Serum vitamin D and anti- mullerian hormone levels in Iraqi infertile women at Baghdad city

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Abstract

Background: Reproductive failure is a significant public health concern. Although relatively little is known about factors affecting fertility, a growing body of literature suggests that environmental & lifestyle factors play an important role. Recent studies suggest that vitamin D may play a role in human reproduction. Exact mechanisms whereby vitamin D may participate in the regulation of reproductive physiology remains far from clear.

Objective: To validate the potential of vitamin D as a biomarker for prediction of female infertility and to study the correlation between vitamin D and Anti- Mullerian hormone.

Methods: A case- control study was conducted at Department of Chemistry and Biochemistry, College of Medicine, Al- Nahrain University, Al- Kadhmiya, Baghdad, Iraq from June 2013 till July 2014. Seventy three subjects were enrolled in this study. Thirty five primary infertile women with mean age (25.4± 4.91 years), and thirty eight age-matched apparently healthy women as controls with mean age (27.3±3.72 years). Fasting blood samples were collected in the morning at 8:00 am from all participants. Vitamin D was measured by high-performance liquid chromatography technique and Anti- Mullerian hormone was measured by enzyme- linked immunosorbent assay.

Results: Vitamin D levels was significantly decreased in the infertile women group compared with fertile control group (35.38±5.83 ng/ mL) versus (49.99±12.90 ng/ mL) respectively. Vitamin D correlated significantly ($r = +0.86$; $p \leq 0.05$) with anti-mullerian hormone and anti- mullerian hormone shows a significant negative correlation with body mass index ($r = -0.78$; $p \leq 0.05$).

Conclusion: A novel relationship was found between circulating Anti- Mullerian hormone and vitamin D in plasma samples of infertile women.

Keywords: *Vitamin D; Female Infertility; Anti- Mullerian Hormone; Mullerian Inhibiting Substance; Ovarian Reserve.*

1. Introduction

Vitamin D is a steroid substance plays a key role in calcium and phosphorus metabolism [1-3]. Its main actions include intestinal calcium absorption and renal calcium reabsorption, as well as a direct effect on chondrocyte and osteoblast differentiation and subsequent bone formation [3]. The vitamin D precursor (7- dehydrocholesterol) is a normal intermediary in the cholesterol pathway and is present in the skin [1].Ultraviolet-B Ultraviolet-B radiation induces conversion of 7-dehydrocholesterol to pro vitamin D_3, which spontaneously isomerizes to vitamin D_3 (cholecalciferol) [1].

Vitamin D_3 is released into circulation and transported by the vitamin D-binding protein (VDBP). Approximately 80-90% derive from sunlight-induced production in the skin. There is also a small amount of the body's total vitamin D derived from diet and or supplements [1]. This may derive from plants or fungi containing vitamin D_2 (ergocalciferol) or flatly fish or cod-liver oil containing vitamin D_3 (cholecalciferol) [1].

In north America and western Europe only small amounts of vitamin D, a fat soluble steroid hormone, enter the metabolic circle via dietary uptake (eg. from fish) [4].

The main source (about 95%) is vitamin D_3 (cholecalciferol) that is photochemically synthesized in the skin by ultra – violet radiation. Thermal conversion of pro- vitamin D_3 (7-dehydrocholesterol) leads to pre-vitamin D_3, which isomerizes into cholecalciferol. Cholecalciferol is bound to serum vitamin D binding protein (DBP) and through a two steps enzymatic pathway involving 25- hydroxylase of the liver and 1α-hydroxylase (CYP 27B1) of the kidney &extra renal tissues, it is converted to the biologically active hormone, calcitriol ($1α,25(OH)_2$ 25(OH)two D_3) [4, 5].].

Biological actions of vitamin D are mediated through the vitamin D receptor (VDR) that is distributed across various tissues including skeleton, parathyroid glands, as well as reproductive tissues [1].

A seasonal distribution in human natural conception and birth rates has been consistently demonstrated, showing a peak conception rate during summer in northern countries with strong seasonal contrast in luminosity [4].Experimental Experimental studies have demonstrated that the ovary is a target organ for 1, 25 (OH) $_2$D3raisingthe possibility that this active metabolite of vitamin D_3 might play a role in modulating ovarian activity [6].

Experiments investigating the significance for fertility and reproductive capacity, demonstrate that 25(OH) D3deficient female rats had reduced fertility rates, decreased litter sizes and compromised mating behavior [7].

Infertility is the inability of a non- contracepting couple to achieve pregnancy within one year of regular unprotected sexual intercourse [8], [9]. About 25% of couples do not achieve pregnancy within one year, 15% of these couples seek medical treatment for infertility and less than 5% remain unwilling childless [9].

Infertility is a major cause of concern during recent times and can be due to male or female factors [9], [10]. However, in many couples, both male and female are implicated with the predominancy of female factor [10, 11]. In an extensive review, effects of vitamin D on fertility of males and females, have been analyzed, and it has been concluded that optimum level of this vitamin is crucial for the healthy reproductive state of both the sexes [10], [12].

Among women, vitamin D has been reported to regulate the concentration of Anti- Mullerian hormone (AMH) in blood [10], [13].Anti- Anti Mullerian hormone, also known as the Mullerian inhibiting substance, is a dimeric glycoprotein exclusively produced by granulosa cells of preantral (primary and secondary) and small antral follicles [14], [15]. The number of small antral follicles is directly related to the total size of the primordial follicle pool [15]. Measurable quantities of AMH appear in serum and with the decrease in the number of antral follicles with age, AMH serum levels also become diminished [15], [16]. AMH serum levels represent ovarian quantitative reserve in vitro fertilization (IVF) patients and may provide an index of age at menopause [15], [17-, 19]. However, it is currently not known if serum AMH levels also reflect oocyte quality and the chance of successful live birth pregnancy [19].

The purpose of this study was to elucidate the role of vitamin D in female reproduction and to assess if any associations existed between vitamin D levels and ovarian reserve.

2.   Methods

This case- control study was approved by the ethics committee of the College of Medicine, AL-Nahrain University. The written informed consent was obtained from patients and controls. The study was conducted in the Department of Chemistry & Biochemistry, College of Medicine, AL-Nahrain University, Baghdad –Iraq from June 2013 to July 2014. Seventy three participants were included in this study. The cases were 35 women suffering from primary infertility, and 38women were taken as age- matched apparently healthy controls. The control group consisted of healthy women with one or more successful pregnancies, without any obstetrical or medical problems like hypertension or diabetes mellitus.

Study group consisted of all those women who were infertile for more than one year, have body mass index (BMI) less than 25, regular menstrual cycle, normal pelvic examination findings and no post history of pelvic inflammatory disease. Exclusion criteria were male factor subfertility.

A venous blood sample (five ml) was collected in fasting state from antecubital vein of each case and control subjects. The blood sample was kept undisturbed for at least 15 minutes & was then centrifuged at 3000 rpm for 10min. Serum samples then were stored at - 20ºC for analysis of vitamin D and Anti- Mullerian hormone.

Reversed phase HPLC (LC-10AD, Shimadzu, HPLC 1991 model -7125, Kyoto-Japan) was used as follows: the vitamin D was isolated from the serum by liquid –liquid extraction using n-hexane solvent, concentrated by evaporation under nitrogen stream and reconstituted with HPLC grade ethanol. The reconstituted sample (50 μl) was injected into the chromatography system on a C18- CLC-ODS column of diameter 4. 6mm (Shimadzu, Japan) with a mobile phase of methanol: water (95:5). Vitamin D was detected spectrophotometrically at 254nm. The column was re- equilibrated with the mobile phase for 5 minutes before injection of the next sample. In order to check the reproducibility of the method, some samples were injected consequently, twice. To verify the assay accuracy, standard analytes were injected for every 15-20 test samples. Serum Anti- Mullerian hormone (AMH) was assayed using the enzyme- linked immunosorbent assay (ELISA) kit (Cusabio, Cosmo Bio, Carlsbad, CA). The assay sensitivity for AMH was 0.375 ng/ mL, and the intra- and inter assay coefficients of variation were 7.1%and 9.8 % respectively.

3.   Statistical analysis

Continuous data are presented as mean ± SD. Differences between the patients and the control group for continuous variables was assessed with a student's t- test. Correlation between vitamin D and anti- Mullerian hormone level was evaluated with a Pearson correlation coefficient. Statistical analysis was performed by SPSS software (Statistical

package for the social sciences, version 18.0, SPSS Inc, Chicago IL). A p value of ≤ 0.05 were considered statistically significant.

4. Results

In total, 73 subjects were enrolled in this study, including 35 infertile women and38 healthy women. Among the infertile women, all were complaining of primary infertility. Demographic profile of both the groups is shown in table (1).

Table 1: Demographic Profile (N= 73)

Variables	Primary infertile women (n=35)	Controls (n=38)	P value
Age (years)	25.4±4.91	27.3±3.72	0.11
BMI(kg/m^2)	24.31±3.78	24.8±2.06	0.66
Duration of marriage (years)	3.5±2.1	4.6±3.1	0.09
Smoking *	(0%)	(0%)	
Education*			
Educated	21(60%)		
Uneducated	14(40%)		
Residence*			
Rural	8(32%)	6(26%)	
Urban	17(68%)	17(0%)	
Occupation* Housewife Working	20(0%)	16(0%)	
	5(0%)	7(0%)	

- (*)Result is expressed as frequency and percentages.

Table 2 shows the mean fasting serum vitamin D level which was significantly lower (p< 0.05) in primary infertile group (35.38±5.83 ng/ mL) in comparison with this level in controls (49.99±12.90 ng/ mL).
Anti- Mullerian hormone (AMH) was significantly (p < 0.05) decreased in infertile group (1.21±0.16 ng/ml) compared with its level in control group (1.54± 0.26ng/ml) as shown in table (2).

Table 2: Biochemical Parameters

parameter	Primary infertile women (n=35)	Controls (n=38)	P value
Vitamin D (ng/ml)	35.38±5.83	49.99±12.90	< 0.05
AMH (ng/ml)	1.21± 0.16	1.54± 0.26	<0.05

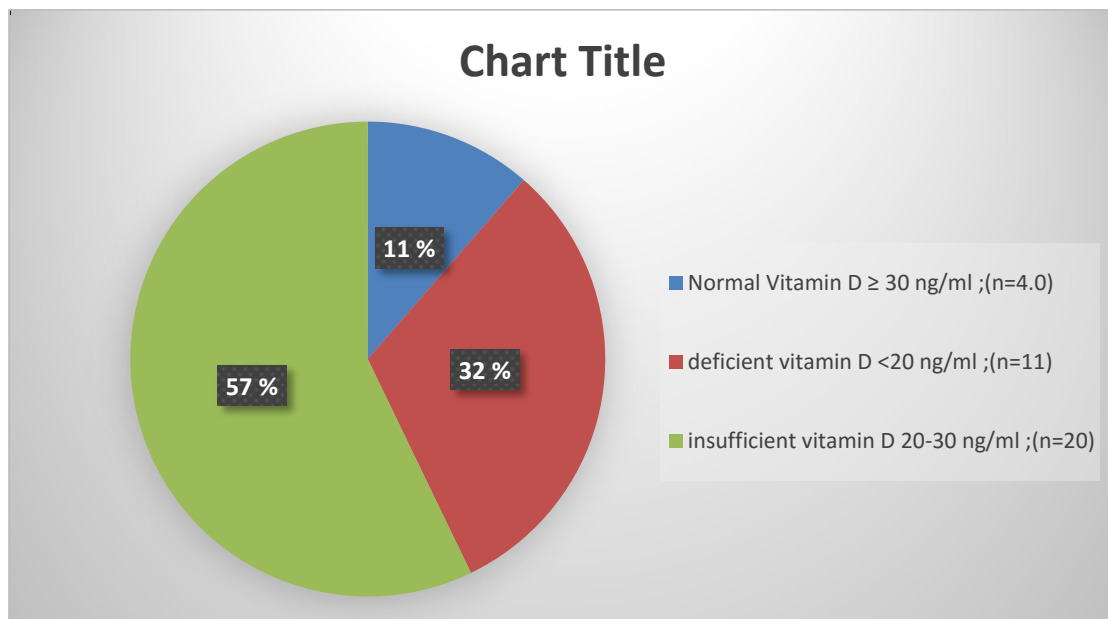The infertile patients were categorized to different groups according to the level of vitamin D as shown in figure (1)

Fig. 1: Pie Graph of Vitamin D Levels in Infertile Women Group According to Different Concentration Categories.

BMI correlated negatively with Anti- Mullerian hormone level (r = -0.78; p< 0.05) as shown in figure (2)
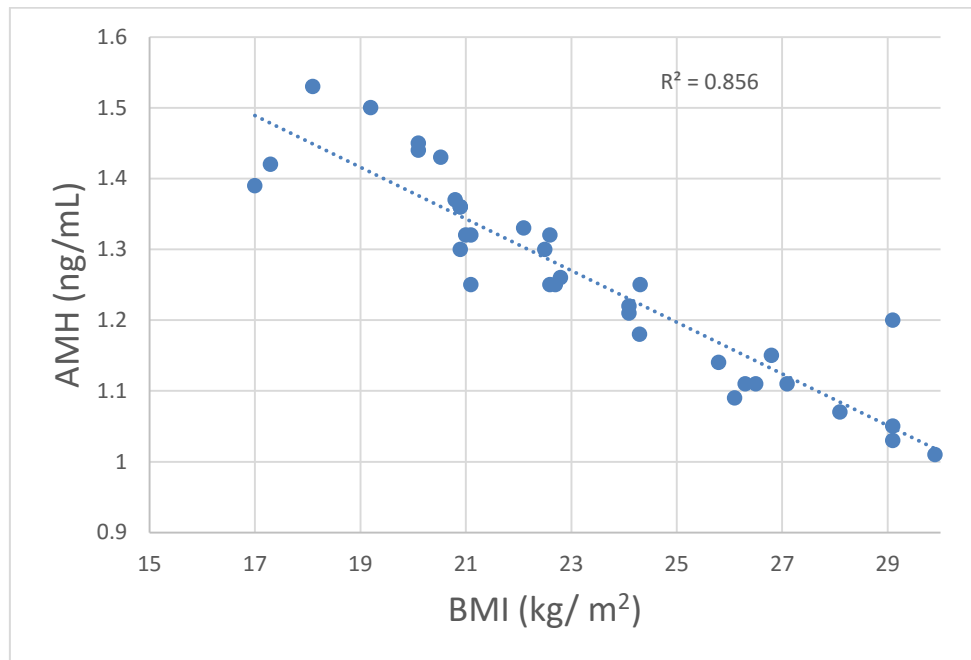
Fig. 2: Correlation between BMI and AMH In Infertile Group.

Figure (3) illustrates that there is a significant correlation between vitamin D and Anti- mullerian hormone levels in infertile women group (r = 0.86; p< 0.05)

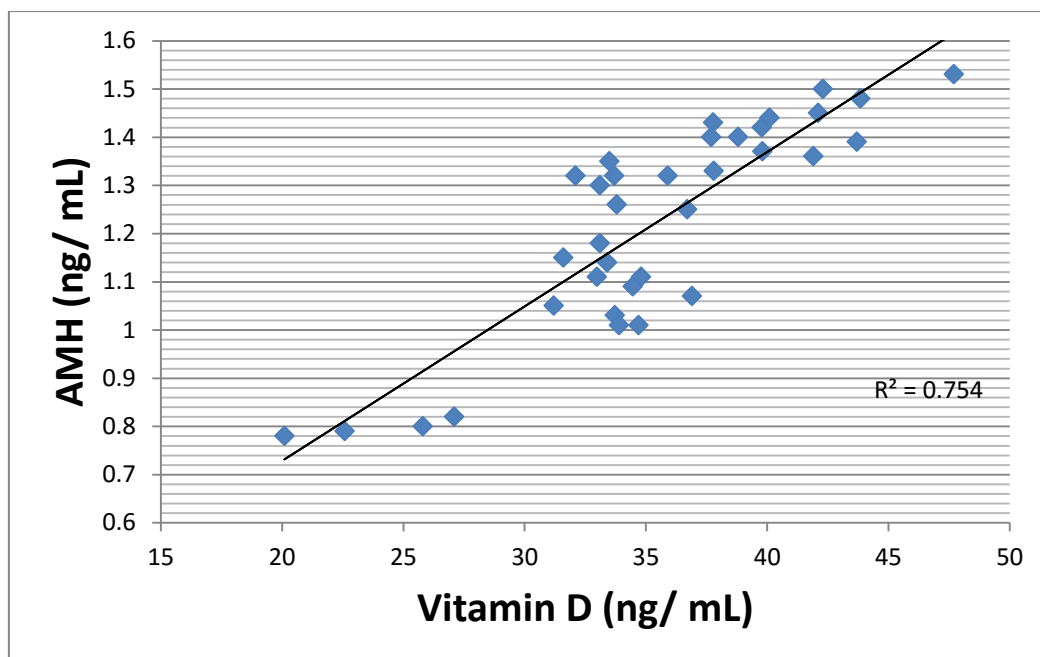**Fig. 3:** Correlation between Vitamin D and AMH.

5. Discussion

Low vitamin D levels have been linked to an increased risk of cancer [20] , autoimmune diseases, diabetes, cardiovascular diseases, and polycystic ovarian syndrome [20- 23] indicating the importance of sufficient vitamin D levels. Although, there is no consensus an optimal vitamin D level, a level of 30ng/mL can be considered to indicate sufficient vitamin D status [21].Many investigators reported that the prevalence of vitamin D insufficiency (20.1- 30 ng/ml) or deficiency (less than 20 ng/ml) was 79% in a population of women undergoing in vitro fertilization [21], [24], [25], [29]. In this study (88.5 %) of infertile women showed vitamin D values below this recommended level (30 ng/ml). High levels of maternal vitamin D have been associated with improved fertility[26], lower rise of gestational diabetes [27], lower rise of cesarean section [28], improved birth outcomes such as birth weight and early growth [29, 30], of note, one study found the follicular fluid 25(OH) D increased the likelihood of clinical pregnancy of 7%. And

follicular fluid 25 (OH) D was well correlated with serum levels [26]. It was hypothesized that vitamin D may improve ovarian steroidogenesis increased endometrial receptively [20], [31].

Throughout this study it was demonstrated that circulating vitamin D was positively correlated with serum Anti-Mullerian hormone levels (ovarian reserve marker) in female infertility group (r =+ 0.86; p ≤0.05). Recently many scientists declared that vitamin D was a target for AMH gene expression [13], [32].

The AMH gene was upregulated by vitamin D via functional vitamin D response elements that bind the vitamin D receptor [13], [32]. Because vitamin D induces the AMH gene [32] and because the vitamin D and AMH are positively correlated as shown in figure (3), it is possible to conclude that vitamin D contributes to AMH actions by increasing AMH production by granulosa cells.

The results of current study indicated that low vitamin D levels are significantly associated (p<0.002) with primary infertility women. This finding may be explained by that vitamin D has a biological plausible role in female reproduction.

In human ovarian tissue, vitamin D stimulated progesterone production by 13%, estradiol production by 9% and estrone production by 21%[33].On the other hand, vitamin D regulates human chorionic gonadotropin expression and secretion in human syncytiotrophoblasts [34], [35] and increases placental sex steroid production.

Previous studies have demonstrated that calcitriol promotes calcium transport in the placenta, stimulates placental lactogen expression, and regulates HOXA 10 protein (homeobox A10 protein) expression in human endometrial stroma cells [36]. HOXA 10 expression is important for the endometrial development, allowing uterine receptivity to implantation [37].

Our data might lead to new therapeutic approaches such as vitamin D supplementation in the treatment of female infertility. Throughout this study, a negative correlation was found between BMI value and vitamin D levels in both control and infertility women groups. This finding was similar to those reported previously. Panidis et al [38] found an inverse association of vitamin D with obesity as well as a BMI-dependent association with insulin resistance. There is evidence that low vitamin D levels are associated with obesity [39] and vice-versa low vitamin D intake might be an independent predictor of obesity [40].

There are however mechanisms beyond obesity that might explain the association of vitamin D deficiency with insulin resistance. First vitamin D may have a beneficial effect on insulin action by stimulating the expression of insulin receptor and thereby enhancing insulin responsiveness for glucose transport [41]. The vitamin D responsive element is found in the promoter of the human insulin gene [42] and transcription of the human insulin gene is activated by $1,25$ $(OH)_2 D_3$ [43].Second vitamin D regulates extracellular and intracellular calcium which is essential for insulin mediated intracellular processes in insulin-responsive tissues such as skeletal muscle and adipose tissue [41].

To our knowledge, this is the first study of measuring vitamin D in infertile Iraqi women. Our overall results suggest that decreasing serum vitamin D levels are associated directly and significantly with female infertility.

These results generally are consistent with those observed previously, Ozkan et al. showed higher pregnancy and implantation rates across tertiles of 25 (OH) D3 in follicular fluid of 84 infertile women undergoing IVF and proposed follicular fluid 25 (OH) D3 levels as an independent predictor to success of an IVF cycle [26]. From the results of this study, it was concluded that normal plasma vitamin D levels is important to maintain normal fertilizing ability and conceive.

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