

ASSOCIATION BETWEEN VITAMIN D, IL-6 LEVELS AND AMH IN IRAQI WOMEN WITH POLYCYSTIC OVARIAN SYNDROME

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Abstract:

The current study aimed to investigate whether serum vitamin D and IL-6 levels are associated with AMH in women with PCOS. We also aimed to investigate which of the above parameters has better specificity and sensitivity for predicting PCOS. **Methods:** Forty-five plus 65 women diagnosed with PCOS were enrolled in the study. The research period has been extended from December 2021 to May 2022. Study participants' serum vitamin D levels, interleukin-6 (IL-6) and serum anti-Müllerian hormone (AMH) levels were estimated. Comparison of data between two groups was performed with an independent-samples t-test. Correlations were evaluated with multiple linear regression and ROC curves were applied to find the optimal cutoff points for vitamins and IL-6 for diagnosing PCOS. **Results:** Compared to controls, AMH and IL-6 levels were higher in the PCOS group ($p < 0.001$), while vitamin D levels were scientifically decreased in women with PCOS compared to the control group ($P < 0.001$). There was a significant correlation between serum vitamin D and AMH, but no significant correlation was observed between IL-6 and AMH. These results were independent of BMI and WHR. PCOS with an AMH cut-off point of 5.18 ng/ml had a sensitivity of 93.8% and a specificity of 65.1%, and PCOS with an IL-6 cut-off point of 33.93 pg/ml had a sensitivity of 90.6%. %, with a specificity of 65.1%. **Conclusion:** Vitamin D is inversely related to AMH. This suggests that chronic low vitamin D levels may have a more lasting negative effect on ovarian reserve. should be investigated to see if it contributes to the reduction of According to the ROC curve results, measuring both AMH and IL-6 together was more useful in distinguishing between women with PCOS and women without PCOS.

Keywords: Vitamin D, AMH, IL-6, PCOS.

Introduction

Polycystic ovary syndrome (PCOS) affects 8% to 18% of women of childbearing age. It is a condition in which appearance and medical history are mixed. Many women are diagnosed during puberty when they are surveyed for symptoms such as hirsutism, persistent acne, oligomenorrhea, and amenorrhea, but other patients are unaware of their diagnosis until they seek help with visualization. may not be noticed ¹. They also have High risk of metabolic syndrome ² and insulin resistance leading to type 2 diabetes and cardiovascular disease ³. Psychological effects of this syndrome include increased rates of anxiety, eating disorders and depression ⁴.

Obesity associated with PCOS was first reported by Stein and Leventhal. All the symptoms and signs of PCOS can be exacerbated by weight gain, and as obesity becomes more common, diagnosis of clinically dependent syndromes is also amplified. Even a 5% to 10% dose reduction may progress symptoms and restore ovulatory menstrual cycles⁵. It is important to recognize a population of lean PCOS women who are hyperandrogenic and anovulatory and may be the most insulin-resistant, regardless of body mass index (BMI)⁶.

Vitamin D deficiency is of concern as a possible symptomatic relief problem for PCOS^{7,8}. Vitamin D supplementation has been shown to reduce serum triglycerides, androgen levels, liver indices, insulin resistance, hirsutism, and normalize the menstrual cycle in adult PCOS women^{7-9,10}. Vitamin D insufficiency (20-29 ng/mL) and deficiency (<20 ng/mL) are equally well defined in adolescents and adults¹¹. The link between vitamin D deficiency in adolescents and PCOS is not so straightforward, and several studies have failed to find a link^{12,13}.

Anti-Müllerian hormone (AMH) is formed primarily in ovarian granulosa cells of preantral and antral follicles. By interfering with folliculogenesis by desensitizing follicles to follicle-stimulating hormone (FSH) and inhibiting follicle growth and recruitment, it serves as a marker of ovarian dysfunction¹⁴. Several studies have reported elevated AMH levels in PCOS^{15,16}. This may be related to an increased number of preantral and antral follicles, or increased production of AMH from these follicles¹⁷.

The mechanisms leading to increased AMH production in PCOS are poorly understood and have been attributed to insulin resistance (IR), obesity, hyperandrogenism, gonadotrophins and their complex interactions^{15,16}. (IR) enhances luteinizing hormone (LH) and androgen production, both intrinsic to and impaired by obesity¹⁶. Hyperandrogenism promotes the growth of ovarian preantral and antral follicles, and elevated LH causes premature luteinization and follicular arrest¹⁸ Driving increased levels of AMH. It is also believed that obesity has a particular inhibitory effect on gonadotropin release due to increased androgen aromatization in adipose tissue as a result of LH suppression, resulting in suppression of dominant follicles¹⁹. There is little consensus among researchers about the primary mechanisms associated with AMH alterations in PCOS¹⁶. There are also narrow studies investigating the contribution of these potential regulatory functions, particularly using gold standard methods such as the euglycemic-hyperinsulinemia clamp and computed tomography (CT)²⁰.

Interleukin-6 (IL-6), a pleiotropic cytokine, plays an important role in the endocrine system, particularly in relation to ovarian maturation, fertilization, and implantation. IL-6 has also been shown to regulate ovarian function and development. Therefore, it may be a major mediator of mild chronic inflammation in PCOS²¹. Furthermore, indicators of chronic subclinical inflammation, such as IL-6, are independent risk predictors of developing type 2 diabetes²². Many studies have found elevated IL-6 levels in women with PCOS, and these studies support the theory that PCOS increases the risk of developing diabetes by activating chronic inflammation²³.

Materials and Methods

Participants

This study was performed for random 110 PCOS & Control groups, who attended the High Institute of Infertility diagnosis and assisted reproductive technologies, Al-Nahrain University in Baghdad-Iraq. The duration of the study extended from December 2021 to May 2022.

Statistical analysis

SPSS 23.0 was used to perform statistical analyses.. The association between vitamin D and IL-6 with AMH was analyzed through multivariable linear regression. ROC curve was applied to find the best cutoff point of vitamin and IL-6 to diagnose PCOS.

Results

Compared to control, the levels AMH and IL-6 were higher in the PCOS group ($p < 0.001$), while vitamin D was lower in the PCOS than control group ($p < 0.001$), as shown in (table 1).

Table1: The information and studied parameters of PCOS and control groups.

Variable(mean± SD)	PCOS	Control	p-value
Age	30.56 ±7.06	31.02 ± 7.75	0.751
BMI	32.21±4.18	24.27±2.37	0.000
WHR	46.8750±5.18	40.8837±3.83	0.000
WWR	43.5781±4.64	37.5581±3.69	0.000
Vit. D	14.52±4.65	40.74±8.56	0.000
AMH	8.54±2.46	4.93±1.49	0.000
IL-6	82.37 ± 49.83	27. 22±13. 76	0.000

Multivariate regression

The correlations of AMH and Vitamin D and IL-6 in the studied groups are shown in Table 2 .

Table 2: Multivariate regression analysis of AMH with Vitamin D in control and PCOS groups.

Variable	B ± SE	95% CI	Beta	t	P-value
Vitamin D	-0.087±0.024	(-0.128 - -0.046)	-0.505	- 4.239	0.000
IL-6	0 .002±0.007	(-0.012 - 0.017)	0.039	0.331	0.742

SE, standard error, CI, confidence of intervals. Dependent variable: AMH.

There was a significant correlation between serum Vitamin D and AMH, meanwhile, no significant correlation was observed between IL-6 and. These results were independent of BMI, WHR.

ROC Curve

The cut-off point of AMH and IL-6 were chosen to recognize PCOS subjects, with the better sensitivity and specificity. The sensitivity for PCOS with a cut-off point for AMH of 5.18ng/ml was 93.8% and the specificity was 65.1%, meanwhile, the sensitivity for PCOS with a cut-off point for IL-6 of 33.93 pg/ml was 90.6% and the specificity was 65.1%. The results were illustrated in Figure 1 and Table 3

Figure 1: A ROC curves of AMH and IL-6 as a diagnostic tool for the diagnosis of PCOS

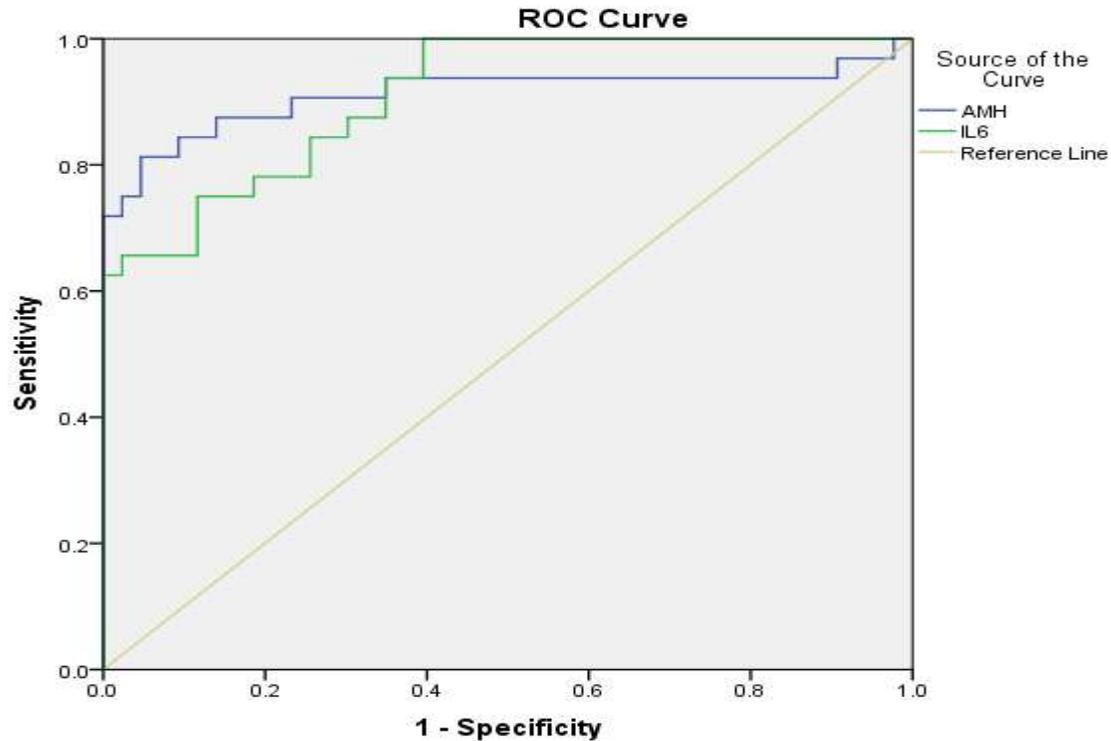


Table 3: Information of ROC curves output of AMH and IL-6.

Variable	AUC	Cut-off point	Sensitivity%	Specificity%
AMH	0.912	5.18	93.8	65.1
IL-6	0.911	33.93	90.6	65.1

AUC = Area under the curve

Discussion

Vitamin D is a steroid hormone that is involved in calcium metabolism and bone structure and plays an important role in controlling many diseases such as cancer, hypertension, autoimmune diseases, obesity and diabetes ²⁴. Surprisingly, accumulating evidence suggests a link between vitamin D and PCOS etiology, symptoms, and signs ^{25,26}. Vitamin D is a key factor in estrogen biosynthesis and can induce the synthesis of progesterone, estrone and estradiol in vitro ²⁷.

In addition, vitamin D plays an important role in regulating AMH expression in granulosa cells and follicle selection in chickens²⁸. AMH plays an important role in folliculogenesis and ovarian dysfunction. PCOS women have higher serum AMH levels compared to healthy women, and AMH is considered a diagnostic and prognostic marker for PCOS²⁹. Vitamin D treatment reduced serum levels of AMH in PCOS patients³⁰, It means that vitamin D helps folliculogenesis in PCOS. Additionally, the study revealed that PCOS patients had lower follicular fluid vitamin D levels compared to controls³¹.

To date, there have been various observational and inadequate intervention studies aimed at assessing the association between vitamin D and AMH levels, with large and inconsistent results³². Maintaining a biological balance with proper AMH and vitamin D levels is a very difficult process. A very popular study observed an important relationship between vitamin D and levels. Naderi and colleagues suggested that vitamin D supplementation led to increased AMH levels³³. Slightly different results were obtained by Irani and colleagues who showed that vitamin D administration in women with PCOS may lower levels of AMH³⁴. However, some studies have not detected an association between AMH and vitamin D³⁵. Shapiro and colleagues showed that vitamin D levels are poor predictors of AMH³⁶. Merhi et al. Finding a positive relationship between vitamin D and AMH levels, suggesting vitamin D deficiency may lead to decreased ovarian reserve in women with PCOS³⁷. Dennis et al. We also suggested that vitamin D may have a positive impact on AMH synthesis in adults³⁸. On the other hand, a recent cross-sectional study of 283 infertile women found no significant relationship between vitamin D and ovarian reserve markers such as AMH³⁹.

Some earlier research suggested that low vitamin D levels may be caused by obesity or the metabolic syndrome, which is frequently linked to PCOS. However, the significance of vitamin D deficiency in AMH control or the pathogenesis of PCOS is still unknown at this time^{40,41}. Our study showed that vitamin D levels were negatively correlated with AMH. This is consistent with a study of Chinese patients by Xuemei and colleagues⁴². Different study results may result from differences in study design, differences in participant characteristics such as gender, age and health status, and differences in study quality. This study is the first to report the relationship between serum vitamin D levels and AMH Iraqi women.

Over the past decades, PCOS researchers have focused on the chronic low-grade asymptomatic inflammatory state and how it is associated with metabolic, endocrine and reproductive impairments in PCOS patients. IL-6, which is a key pro-inflammatory cytokine in chronic inflammation, is closely linked with PCOS and IR and may be the precursor to the development and IR and their metabolic consequences⁴³. Genetic variations in genes encoding inflammation-associated mediators underpin PCOS development, and their interaction with environmental factors may explain the disparate clinical phenotypes of PCOS⁴⁴. In the present study, serum IL-6 were significantly higher in PCOS women. This result agreed with the results who displayed that IL-6 may be a marker of early low-grade chronic inflammation among patients with PCOS⁴⁵. Recently, PCOS was significantly linked to IL-6 levels, perhaps because of chronic low-grade inflammation⁴⁶, IL-6 can rise indirectly the activity of the androgen receptor⁴⁷. No

correlation was shown between the follicular levels of IL6 and AMH. Our results agreed with study that did not find an association between IL-6 and AMH⁴⁸. Although IL-6 indirectly promote the synthesis of testosterone secreted by follicular cell, it can increase androgen receptor expression in ovarian tissue, thus indirectly increasing the androgen activity and becoming one of the mechanisms of PCOS incidence⁴⁹.

Evaluation of indicative tests is a matter of concern in modern medicine not only for confirming the occurrence of disease, but also to rule out the disease in healthy people⁵⁰. ROC plays a dominant role in assessing diagnostic ability of tests to differentiate the true state of subjects, finding the ideal cut-off point, and comparing two alternative indicative tasks when each task is made on the same subject^{51,52}. AUC reflects the general sensitivity and specificity of a specific index in disease diagnosis, as well as the overall diagnostic accuracy of this indicator: $0.5 < AUC \leq 0.7$ means a small diagnostic accuracy, $0.7 < AUC \leq 0.9$ means intermediate diagnostic accuracy, $0.9 < AUC < 1.0$ means a great diagnostic accuracy⁵³. ROC was made to assess sensitivity and specificity of AMH and IL-6 as a diagnostic test for PCOS. The results in Table 3 show that in general, the AUC for AMH to diagnose PCOS is 0.912, meanwhile, the AUC for IL-6 to diagnose PCOS is 0.911. ROC curves as mentioned above was used to select the best cut-off points of each of the three parameters to diagnose PCOS. The results of our study indicate that the specificity and sensitivity are close for both AMH and IL-6, but it is slightly in favor of AMH. According to the results, both can be used screening and diagnosis of PCOS. This study demonstrated that these two parameters are all valuable for diagnosis of PCOS.

Conclusions

In the current study, a new relationship between serum vitamin D and AMH is described here, suggesting that vitamin D is associated with ovarian reserve in women of late reproductive age. Prospective studies should investigate whether low vitamin D levels and high levels of IL-6 contribute to decreased ovarian reserve. According to the ROC curve results, measuring both AMH and IL-6 together was more useful in distinguishing between women with PCOS and women without PCOS.

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