Original Article

Relationship between vitamin D level and ovarian reserve markers in infertile women in Diyala province, Iraq

Enas Jaleel Alobaidy,[1] Raaked Kamel Saadi,[2] Shaymaa Kanaan Mansoor[3]

Department of Obstetrics and Gynecology, College of Medicine,[1] University of Al-Diyala,[2] Al-Batool Teaching Hospital,[3] Diyala, Iraq

Objective: To investigate the relationship between vitamin D and antral follicles (AFC) and anti-Mullerian hormone (AMH).

Methodology: This cross-sectional study was conducted on infertile women. Patients had to be married, between the ages of 18 and 40, without a history of chronic illnesses, abstain from drug use, not have any specific gynecological diseases, as determined by the gynecologist who oversaw the project, and have an AFC count of less than 10 in sonography between less than 10 in sonography during early follicular period. Additionally, their AMH levels were lower.

Results: The study included 99 females with mean age of 29 ± 6 years. Majority were over 21; 46.9% had college degrees, 53.1% were housewives, 83.7% were nonsmoking. Overweight women were 48.48% and obese were 3.13%. AFC and AMH significantly rose with vitamin D supplementation. Also, mean vitamin D levels fluctuated following supplementation.

Conclusion: Vitamin D has a promising influence on AMH expression. Vitamin D boosts AMH levels with increase in the number of antral follicles or ovarian reserve.

Keywords: Vitamin D status, ovarian reserve markers, infertile women.

INTRODUCTION

Women beyond the age of 30 often have diminished ovarian reserve (DOR), a physiological phenomenon that reduces both egg production and egg quality.[1] However, in some women, DOR occurs before age 30 and causes early infertility, a condition known as pathological DOR.[2] Clinically, DOR is defined as symptoms of insufficient anti-Mullerian hormone (AMH), a low number of antral follicles (AFCs), and a negative clomiphene citrate test in women with a regular menstrual cycle.[3] The granulosa cells of antral and pre-antral follicle development are where the ovarian reserve biomarker AMH is most evident.[4] Therefore, a low blood AMH level is an indicator of DOR.[4]

Sunlight converts a fat-soluble vitamin D into a hormone called calcitriol. The first is the production of 25-hydroxyvitamin D, which is mediated by the liver enzyme 25-hydroxylase (presumably cytochrome P450 2R1 [CYP2R1]) (hereafter referred to as 25OHD). A second process, mediated by 1-hydroxylase (CYP27B1) in the kidney, transforms 25OHD to the active hormone calcitriol (1,25-dihydroxyvitamin D). Although the 1-hydroxylase gene is present outside of the kidneys, its role in the production of calcitriol remains unclear. The active form of vitamin D in the body is 25OHD, the precursor of calcitriol, which circulates coupled to a plasma carrier protein called vitamin D binding protein (DBP). DBP is also responsible for carrying calcitriol and vitamin D.[5] Since vitamin D receptors (VDR) are found in many tissues and organs, this vitamin plays an important role throughout the body and acts an important contributor to insulin resistance.[6] Since VDR is expressed in the reproductive systems of both sexes, it is possible that vitamin D plays a role in infertility.[7,8]

A study of 2700 infertile women found a significant correlation between exposure to vitamin D-rich environments and an increase in oocyte quality.[9] Ovarian reserve was evaluated by ultrasonic counting AFC.[10] The measurement of ovarian reserve using AMH is more accurate than using AFC,[11] and is widely used in assisted reproduction. Vitamin D and acromial microsomal levels have been linked to a variety of infertility causes, including endometriosis and polycystic ovary syndrome (PCOS).[12] The purpose of this study was to look for any links between vitamin D and adrenocorticotropic hormone (AMH/AFC)

METHODOLOGY

This cross-sectional included 99 infertile females, (infertility described as a lack of pregnancy after 12 months of trying without birth control or fertility medicines) referred to independent private infertility centers in Diyala province/Iraq from 15th May 2020 to 1st October 2021. The study was approved by an independent ethics committee, and it followed the Declaration of Helsinki's guidelines for conducting scientific experiments on human subjects. An informed consent was obtained from all participants.

Endometriosis, pelvic surgery, smoking, recreational drug use, prior chemotherapy or pelvic radiation, and prior hormonal treatment within the preceding 6 months were all disqualifying factors. Patients ranged in age from 18 to 40, were married and had no history of chronic diseases, drug use, or specific gynecological diseases like endometriosis, pelvic surgery, smoking, history of hormonal treatment for previous 6 months ago, uterine or ovarian surgery, chemotherapy, or radiation treatment. Those who had used vitamin D supplements regularly during the three months before to inclusion were ineligible. Patients had an AFC count of less than 10 on sonography performed between days 3 and 10 of their menstrual cycle, an AMH level of less than 1 ng/ml.

Variables such as age, employment, education, smoking status, and body mass index (BMI) were collected. An analysis of AMH, AFC and serum 25-hydroxyvitamin D levels was done. Every patient took 50,000 international units (IU) of vitamin D orally weekly for three months. Both the adrenocorticotropic hormone (AMH) and vitamin D levels were rechecked after treatment. Participants were requested to return both full and empty prescription bottles at the conclusion of the trial.

Statistical Analysis: Data analysis were analyzed using SPSS version 22. Pearson correlation displays the relationship between continuous data, whereas Chi-square tests the connection between variables. The T test was used to compare continuous variables at the mean and the median. $p<0.05$ was considered significant.

Table 1: Distribution of patients according to age groups, education, working and smoking status.

Variable		Frequency	Percentage
Age groups	20 and less	7	7.1
	21 – 30	50	50.5
	> 30	42	42.4
Education	Primary	30	30.3
	Secondary	26	26.3
	College	43	43.4
Work	Housewife	56	56.6
	Employment	43	43.4
Smoking status	No	91	91.9
	Yes	8	8.1

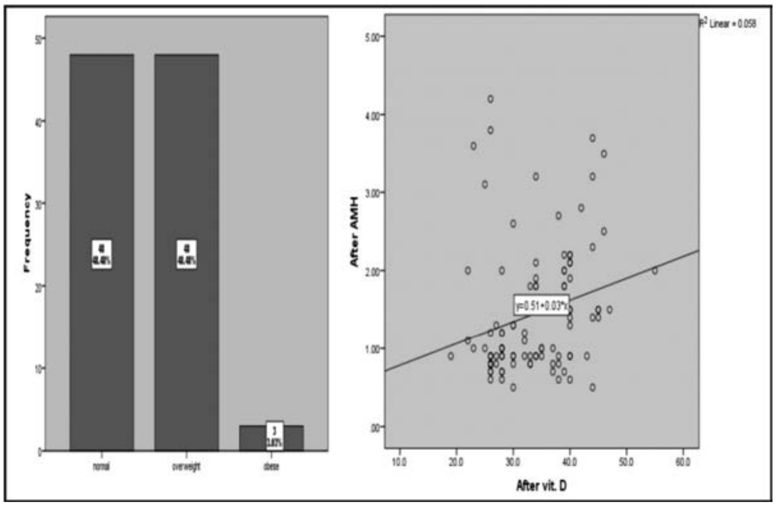

Fig. 1(A): Distribution of patients according to BMI. (1B): After supplementation correlation.

RESULTS

The study had 99 females with mean age of 29 ± 6 years. Demographic features are shown in Table 1 and Fig. A and B.

There were significant differences between mean of Vit. D before supplement and after supplement. There was significant increase in Vit D level after Vit D supplement (Table 2). There was significant positive correlation between Vit D level after supplement and AMH, when given Vit D. as supplement for 3 months, there is increase in AMH (Table 3 and Fig. 1a,b).

Table 2: Differences between mean of AFC, AMH and Vit D before and after.

Group	Mean	Std. Deviation	p-value
Before AFC	7.18	1.97	0.0001
After AFC	14.45	3.70	
Before AMH	1.09	0.83	0.0001
After AMH	1.45	0.83	
Before Vit. D	14.30	3.07	0.0001
After Vit. D	33.86	7.16	

Table 3: Correlation between AFC, AMH and Vit D level after Vit D supplement.

		AMH after	Vit D. After
AFC after	R	0.007	0.179
	P.V	0.948	0.077
AMH after	R	–	0.240
	P.V		0.017

DISCUSSION

Patients with vitamin D deficiency had a considerable improvement in their blood vitamin D levels and their serum AMH levels following intervention, as shown in the current study. Vitamin D and AMH levels have conflicting results.[10] Modified granulosa cells and ovarian follicle AMH expression,[12] have been linked to vitamin D's ability to modulate AMH levels in vitro, where the hormone is produced. The studies linking vitamin D and ovarian stimulating hormone (AMH) in women are controversial. A recent comprehensive review,[10] found that most studies had no meaningful correlation between vitamin D status and adrenocorticotropic hormone (AMH).

Vitamin D insufficiency linked to reduced AMH blood levels, according to a separate research of women with PCOS.[13,14] Other investigations, notably those involving infertile women with limited ovarian reserve, found that the benefits of vitamin D on AMH levels were mitigated.[15] Some research focuses on females with normal infertility and ovulation, whilst others investigate women with PCOS or diminished ovarian reserve. Moreover, individual vitamin D levels are altered by race, geographic location, and sun exposure and have a role in ovarian reserve alterations.[16]

In the present research, the mean age of patients is comparable to the mean age of participants in another study,[17] which was 34.5. In a trial of infertile PCOS females with vitamin D insufficiency, participants took 50,000 IU of vitamin D per week or a placebo for eight weeks. When comparing the vitamin D group to the placebo group, the latter saw a substantial decrease in AMH levels.[18] Results of a study comparing the effects of vitamin D “50,000 IU, administered on the first day of the menstrual cycle” to a placebo on AMH levels in 49 young women with normal menstrual cycles are reported by Dennis et al. An abnormally high number of antral ovarian microfollicles were produced in women with PCOS because of fluctuating blood AMH levels.[19]

Vitamin D supplementation has been shown to improve PCOS symptoms in a number of studies.[20,21] Because of this, it should come as no surprise that the decline in AMH in this group is associated with better folliculogenesis and ovulation status. Limitations of the study are that sample size is small. Because vitamin D 25-OH is a well-established biomarker for vitamin D,[22] we didn't check for vitamin D-binding protein, which has never been measured in any previous studies. As with previous studies, some dietary patterns, such as the Mediterranean diet, have been linked to higher levels of vitamin D in the blood, which may confound our findings.[23] Due to the nature of the inclusion criteria (vitamin D deficiency), a control group was not an option for this research, and a before-and-after interventional study was selected as the method of analysis. Thus, it is not known whether or not the vitamin D intervention triggered the noted shift.[24]

CONCLUSION

The vitamin D receptor has a promising impact on the AMH, increasing AMH expression. Vitamin D boosts AMH levels with increase in the number of antral follicles or ovarian reserve.

Author Contributions:
Conception and design: Enas Jaleel Alobaidy, Raaked Kamel Saadi.
Collection and assembly of data: Raaked Kamel Saadi.
Analysis and interpretation of data: Raaked Kamel Saadi.
Drafting of the article: Shaymaa Kanaan Mansoor.
Critical revision of article for important intellectual content: Shaymaa Kanaan Mansoor.
Statistical expertise: Shaymaa Kanaan Mansoor.
Final approval and guarantor of the article: Shaymaa Kanaan Mansoor.
Corresponding author email: Enas: enas@uodiyala.edu.iq
Conflict of Interest: None declared.
Rec. Date: Nov 23, 2022 Revision Rec. Date: Jan 11, 2023 Accept Date: Jan 16, 2023.

REFERENCES

1. Youssef MA, van Wely M, Mochtar M, Fouda UM, Eldaly A, El Abidin EZ, et al. Low dosing of gonadotropins in in vitro fertilization cycles for women with poor ovarian reserve: systematic review and meta-analysis. Fertil Steril 2018;109:289-301.
2. Morin SJ, Patounakis G, Juneau CR, Neal SA, Scott RT, Seli E. Diminished ovarian reserve and poor response to stimulation in patients < 38 years old: a quantitative but not qualitative reduction in performance. Hum Reprod 201;33:1489-98.
3. Ferraretti AP, Gianaroli L. The Bologna criteria for the definition of poor ovarian responders: is there a need for revision? Hum Reprod 2014;29:1842–5.
4. Moolhuijsen LME, Visser JA. Anti-Müllerian Hormone and Ovarian Reserve: Update on Assessing Ovarian Function. J Clin Endocrinol Metab 2020;105:3361–73.
5. Dilaver N, Pellatt L, Jameson E, Ogunjimi M, Bano G, Homburg R, et al. The regulation and signalling of anti-Müllerian hormone in human granulosa cells: relevance to polycystic ovary syndrome. Hum Reprod

2019;34:2467-79.
6. Fondjo LA, Owiredu WKBA, Sakyi SA, Laing EF, Adotey Kwofie MA, Antoh EO, et al. Vitamin D status and its association with insulin resistance among type 2 diabetics: A case-control study in Ghana. PloS One 2017;12:e0175388.
7. Ranjana H. Role of vitamin D in infertility. J Public Health Policy Plann2017;1:8–10.
8. Anagnostis P, Karras S, Goulis DG. Vitamin D in human reproduction: a narrative review. Int J Clin Pract 2013;67:225-35.
9. Chu J, Gallos I, Tobias A, Tan B, Eapen A, Coomarasamy A. Vitamin D and assisted reproductive treatment outcome: a systematic review and meta-analysis. Hum Reprod 2018;33:65–80.
10. Khan HL, Bhatti S, Suhail S, Gul R, Awais A, Hamayun H, et al. Antral follicle count (AFC) and serum anti-Müllerian hormone (AMH) are the predictors of natural fecund ability have similar trends irrespective of fertility status and menstrual characteristics among fertile and infertile women below the age of 40 years. Reprod Biol Endocrinol 2019;17:20-6.
11. Fleming R, Seifer DB, Frattarelli JL, Ruman J. Assessing ovarian response: antral follicle count versus anti-Müllerian hormone. Reprod Biomed Online 2015;31:486–96.
12. Garg D, Tal R. The role of AMH in the pathophysiology of polycystic ovarian syndrome. Reprod Biomed Online 2016;33:15–28.
13. Mridi I, Chen A, Tal O, Tal R. The association between vitamin D and antiMüllerian hormone: a systematic review and meta-analysis. Nutrients 2020;12:1567-9.
14. González-Duarte RJ, Cázares-Ordoñez V, Díaz L, Ortíz V, Larrea F, Avila E. The expression of RNA helicase DDX5 is transcriptionally upregulated by calcitriol through a vitamin D response element in the proximal promoter in SiHa cervical cells. Mol Cell Biochem 2015;410:65-73.
15. Merhi Z, Doswell A, Krebs K, Cipolla M. Vitamin D alters genes involved in follicular development and steroidogenesis in human cumulus granulosa cells. J Clin Endocrinol Metab 2014;99:1137–45.
16. Irani M, Merhi Z. Role of vitamin D in ovarian physiology and its implication in reproduction: a systematic review. Fertil Steril 2014;102:460–8.
17. Naderi Z, Kashanian M, Chenari L, Sheikhansari N. Evaluating the effects of administration of 25-hydroxyvitamin D supplement on serum anti-mullerian hormone (AMH) levels in infertile women. Gynecol Endocrinol 2018;34:409–12.
18. Tal R, Seifer DB. Potential mechanisms for racial and ethnic differences in antimullerian hormone and ovarian reserve. Int J Endocrinol 2013;2013:818912.
19. Aramesh S, Alifarja T, Jannesar R, Ghaffari P, Vanda R, Bazarganipour F. Does vitamin D supplementation improve ovarian reserve in women with diminished ovarian reserve and vitamin D deficiency: a before-and-after intervention study? BMC Endocr Disord 2021;21:126-9.
20. Dastorani M, Aghadavod E, Mirhosseini N, Foroozanfard F, Zadeh Modarres S, Amiri Siavashani M, et al. The effects of vitamin D supplementation on metabolic profiles and gene expression of insulin and lipid metabolism in infertile polycystic ovary syndrome candidates for in vitro fertilization. Reprod Biol Endocrinol 2018;16:94-8.
21. Dennis NA, Houghton LA, Pankhurst MW, Harper MJ, McLennan IS. Acute supplementation with high dose vitamin D3 increases serum anti-müllerian hormone in young women. Nutrients 2017;9:719-23.
22. Garg D, Tal R. The role of AMH in the pathophysiology of polycystic ovarian syndrome. Reprod Bio Med Online 2016;33:15–28.
23. Lagowska K, Bajerska J, Jamka M. The role of vitamin D Oral supplementation in insulin resistance in women with polycystic ovary syndrome: a systematic review and meta-analysis of randomized controlled trials. Nutrients 2018;10:1637-9.
24. Zhang H, Huang Z, Xiao L, Jiang X, Chen D, Wei Y. Meta-analysis of the effect of the maternal vitamin D level on the risk of spontaneous pregnancy loss. Int J Gynaecol Obstet 2017;138:242–9.