMH and each factor (physical activity, smoking, TSH and BMI) did not significantly differ according to the statistical tests (P>0.05), but there was a clear statistical difference (P<0.05) in the relationship of AMH with age, showing that AMH decreased with advancing age. The association between AMH and AFC, where AMH was the quantitative expression of the antral follicle count (AFC), also showed a significant difference.

➢ ***Conclusion:***

Smoking and physical activity had no impact on AMH levels, and neither did BMI or TSH levels. However, age clearly had an impact on AMH, and AMH was the quantitative representation of AFC.

Keywords:- *Polycystic Ovary Syndrome, Anti-Müllerian Hormone, Antral Follicle, Fertility.*

I. INTRODUCTION

In women of reproductive age, PCOS is the most prevalent endocrine disorder, with an estimated prevalence of 8-13%. Infertility is most commonly caused by it 1. Both adolescent girls and adults are affected by a heterogeneous disorder that has multiple phenotypes. Irving Stein and Michael Leventhal, two physicians, first described the syndrome in 19352. Women with PCOS have a variety of clinical symptoms (Oligomenorrhea or amenorrhea, hirsutism, acne, male-pattern hair loss), psychological problems (decreased quality of life, poor self-esteem, depression, anxiety), reproductive problems (infertility and pregnancy complications), and metabolic complications (insulin resistance, metabolic syndrome, IGT, DM2 and possibly CVD)3.

PCOS, a chronic condition with psychological manifestations, usually starts in adolescence and progresses to infertility and increasing metabolic complications over time. If the syndrome is associated with obesity at a younger age, metabolic complications like IGT and DM2 can develop in adolescence 4.

The term polycystic ovary syndrome is not fully defined. The ovaries' appearance is mainly due to the multiplicity of antral follicles, as there are no epithelial abscesses or cysts5. Due to its heterogeneous and complex nature, the pathophysiology of PCOS is still unclear. A group of factors interact and exacerbate each other creating a vicious circle, such as hyper-androgenism is a result of a fundamental defect in theca cells in the ovary or as a result of a defect in the hypothalamic-pituitary-ovarian axis3, This results in a defect in the secretion of GnRH Pulsation, leading to an abnormal secretion of reproductive hormones (increased LH and increased ratio of LH/FSH). Poor Ovarian function and defective growth of follicles are the consequences of an imbalance in these hormones. An increase in AMH could also lead to hyperinsulinemia and insulin resistance, which are linked to visceral obesity and dyslipidemia6.

The heterogeneous of the disease is due to the different percentage of participation of each of these factors among women 7. PCOS has no single diagnostic test; therefore, several diagnostic criteria have been proposed, including the National Institutes of Health (NIH) criteria, the Rotterdam criteria for 2003 and the Androgen Excess and PCOS Society criteria (AE-PCOS) 8,9.

The 2003 Rotterdam Criteria are now recognized as a gold standard for PCOS diagnosis on a global scale. Based on the presence of two of the following three criteria:

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1-Oligomenorrhea (Oligo/Amenorrhoea).2-Biochemical and/or clinical hyperandrogenism (HA). 3-The presence of more than 12 follicles with a diameter of 2-9 mm in each ovary or when the volume of the ovary was larger than (10 cm3) where indicators of polycystic ovaries (PCOM) as shown by ultrasonography.

Anti-Mullerian Hormone (AMH) is a diglycoprotein and a member of the transforming growth factor beta (TGF-β) family. The AMH gene is encoded by the 2.75 kbp gene, which has five exons on chromosome 19 13.3 p 10.

Male Sertoli cells in the testis release AMH early and in large quantities throughout the development of the embryo. This hormone causes the retraction of the Muller ducts, which stops the development of female organs. For female fetuses, granulosa cells in the ovary secrete AMH hormone at a late stage and in little amounts. The female reproductive system develops as a result of this hormone's absence10,11. Early follicular recruitment is suppressed and early follicular reserve depletion is inhibited by AMH.

AMH is currently being applied in clinical settings to evaluate ovarian reserve and enhance infertility care11. An increase in ovarian follicles across the board during all phases of development is a hallmark of PCOS. Particularly in pre-antral and small antral follicles, this rise was noticeable.

AMH is first produced by these follicles. AMH is released by granulosa cells (GCs) of antral follicles and quantifiable blood levels are produced. These serum levels appear to be proportional to the number of follicles that are developing in the ovaries11,12.

AMH concentrations in blood serum could be measured with sensitive assays, and it was discovered that these concentrations were 2-4 times higher in women with polycystic ovary syndrome compared to healthy women. Initially, this high serum AMH level was thought to reflect the increased reserve of preantral and small antral follicles within the polycystic ovary; however, the increased production of AMH is qualitative, meaning that each granulosa cell in a polycystic ovary can produce 75 times more AMH compared to a granulosa cell in a normal ovary13.

II. MATERIALS AND METHODS

This research was conducted in the laboratory of Dr. Youssef Al-Hussein Center for Fertility and Infertility Treatment in Tartous, during the period from July 2022 to the end of October 2022, where the study included two groups, the patients and the healthy (Figure 1), and the study sample was distributed as follows:

The first group: (83) patients with polycystic ovary syndrome, and their ages ranged between (21-39) years.

The second group: (67) healthy women, whose ages ranged from (23-45) years, for the purpose of making a comparison with the patients group.

➢ *Exclusion Criteria: It Included the following:*

- Thyroid disorders - Cushing's syndrome
- Congenital adrenal hyperplasia
- Removing one or both ovaries
- Hyperprolactinemia
- Radiation or chemotherapy

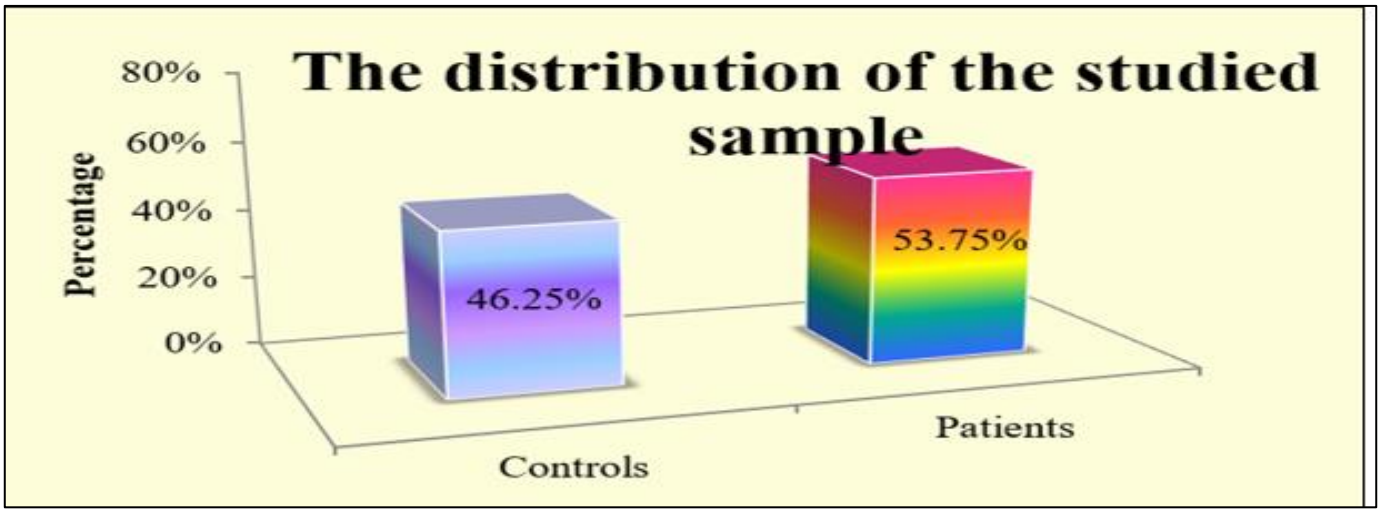

Fig 1: The Studied Sample is Distributed According to the Two Groups of Patients and Healthy Women

The questionnaire was filled up with information from healthy and patients women, 5 ml of venous blood was taken in dry sterile tubes, and the serum was separated using a tube separator at a speed of 3000 round per minute for 10 minutes . The serum was examined biochemically as soon as it was collected.

Serum AMH levels measurement was performed using the electrochemiluminescence immunoassay (Elecsys Cobas e411 analyzers, Roche Diagnostics GmbH, Mannheim, Germany). The measurement range for serum AMH was (0.03-23 ng/ml). Serum TSH levels were assayed through immunofluorescence using the I-chroma Boditech/Korea analyzer.

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All women underwent ultrasonography and the number of antral follicles was calculated (day 2-4) using an endovaginal probe (Mindray DC-7 MX29003997 China, 5–8 mHz) by a specialized doctor. Antral follicles with a diameter between 2-9 mm were measured in both ovaries, and follicles greater than 10 mm were ignored.

III. STATISTICAL METHODOLOGY

The results were analyzed with the help of the Statistical Package for Social Sciences (SPSS 24), using the following tests: t-test for two independent samples to compare the averages of two independent samples, chi-square test to determine the relationship between nominal or rank variables, Pearson correlation to determine the value and significance of the association between variables amount.

Where the differences at the significance threshold (P value<0.05) were considered statistically significant.

IV. RESULTS AND DISCUSSION

The relationships between AMH levels and the variables in PCOS patients and healthy women are discussed below:

A. The Relationship between AMH Level and Physical Activity

The effect of physical activity on the median AMH level has been investigated in patients with polycystic ovary syndrome and in healthy participants. Table (1) shows that there were no statistically significant differences because the significant difference was greater than 0.05.

The findings of this study are in opposition to those of the Moran study (Australia, 2011) and the Kazeminia study (Iran, 2022). These studies found that levels of AMH decrease with good exercise because it is expected that ovarian function will improve 14,15.

These studies determined the type of physical activity (exercise or the use of specific sports equipment), its duration, and its frequency throughout the week or month, whereas the current study limited the physical activity to (yes or no), as it indicated that the answer is yes to the presence of physical effort, whether by working indoors or going to a gym, while the answer is no, there is no physical effort.

Table 1: The Relationship between AMH and both Physical Activity and Smoking

			Patients			Controls		
			Mean	**standard deviation**	**P-value**	**Mean**	**standard deviation**	**P-value**
AMH	physical activity	a little	3.91	7.6	0.856	1.96	3.3	0.497
		middle	3.21	7.3		1.98	3.8	
	smoking	non smoker	3.21	7.8	0.642	1.72	3.4	0.606
		smoker	3.85	7.3		2.16	3.7	

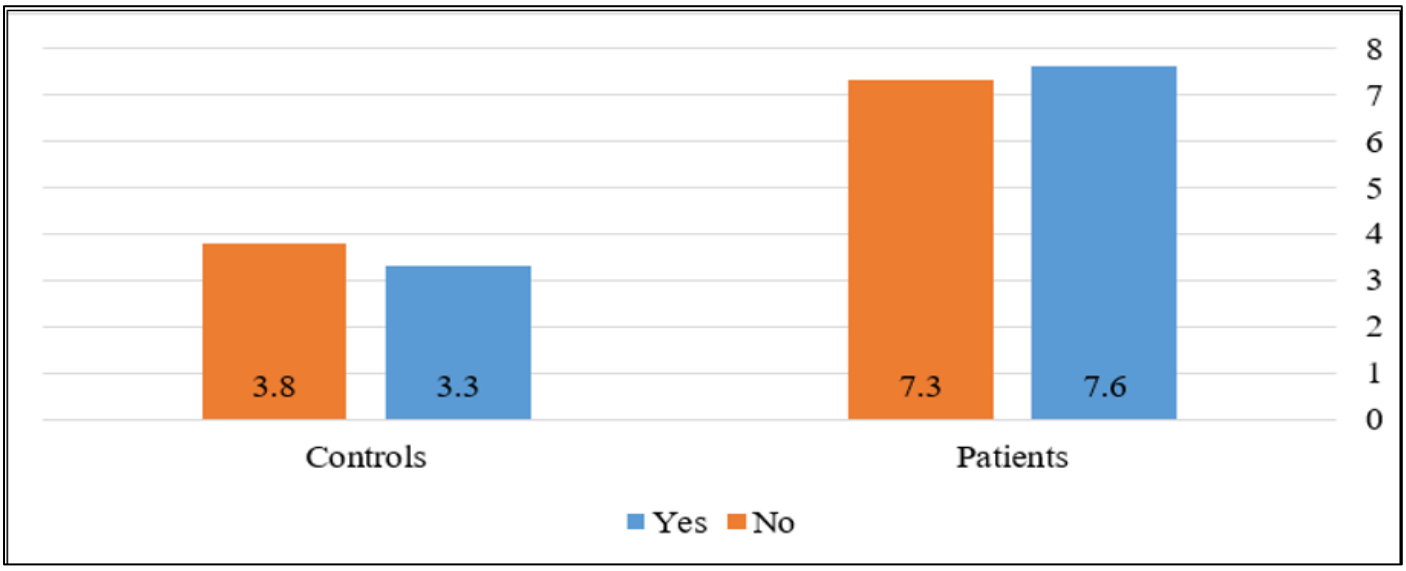

Fig 2: The Relationship between AMH and Physical Activity between the Two Groups of Patients and Control

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B. The Relationship Between AMH Level and Cigarette Smoking

The purpose of this study was to clarify the relationship between AMH levels and smoking status in patients and healthy women. Table (1) makes it evident that there were no statistical differences between AMH and smoking because the P-value was greater than 0.05. This may concur with the study carried out by Bhide et al., (the United Kingdom, 2021)16.

While I disagreed with the findings of the studies by Plante et al. (America ,2010), and Dólleman (the Netherlands, 2013), both studies showed that current smoking is associated with lower levels of the hormone AMH, suggesting an immediate associaton between smoking and the depletion of ovarian follicles17,18.

The reason for difference is that these studies concentrated on examining the relationship between AMH and the smoking factor and they classified smokers as passive, active, or former by counting how many cigarettes they smoked each day, how many packs they consumed annually, and when they gave up.

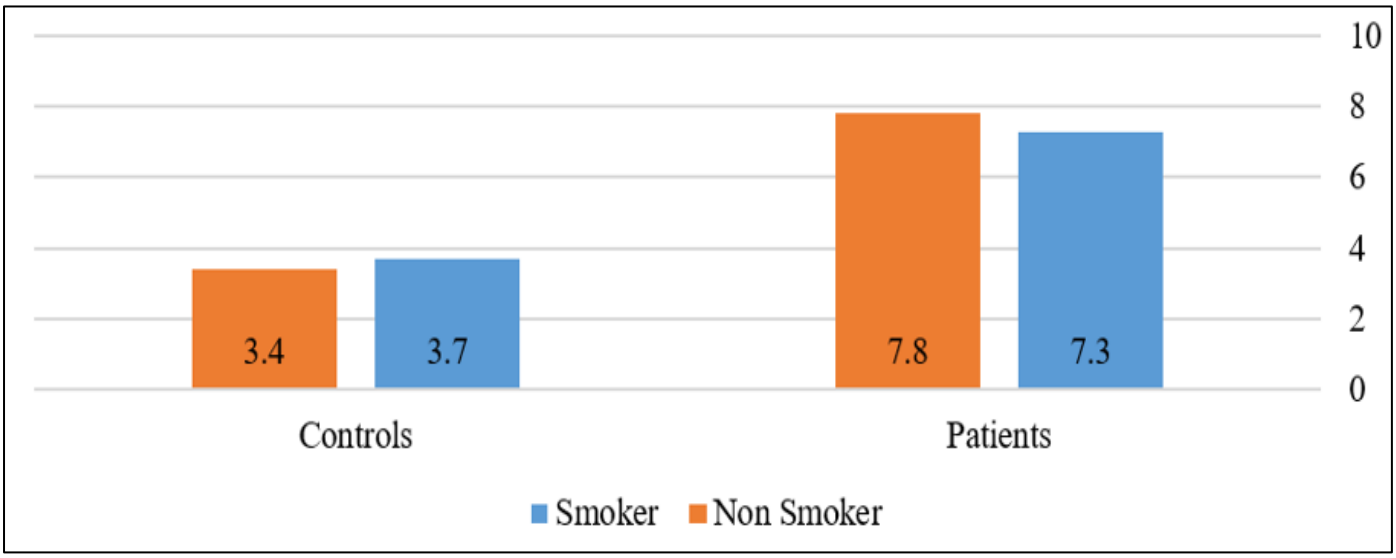

Fig 3: The Relationship between AMH and Smoking between the Two Groups of Patients and Control

C. The Relationship Between AMH and TSH Levels

It was discovered from table (2) that the P-value is greater than 0.05, and this indicates that there is no significant difference between the levels of TSH and AMH. This result was in contrast to Kabodmehri's research, which was conducted in (Iran, 2021) and another study conducted by Kareem AL-Jaff in (Iraq, 2018), which found that AMH decreases with an increase in TSH 19,20.

This difference is explained by the fact that individuals with thyroid disorders were not included in our investigation; whereas, patients with polycystic ovary syndrome and thyroid disorders were included in other studies.

Table 2: Relationship of AMH with (TSH-AFC - Age - BMI)

	AMH			
	Patients		**Controls**	
TSH	0.830	0.037	0.646	0.072
AFC	0.000	0.955	0.000	0.894
Age	0.048	0.327-	0.283	0.167
BMI	0.261	0.190	0.940	0.012

D. The Relationship between AMH and BMI

The current study also explained the relationship between the level of AMH and BMI, which is shown in table (2). It was noted that the P-value was greater than 0.05, within both the patient sample and the control sample, meaning that there were no statistical differences between the level of AMH and BMI, because most of the women who visited the center had already started the first line of treatment to lose weight, their weights were closer to normal. This is line with Kloos study and the Park study in (Korea,2010), that found no relationship between AMH and BMI21,22.

E. The Relationship between AMH Level and Age

Due to its significant impact on fertility, the relationship between AMH level and age is one of the more intriguing topics. After statistical analysis, we came to the conclusion that there is a significant negative correlation between AMH and age in control sample ($P < 0.05$, $r = -0.327$) and that AMH decreased with advancing age (Figure 3). These results support the scientific belief that AMH is an indicator of ovarian ageing 21.

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AMH concentrations are already high in patients, so the decline in AMH is slower with age, but the concentration of AMH starts to decrease at a faster rate at the age of 38 and beyond, as shown in (Figure 4), so there was no significant correlation between the level of AMH and age in patients with polycystic ovary syndrome. Because most of the patient samples in our study were 38 years of age or younger, with one patient being 39 years old, this decline was not noticed 22.

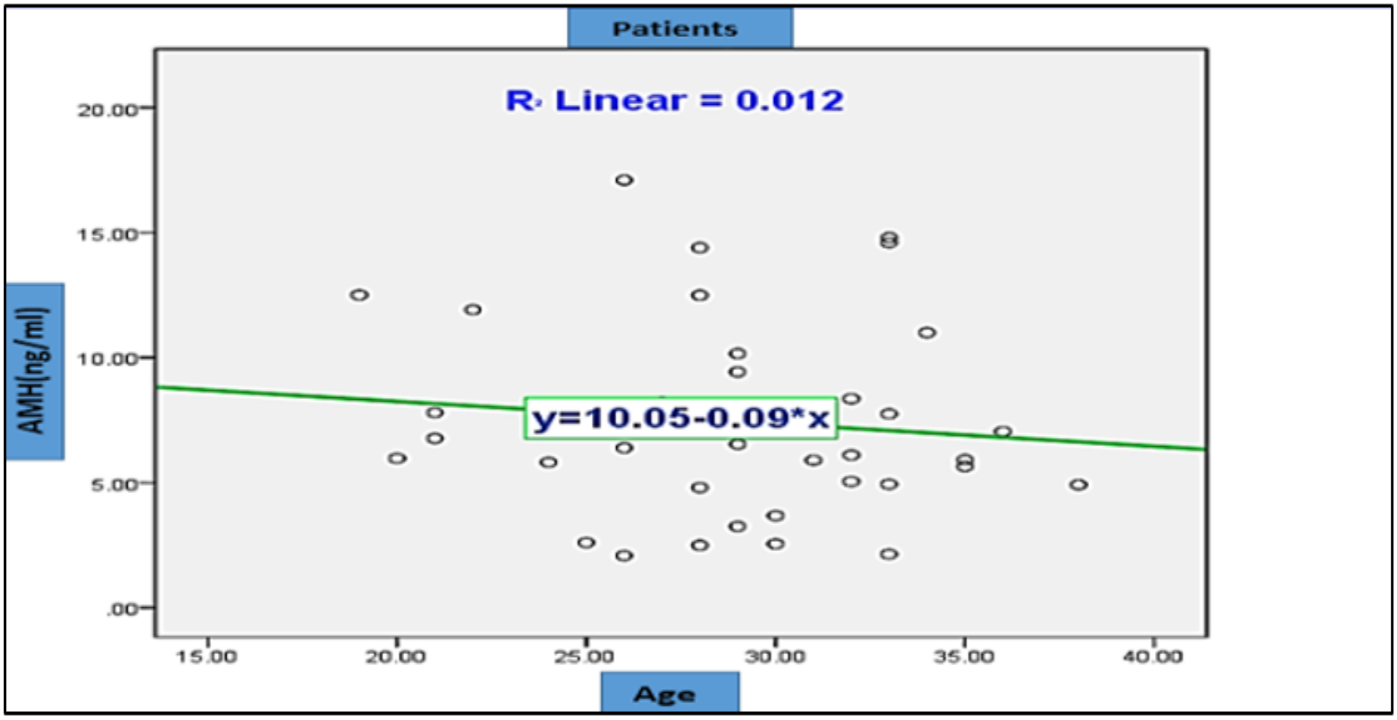

Fig 4: The Relationship between AMH Levels and Age in Controls

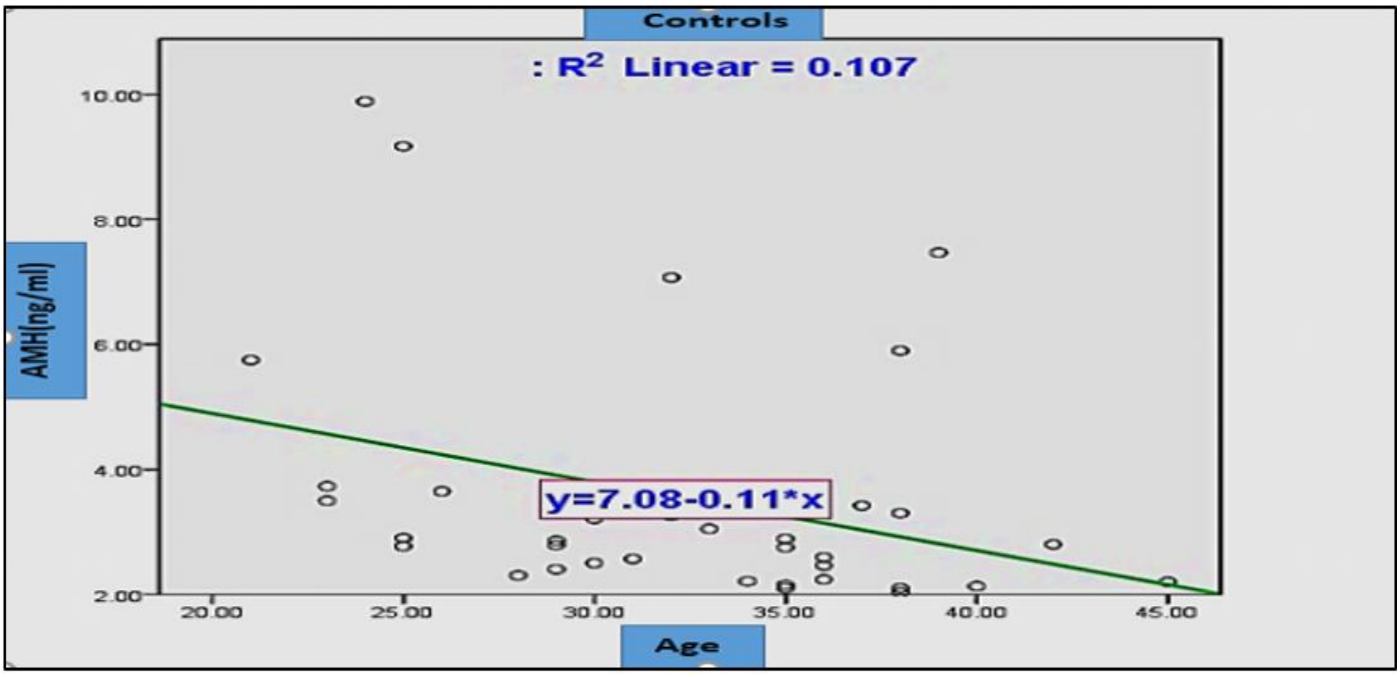

Fig 5: The Relationship between AMH Levels and Age in Patients with Polycystic Ovary Syndrome

F. The Relationship Between AMH Concentration and AFC

There is a close relationship between AMH levels and the number of antral follicle count (AFC) since the AMH is a quantitative indication of the AFC. Table 2 clearly shows that there was a highly significant positive correlation between AMH and AFC ($P<0.05$). The correlation coefficients for the control group and patients group were ($r=0.955$, $r=0.894$) respectively. This association was explained in control group (Figure 5), by the fact that AMH is typically secreted by ovaries from the preantral and small antral follicles, which are the follicles visible on ultrasound imaging (AFC).

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This finding In the patient group (Figure 6), is explained by the fact that polycystic ovarian syndrome prevents follicles development at the point where the dominant follicle is chosen, as it does in healthy state, and that this prevents follicle maturation during later stages development, which leads to the accumulation of many immature follicles (antral and pre-antral), which could account for the elevated levels of AMH. In other words, higher AMH production results from an increase in follicles, which is a characteristic of PCOS 23.

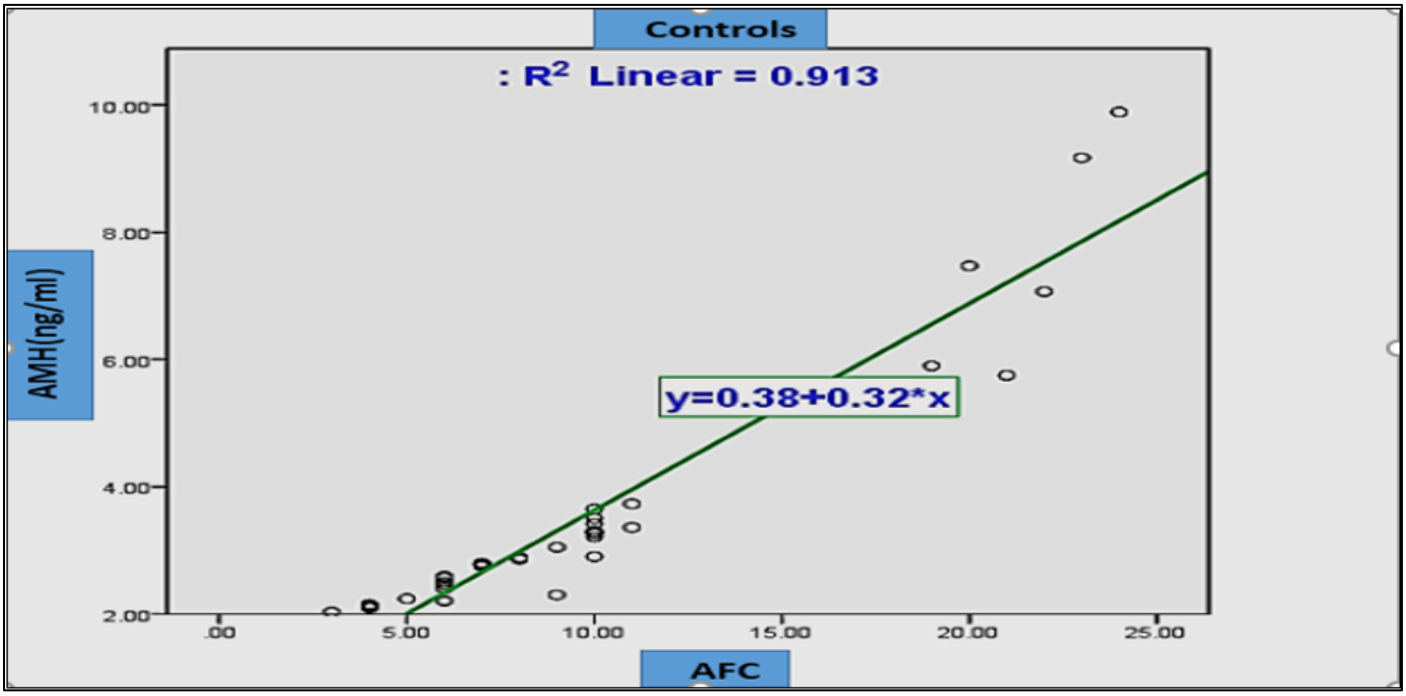

Fig 6: The Relationship between AMH Levels and AFC in Controls

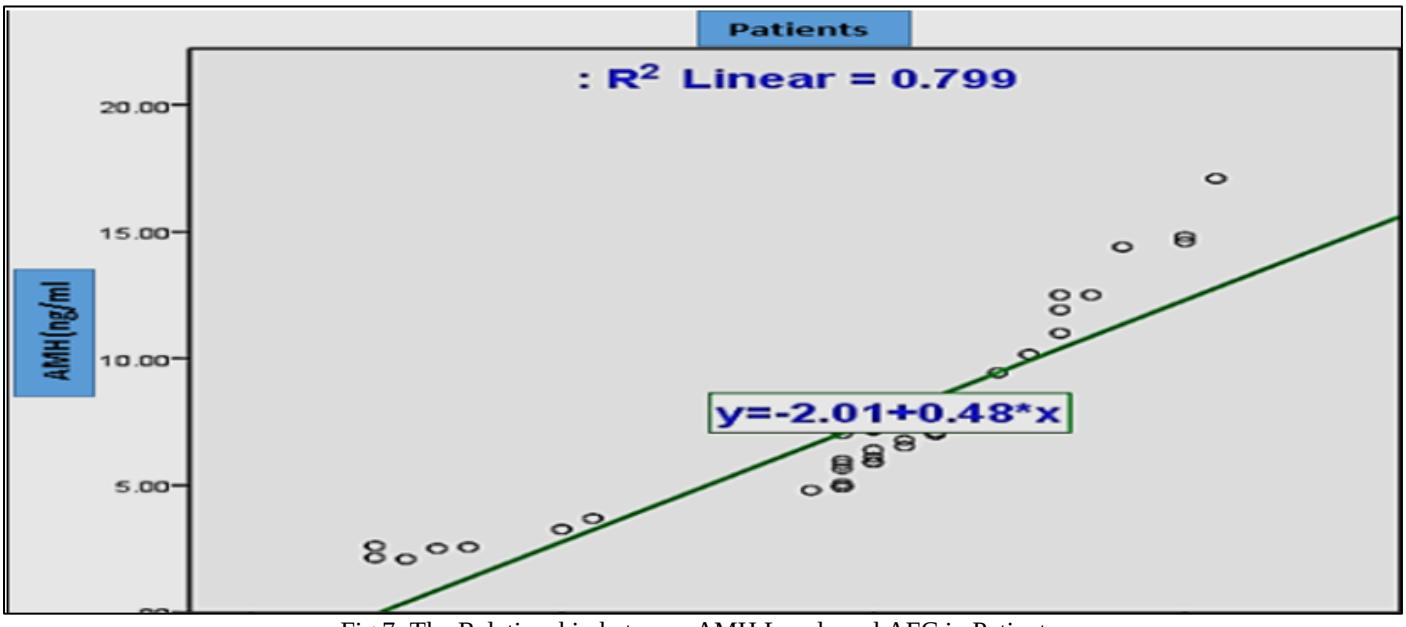

Fig 7: The Relationship between AMH Levels and AFC in Patients

V. CONCLUSION AND RECOMMENDATIONS

This study shown that there is a definite association between AMH concentration and age in healthy women, but there is no effect of age on AMH level in patients. No correlations were found between AMH and any of the following factors: smoking, physical activity, BMI or TSH. There has also been evidence of a significant correlation between AMH levels and AFC.

In order to preserve ovarian reserve and fertility for as long as possible during a woman's life, the study advises further investigation into the relationship between AMH and other factors like insulin resistance, lipid profile, and alcohol to ascertain how they affect AMH.

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➢ *List of Abbreviations*

- PCOS: Poly Cystic Ovary Syndrome
- BMI: Body Mass Index
- AFC: Antral Follicle Count
- TSH: Thyroid Stimulating Hormone
- AMH: Anti Mullerian Hormone
- FSH: Follicle Stimulating Hormone
- LH: Luitinizing Hormone
- IGT: Impaired Glucose Tolerance
- DM2: Diabetes Mellitus type2
- OA: Oligo/Amenorrhea
- HA: Hyperandrogenism
- PCOM: Polycystic Ovarian Morphology
- GCs: Granulosa Cells
- TGF-β: Transforming Growth Factor-β

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