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Association between Vitamin D Deficiency and Serum Anti-mullerian Hormone in Infertile Women: A Cross Sectional Comparative Study

S DEY[a], S ISHRAT[b], P FATIMA[c], F ISLAM[d], R ARA[e], ER NANDI[f]

Abstract

Background: ***Reproductive failure is a significant public health concern. Vitamin D influences steroidogenesis and ovarian follicular development. AMH has a crucial role in the regulation of follicular recruitment and oocyte development. Several studies have recently shown that serum AMH is affected by several factors including obesity, serum leptin and serum vitamin D. Results from studies are conflicting with some suggesting that vitamin D status is associated with ovarian reserve, whereas other studies have not found any significant correlation between vitamin D and AMH levels.***

Objective: To evaluate the association between vitamin D deficiencies with serum anti-mullerian hormone in infertile women.

Materials and methods: The study population was the infertile women of 20-34 years of age. The women having serum anti-mullerian hormone levels d" 1 ng/ml were grouped as diminished ovarian reserve and those having serum anti-mullerian hormone levels > 1 ng/ml were grouped as normal ovarian reserve. Serum anti-mullerian hormone and serum vitamin D levels were measured at the same day.

Result: Vitamin D deficiency was defined when vitamin D levels were d" 20ng/ml. All infertile women with normal and diminished ovarian reserve are deficient in vitamin D. The mean serum AMH was 1.42±1.49 in vitamin D level d"20 ng/ml and 3.14± .45 in vitamin D level >20 ng/ml. Bivariate analysis was done to see that vitamin D deficiency was significantly more (calculated odds ratio 4.40) in those with diminished ovarian reserve. There was significant positive correlation between serum vitamin D level and serum anti-mullerian hormone.

Conclusion: Vitamin D deficiency is associated with low serum anti-mullerian hormone in infertile women.

Key words: Vitamin D deficiency, infertile women, Anti-mullerian hormone (AMH), Diminished ovarian reserve.

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a. Dr Sharbari Dey, Consultant (Gynae), Trainee, Department of Reproductive Endocrinology and Infertility, Bangabandhu Sheikh Mujib Medical University.

b. Dr Shakeela Ishrat, Associate Professor, Department of Reproductive Endocrinology & Infertility, Bangabandhu Sheikh Mujib Medical University.

c. Prof. Parveen Fatima, Ex-Chairman, Department of Reproductive Endocrinology & Infertility, Bangabandhu Sheikh Mujib Medical University; Chairman, Care Medical College & Hospital Limited.

d. Dr. Farzana Islam, Consultant (Gynae), Trainee, Department of Reproductive Endocrinology and Infertility, Bangabandhu Sheikh Mujib Medical University.

e. Dr. Rowshan Ara, Consultant (Gynae), Trainee, Dept. of Reproductive Endocrinology and Infertility, Bangabandhu Sheikh Mujib Medical University.

f. Dr. Eva Rani Nandi, Medical Officer (Gynae), Trainee, Department of Reproductive Endocrinology and Infertility, Bangabandhu Sheikh Mujib Medical University.

Address of Correspondence: Dr. Sharbari Dey, Consultant (Gynae), Trainee, Department of Reproductive Endocrinology and Infertility, Bangabandhu Sheikh Mujib Medical University. Cell: +01711167836,
Email: sharbaridey8@gmail.com, sukriti66@yahoo.com

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Introduction:

Diminished ovarian reserve reflects reduced quantity and quality of the remaining oocyte pool in infertile women. The markers of ovarian reserve, that are commonly used, are serum anti-mullerian hormone (AMH), antral follicle count (AFC) and serum follicle stimulating hormone (FSH). Diminished ovarian reserve characterized by low AMH, low AFC and high FSH can predict poor ovarian response to stimulation and poor pregnancy rate [1,2]. Among the biochemical tests basal (D2/3) serum FSH (Follicle stimulating hormone) is the most commonly used ovarian reserve test, but due to it's inter and intra cycle variability it may be a less reliable predictor as an ovarian reserve marker now a days as anti-mullerian hormone (AMH) is better predictor of ovarian reserve than FSH. Ultrasonographically measured, AFC (antral follicle count) is another good predictor of ovarian reserve.

Vitamin D is a steroid hormone, responsible for various functions in metabolism in almost all organs in the body as well as in reproductive system. Actions like prodifferentiation, anti-proliferation, pro-apoptosis, immune suppression and anti-inflammation have been attributed to vitamin D[3]. Vitamin D receptor m-RNA expression has been identified in human ovaries, mixed ovarian cell cultures and granulosa cell cultures[4].

Vitamin D and ovarian reserve markers have been studied in both fertile and infertile populations, yielding conflicting results. Some studies[5-7]have found associations between vitamin D levels and ovarian reserve markers while others [8,9] have not. Some have tried vitamin D supplementations to increase ovarian reserve markers [10,11].

If association is established between vitamin D deficiencies and diminished ovarian reserve, it is reasonable to improve ovarian reserve by vitamin D supplementation. The objective of our study is to explore if there is any association between serum anti-mullerian hormone and vitamin D deficiency.

Material And Methods:

This cross-sectional comparative study was carried out in the Department of Reproductive Endocrinology and Infertility in Bangabandhu Sheikh Mujib Medical University from July 2018 to June 2019. Sample size was calculated at 80% power and 5% level of significance. The hypothetical proportion of controls with exposure was 40.1 according to a previous study[12]. The Institutional Review Board of Bangabandhu Sheikh Mujib Medical University approved the study. The study population was infertile women in the age range 20-34 years. The women with diminished ovarian reserve (AMH< 1ng/ml) were group I, and women with normal ovarian reserve (AMH e"1ng/ ml) were group II. Women who had endometriosis, ovarian surgery, radiotherapy and/ or chemotherapy, had vitamin D supplementation or taking oral contraceptive pill or any medications for systemic diseases in last 6 months were excluded.

The women were briefed in detail regarding the objectives, rationality and potential benefits of the study and informed written consent was taken. Fasting blood samples were taken for measurements of AMH and vitamin D. Serum AMH levels were measured with an ELISA kit (AMH Gen II ELISA: Beckman Coulter and R & D Systems) in the Department of Microbiology and Immunology. Serum 25(OH) D, a vitamin D metabolite, was measured in the Department of Biochemistry and Molecular Biology using Architect 25-OH vitamin D assay (Abbott Ci4100), a chemiluminiscent micro particle immunoassay (CMIA) for the quantitative determination of 25-hydroxyvitamin D in human serum and plasma. Seasonal variation of vitamin D levels was not a confounding factor as blood samples for vitamin D and AMH estimation was drawn at the same time.

Serum 25(OH) D concentration d" 20ng/ml is considered as vitamin D deficiency, 21-29 ng/ml as vitamin D insufficiency and 30 ng/ml or higher as sufficient. Values <10ng/ml is considered as severe deficiency[13].

Statistical analyses carried out by using the Statistical Packages for Social Sciences version 22.0 for Windows (SPSS Inc., Chicago, Illinois, USA). Inferential analysis was done to find out the association of ovarian reserve status with vitamin D level and other exposure variables by performing bivariate chi- square test and unpaired student T test. Binary logistic regression analysis was done to calculate odds ratio with 95% confidence intervals. Correlation between vitamin D and AMH was done by Pearson's correlation coefficient test. The test was two sided and p values <0.05 was considered statistically significant.

Results:

In this cross-sectional comparative study, 156 infertile women were enrolled as study population of which 78 women with diminished ovarian reserve were group I and 78 women with normal ovarian reserve were group II. The demographic and clinical characteristics shown in the table I. Variables like age and family history of early menopause that could affect ovarian reserve and variables like BMI, occupation and habitat that could affect vitamin D levels were matched between the groups.

The mean serum anti mullerian hormone (AMH) was 0.35±0.29 in group I and 2.91±1.6 in group II. The difference was statistically significant (p<0.05) between two groups. The mean vitamin D level in women with normal ovarian reserve was 15.27±4.95 ng/ml and mean vitamin D level in women with diminished ovarian reserve was 12.02±6.88 ng/ml. Both groups were deficient in vitamin D according to the threshold of 20 ng/ ml, However, the difference between the mean levels of vitamin D in the two groups was significant (p value 0.001) (Table II).

Vitamin D deficiency was significantly more in those with diminished ovarian reserve (p value 0.007). Calculated odds ratio revealed that vitamin D deficiency is 4.40 times more common in women with diminished ovarian reserve (Table III).

Table-I

Showing comparison of demographic and clinical characteristics between two groups (n=156)

Characteristics	Diminished ovarian reserve (Group I)	Normal ovarian reserve (Group II)	P value
Mean age (years)	31.28	30.35	0.096
Family history of early menopause			
Yes (%)	9	3.8	0.191
No (%)	91	96.2	
Socio economic status			
Lower middle (%)	67.9	55.1	0.251
Middle (%)	30.8	43.6	
High (%)	1.3	1.3	
Occupation			
Housewife (%)	82.1	79.5	0.685
Service (%)	17.9	20.5	
Education			
Illiterate (%)	5.1	2.6	0.482
Below SSC (%)	48.5	39.8	
SSC (%)	19.2	17.9	
HSC (%)	9.0	15.4	
Graduate and above (%)	18.0	24.4	
Habitat			
Urban (%)	66.7	76.9	0.155
Rural (%)	33.3	23.1	
Mean Body mass index	24.67	25.39	0.176

Table-II

Comparison of laboratory parameter between two groups of study populations (n=156)

Laboratory parameter	Diminished ovarian reserve (Group I)	Normal ovarian reserve (Group II)	P value
Vitamin D level (ng/ml)			
≤20 (%)	94.9	80.8	
>20 (%)	5.1	19.2	
Mean serum vitamin D (ng/ml)	12.02	15.27	0.001^{s}
Serum anti mullerian			
hormone (AMH) (ng/ml)	14.1	0.0	
<0.5 (%)	85.9	0.0	
0.5-0.99 (%)	0.0	69.2	
1-3.5 (%)	0.0	30.8	
>3.5 (%)			
Mean serum AMH (ng/ml)	0.35	2.91	0.001^{s}

Table-III

Bivariate analysis showing comparison of serum vitamin D level among two groups (n=156)

Vitamin D level	Diminished ovarian reserve (Group I)	Normal ovarian reserve (Group II)	Odds ratio (95 % Confidence interval)	P value
≤20ng/ml (%)	94.9	80.8	4.40 (1.28-16.64)	0.007[s]
>20ng/ml (%)	5.1	19.2		

Figure 1 presents the bar diagram showing the proportion of diminished ovarian reserve and normal ovarian reserve in women with severe deficiency (<10ng/ml), moderate deficiency (10-29 ng/ml) and insufficiency (21-29.9 ng/ml). Significantly more women with diminished ovarian reserve had severe vitamin D deficiency in comparison to women with normal ovarian reserve. Figure 2 shows significant positive correlation between serum vitamin D level and serum AMH.

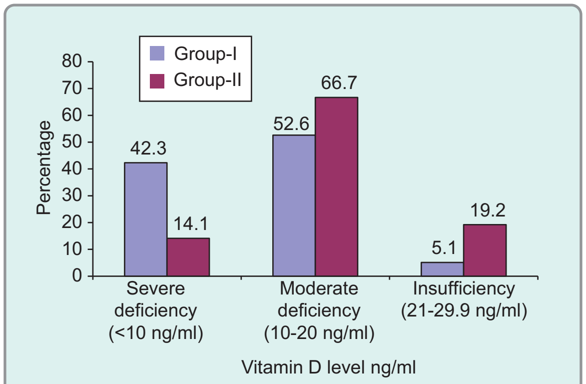

Fig.-1: *Bar diagram showing vitamin D levels in different categories in relation to ovarian reserve.*

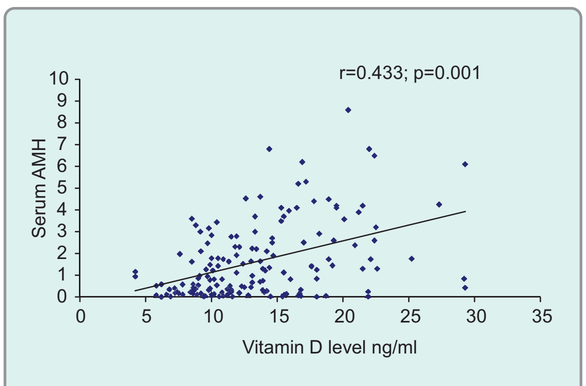

Fig.-2: *Scatter diagram showing positive significant correlation (r=0.433; p=0. 001) between serum vitamin D level and serum AMH*

Discussion:

The cross-sectional comparative study was done to see the association of vitamin D deficiency with serum AMH level. All the women with or without diminished ovarian reserve were deficient in vitamin D. There was significant lower serum vitamin D level in women with diminished ovarian reserve. Vitamin D deficiency (d"20ng/ml) was 4.4 times more common in women with diminished ovarian reserve. The difference was more marked in women with severe vitamin D deficiency (<10ng/ml).

In women with diminished ovarian reserve the mean vitamin D level in our study was 12.02 ng/ml and 15.27 ng/ml in women with normal ovarian reserve. Mean concentration of serum vitamin D level of 15.46ng/ml was also found in an observational study[15] in infertile Iranian women. In our study 80.8% of the infertile women with normal ovarian reserve were deficient and 19.2% of them were insufficient in vitamin D levels. This is supported by the findings that hypovitaminosis D was observed in 77.7% of Bangladeshi women[14]. The possible reasons behind are traditional clothing habits or inadequate outdoor activities and sunshine exposure. Similar reasons may apply to the Iranian women[15], also deficient in vitamin D.

Positive correlation between serum vitamin D levels and serum AMH has been reported by many studies [5,6,9-11]. Low vitamin D levels may have an adverse effect on ovarian reserve[16-19].

Honda et al [6] investigated the associations of anti-mullerian hormone levels with vitamin D levels as well as nutritional parameters of healthy women of reproductive age. Decreased AMH levels were associated with vitamin D deficiency but not with nutritional parameters. The case control study of Abdul-Rashid et al [7] revealed that vitamin D levels were significantly lower in infertile women compared to fertile women and serum vitamin D levels had significant correlation with serum AMH levels. Regression analysis after adjusting for all covariates by Merhi et al [16] reported significant correlation between vitamin D and AMH in older but not in younger women, suggesting that vitamin D deficiency may be associated with lower ovarian reserve in late reproductive age.

Gorkem et al [8] documented no correlation between vitamin D and AMH levels in women with adequate ovarian reserve, polycystic ovary syndrome and diminished ovarian reserve. Logistic regression analysis after adjusting for potential confounders by Drakapoulos et al [9] did not find significant correlation between vitamin D and AMH in infertile women. However, among them only 30.7% was vitamin D deficient. The studies were among Caucasians with different dress code and sunshine exposure. The racial, regional and cultural variations may account for the difference from our findings.

Dennis et al [10] determined serum AMH of women who received daily supplementation of vitamin D for 6 months. In women change in AMH levels correlated with the initial AMH level and the magnitude of change in vitamin D levels. The vitamin D supplementation prevented seasonal AMH change. They suggested that vitamin D deficiency may confound clinical decision based on AMH, and so vitamin D deficiency should be considered when serum AMH levels are obtained for diagnosis of diminished ovarian reserve. A randomized controlled double-blind study by Dennis et al [20] tested the hypothesis that vitamin D influences ovarian production of serum AMH. Circulating AMH levels increased progressively in the week following acute supplementation with an oral dose of 50,000 IU of vitamin D. Appropriate vitamin D supplementations of women with diminished ovarian reserve may improve ovarian reserve and treatment outcome.

The study was compromised by factors like small size, single center, short period and absence of random sampling. Women with polycystic ovary syndrome who have high ovarian reserve in terms of AMH and also prevalence of vitamin D deficiency were excluded. Conclusions cannot be drawn about this specific group of infertile women. Since this is a cross sectional comparative study and cannot generate or exclude any cause effect hypothesis, longitudinal cohort studies are needed. Since all infertile women with or without diminished ovarian reserve were deficient in vitamin D, threshold values for both vitamin D deficiency and diminished ovarian reserve may have to be reviewed for our population.

Conclusion:

Several studies revealed the vital role of vitamin D in human reproduction. Ovarian reserve is a crucial and important factor in reproductive function. The study suggests a significant association of serum vitamin D with anti-mullerian hormone levels in women with diminished ovarian reserve. All infertile women with normal and diminished ovarian reserve are deficient in vitamin D. But those with diminished ovarian reserve are severely deficient. Supplementation of vitamin D may improve the circulating AMH in women with diminished ovarian reserve for a better response to stimulation protocol for better fertility outcome.

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