Original Research Article

The Associations of Serum AMH, Vitamin D, FSH and AFC in Different Age Groups of Infertile Women

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A B S T R A C T

The objective of our study was to identify the associations between the tests that used in ovarian reserve assessment: Anti-Mullerian hormone (AMH), follicle stimulating hormone (FSH) and antral follicle count (AFC) and to distinguish the most reliable markers for ovarian reserve in order to select an adequate strategy for the initial stages of infertility treatment and identify the effect of vitamin D on the ovarian reserve and its correlation with AMH. Anti-mullerian hormone (AMH) is expressed only in the gonads. In female it's secreted by adult granulosa cells of the ovary. The levels of AMH reflect the number of preantral follicles and thus as a marker of oocyte pool which is a germinal reserve of the ovary for reproduction. Vitamin D is a fat-soluble vitamin that belongs to the family of steroid hormones. It has a biologically plausible role in female reproduction. In this paper AMH, FSH and Vitamin D was determined by enzyme linked immunosorbent assay in: 60 infertile women with PCOS (cases) undergoing IVF and 30 healthy women had one child at least (control). The antral follicle count (AFC) was recorded for each female in case group. The AMH and 25(OH) D levels in cases were lower than that of control, while FSH level in cases was higher than control group. The AMH in cases was significantly decreased with increasing age. The AFC was inversely associated with age. There was a significant positive association between ovarian response in terms of the antral follicle count and AMH levels, there was no association between ovarian response in terms of the AFC with 25(OH)D and FSH implying that AMH can be used as a good predictor of ovarian reserve and ovarian response. The correlation between 25(OH)D and AMH among women ages 36-42 showed that AMH levels decreased significantly with increasing 25(OH)D levels, and was found no statistically significant correlation between 25(OH)D and AMH among women under the age of 36.

Introduction

AMH is an important member of the TGF-b super family secreted by granular cells (GCs) and plays an important role in the folliculogenesis. It has the highest expression in small antral follicles and major suppresses primordial follicles into the growth phase (Goodarzi *et al.,* 2011). Infertility is a complex issue with significant medical, psychosocial, and economic problems. Polycystic ovarian syndrome (PCOS) is one of the most common endocrine disorders (Norman *et al.,* 2007), affecting up to 5 to 10% reproductive-aged

women (Qiao *et al.,* 2013) and is one of the most major cause of infertility. The major clinical features of PCOS contain the four common symptoms: menstrual disorders, Infertility hyperandrogenemia, and metabolic syndrome- (Trikudanathan *et al.,* 2015). FSH in females, initiates follicular growth, specifically affecting GCs. It is thought that each follicle has its own threshold FSH concentration varying from hypo-response to a risk of ovarian hyperstimulation and this concentration has to be exceeded to ensure dominant follicle selection. It is reported that AMH inhibits FSH-stimulated follicle growth (Durlinger *et al.,* 2001) and is one of the factors restrains the sensitivity of ovarian follicles for FSH, thus preventing follicle selection and resulting in follicle arrest at the small antral phase, with failure of dominance (Grossman *et al.,* 2008). It is suggested the GCs from polycystic ovaries continue to produce elevated levels of AMH, possibly because of impaired access of FSH to follicles (Desforges-Bullet *et al.,* 2010). AMH seems to be involved in the inhibition of FSH action by repressing the FSH-dependent aromatase activity. So in anovulatory patients, although the serum FSH is at low/normal concentrations, AMH level is not low sufficient to allow the expression of aromatase (Catteau-Jonard *et al.,* 2013).

Vitamin D

It is an essential steroid hormone classically known for its role in maintenance of calcium and phosphatehomeostasis. Vitamin D is largely generated in the epidermis with exposure to ultraviolet radiation. Two different forms of vitamin D from dietary sources are vitamin D_2 (ergocalciferol) derived from plants and vitamin D_3 (cholecalciferol) derived from animals (Bouillon *et al.,* 2015). The cutaneous precursor of vitamin D, previtamin D_3 (7-dehydrocholesterol), is derived from cholesterol in food. After exposure to short

wave UVB radiation the B ring of 7-dehydrocholesterol is transformed into previtamin D_3 (cholecalciferol) or converted into two inactive products. It undergoes two hydroxylation steps by P450 mixed function mono-oxidases. In the liver, vitamin D hydroxylation into 25-hydroxyvitamin D (25(OH)D) is modulated by the mitochondrial CYP27A1 or the microsomal CYP2R1.Further hydroxylation takes place in the kidney in the proximal convoluted tubule to the physiologically active form, 1 α,25-dihydroxyvitamin D3 (1,25(OH)2D3),by CYP27B1.

Active vitamin D binds to VDBP and is transported to target cells. Once the complex reaches the target cell, vitamin D is released from the VDBP and $1,25(OH)_2D_3$ binds to vitamin D receptors (VDR) present in the cytoplasm. VDR transports vitamin D into the nucleus. The female reproductive system, as the male reproductive system, is composed of central regulators including the hypothalamus and the pituitary gland and peripheral organs such as the ovary, uterus, and during pregnancy the placenta. *In vitro* studies have shown a direct modulation by vitamin D of estradiol, estrone, and progesterone production in human ovarian cells (Parikh *et al.,* 2010) in female reproductive physiology. Anti-Mullerian hormone (AMH) is a marker of ovarian reserve.

There have been several studies suggesting modulation of AMH levels by vitamin D. A functional VDRE has been noted in the promoter region of the AMH gene (Molly *et al.,* 2009).

Association studies in humans have shown a positive correlation between vitamin D and AMH levels. One study in women with regular menstrual cycles has shown decreased AMH levels with lower vitamin D levels (Merhi *et al.,* 2014).

Materials and Methods

The present study comprised of 60 infertile women with symptoms of PCOS undergoing IVF and 30 healthy women. The studied population was divided into three subgroups according to their age: ≤25, 26-36 and >36 years. Blood samples were collected from individual on 2-3 days of their spontaneous menstrual cycles the numbers of antral follicles that measured 2-10 mm in size were counted in each ovary. The sum of both counts was the AFC.

Samples were hospitalized at laboratories in the Al-Zahraa Al-Batool private hospital. the samples were collected from October 2017 to February 2018,blood samples were collected (5 ml) and centrifuged at [4000 rpm] for 10 min after clotting, to separate the serum from the cells to determine human serum AMH, FSH and Vitamin D levels. The resultant serum was separated and stored at [-20] °C until time of analyses.

Human serum Anti-Mullerian Hormone (AMH), Follicle Stimulating Hormone (FSH) and Vitamin D were measured by using (ELISA) an enzyme-linked- immune-sorbent assay kit (Sandwich) technologies for individual using commercially available kits AMH, FSH and 25-OHvitaminD (YHLO Biotech, South Korea).

Statistical analysis

Statistical analysis of data, was performed using Mini-Tab-System version 18.1). One-way ANOVA test was used for analysis of variance for average hormone level as quantitative variable by qualitative variable. Correlation coefficient (r) between vitamin D and AMH was used. The results in all the above mentioned procedures were accepted as statistically significant when the p-value was less than 5% ($p < 0.05$).

Results and Discussion

The mean levels of AMH, FSH, and 25(OH)D recorded in the present study for cases were more or less close to other study (Ficicioglu *et al.,* 2006) When compared to controls AMH and 25OH-D where significantly lower than those encountered in the controls (3.738±2.279 vs. 2.568± 1.960ng/mL and 31.77±7.48 vs. 10.511±3.041pg/ml, respectively).

FSH was significantly higher in cases than controls. Consequently, the lower level of AMH observed in most women with PCOS undergoing IVF. Although FSH levels in the cases were higher than those of controls, they are still within the normal range of 3-11 mIU/ml (Tietz *et al.,* 1995) indicating that FSH alone is not sufficient to predict the female reproductive potential (Grossman *et al.,* 2008) and support the hypothesis that there is a reverse relationship between AMH and FSH. Statistical data suggested that among infertile women there is a high incidence of Vitamin D deficiency.) A study found that 90.8% of women being worked up for infertility had insufficient (68.6%) or deficient (22.2%) vitamin D levels (Schriock *et al.,* 2012). Likewise, another study by (Anifandis *et al.,* 2010) from Greece reported 79 %of women undergoing in vitro fertilization (IVF) were vitamin D insufficient or deficient.

Our study reports 66.6% of infertile women having vitamin D deficiency. There is some evidence that vitamin D deficiency and its effects on fertility may be indirect. Without vitamin D, the body absorbs up to 30% less calcium and 20% less phosphorus. In experimental conditions, when the hypocalcaemia and hypophosphatemia were corrected in the female their fertility returned (Johnson *et al.,* 2010). It's possible the primary cause of infertility may be

hypocalcaemia-and/or hypophosphatemia. We examined the relationships between age and ovarian reserve indicators in the case group. Age showed a significant negative association with AMH level (F= 5.2, p<0.008) and AFC (F= 3.61, p<0.03) and significant positive association with 25(OH)D. There was no association between age and FSH (F=0.630, p= 0.538). AMH showed a positive correlation with AFC (F=7.31, p<0.0001). There was no association between AFC with vitamin D (F=1.06, p<0.375) (Fig. 1–3 and Table 1).

The relationship between AMH, FSH, AFC and vitamin D levels and age

The mean levels of AMH in relation to the age of the study population are illustrated in Table 2. According to their age, the study population was divided into three groups: ≤25, 26-36 and >36 years. The mean levels of AMH in cases was significantly decreased with increasing age (3.656± 2.675 ng/mL, 2.175 ±1.249 ng/mL and 1.536 ± 1.376 ng/mL at ≤25, 26-36 and >36 years, respectively; F=5.20 and p=0.008) (Table 2).

Table.1 The mean levels of AMH, FSH and vitamin D in cases as compared to controls

Parameters	Case (N=60)	Control (N= 30)	p-value
AMH (ng/mL)	2.568±1.960	3.738±2.279	0.023*
FSH (mIU/mL)	8.026±3.084	6.63±2.467	0.034*
25(OH)D (ng/mL)	10.511±3.041	31.77±7.48	0000*

Note: Values are represented with means and ± SD; p*; significant MH; Anti-Mullerian Hormone; FSH: Follicle Stimulating Hormone; 25(OH)D: 25-hydroxyvitamin D

Table.2 The relationship between AMH, FSH and vitamin D levels and age

| AGE (year)	≤25	26-36	>36	F	P-value
AMH (ng/mL)	3.656 ± 2.675	2.175 ± 1.249	1.356 ± 1.376	5.20	0.008*
FSH (mIU/mL)	7.778 ± 2.892	8.006 ± 3.132	7.383 ± 3.493	0.630	0.538
AFC	19.21 ± 6.70	14.71 ± 6.06	13.00 ± 8.83	3.61	0.033*
25(OH)D (ng/mL)	9.02 ± 2.539	11.116 ± 3.189	11.556 ± 2.338	3.60	0.034*

Note: Values are represented with means and ± SD; p*; significant; AMH; Anti-mullerian Hormone; FSH: Follicle Stimulating Hormone; 25(OH)D: 25-hydroxyvitamin D, AFC: antral follicle count

**Table.3** The correlation between the vitamin D and AMH

Serum 25(OH)D (ng/ml)	Age	< 10 ng/ml	11-20 ng/ml	21-80 ng/ml	P
AMH (ng/mL)	20-36	2.887± 1.930	2.305± 2.025	3.738± 2.279	0.859
Serum 25(OH)D (ng/ml)	Age	< 10 ng/ml	11-20 ng/ml	21-80 ng/ml	P
AMH (ng/mL)	36-42	1.077± 0.665	1.880± 1.767	6.47±6.44	0.006*

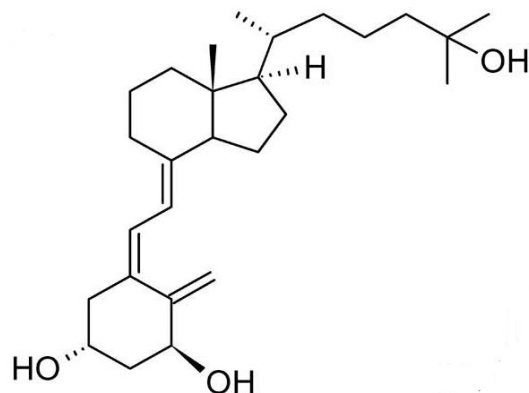Note: Values are represented with means and ± SD; p*; significant

Fig.1 The chemical structure of vitamin D_2 and D_3

Vitamin D2 (Ergocalciferol)   Vitamin D3 (Cholecalciferol)
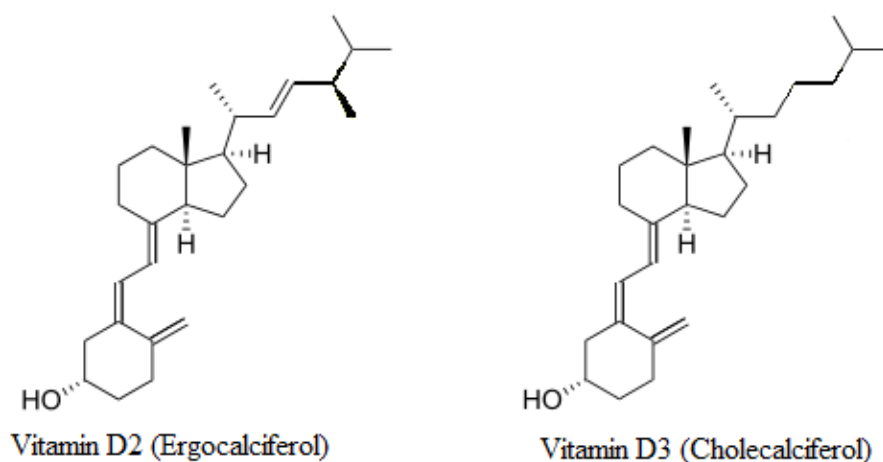
Fig.2 The chemical structure of 25-hydroxy vitamin D_3
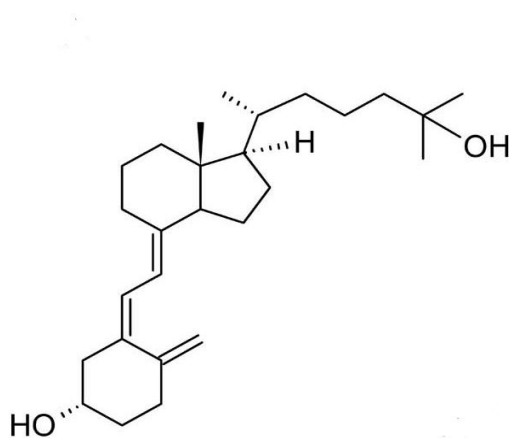
Fig.3 The chemical structure of 1,25-hydroxy vitamin D_3

The mean levels of AMH, FSH, and vitamin D recorded in the present study for cases were more or less close to those reported by another study (Qiao *et al.,* 2013). Table 2 lists the differences between groups for the mean ± standard deviation AMH, FSH, 25(OH)D and AFC values. There were significantly higher AMH levels in group I compared with groups II and III. This value was also higher in group II compared to group III. The antral follicle count

(AFC) were significantly higher in group I compared with group III, FSH and 25(OH)D levels were significantly higher only in group II compared to group I. Data presented in this study showed that the mean levels of AMH in cases decreased with increasing age. This inverse relationship is in agreement with that found by another study (Van Rooij *et al.,* 2005) which is reported that serum AMH levels decline with age in women with proven fertility. They added that serum AMH represents the best endocrine marker with their age groups (the young, the adult and the elderly group) was investigated. In this study, the relationship between the serum vitamin D level of patient with their age groups (the young, the adult and the elderly group) was investigated. And its showed a significant relationship of vitamin D with age of cases (P=0.03). In line with our study, another study found serum 25 dihydroxy-vitamin D levels in the older age groups were higher when compared with the adults (Steingrimsdottir *et al.,* 2005). Likewise, a significant relationship of serum vitamin D levels with age of the patient was detected too by (Baradaran A *et al.,* 2012). Accordingly, with another study found a positive relationship between serum 25(OH)D levels and age of patient (Bischoff-Ferrari *et al.,* 2004).

The correlation between the vitamin D and AMH

The present study found that 66.6% of the study population was suffering from either vitamin D insufficiency or deficiency according to Holick's classification (Holick *et al.,* 2007) and decreased serum AMH level was associated with vitamin D deficiency. Although previous studies have investigated serum 25(OH)D concentrations in infertile women by age group (Merhi *et al.,* 2014) and they observed that vitamin D treatment down-regulated AMH receptor (AMHR) gene expression and signaling by interruption of Smad 1/5/8 phosphorylation and its nuclear translocation., an inverse correlation existed

between follicular fluid 25(OH)D levels and AMH receptor-II (AMHR-II) mRNA gene expression. Women with insufficient/deficient levels of 25(OH)D in follicular fluid displayed a 2-fold increase in AMHR-II mRNA expression levels compared to those with sufficient 25(OH)D levels. At the serum level, (Merhi *et al.,* 2014) levels observed a weak negative association between vitamin D and AMH among women under 35 years of age and a weak positive relationship above 40 years of age. Another study suggested that vitamin D may influence the ovarian reserve. A study of group of women found that women <30 years old had significantly lower mean serum 25(OH)D levels compared with women ≥30 years old, and 42.1% of these younger women were vitamin D-deficient (Nakamura *et al.,* 2014). In the present study showed an AMH levels decreased significantly with increasing 25(OH)D levels among women ages 36-42, and found no statistically significant correlation between 25(OH)D and AMH among women under the age of 36 (Ross *et al.,* 2014) (Table 3).

In conclusion, through our research, we found that the serum AMH levels are strongly related with the antral follicle count, this relationship is more significant than other ovarian reserve parameters. These results also indicate that the serum AMH measurement is a better predictor for the number of early antral follicles compared to conventional hor-mone measurements. Measuring AMH levels in combination with AFC may improve the assessment of ovarian reserve for evaluating fertility potential and monitoring infertility treatment. In the present study showed an inverse relationship between 25(OH)D and AMH among women ages 36-42, and no statistically significant correlation between 25(OH)D and AMH among women under the age of36. The prevalence of vitamin D deficiency was very high among the patients who participated in this study. However, no

significant correlation was found between ovarian response with vitamin D and FSH levels.

Acknowledgement

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