e-ISSN: 0975-1556, p-ISSN:2820-2643

Available online on www.ijpcr.com

International Journal of Pharmaceutical and Clinical Research 2023; 15(8); 1648-1652

Original Research Article

Study the Vitamin D Levels in Infertile Females and Correlation of Vitamin D Deficiency with Anti-Mullerian Hormone Levels in Comparison to Fertile Females

Priyanka[1], Madhu Priya[2], Kumari Bibha[3]

[1,2]Senior Resident, Department of Obstetrics and Gynaecology, Sri Krishna Medical College and Hospital, Muzaffarpur, Bihar

[3]Professor, Department of Obstetrics and Gynaecology, Sri Krishna Medical College & Hospital, Muzaffarpur, Bihar

Received: 25-06-2023 / Revised: 28-07-2023 / Accepted: 30-08-2023

Corresponding author: Dr. Madhu Priya

Conflict of interest: Nil

Abstract:

Background: Low vitamin D (25-hydroxyvitamin D) status has been linked to endometriosis, polycystic ovarian syndrome, and poor fertility in both humans and animals, according to research. Antimullerian hormone (AMH), FSH, mRNA, and gene expression in reproductive organs are regulated by vitamin D, suggesting a function in female reproduction. The purpose of this study is to determine the vitamin D levels of infertile women and the relationship between vitamin D deficiency (VDD) and serum AMH in infertile women as compared to fertile women.

Methods: From May 2022 to April 2023, this prospective study was conducted in Department of Obstetrics and Gynaecology, SKMCH, Muzaffarpur, Bihar. Out of a total of 70 infertile women who met the inclusion and exclusion criteria, 45 were discovered to have VDD. 35 of these patients were selected as cases, and the AMH levels in these patients were tested. 35 healthy, reproductive females were used as the control group, and vitamin D and AMH levels were measured. VDD and AMH connection was investigated in both groups.

Results: Of infertile females, 64.28% had the VDD. The mean for vitamin D in cases with vitamin D deficiency was 6.18±2.09, while the mean for AMH was 1.94±1.30. The mean for vitamin D was 4.85±3.02 and for AMH was 3.47±2.59 in vitamin D-deficient controls. AMH levels were lower in cases than in the control group (P = 0.003), and vitamin D levels were significantly lower in fertile than infertile females. Two sample t tests were utilized to compare the two groups in the statistical analysis of the link between vitamin D and AMH linear regression test.

Conclusion: Of infertile females, 64.28% had the VDD. VDD and AMH levels were not significantly correlated in either group.

Keywords: AMH, fertile, infertile, outcome, vitamin D deficiency.

Introduction

A serious global health issue, vitamin D deficiency (VDD) affects both industrialized and poor nations. Recent epidemiologic investigations have demonstrated links between low vitamin D levels and numerous disease states and their detrimental impact on many systems.

A role for vitamin D in female reproduction is suggested by the fact that a significant number of genes are expressed by vitamin D in reproductive organs. Evidence from both humans and animals suggests that endometriosis and poor fertility are linked to low vitamin D levels. Since serum 25(OH)D has a half-life of around three weeks, it is the most reliable indication of vitamin D status and offers the greatest overall evaluation of vitamin D status.[1] Additionally, vitamin D promotes cell differentiation and suppresses cell division. The vitamin D receptor (VDR) is the main site of action for this hormone. Vitamin D can alter the transcription of genes, the synthesis of proteins, and messenger ribonucleic acid (mRNA) through its receptor. Dietary VDD decreases total fertility by 25% in animal studies.[2] The involvement of VDD has been linked in numerous studies to polycystic ovarian syndrome (PCOS) and a wide range of diseases associated with pregnancy.[3]

A dimeric glycoprotein called antimullerian hormone (AMH) is produced by granulosa cells in the preantral and antral follicles of the ovary in women. AMH's primary physiological functions in the ovary appear to be restricted to preventing the early stages of follicular development, preserving

ovarian reserve, and altering the way follicles react to follicular stimulating hormone (FSH).[4] AMH also lessens follicle sensitivity to FSH in vivo and inhibits Preantral follicle development produced by FSH in vitro.[5] The AMH levels are used in reproductive medicine to assess PCOS status and ovarian reserve (low levels).[6]

From complementary deoxyribonucleic acid (cDNA) microarray investigation of a prostate cancer cell line, AMH was identified as a target gene controlled by vitamin D (1,25-dihydroxy vitamin D3).[7] These researchers discovered a functional VDR element in the human AMH promoter after discovering that the AMH-mRNA expression is upregulated in response to vitamin D in vitro, proving that vitamin D directly affects AMH expression. Additionally, vitamin D controls mRNA, FSH, AMH, and is likely involved in the process of follicle selection.[8] There is now a great deal of disagreement in the research as to whether vitamin D has any impact on AMH synthesis or fertility.

Therefore, we designed this study to compare ovulatory fertile females to infertile females in terms of serum AMH and vitamin D status, as well as to explore the range of vitamin D levels in infertile females.

Materials and Methods

From May 2022 to April 2023, a prospective study was conducted on patients who visited the Obstetrics and Gynecology outpatient department (OPD) at Sri Krishna Medical College and Hospital in Muzaffarpur, Bihar. The total of 70 infertile individuals who met the inclusion criteria were taken as cases after receiving written informed consent and following best clinical practice recommendations.

Vitamin D levels in these patients were measured. 45 of the total 70 infertile females tested positive for VDD. 35 of these patients were selected as cases, and the AMH levels in these patients were tested. The AMH levels in these patients were evaluated. According to the Institute of Medicine and studies by EURONUT SENECA,[9] SUVIMAX,[10] and Goswami et al.[11], vitamin D levels were divided into three categories: deficiency, insufficiency, and sufficiency. Deficiency <10 ng/ml, insufficiency 10 to 20 ng/ml, and sufficient levels were taken >20 ng/ml are the reference levels for vitamin D (25(OH) D) in serum (blood). The normal working staff members and fertile females who visited our OPD for consultation due to other reasons were tested for vitamin D levels, and those who were determined to be vitamin D deficient were recruited as controls. AMH levels were also evaluated in the 35 fertile ladies with VDD.

1. Patient N = 70 (enrolled to study vitamin D levels in infertile females with unexplained infertility)
2. Cases N1 = 35 (infertile females with VDD, for AMH levels assessment)
3. Control N2 = 35 (fertile females with VDD, for AMH levels assessment).

This study included infertile women with unexplained infertility who were between the ages of 18 and 40 as cases and fertile, healthy women in the same age range as controls.

The following conditions were not included in this study: endometriosis, thyroid problems, autoimmune disease, tubal factor, male factor, or polycystic ovarian syndrome. History of smoking (tobacco use), oral contraceptive pill, any hormonal or steroid drug usage, known VDD, obesity (body mass index, BMI > 35).

Data was analyzed using a linear regression test to determine the relationship between vitamin D and AMH, and two sample t tests were performed to compare the two groups.

Results

In this study, the majority of patients had primary infertility (55.71%) lasting 1 to 5 years (95.71%) and had graduated from high school or higher (58.57%) as their educational level.

Inquiries concerning the professions of all patients revealed that 87.50% were nonprofessional (housewives), as shown in Table 1.

Table 1: Demographic profile and infertility characteristics of cases, infertile group (n=70)

Age distribution (years)		
• 18-25	18	25.71%
• 26-30	25	35.71%
• 31-35	22	31.42%
• 36-40	5	7.14%
Education Level		
• Upto 12th class	29	41.42%
• Graduate and above	41	58.57%
Occupation		
• Nonprofessional	60	87.50%
• Professional	10	14.20%
Type of infertility		

• Primary	39	55.71%
• Secondary	31	44.28%
Infertility duration		
• Upto 5 years	67	95.71%
• 6-10 years	2	2.86%
• 11-15 years	1	1.42%

To examine the range of vitamin D levels, our study was conducted on a population of infertile females. Overall, 64.28% of infertile females had VDD (up to 10 ng/ml), 30.0% had vitamin D deficiency (10–20 ng/ml), and 5.71% had appropriate vitamin D levels (>20 ng/ml). According to Table 2, the median level of vitamin D was 9.30± 5.59 ng/ml.

Table 2: Vitamin D levels in infertile group (n=70)

Vitamin D levels	Infertile group (n=70)	Percentage
Deficient group (<10 ng/ml)	45	64.28%
Insufficient (10-20 ng/ml)	21	30.0%
Sufficient (>20 ng/ml)	4	5.71%

The average levels of vitamin D and AMH in cases of vitamin D deficiency (infertile females) were 6.18±2.09 and 1.94±1.30, respectively. The average levels of vitamin D and AMH in controls with inadequate vitamin D (fertile females) were 4.85±3.02 and 3.47±2.58, respectively. On comparison of these two groups infertile group had significantly Table 3.

Table 3: Age profile of vitamin D deficient cases (n1=35) and control (n2=35)

Age group (years)	Cases	Controls
18-25	6(17.1%)	2(5.71%)
26-30	13(37.1%)	14(40.0%)
31-35	11(31.4%)	13(37.1%)
36-40	5(14.2%)	6(17.1%)

Table 4: BMI profile of vitamin D deficient cases (n1=35) and control (n2=35)

BMI (kg/m^2)	Cases	Controls
Underweight (18.5)	4(11.4%)	3(8.57%)
Normal weight (18.5-24.9)	18(51.4%)	21(60.0%)
Overweight (25-29.9)	10(28.5%)	8(22.8%)
Obese (≥30)	3(8.57%)	3(8.57%)

Compared to the fertile group in our study, Table 4 and Table 5 of AMH (P = 0.003) but greater levels of vitamin D (P = 0.04) [Table 6]. Vitamin D and AMH levels, however, did not significantly correlate in either group.

Table 5: AMH levels in vitamin D deficient infertile and fertile women

AMH levels	Cases (infertile group) n1=35	Percentage	Cases (fertile group) n1=35	Percentage
Upto 1.0 ng/ml	12	34.28%	6	17.14%
1.1-3.5 ng/ml	19	54.28%	16	45.71%
>3.5 ng/ml	3	8.5%	13	37.14%

Table 6: Mean values of AMH and vitamin D levels in infertile and fertile women

Mean value	Cases (infertile group)	Control (fertile group)
Vitamin D	6.18±2.09 ng/ml (p=0.04)*	4.85±3.02 ng/ml
AMH	1.94±1.30 ng/ml (p=0.003)	3.47±2.59 ng/ml

P<0.05 by t-test.

Discussion

The range of vitamin D levels in women of reproductive age who experienced primary infertility with unknown etiology and the prevalence of VDD in infertile females were observed in the current investigation. In our research, we discovered that 64.28% of infertile females had VDD. In a study, vitamin D levels were low or insufficient in 81.3 to 98.2% of the women with reduced fertility. [12] The little disparity between our results and these results may be due to population and geographic variations.

In our study comparing these two groups, vitamin D levels were significantly (P = 0.04) lower in the control group (fertile females) than in the cases group (infertile females). AMH levels were lower in the cases group compared to the control group (P = 0.003), which may contribute to female infertility. AMH is a predictor of ovarian reserve and ovarian responsiveness, which, excluding other

reasons of infertility, directly affect a woman's fertility.

Anti-rickets factor or sunlight vitamin is other names for vitamin D. With the exception of vitamin D supplementation, dietary intakes typically have little impact on blood levels.[13] Even in tropical nations, where there is enough of sunlight (necessary for the body to produce vitamin D on its own), VDD affects people of all ages and is prevalent in a range of 50 to 90%.[14] Age or conditions linked to infertility had no impact on vitamin D levels.[12] According to another study, there were substantially more VDD cases in the subfertility group than in the control group (59.0 versus 40.4%; P <0.01).[15] A positive correlation between serum AMH levels and vitamin D was only seen in one study.[16] However, because there were such a small number of participants, this study acknowledges a serious methodological flaw.

A study found that taking vitamin D supplements could lower excessive AMH production and restore normal ovulation by increasing follicular sensitivity to FSH.[17] According to an observational study, there is no significant relationship between serum vitamin D levels and AMH in young people, although there may be a weak positive relationship in women who are 40 years of age or older. There is no clear-cut pattern for how vitamin D might influence AMH production or serum levels in the currently available literature. Our prospective study's main conclusion in reproductive-age women is that serum vitamin D levels don't seem to be connected to AMH levels. Merhi et al. had also reported, no relationship between serum vitamin D and AMH levels in women aged 35 to 40 years, with similar results.[18]

There is disagreement on how vitamin D might impact AMH production or whether vitamin D actually plays any part in AMH production. The in vitro results examining the impact of vitamin D on AMH synthesis are likewise conflicting, much like the in vivo investigations. Early research involving a cell line from prostate cancer demonstrated that vitamin D could raise AMH.[8,19] Women with darker skin pigmentation have also been said to have lower serum AMH levels.[20] Additionally, it has been observed that women with darker complexion lose AMH more quickly than those with lighter skin, which causes menopause to start earlier.[21,22]

Theoretically, vitamin D administration in VDD could enhance ovarian reserve and postpone the onset of menopause because vitamin D stimulates granulosa cell production of AMH.[23]

Conclusion

VDD was 64.28% common in groups of infertile women. In groups of both fertile and infertile women, there was no association between VDD and AMH levels.

It is concerning how frequently VDD occurs in women with decreased fertility. Prospective studies are urgently required to establish a causal link and explore the therapeutic potential of vitamin D supplementation in this population.

References

1. Feldman D, Pike JW, Glorieux FH. Vitamin D. 2nd ed. San Diego, CA: Elsevier Academic Press; 2005; 15-36.
2. Bakhshalizadeh S, Amidi F, Alleyassin A, Soleimani M, Shirazi R, Shabani Nashtaei M. Modulation of steroidogenesis by vitamin D3 in granulosa cells of the mouse model of polycystic ovarian syndrome. Syst Biol Reprod Med. 2017; 27:1-12.
3. Irani M, Merhi Z. Role of vitamin D in ovarian physiology and its implication in reproduction: a systematic review. Fertil Steril. 2014; 102:460-68.
4. Dewailly D, Andersen CY, Balen A, Broekmans F, Dilaver N, Fanchin R, et al. The physiology and clinical utility of antimullerian hormone in women. Hum Reprod Update. 2014; 20:370-85.
5. Durlinger AL, Gruijters MJ, Kramer P, Karels B, Ingraham HA, Nachtigal MW, et al. Anti-Mullerian hormone attenuates the effects of FSH on follicle development in the mouse ovary. Endocrinology. 2001; 142:4891-9.
6. Hazout A, Bouchard P, Seifer D, Aussage P, Marie Junca A, Cohen-Bacrie P. Serum antimüllerian hormone/müllerian-inhibiting substance appears to be a more discriminatory marker of assisted reproductive technology outcome than follicle-stimulating hormone, inhibin B, or estradiol. Fertil Steril. 2004; 82:1323-29.
7. Krishnan AV, Moreno J, Nonn L, Malloy PJ, Swami S, Peng L, et al. Novel pathways that contribute to the anti-proliferative and chemopreventative activities of vitamin D in prostate cancer. J Steroid Biochem Mol Biol. 2007; 103:694-702.
8. Malloy PJ, Peng L, Wang J, Feldman D. Interaction of the vitamin D receptor with a vitamin D response element in the Mullerianinhibiting substance (MIS) promoter: regulation of MIS expression by vitamin D in prostate cancer cells. Endocrinology. 2009; 150: 1580-7.
9. Van der Wielen RP, Löwik MR, van den Berg H, de Groot LC, Haller J, Moreiras O. Serum vitamin D concentrations among elderly people in Europe. Lancet. 1995; 346:207-10.
10. Chapuy MC, Preziosi P, Maamer M, Arnaud S, Galan P, Hercberg S. Prevalence of vitamin D

insufficiency in an adult normal population. Osteoporos Int. 1997; 7:439-43.
11. Goswami R, Vatsa M, Sreenivas V, Singh U, Gupta N, Lakshmy R, et al. Skeletal muscle strength in young Asian Indian females after vitamin D and calcium supplementation: a double-blind randomized controlled clinical trial. J Clin Endocrinol Metab. 2012; 97:4709-16.
12. Dressler N, Chandra A, Aguirre Dávila L, Spineli LM, Schippert C, von Versen-Hoynck F. BMI and season are associated with vitamin D deficiency in women with impaired fertility: a two-centre analysis. Arch Gynecol Obstet. 2016; 293:907-14.
13. Dixon KM, Mason RS. Vitamin D. Int J Biochem Cell Biol. 2009; 41:982-5.
14. Mehlawat U, Singh P, Pande S. Review article current status of vitamin-D deficiency in India. Innov Pharm Pharmacother. 2014;2: 328-35.
15. Al-Jaroudi D, Al-Banyan N, Aljohani NJ, Kaddour O, Al-Tannir M. Vitamin D deficiency among subfertile women: case-control study. Gynecol Endocrinol. 2016; 32:272-5.
16. Dennis NA, Houghton LA, Jones GT, van Rij AM, Morgan K, McLennan IS. The level of serum anti-Müllerian hormone correlates with vitamin D status in men and women but not in boys. J Clin Endocrinol Metab. 2012; 97:2450-5.
17. Irani M, Minkoff H, Seifer DB, Merhi Z. Vitamin D increases serum levels of the soluble receptor for advanced glycation end products in women with PCOS. J Clin Endocrinol Metab. 2014; 99: 886-90.
18. Merhi ZO, Seifer DB, Weedon J, Adeyemi O, Holman S, Anastos K, et al. circulating vitamin D correlates with serum antimüllerian hormone levels in late-reproductive-aged women: Women's Interagency HIV study. Fertil Steril. 2012; 98:228-34.
19. Wojtusik J, Johnson PA. Vitamin D regulates anti-Mullerian hormone expression in granulosa cells of the hen. Biol Reprod. 2012; 86:91.
20. Seifer DB, Golub ET, Lambert-Messerlian G, Benning L, Anastos K, Watts DH, et al. Variations in serum müllerian inhibiting substance between white, black, and Hispanic women. Fertil Steril. 2009; 92:1674-8.
21. Gold EB. The timing of the age at which natural menopause occurs. Obstet Gynecol Clin North Am. 2011; 38:425-40.
22. Tal R, Seifer DB. Potential mechanisms for racial and ethnic differences in antimüllerian hormone and ovarian reserve. Int J Endocrinol. 2013; 2013:818912.
23. Schoenaker DA, Jackson CA, Rowlands JV, Mishra GD. Socioeconomic position, lifestyle factors and age at natural menopause: a systematic review and meta-analyses of studies across six continents. Int J Epidemiol. 2014; 43:1542-62.