Impact of Serum Vitamin D Level in Infertile Women with Diminished Ovarian Reserve

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Abstract—Background: Vitamin D deficiency is reported to be common worldwide. Reduction in ovarian reserve even in young women is much alarming now a day. There is an increasing awareness that vitamin D plays an important role in reproduction. It is hypothesized that vitamin D has a direct effect on AMH production and, thus, patients with higher concentrations of vitamin D are able to maintain their ovarian reserve for longer. Objective of the study was to evaluate association between serum vitamin D with diminished ovarian reserve.

Materials and methods: A case control study was conducted at Department of Reproductive Endocrinology and Infertility, Bangabandhu Sheikh Mujib Medical University, Dhaka from July 2018 to June 2019.156 subjects were enrolled in the study of which 78 were diagnosed as diminished ovarian reserve (DOR), as case and 78 with normal ovarian reserve (NOR) of same age group (20-34) as control, Baseline TVS was done on D2-5 and antral follicle count (AFC) was documented. Patients were advised for serum AMH and serum vitamin D level measurements at the same day. Then association between AMH, AFC and serum vitamin D was analyzed.

Result: Vitamin D deficiency is defined as level ≤ 20 ng/ml. The mean vitamin D level was significantly lower in DOR women than controls (12.02±6.88 vs 15.27±4.95 ng/ml).There were positive significant correlation (r=0.433; p=0.001) between vitamin D level and serum AMH and positive significant correlation (r=0.419; p=0.001) between vitamin D level and AFC too.

Conclusion: Vitamin D deficiency is found to be associated with diminished ovarian reserve group and shows significant positive correlation with serum AMH and AFC.

Keywords— Vitamin D, Diminished Ovarian reserve, Anti-Mullerian hormone, Antral follicle count.

I. INTRODUCTION

Diminished ovarian reserve (DOR) describes women of reproductive age having menses whose response to ovarian stimulation or fecundity is reduced compared with women of comparable age (Practice Committee of the American Society for Reproductive Medicine, 2015)[1]. AMH remain one of the most reliable markers of ovarian reserve and commonly used as a predictor of ovarian response in ovarian stimulation protocol. The AMH gene is up regulated by vitamin D via functional vitamin D response elements that bind the vitamin D receptor[2,3]. Antral Follicle Count (AFC) is considered another reliable ovarian reserve marker. AFC is commonly the ovarian reserve test of choice because of availability of ultrasound machine[4,5]. Vitamin D is a steroid hormone classically known for its role in maintenance of calcium and phosphate homeostasis. In vitro studies have shown a direct modulation by vitamin D of estradiol, estrone and progesterone production in human ovarian cells[6]. There have been several studies suggesting modulation of AMH levels by vitamin D. A functional vitamin D receptor element has been noted in the promoter region of AMH gene[7]. There were conflicting studies in existing literature about the influence of vitamin D on ovarian reserve markers. Some have found positive correlation[8,9,10,11,12,13] and others found no association[14,15,16]. DOR prevalence is increased from 19% to 26% from 2004 to 2011[17]. About 42.1% of younger women were vitamin D deficient[18]. Thus this study was conducted to evaluate any association

between vitamin D on ovarian reserve markers in young women with DOR in Bangladesh with a view that correction of vitamin D status may improve the circulating AMH levels and AFC in DOR women for a better response to stimulation protocol for better fertility outcome.

II. MATERIALS AND METHODS

This Case control study was conducted over July 2018 to June 2019 in the Department of Reproductive Endocrinology and Infertility, Bangabandhu Sheikh Mujib Medical University, Dhaka. The study population was infertile women of reproductive age (20-34 years), who were diagnosed as diminished ovarian reserve (DOR), as case (group I) and patients with normal ovarian reserve (NOR) of same age group as control (group II). Sample size was calculated at 80% power. and 95% level of confidence. The hypothetical proportion of controls with exposure was 40.1 according to a previous study[19]. Ethical approval was taken from Institutional review board (IRB) of BSMMU. Inclusion criteria were all the infertile women aged 20-34 years and diagnosed as diminished ovarian reserve (DOR) by their serum AMH level (<1 ng/ml). Women with AMH level ≥1ng/ml regarded as normal ovarian reserve (NOR) and **e**xclusion criteria were women >34 years of age, taking vitamin D supplementation or medication for any systemic disease, who had under-went for ovarian surgery or receiving radio &/or chemotherapy, with endometriosis, recent oral contraceptive pill users (last 6 months). The patients were briefed in detail regarding the study and informed written consent was taken. Baseline TVS (D2-5) was done and antral follicle count (AFC) was documented. AFC was detected using KONTRON MEDICAL ultrasonography machine with a 7.5-MHz vaginal transducer by the same clinician. AFC are defined as those measuring 2-6 mm in largest mean diameter on 2-dimentional planes. Cut-off value was of 5 follicles per ovary[20]. Patients were advised for serum AMH and serum vitamin D level measurements at the same day. A venous blood sample (five ml) was collected in fasting state from antecubital vein of each case and control. Blood samples were processed and then analysis of vitamin D and AMH was done. Serum AMH levels were measured with an ELISA kit (AMH Gen II ELISA: Beckman Coulter and R & D Systems). Serum 25 (OH) D was measured using Architect 25-OH vitamin D assay, (Abott.Ci4100) is a chemiluminescent Microparticle Immunoassay (CMIA) for the quantitative determination of 25- hydroxyvitamin D in human serum and plasma. Vitamin D is ≤ 20 ng/ml was regarded as deficiency and vitamin D 21-29.9 ng/ml was regarded as insufficiency. 30ng/ml or more was in sufficient group. 10-20 ng/ml regarded as moderate deficiency. Values < 10 ng/ml regarded as severe deficiency[21,22].

III. STATISTICAL ANALYSIS

Statistical analyses were carried out by using the Statistical Package for Social Sciences version 22.0 for Windows (SPSS Inc., Chicago, Illinois, USA). The results of the study were presented in tables, figures and diagrams. Descriptive analysis was done to explore the respondent's characteristics. Then inferential analysis was done to find out association of the ovarian reserve status with vitamin D level and other exposure variables by performing chi-square test and student t test. Binary logistic regression analysis was done to calculate odds ratio (OR) with 95% confidence intervals. Correlation between vitamin D and ovarian reserve markers was evaluated with a Pearson correlation coefficient (r). The tests were two sided and P values <0.05 was considered as statistically significant.

IV. RESULTS

156 infertile young women were enrolled in this case control study of which 78 were cases of diminished ovarian reserve (DOR) and 78 were normal ovarian reserve (NOR) as controls. The cases and controls were matched in terms of demographic as well as clinical characteristics shown in table I.

Table I: Distribution of the study population by demographic and clinical characteristics (n=156)

Characteristics	Diminished ovarian reserve cases	Normal ovarian reserve controls	P value
Mean age (years)	31.28	30.35	0.096
Socio economic status Lower middle (%) Middle (%) High (%)	 67.9 30.8 1.3	 55.1 43.6 1.3	0.251
Occupation House wife (%) Service (%)	 82.1 17.9	 79.5 20.5	0.685
Education Illiterate (%) Below SSC (%) SSC (%) HSC (%) Graduate and above (%)	 5.1 48.5 19.2 9.0 18.0	 2.6 39.8 17.9 15.4 24.4	0.482
Habitat Urban (%) Rural (%)	 66.7 33.3	 76.9 23.1	0.155
Mean Body mass index	24.67	25.39	0.176

Table II: Comparison of serum vitamin D level and ovarian reserve markers between two groups (n=156)

Parameters	Diminished ovarian reserve	Normal ovarian reserve	P value
Mean Serum Vitamin D level	12.02	15.27	
Mean Serum Anti Mullerian Hormone (AMH) (ng/ml)	0.35	2.91	0.001*
Mean Antral Follicle Count (AFC)	6.08	10.62	

Table II shows mean serum AMH 0.35 ng/ml and 2.91 ng/ml in group I and II and mean serum AFC is 6.08 and 10.62 in group I and II respectively. The mean vitamin D level in women with DOR was 12.02 ng/ml and was 15.27 ng/ml in NOR women. Considering the threshold of 20 ng/ml, both groups are deficient in vitamin D.

Table III: Comparison of ovarian reserve markers in study population with serum vitamin D level (n=156)

Parameters	Vitamin D level ng/ml		P value
	Deficiency ≤20 ng/ml (n=137)	Insufficiency >20 ng/ml (n=19)	
	Mean ± SD	Mean ± SD	
Serum AMH Range (min-max)	1.42±1.49 0.01-6.80	3.14±2.45 0.02-8.60	0.001[S]
AFC Range (min-max)	8.00±3.25 1-17	10.84±4.03 4.0-17	0.001[S]

Table III shows mean serum AMH was 1.42±1.49 in vitamin D level ≤ 20 ng/ml and 3.14±2.45 in vitamin D level >20 ng/ml. The mean AFC was 8.00±3.25 in vitamin D level ≤ 20 ng/ml and 10.84±4.03 in vitamin D level >20 ng/ml. The difference of serum AMH and AFC were statistically significant (p<0.05).

Table IV: comparison of serum vitamin D level between two groups (n=156)

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

Bivariate analysis was done to see that 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. 94.9% DOR women and 80.8% NOR women had vitamin D deficiency. However, the difference between the levels of vitamin D is significant (p value 0.001) between two groups.

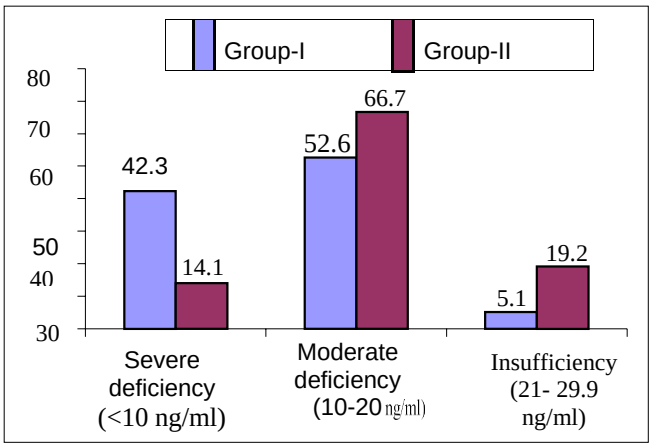

Figure 1: Bar diagram showing serum vitamin D level of the study population

Figure 1 Bar diagram showing serum vitamin d level in study population. 52.6% population had moderate deficiency in group I and 66.7% in group II. On the other-hand 42.3% population belonged to severe deficiency state in group I in comparison to 14.1% in group II. (p<0.05)

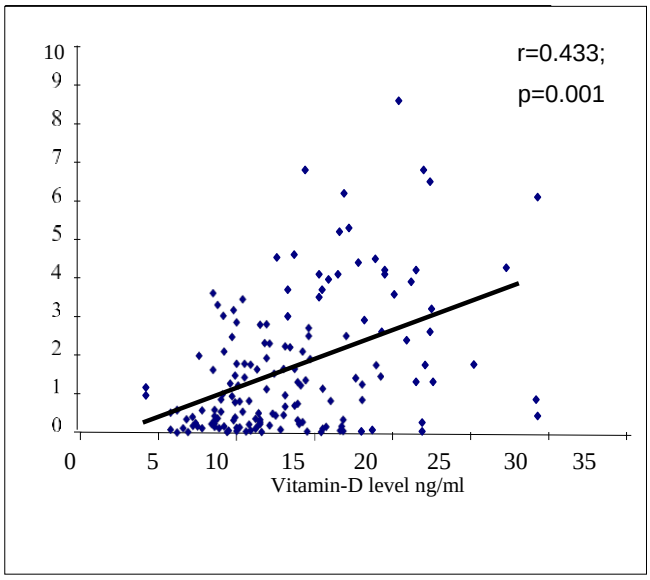

Figure 2: Scatter diagram showing positive significant correlation (r=0.433; p=0.001) between serum vitamin D level and serum AMH (Anti-Mullerian hormone).

Figure 2 shows significant positive correlation between serum vitamin D level and serum AMH.

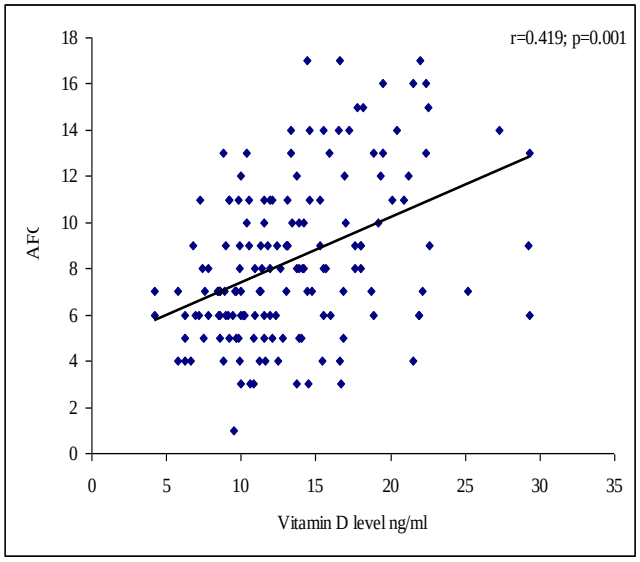

Figure 3: Scatter diagram showing positive significant correlation (r=0.419; p=0.001) between serum vitamin D level and AFC (Antral follicle count).

Figure 3 shows significant positive correlation between serum vitamin D levels and AFC.

V. DISCUSSION

The case control study was carried out to evaluate an association of vitamin D with DOR in young infertile women. All women in both groups were deficient in vitamin D. However serum vitamin D levels were significantly lower in women with diminished ovarian reserve (94.9% in DOR and 80.8% in NOR group). Vitamin D deficiency (≤ 20ng/ml) was 4.4 times more common in women with diminished ovarian reserve. The difference was more marked with severe vitamin D deficiency. The mean vitamin D level in our study was 12.02 ng/ml in women with diminished ovarian reserve and 15.27 ng/ml in women with normal ovarian reserve. The observational study[8] on infertile Iranian women also found out a mean concentration of serum vitamin D level at 15.46 ng/ml. 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 both veiled with black cloak and unveiled[23]. The possible reasons behind are traditional clothing habits or inadequate outdoor activities and sunshine exposure. Similar reasons may apply to the Iranian women, also deficient in Vitamin D. Mean AMH and mean AFC , both are significantly lower in DOR study group which are similar to the study findings of Arefi et al and Gorkem et al.[8,15] and both were significantly decreased in vitamin D deficiency group (≤ 20 ng/ml). The present findings agree with human studies have reported positive correlations between serum vitamin D and AMH level[2,11,12,13] and vitamin D and AFC[8]. Conversely, some studies[14,151,16] observed no correlation between vitamin D and AMH and AFC. The contradictory finding between the studies may be attributed to the different ethnicities of the study population, having different sociocultural religious habits and different dress codes. Drakopoulos et al.[16] evaluated women with Caucasian ethnicity. The study of Fabris et al.[14] included only oocyte donors, who are healthy young and don't have any infertility problems and belonged to Caucasian ethnicity too. Gorkem et al.[15] study was held in Turkey. Ethnicity was mixed as Caucasian, west Asian and Kurd. Their study included relatively small sample size (n=32). In this study 94.9% cases are vitamin D deficient with a mean vitamin D level 12.02 ng/ml which is similar to Arefi et al.[8] which was 71.4% and 15.48 ng/ml respectively. In contrast, mean vitamin D levels were not mentioned in Drakopoulos[16] and Fabris study[14]. Less than 50% of their study population were vitamin D deficient (30.7% and 18 % respectively). There-fore the severity of vitamin D deficiency in their study, when compared to this study was less and may be the reason why no correlation between vitamin D deficiency and ovarian reserve was identified. A randomized controlled double-blind study by Dennis et al[13] 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 supplementation of women with diminished ovarian reserve may improve ovarian reserve and treatment outcome.

VI. CONCLUSION

Currently available data identifies the vital role of vitamin D in reproduction. One such area of reproductive function is ovarian reserve. This study suggests a significant association of serum vitamin D with ovarian reserve markers in women with diminished ovarian reserve. All infertile women with normal and diminished ovarian reserve are deficient in vitamin D by recognized threshold (20ng/ml). But those with diminished ovarian reserve are severely deficient. Supplementation of vitamin D may have a favorable effect on ovarian reserve in infertile women with diminished ovarian reserve.

VII. CONFLICT OF INTEREST

Authors declare no conflict of interest.

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