Association Between Vitamin D Deficiency and Unexplained Infertility

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ABSTRACT

Background: The causes of female infertility are different and include genetic and anatomic abnormalities as well as endocrine and autoimmune disorders (ADs). Vitamin D might influence steroidogenesis of both estradiol and progesterone in healthy women where low levels of 25(OH)D levels might be associated with infertility.

Aim of the Work: To elucidate if there is relationship between vitamin D and unexplained infertility and if it more prevalent in them.

Patients and Methods: This case-control study was conducted at Ain Shams University Maternity Hospital during the period between October 2018-april 2019. A total of 70 women with prior history of unexplained infertility were included as group A (study group). A Second set of 70 fertile control group were included as group B (control group).

Results: Our study indicated that 25(OH) vitamin-D was significant lower in unexplained infertility group than control group (according to vitamin D level 39 out of 70 in unexplained infertility patient (55.7) had deficiency, while 30(42.9%) had insufficiency and 1(1.4) had sufficiency, in comparison to control group 21(30.0%) had deficiency, while 43(61.4) had insufficiency and 6(8.6%) had sufficiency), there were no significant correlation between 25(OH) vitamin D and age and BMI.

Conclusion: Vitamin-D deficiency had a role in unexplained infertility, nevertheless it is not a good diagnostic test. Unexplained infertility is multifactorial; Vit-D is only one of many factors/ theories.

Key Words: Subfertility, unexplained infertility, vitamin D deficiency.

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INTRODUCTION

Infertility is defined as failure to conceive after regular unprotected vaginal intercourse for 1 year^[1]. This definition reflects the prognostic approach of this condition, based on the knowledge that, in general population, 84% of all women are expected to conceive within 1 year of regular unprotected sexual intercourse and 92% after 2 years and 93% after 3 years (Te Velde *et al.*, 2000)^[2].

Unexplained infertility refers to the absence of a definable cause for a couple's failure to achieve pregnancy after 12 months of attempting conception despite a thorough evaluation, or after six months in women 35 and older^[3].

Several possibilities have been proposed to explain why some couples fail to conceive in the absence of an identifiable cause. Subtle changes in follicle development, ovulation, and the luteal phase have been reported in some of these women^[4]. In other couples, the male partner's semen analysis shows sperm concentration and motility at the lower end of the normal range^[5], Implantation failure, subtle cervical factors, or problems with sperm and egg transport or interaction are other possibilities, Many cases of unexplained infertility are probably caused by the presence of multiple factors (e.g., female partner over 35 years of age with diminished ovarian reserve and male partner with low normal semen parameters), each of which on their own do not significantly reduce fertility, but can reduce the pregnancy rate when combined^[6].

During the last years, vitamin D has attracted increased attention from the scientific community, the food industry, policy makers and the public, this is mainly due to new discovers about the impact of vitamin D on several health outcomes beyond it's known as metabolic actions in the bone and mineral metabolism specifically, the presence of the vitamin D receptor in many body tissues supported evidence linking vitamin D deficiency to increased risk of certain auto-immune diseases, cancers, cardiovascular disease, diabetes and psychiatric disorders^[7].

In addition to its effects on health and metabolism, vitamin D has raised interest because of the large variation in the prevalence of vitamin D deficiency across countries worldwide with estimates ranging from 2 to 90%^[7].

Vitamin D has also role in the regulation of sex hormone steroidogenesis, increasing evidence suggest that vitamin

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D might have regulatory role in polycystic ovary syndrome (PCOS)- associated symptoms including ovulatory dysfunction, insulin resistance and hyperandrogenism^[8].

Vitamin D deficiency, defined as serum concentration of 25-OHD of less than 20ng/ml, is commonly noted in fertility clinic patients, it is global trend that is observed in all age groups, it is reported in the literature that women with significant level of vitamin D had brighter chances of clinical pregnancies after ART^[8].

In contrast, it is also reported that couple under taking ICSI procedure (euploid blastocyst transfer) had no impact on outcome where substantial levels of vitamin D were concerned, however presence of vitamin D receptor (VDR) in critical reproductive organs has given edge to the positive physiological role of vitamin D in reproduction and ART in particular^[9].

Additionally, low 25 hydroxyvitamin D (25(OH)D) levels have been associated with obesity, metabolic, and endocrine disturbances in PCOS women and vitamin D supplementation has been suggested to improve menstrual frequency and metabolic disturbances in those women^[8].

Moreover, vitamin D might influence steroidogenesis of both estradiol and progesterone in healthy Women where low levels of 25(OH) D levels might be associated with infertility and high levels might be associated with endometriosis, The most up to date vitamin D studies outside its conventional role of calcium homeostasis in the Middle East so far have covered most of the obesity-related diseases including diabetes mellitus, hypothyroidism, and the full metabolic syndrome, Despite the abundance and overflow of recent literature with respect to vitamin D role in health and disease, there are an equal overwhelming number of questions left unanswered and this probably explains the rationale to conduct more research on vitamin D, namely the reported association between vitamin D deficiency and infertility^[10].

AIM OF THE WORK

To assess the vitamin D status in unexplained infertile women

PATIENTS AND METHODS

The is a case-control study had been conducted at Ain Shams Maternity hospital, from women attending outpatient infertility clinic and family planning clinic enrolled 140 women into two groups: each group containing 70 with unexplained infertility and the control group with fertile women coming for family planning during the period from October 2018 until April 2019.

The study included patients with unexplained infertility: age between 20-35 years, normal semen analysis, normal hormonal profile (FSH, LH and prolactin, TSH, AMH), normal transvaginal ultrasound (normal appearance of adnexa), no evidence of endocrine disorder and normal hysterosalpingography or laparoscopy. Medical condition that can cause infertility such as diabetic mellitus, polycystic ovary, hypothyroidism, hypertension, liver or renal disease, Uncorrected Congenital or acquired uterine abnormality, Patient who refused, Current use of contraceptive, Patient less than 20 years or more than 35 years, Male factor infertility and patient under vitamin D therapy were excluded from the study.

- Group A (Cases): women with unexplained infertility
- **Group B (Control):** non pregnant fertile women from family planning clinic as control group

Methods of randomization

Simple random sampling.

Methods and material

All included women after informed consent was subjected to: Consent: a written informed consents were taken from all cases of two groups after full explanation of steps and significant of this study. Full history taking with especial emphasis to maternal age, as well as presence of any disease. General examination including BMI, Abdominal and pelvic examination. Revising of criteria for diagnosis of unexplained infertility. Unexplained infertility was diagnosed according to World Health Organization^[11] criteria, all women was undergone hormonal assessment to evaluate their ovulatory cycle, thyroid function test, circulating prolactin levels. The ovarian reserve was checked by measurement of serum level FSH and luteinizing hormone. Screening of infertility also include transvaginal ultrasound and hysterosalpingography to exclude possible uterine malformation or pathology and to assess the potency of the fallopian tube.

Laboratory investigation

Sample collection

Venous blood sample (5ml) blood was collected using sterile syringe from every participant at any day of menstrual cycle then vitamin D level was measured by using enzyme linked immunosorbent assay technique (lot n. vds4203; calbiotech Inc., Austin drive, spring vall, California, USA) the procedure was carried out according to the manufactures instruction as supplied with kit. All biochemical measures will perform in single batch and comparable number of cases and control samples was always assayed simultaneously in same enzyme linked assay plate. The kit recorded the result as deficient if vitamin D is less than 20ng/ml, insufficient 20-30ng/ml and sufficient >30ng/ml.

Principles of the test

The kit is a solid phase enzyme-linked immunoassay (ELISA), based on the principles of competitive binding. Anti-vitamin D antibody coated wells are incubated with vitamin D standards, controls, samples and vitamin D – biotin conjugate at room temperature for 90 minutes.

during the incubation a fixed amount of biotin –labelled vitamin D competes with the endogenous vitamin D in the sample, standard or quality control serum for fixed number binding sites on the anti-vitamin D antibody. Following a wash step, bound vitamin D biotin is detected with streptavidin – horseradish peroxidase (SA-HRP). The total assay procedure run time is 2.5 hours.

Procedures

All reagent and specimens were allowed to come to room temperature before use. All reagents were gently mixed without foaming. Once the procedure has started all steps were completed without interruption.

Vitamin D status was classified as following:

| Vitamin D level | Vitamin D status | | |
|-----------------|------------------|--|--|
| <20ng/ml | Deficient | | |
| 20-30ng/ml | Insufficient | | |
| 30-100 ng/mL | Sufficient | | |

Ethical consideration: Only patient initials were recorded, and if patient name appears on any other document, it was kept in privacy by the investigator. Before the beginning of the study and in accordance with local regulation followed, the protocol and all the corresponding documents were declared for ethical and research approval by the council of obstetrics and gynecology department, Ain Shams University.

Data management and analysis

The collected data was revised, coded, tabulated and introduced to PC using statistical package for social science (IBM Corp. Released 2011, IBM SPSS Statistics for windows, version 20.0. Armonk, NY: IBM Corp.). Data was presented and suitable analysis was done according to the type of data obtained for each parameter. P value:level of significance: P > 0.05: Non significant (NS), P < 0.05: significant (S) and P < 0.01: highly significant (HS).

RESULTS

The current study was conducted at Ain Shams University Maternity Hospital during the period between october2018-april 2019. Total 70 women with prior history of unexplained infertility were included as group A (study group). A Second set of 70 fertile control group were included as group B (control group).

Blood sample (5ml) were collected at any time of menstrual cycle and allowed for clotting then centrifuged at laboratory of microbiology and immunology department at faculty of medicine Ain Shams University to be frozen till time of testing then vitamin D level was measured by using enzyme linked immunosorbent assay technique The kit recorded the result as deficient if vitamin D is less than 20ng/ml, insufficient 20-30ng/ml and sufficient >30ng/ml.

Data of all patients in both groups were allocated and scheduled in the master sheet for statistical evaluation (Table 1).

| Table 1: Demographic | characteristics | among the | studied | groups |
|----------------------|-----------------|-----------|---------|--------|
|----------------------|-----------------|-----------|---------|--------|

| Variables | | Case (N=70) | Control (N=70) | Р |
|---------------------|-----------|----------------|-------------------|--------|
| Age | Mean±SD | 27.8±2.9 | 27.8±2.8 | AD 052 |
| (years) | Range | 22.0-34.0 | 20.0-34.0 | ~0.955 |
| BMI | Mean±SD | 24.7±2.0 | 24.2±1.8 | AQ 205 |
| (kg/m^2) | Range | 19.2–29.6 | 19.9–29.7 | ^0.205 |
| Duration of non- | Mean±SD | 2.6±0.8 | | AD 754 |
| pregnancy (years) | Range | 1.0 - 5.0 | | ^0.754 |
| Type of infertility | Primary | 43 (61.4%) | | |
| (n, %) | Secondary | 27 (38.6%) | | - |

^Independent t-test,

This Table shows that: the mean age among both groups was 27.8 ± 2.9 with a range of of 22.0-34.0, the mean BMI was 24.7 ± 2.0 with range 19.2-29.6 .Mean duration of infertility 2.6 ± 0.8 with range 1.0-5.0, according to type of infertility primary infertility 43 (61.4%) while secondary infertility 27 (38.6%) this table shows that there was No statistical significant difference between case and control groups regarding demographic characteristics (Table 2).

Table 2: Hormonal profile among the studied groups

| | Variables | | Case (N=70) | Control (N=70) | P |
|-----------|-----------|---------|----------------|-------------------|---------|
| FSH | | Mean±SD | 7.6 ± 0.9 | 7.4±1.0 | 0.260 |
| (mIU/mL) | | Range | 5.3-9.8 | 4.8–9.6 | 0.309 |
| LH | | Mean±SD | 5.5±1.2 | 5.2±1.0 | 0.140 |
| (mIU/mL) | | Range | 3.6–9.1 | 2.5-7.7 | 0.140 |
| Prolactin | | Mean±SD | 13.1±4.8 | 12.6±4.7 | 0.540 |
| (ng/mL) | | Range | 4.0–19.7 | 3.8–19.6 | 0.549 |
| E2 | | Mean±SD | 55.3±9.8 | 55.6±9.9 | 0.950 |
| (pg/mL) | | Range | 38.9-78.1 | 38.0-81.8 | 0.830 |
| TSH | | Mean±SD | $1.9{\pm}0.4$ | 1.8 ± 0.4 | 0 4 4 7 |
| (mIU/mL) | | Range | 0.8–3.0 | 0.9–2.8 | 0.447 |

^Independent t-test.

This Table shows that: there was No statistical significant difference between case and control groups regards hormonal profile (Table 3).

Table 3: Partner's semen analysis among case group

| Variables | Mean±SD | Range |
|--|-----------|------------|
| Volume (mL) | 2.9±0.6 | 1.5-7.0 |
| Sperm concentration (x10 ⁶ /mL) | 19.7±10.3 | 15.7-88.0 |
| Sperm total count (x10 ⁶) | 57.7±36.9 | 39.1–294.0 |
| Motility % | 54.6±4.5 | 47.2-80.0 |
| Progressive motility % | 41.5±5.1 | 31.3-56.0 |
| Normal morphology % | 66.4±8.6 | 48.0–78.0 |
| Total=70 | | |

This Table shows that: Partner's semen analysis among case group (Table 4).

Table 4: Vitamin-D grades among the studied groups

| Grades | Case (N=70) | Control (N=70) | ^P | OR (95% CI) |
|--------------|----------------|-------------------|--------|-------------|
| Defficiency | 39 (55.7%) | 21 (30.0%) | | |
| Insufficieny | 30 (42.9%) | 43 (61.4%) | 0.003* | 2.94 |
| Sufficiency | 1 (1.4%) | 6 (8.6%) | | (1.20-2.54) |

#Fisher's Exact test, *Significant, OR: Odds ratio, CI: Confidence interval

(Table 5) shows that: there was significant association between vitamin D level and unexplained infertility as 55.7% of unexplained infertility were having vitamin D deficency while 30.0% in control group with *p* value =0.003.

 Table 5: Diagnostic performance of Vitamin-D in diagnosis of unexplained infertility

| Factors | AUC | SE | Р | 95% CI | Cut off |
|-----------|-------|-------|----------|-------------|---------|
| Vitamin-D | 0.719 | 0.043 | < 0.001* | 0.635-0.802 | ≤13.0 |

AUC: Area under curve, SE: Standard error, CI: Confidence interval, *significant

This table shows that Vitamin-D had significant low diagnostic performance in diagnosis of unexplained infertility with cut off. ≤ 13.0 (Figure 1).



Fig. 1: Receiver-Operating Charactristics (ROC) curve derived from multiple logistics regression model. Area under the ROC curve (AUC) =0.719 (SE=0.043,95%cl0.635–0.802, *P*<0.0001).

The ROC curve derived from regression model, and the parameters of the curve is shown in table the area under ROC curve (AUC) = 0.719 (SE=0.043, 95%cl0.635-0.802, P<0.0001) denoting a good prediction.

DISCUSSION

Infertility is a common condition that affects 9%-18% of the general population. For healthy young couples, the probability of getting pregnancy per a reproductive cycle is about 20% to 25%^[12].

In present study, the mean age of included women of group A(case) from 22-34 and mean \pm SD 27.8 \pm 2.9 years while group B(control) from 20-34 and mean \pm SD 27.8 \pm 2.9 years and study found there were no significant correlation between 25(OH) vitamin D and age.

This result is supported by Luke *et al.*,^[13] conducted case control study and there was no significant difference between mean age of cases 29.5 ± 5.5 years versus control 29.9 ± 5.2 (P+0.64).

In other hand Fatini *et al*^[14] who found that mean age was 35 years, ranging from 18 to 46 years of age. This may attributed to early marriage in our population.

In present study the mean BMI in case group was 19.9-29.7 and mean \pm SD (24.7 \pm 2.0) while control group was 19.9-29.7 and mean mean \pm SD (24.2 \pm 1.8) and showed there is no statistically significant.

Against our result Kremer *et al.*,^[15] evidenced a significant positive correlation between BMI and 25(OH)D level, which was confirmed at multivariable analyses mean BMI was 14-40 and mean \pm SD (22.4 \pm 3.6).

The mean age of Duration of infertility in present study in women with unexplained infertility was 2.6 \pm 0.8 years, in other hand Fatini *et al.*,^[14] who found that median age of infertility was 4 years.

It was important to study the hormonal profile in both groups with special emphasis on FSH, LH, prolactin, and estradiol, TSH. The mean FSH among cases (7.6 ± 0.9) while control group was (7.4 ± 1.0) . The mean LH among case (5.5 ± 1.2) while control was (5.2 ± 1.0) . The mean prolactin among case was (13.1 ± 4.8) while control was (12.6 ± 4.7) . The mean estradiol among case was (55.3 ± 9.8) while 55.6 ± 9.9). The mean TSH among case (1.9 ± 0.4) while control group (1.8 ± 0.4)

All hormonal profile has shown that there was no statistically significant between cases and control group in our study.

Against our study blacker *et al.*,^[16] it was found that serum FSH and LH , estradiol, prolactin and TSH was significant higher in cases compared to control group with *P value* of <0.001.

Our study found that there is association between vitamin D deficency and unexplained infertility compared to control group with *p-value*=0.003.

Triggianese *et al.*,^[17] found that vitamin D is lower in infertile (70 cases then control group (28) with *p*-value=0.03.

However vitamin D had significant low diagnostic performance in diagnosis of unexplained infertility with p-value <0.001.

Also our study found that vitamin D level has good prediction of unexplained infertility with Area Under Curve (AUC) = 0.719 (SE=0.043, 95cI 10.635-0.802,) with *p*-value<0.0001 and with Cut-off ≤ 13.0 .

This study showed Vitamin D level ≤ 13.0 mg/ml had moderate sensitivity, but low other diagnostic characteristics in diagnosis of unexplained infertility.

Our study indicated that 25(OH) vitamin-D was significant lower in unexplained infertility group than control group (mean vitamin D level in study group was 9.5 ± 4.5 while control group was 13.2 ± 4.8 and *P value* was <0.001 which was significant) but not sufficient to be diagnostic tool for unexplained infertility.

Raja Kumari *et al.*,^[18] study that studied the role of sunshine vitamin "D" sufficiency in male and female infertility partially in line with our study and found that women with sufficient levels of vitamin D show higher implantation rates, higher rates of clinical pregnancy in IVF and ET cycles. Its most important role in reproduction may be at the endometrial level.

Against our results, Butts *et al.*,^[19] study which studied poor ovarian stimulation outcome in PCOS or unexplained infertility are associated with vitamin D deficiency or not disagreed with us and found the association between vitamin D deficiency and diminished live birth relied on carrying the diagnosis of PCOS and was not observed in unexplained infertility. In PCOS, subjects with vitamin D deficiency [25(OH)D, 20 ng/mL or 50 nmol/L] were less likely to ovulate and experienced a 40% lower chance of live birth than those not deficient. In pregnant subjects from both studies, vitamin D deficiency was associated with elevated risk of early pregnancy loss.

Another study support our results, E Lerchbaum and Obermayer-Pietsch,^[20] study which assessed the association between vitamin D and fertility in women and men as well as in animals support our results and found that mice with vitamin D deficiency have significant gonadal insufficiency, decreased sperm count and motility, and histological abnormalities of testis, ovary and uterus. In PCOS women, low 25-hydroxyvitamin D (25(OH)D) levels are associated with obesity, metabolic, and endocrine disturbances and vitamin D supplementation might improve menstrual frequency and metabolic disturbances in those women. In men, vitamin D is positively associated with semen quality and androgen status. Moreover, vitamin D treatment might increase testosterone levels.

Monteiroa *et al.*,^[21] study which evaluated if vitamin D deficiency have a role in the reduced fertility supported our results and revealed a positive correlation between circulating vitamin D levels and semen quality parameters. Vitamin D supplementation is being considered to improve semen quality in at least some of the idiopathic cases of male infertility.

Voulgaris *et al.*,^[22] study which assessed Vitamin D and aspects of female fertility agreed with our results and strongly implied that vitamin D is implicated in female reproduction and might represent a beneficial and inexpensive therapeutic approach, in combination with first-line medical treatments, to female infertility.

Pagliardini *et al.*,^[23] study that found high prevalence of vitamin D deficiency in infertile women referring for assisted reproduction in line with our results and stated that serum 25(OH)D levels are highly deficient in women seeking medical help for couple's infertility. Levels are significantly associated with body composition, seasonal modifications and causes of infertility. Importantly, this deficiency status may last during pregnancy with more severe consequences.

Another study against our results, Abbas *et al.*,^[24] study which assessed the effect of vitamin D insufficiency treatment on fertility outcomes in frozen-thawed embryo transfer cycles did not show any significant difference between vitamin D insufficient and treated women in term of chemical or clinical pregnancy rates. Vitamin D insufficiency treatment is not associated with higher pregnancy rate in frozen-thawed embryo transfer cycles.

Finally, Yilmaz *et al.*,^[25] study that assess if serum vitamin D levels have any effect on intrauterine insemination success disagreed with us and found that there is no association between female infertility and serum vitamin D levels. Vitamin D does not predict pregnancy in infertile women undergoing IUI. Further research which will provide a comparison between much more women who have deficient and sufficient 25 (OH) D3 levels is warranted.

CONCLUSION

Vitamin-D deficiency had a role in unexplained infertility, nevertheless it is not a good diagnostic test. (Unexplained infertility is multifactorial) Vit-D is only one of many factors/ theories).

CONFLICT OF INTERESTS

There are no conflicts of interest.

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