International Journal of Current Advanced Research
ISSN: O: 2319-6475, ISSN: P: 2319-6505, Impact Factor: 6.614
Available Online at www.journalijcar.org
Volume 9; Issue 04 (A); April 2020; Page No.21809-21812
DOI: http://dx.doi.org/10.24327/ijcar.2020.21812.4296

Research Article

OVARIAN RESERVE MARKERS AND VITAMIN D LEVEL IN WOMEN WITH DIMINISHED OVARIAN RESERVE

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A R T I C L E I N F O

Article History:
Received 12th January, 2020
Received in revised form 23rd February, 2020
Accepted 7th March, 2020
Published online 28th April, 2020

Key words:

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

A B S T R A C T

Background: Deficiency in serum vitamin D level is a common problem worldwide. Reduction in ovarian reserve even in young women is much alarming now a days. The mechanism by which vitamin D may affect reproductive physiology is little known; a direct effect of vitamin D in ovarian steroidogenesis and ovarian follicular development has been suggested. Several studies have identified an association between ovarian reserve and serum vitamin D level. Objective of the study was to evaluate association of ovarian reserve markers (AMH, AFC) and serum vitamin D in women with diminished ovarian reserve.

Materials and methods: In this case control study, the study population(156) were infertile women of reproductive age (20-34 years), who were diagnosed as diminished ovarian reserve (DOR) , as case (78) and patients with normal ovarian reserve (NOR) of same age group as control (78) , attending Reproductive Endocrinology and Infertility Department, Bangabandhu Sheikh Mujib Medical University. 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 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 mean vitamin D level was significantly lower in DOR women. 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.

INTRODUCTION

Ovarian reserve can be defined as the quality and the quantity of the remaining follicle pool in the ovaries(1). Diminished ovarian reserve (DOR) is defined as reduced capacity of the ovaries to produce oocytes. The development of DOR generally reflects the process of follicular depletion and decline in oocyte quality(2,3,4).

AMH remain one of the most reliable markers of ovarian reserve and commonly used as a predictor of ovarian response in ovarian stimulation protocol in women with normal ovarian reserve or diminished ovarian reserve (DOR)(5,6,7).

Antral Follicle Count (AFC) is considered another reliable ovarian reserve marker. Follicle count can be determined easily with the help of high-resolution sonographic systems(8,9).

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Vitamin D**:** refers to a fat soluble steroid responsible for regulating calcium, iron, magnesium, phosphate and zinc metabolism. The biologic actions of vitamin D are mediated through the vitamin D receptors (VDR). Vitamin D receptors has been identified not only in calcium regulating tissues but also in various reproductive organs such as ovaries (granulosa cells) uterus, placenta, testis, hypothalamus, and pituitary gland(10). This diverse expression of VDR suggests a potential role of vitamin-D in female reproduction and infertility.

The mechanism by which vitamin-D may affect reproductive physiology is little known. Gonad function may be altered by vitamin-D deficiency, as evidenced by the expression of vitamin d receptor m RNA in human ovaries, mixed ovarian cell cultures, and granulose cell cultures(11). There were conflicting studies in existing literature about the influence of vitamin D on ovarian reserve markers. Some have found positive correlation (12,13,14), but others found no association(15,16).

This study was conducted to evaluate any association between vitamin D on ovarian reserve markers in 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.

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. 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 [17]. Ethical approval was taken from Institutional review board (IRB) of BSMMU. 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). Inclusion criteria were all the infertile women aged 20-34years and diagnosed as diminished ovarian reserve (DOR) by their serum AMH level (AMH <1 ng/ml). Women with AMH level ≥1 ng/ml regarded as normal ovarian reserve (NOR) and exclusion criteria were women > 34 years of age, taking vitamin D supplementation or medication for any systemic disease, who had underwent 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 plane. Cut-off value was of 5 follicles per ovary [18]. 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). Cut-off value of AMH was 1 ng/ml and defined DOR with serum AMH level < 1 ng/ml. AMH ≥ 1ng/ml regarded as NOR.

Vitamin D: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 25hydroxyvitamin 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. Values < 10 ng/ml regarded as severe deficiency [10,19].

Statistical analyses were carried out by using the Statistical Package for Social Sciences version 22.0 for Windows (SPSS Inc., Chicago, Illinois, USA). The 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 unpaired student t test. Binary logistic regression analysis was done to calculate odds ratio (OR) with 95% confidence intervals. Pearson's Correlation Coefficient (r) was calculated to find out the correlation between Vitamin D and AMH and AFC. The tests was two sided and P values <0.05 was considered as statistically significant.

RESULTS

The study population of this case control study was 156 infertile women of which 78 were cases of diminished ovarian reserve and 78 were normal ovarian reserve 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 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 (%)	67.9	55.1	
Middle (%)	30.8	43.6	0.251
High (%)	1.3	1.3	
Occupation			
House wife (%)	82.1	79.5	0.685
Service (%)	17.9	20.5	
Education			
Illiterate (%)	5.1	2.6	
Below SSC (%)	48.5	39.8	
SSC (%)	19.2	17.9	0.482
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

The means vitamin D level in women with diminished ovarian reserve was 12.02±6.88 ng/ml and in normal ovarian reserve was 15.27±4.95 ng/ml. considering the threshold of 20 ng/ml, both groups are deficient in vitamin D. However, the difference between the levels of vitamin D is significant (p value 0.001) between two 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 mean antral follicle count (AFC) was 6.08±1.97 in group I and 10.62±3.14 in group II. The difference was statistically significant (p<0.05) between two groups. (Table II).

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

Parameters	Diminished ovarian reserve Cases	Normal ovarian reserve Controls	P value
Mean Serum Vitamin D level (ng/ml)	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 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	1.42	±1.49	3.14	±2.45	0.001[s]
Range (min-max)	0.01	-6.80	0.02	-8.60	
AFC	8.00	±3.25	10.84	±4.03	0.001[s]
Range (min-max)	1	-17	4.0	-17	

Table III shows the 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 ($p<0.05$) between two groups.

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 (Table IV).

Table V Correlation between vitamin D level and ovarian reserve markers in the study population (n=156)

Parameters	Vitamin D level (ng/ml)	
	r value	p value
Serum Anti-mullerian Hormone (AMH) (ng/ml)	0.433	0.001*
Antral Follicle count (AFC)	0.419	0.001*

***=significant**

Table Vshows significant positive correlation between serum vitamin D level and serum AMH and significant positive correlation between serum vitamin D levels and antral follicle count.

DISCUSSION

The case control study was carried out to evaluate an association of vitamin D with diminished ovarian reserve. All women in both groups were deficient in vitamin D. However, serum vitamin D levels were significantly lower in women with diminished ovarian reserve. 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 (<10ng/ml).

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[12] on infertile Iranian women also found out a mean concentration of serum vitamin D level at 15.46ng/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[20]. 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 Gorkem *et al* and Arefi *et al*[12,15] 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[13,14,21] and vitamin D and AFC Conversely, some studies[17,18,] 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. 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).

A randomized controlled double-blind study by Dennis *et al*[21] 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.

CONCLUSION

Currently available data identifies the vital role of vitamin D in reproduction. One such area of reproductive function is ovarian reserve. 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 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 of infertile women.

Conflict of Interest

Authors declare no conflict of interest.

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How to cite this article:

Dey S , Das S, Ishrat S, Fatima P and Banu J (2020) 'Ovarian Reserve Markers and Vitamin D Level in Women with Diminished Ovarian Reserve', *International Journal of Current Advanced Research,* 09(04), pp. 21809-21812.
DOI: http://dx.doi.org/10.24327/ijcar.2020.21812.4296

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