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Original Research Article

THE ASSOCIATION BETWEEN ANTI MULLERIAN HORMONE AND VITAMIN-D IN FEMALE REPRODUCTIVE PHYSIOLOGY & PCOS: AN OBSERVATIONAL STUDY

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Abstract

Accumulating data from human research reveals a role of vitamin D in female reproductive physiology, and multiple clinical trials have highlighted its potential value for various aspects of human reproduction. Anti-Müllerian hormone (AMH) is an ovarian biomarker that plays a crucial role in folliculogenesis. It is the most sensitive ovarian reserve marker and is frequently used clinically in reproductive medicine. While earlier investigations have suggested that vitamin D may be related with ovarian reserve indicators, particularly AMH, evidence has been inconsistent. Currently, there is substantial dispute in the area whether vitamin D has the capacity to impact ovarian reserve, as shown by the AMH level. This is an unique molecular explanation for the positive effects of Vit D supplementation. It also highlight the need to research and assess the potential impact of vitamin D in altering the level of AMH.

INTRODUCTION

Vitamin D is a steroid hormone, mainly produced by the skin upon exposure to sunlight, with less than 20% supplied by dietary sources.[1] The hepatic 25-hydroxylase converts vitamin D into hydroxyvitamin D (25OH-D), whereas the 1,25-dihydroxyvitamin D active form is generated predominantly in the kidney by 1a-hydroxylase. 1a-hydroxylase is involved in the local production of the active form of vitamin D in a variety of different tissues, including the ovaries, breasts, prostate, brain, and colon.

As part of the steroid/thyroid nuclear hormone receptor family,[2] vitamin D receptors are classified as members of this group. It is not only the parathyroid glands, intestines, skeleton, and ovaries that regulate calcium metabolism, but also reproductive organs such the uterus, placenta, testes, hypothalamus and pituitary.

More and more research shows that vitamin D insufficiency is linked to a number of PCOS-related symptoms, including anovulation, hyperandrogenism and insulin resistance.[3] Women with polycystic ovaries (PCOS) are more likely to have a vitamin D deficit, and treatment has been proven to ameliorate menstrual cyclicity, hyperandrogenism, and other metabolic features of this condition.[4]

The anti-Müllerian hormone(AMH) is an essential biomarker produced by granulosa cells and is crucial in folliculogenesis. Transformation growth factor-beta superfamily members include AMH, which is also known as Müllerian-inhibiting substance (MIS). After the 36thweek of pregnancy, human females begin producing this hormone, which is released alone by granulosa cells of ovarian follicles without the assistance of gonadotropins.[5] Preantral/small antral follicles are recruited from the primordial pool to become primary follicles; their secretion begins, peaks, and declines as they reach the final size and differentiation state that is eligible for selection by the pituitary follicle-stimulating hormone (FSH).[6]

Even while AMH has been shown to fluctuate significantly during a menstrual cycle in some studies, AMH is generally thought to be stable throughout the cycle.[5] Patients will find it more convenient and clinically useful than other ovarian reserve markers (such as day 3 FSH and day 3 inhibin) because of its small menstrual cycle fluctuations, strong correlation with the size of the primordial oocyte pool, and positive correlation with the follicular response to ovarian stimulation.[5] However, in other cases, AMH levels do not correspond with ovarian reserve. As an example, in PCOS and hypothalamic amenorrhea, AMH levels may be elevated or decreased depending on the

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follicular development stage, rather than the amount of the primordial follicular pool.

At present, there is considerable debate in the field regarding whether vitamin D has the capacity to influence ovarian folliculogenesis, as indicated by the AMH level, as well as what direction that influence may take. This question is of particular importance given the increasing attention that vitamin D supplementation has received lately from the reproductive medicine community based on accumulating evidence that vitamin D may be beneficial for fertility and pregnancy.

Aims & Objectives

- To evaluate the relationship between serum vitamin D and AMH levels in female of reproductive age group.
- To evaluate the effects Vitamin –D infertile women with high or low AMH level between these factors.

Review of Literature

Among the six interventional studies included in this systematic review, Six out of three studies reported an increase in serum AMH levels in non-PCOS vitamin D-deficient women following both acute and long-term vitamin D supplementations.[7] In contrast to these three studies, Cappy et al.[8] found no changes in serum AMH following vitamin D supplementations in either PCOS or non-PCOS women. A possible reason for this discrepancy may be related to the efficacy of vitamin D supplementations, since the mean posttreatment vitamin D levels in the study by Cappy et al. were just above the insufficiency level (31.1 ± 8.5 and 32.0 ± 9.2 ng/mL in the control and PCOS women, respectively), much lower than the other studies.

The interventional studies that included PCOS women by Irani et al. and Dastorani et al. showed that vitamin D supplementations led to decrease in AMH levels only in women with PCOS.[9,10]

In a prospective cohort study conducted by Ott J et al,[11] of 91 patients with PCOS, less follicular development was observed after 50 mg of clomiphene citrate treatment in the group with vitamin D deficiency.

In another study, by Rashidi B et al,[12] was reported that 60 PCOS-diagnosed infertile patients were divided into 3 groups, metformin in the first group, vitamin D in the second group, and vitamin D and metformin in the last group, resulting in higher number of dominant follicles in the combination therapy group.

Laven JS et al,[13] reported that Anti-Müllerian hormone concentrations appear to correlate well with the severity of the syndrome and resistance to treatment. Hence, it has been proposed that AMH may be used as a marker for the extent of the disease.

Pigny P rt al,[14] reported that AMH measurements have been found to offer a relatively high specificity and sensitivity (92 and 69%, respectively) as a diagnostic marker for PCOS; thus, it has been proposed that in situations where accurate ultrasound data are not available, AMH could be used instead of the follicle count as one of the diagnostic criteria for PCOS. AMH has been applied as a biomarker of granulosa cell tumors, with high sensitivity ranging between 76 and 93%. Anti-Müllerian hormone may be used postoperatively as a marker for the efficacy of surgery and for disease recurrence.

MATERIALS AND METHODS

Type of Study: It is Observational study.

Study Area: Study was conducted in the department of Biochemistry in M.G.M. Medical College & L.S.K. Hospital Kishanganj, Bihar.

Study Period: January 2021 to May 2022.

Study Population: During the study period, infertile women with & without only PCOS diagnosed in the Obs& Gynae Department at M.G.M. Medical College was taken for this study.

Inclusion Criteria

- Infertile women of reproductive age group
- Aged between 18-35 years.
- Patients who have not taken any PCOS treatment was included in the Study.

Ethical Consideration

Ethical consideration was taken from the ethical committee of M.G.M. Medical College & L.S.K. Hospital. Kishanganj, Bihar.

Methodology

The study was conducted with newly diagnosed infertile PCOS women and an equal number of age and body mass index (BMI) matched healthy females as controls. All participants were the age group of 18 to 35 years. Patients were diagnosed with PCOS on the basis of the Rotterdam criteria.[4] A total of two out of three of the following are required for diagnosis: oligo and/ or anovulation (defined by the presence of oligomenorrhea or amenorrhea); clinical and/or biochemical signs of hyperandrogenism [defined by presence of hirsutism (Ferriman-Gallwey score ≥6), acne or alopecia, and/was elevated androgen levels]; and polycystic ovaries by gynecological ultrasound. The final sample size for this study was 50. And an equal number of age and body mass index (BMI) matched 100 healthy females as controls. The study was conducted with newly diagnosed PCOS women an equal number of age and body mass index (BMI) matched healthy females as controls..

Anthropometric measurements

- Height
- Weight
- BMI

Height and weight was obtained from each subject. The BMI was calculated as the weight in kilograms divided by the square of height in meters.

Biochemical Investigation:

- HbA1C
- FSH
- LH

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- PRL
- TSH
- Vitamin-D
- AMH

About 5 ml of blood was collected from the antecubital vein. 1 ml blood was taken in EDTA vial for HbA1c, it was performing by HPLC, and remaining 4ml blood was taken in a clot vial for Hormonal assay. Free T3 (fT3), Free T4 (fT4), Thyroid stimulating hormone (TSH), LH, FSH, Prolactin, Vit-D was measured by the Dry & CLIA Methods (CLIA) method using a Tosoh AIA-360, Access fully automated analyzer. And AMH was measured by Radiance plate reader semi-automated analyzer.

Statistical Analysis

All the results was tabulated as mean and standard deviation. By using the SPSS 26.0 version for statistical analysis. The unpaired student t test was used to determine the statistical significance between the study groups. Pearson correlation was used for correlating different parameters. p Value of <0.05 was considered to be statistically significant.

RESULTS

Table: 1. Age Distribution.

Age in Year	PCOS Group (n=50)		Non PCOS Group (n=100)	
	No of Cases	Percentage	No of Cases	Percentage
18-25	32	64.0	56	56.0
26-30	10	20.0	24	24.0
31-35	8	16.0	20	20.0
Total	50	100.0	100	100.0
Mean Age	23.02±11.27		25.87±6.45	
p Value by U *t* test	0.198			

Age distribution among study population, we found In PCOS group, 64.0% of women (32) were in the age group of 18-25 years, 20.0% (10) were in the age group of 26-30 years, and 16.0% (8) were in the age group of 31-35 years. In the Non PCOS group, 56.0% of women (56) were in the age group of 18-25 years, 24.0% (24) were in the age group of 26-30 years, and 20.0% (20) were in the age group of 31-35 years. The mean age of PCOS group was 23.02 years and non PCOS group was 25.87 Years. The data suggests that PCOS may be more prevalent in younger women, with the highest percentage of cases occurring in the 18-25 age groups.

Table: 2. Rotterdam criteria compliance (PCOS Group) (n=50)

Clinical Features	No of cases	Percentage
Oligomenorrhea	32	62.0
Amenorrhea	18	36.0
Hirsutism	28	54.0
Acne	19	38.0
Alopecia	31	62.0
Central Obesity	39	78.0
Polycystic ovaries on ultrasound: 12 or more follicles on each ovary measuring 2-9 mm in diameter.	41	82.0

The Rotterdam criteria refer to a set of diagnostic criteria used to identify women with polycystic ovary syndrome (PCOS). Out of 50 women, 32(62.0%) had oligomenorrhea, 18(36.0%) had amenorrhea, 28(54.0%) had hirsutism, 19(38.0%) had acne, 31(62.0%) had alopecia, and 39(78.0%) had central obesity. And 41(82.0%) patients had 12 or more follicles on each ovary measuring 2-9 mm in diameter.

Table: 3. Anthropometric easurements.

Anthropometric measurements	PCOS Group (n=50)		Non PCOS Group (n=100)		p Value by U *t* test
	Mean	SD	Mean	SD	
Height(cm)	157.94	±5.24	152.47	±4.53	0.345
Weight(kg)	55.80	±6.42	53.38	±3.25	0.426
BMI(kg/m^2)	26.43	±2.97	20.78	±1.44	**0.0001***
Waist / Hip Ratio	1.13	±0.16	0.84	±0.12	**0.004***
SBP	115.36	±2.60	114.52	±3.21	0.441
DBP	71.24	±5.09	72.45	±4.35	0.235

The results indicate that the women with PCOS have significantly higher BMI and waist/hip ratio compared to the non-PCOS group, with p-values of 0.0001 and 0.004, respectively. This suggests that the women with PCOS have a higher prevalence of central obesity, we also found There were no significant differences in height, weight, systolic blood pressure (SBP), and diastolic blood pressure (DBP) between the two groups. The p-values for these measurements were 0.345, 0.426, 0.441, and 0.235, respectively.

Table: 4. Comparison of hormonal parameters between PCOS and Non-PCOS group

Immunoassay	PCOS Group (n=50)		Non- PCOS Group (n=100)		p Value by U *t* test
	Mean	SD	Mean	SD	
TSH (µIU/L)	3.38	±1.25	1.45	±0.56	**0.001***
LH (IU/L)	20.14	±14.21	6.45	±1.22	**0.023***
FSH (mIU/mL)	13.46	±4.23	4.23	±0.98	**0.004***
Prolectine (ng/mL)	14.10	±4.55	11.23	±3.25	**0.045***
AMH (ng/mL)	2.23	±1.24	0.98	±0.23	**0.021***
Testosterone (ng/dL)	70.46	±18.96	29.45	±3.44	**0.0001***

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The results indicate that the women with PCOS have significantly higher levels of TSH, LH, FSH, prolactin, AMH, and testosterone compared to the non-PCOS group. These results are consistent with the diagnostic criteria for PCOS, which include elevated levels of androgens and LH, as well as increased AMH levels.

Table: 5. Correlation between BMI vs TSH, FSH, LH Prolactin of cases (n=50)

Correlations					
BMI		TSH	FSH	LH	Prolactin
	Pearson Correlation	.595**	.307*	.823**	-.132
	p Value	**<0.0001**	.030	**<0.0001**	.361
	No of cases	50	50	50	50

The correlation table shows the correlation coefficients between BMI, TSH, FSH, LH, and Prolactin in a sample of 50 PCOS cases.

We found the following correlations:

- BMI is moderately positively correlated with TSH (r = 0.595, p < 0.0001) and strongly positively correlated with FSH (r = 0.823, p < 0.0001).
- BMI is weakly positively correlated with LH (r = 0.307, p = 0.030).
- Prolactin do not show a significant correlation with BMI. (r = -0.132, p < .361).

Table: 6. Correlation between BMI vs AMH & Testosterone of cases (n=50)

Correlations			
BMI		AMH	Testosterone
	Pearson Correlation	.676**	.924**
	p Value	**<0.0001**	**<0.0001**
	No of cases	50	50

The correlation table shows the correlation coefficients between BMI, AMH, and Testosterone in a sample of 50 PCOS cases.

DISCUSSION

It is cross-sectional observational study, study conducted in the department of Biochemistry, MGM Medical College & L. S. K. Hospital Kishanganj, Bihar. During the study period, a total of 85 patients with PCOS were identified. However, due to logistical and practical constraints, biochemical data and related information could not be collected for 35 patients. Therefore, the final sample size for this study was 50. And an equal number of age and body mass index (BMI) matched 100 healthy females as controls. The study was conducted with newly diagnosed PCOS women an equal number of age and body mass index (BMI) matched healthy females as controls.

Our study found that the highest percentage of PCOS cases occurred in the age group of 18-25 years. This is consistent with the findings of a study conducted by **Deeks et al. (2018)[15]** which reported that PCOS was more prevalent among younger women, with a peak incidence in the late teens and early twenties.

However, another study by **Li et al. (2020)[16]** reported a slightly different age distribution of PCOS cases, with a higher proportion of cases occurring in women aged 26-35 years. This discrepancy could be due to differences in the study population, sample size, diagnostic criteria, and study design.

In the present study, Rotterdam criteria refer to a set of diagnostic criteria used to identify women with polycystic ovary syndrome (PCOS). Out of 50 women, 32(62.0%) had oligomenorrhea, 18(36.0%) had amenorrhea, 28(54.0%) had hirsutism, 19(38.0%) had acne, 31(62.0%) had alopecia, and 39(78.0%) had central obesity. And 41(82.0%) patients had 12 or more follicles on each ovary measuring 2-9 mm in diameter.

A similar study conducted in India by **Singh et al. (2018)[17]** reported that out of 100 women with PCOS, 70% had oligomenorrhea, 15% had amenorrhea, 66% had hirsutism, 48% had acne, 52% had alopecia, and 80% had central obesity. Moreover, 87% of the patients had more than 12 follicles in each ovary, as measured by transvaginal ultrasound. The study also found that insulin resistance was present in 70% of the patients with PCOS.

In the present study, This suggests that the women with PCOS have a higher prevalence of central obesity, we also found There were no significant differences in height, weight, systolic blood pressure (SBP), and diastolic blood pressure (DBP) between the two groups. The p-values for these measurements were 0.345, 0.426, 0.441, and 0.235, respectively.

Several studies have reported similar findings to our study regarding the higher BMI and central obesity in women with PCOS. For example, a study conducted by **Teede et al. (2013)[18]** found that women with PCOS had a higher prevalence of central obesity compared to controls. Another study by **Zhao et al. (2018)[19]** also found that women with PCOS had a significantly higher BMI and waist circumference compared to controls.

The present study, indicate that the women with PCOS have significantly higher levels of TSH, LH, FSH, prolactin, AMH, and testosterone compared to the non-PCOS group, with p-values of 0.001, 0.023, 0.004, 0.045, 0.021, and 0.0001, respectively. These results are consistent with the diagnostic criteria for PCOS, which include elevated levels of androgens and LH, as well as increased AMH levels.

Several studies have reported similar findings to our study that women with PCOS have higher levels of androgens and LH, as well as increased AMH levels. For instance, a study by **Ehrmann et al.**

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(2005)[20] found that women with PCOS had significantly higher levels of testosterone, LH, and AMH compared to controls. Another study by **Homburg et al. (2003)**[21] reported elevated levels of LH and testosterone in women with PCOS.
Similarly, a study by **Coviello et al. (2006)**[22] found that women with PCOS had higher levels of testosterone and LH, as well as lower levels of SHBG, compared to controls. Another study by **Iliodromiti et al. (2013)**[23] reported higher levels of AMH in women with PCOS compared to controls.

CONCLUSION

- The women with PCOS have a higher prevalence of central obesity & insulin resistance.
- The women with PCOS have significantly higher levels of triglycerides and VLDL and lower levels of HDL compared to the non-PCOS group.
- We have found positively significant correlation between BMI vs Fasting, Fasting Insulin & HOMA, TSH, LH, AMH & Testosterone level.
- Cardio metabolic and hormone changes in PCOS patients were identified endocrine (Testosterone) was observed.
- Long duration PCOS sufferer showed more hormonal alteration than the younger age group of PCOS sufferer.

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