Original Article

The Relation Between Vitamin D Level and Pregnancy Outcome in Women with Unexplained Infertility Undergoing Induction of Ovulation

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ABSTRACT

Background: Infertility is a unique medical condition because it involves a couple, rather than a single individual. It is defined as inability of a couple to conceive after 12 months of regular intercourse without use of contraception in women less than 35 years of age; and after six months of regular intercourse without use of contraception in women 35 years and older. Vitamin D has profound effects on many biological systems, including the reproductive system. The spectrum of Vitamin D target organs has expanded and the reproductive role of Vitamin D is highlighted by expression of the Vitamin D receptor (VDR) and enzymes that metabolise Vitamin D in testis, the male reproductive tract and human spermatozoa**.**

Aim: To assess the association between serum level of Vitamin D and pregnancy outcome in women with unexplained infertility undergoing an induction of ovulation.

Materials and Methods: This is a cohort study was conducted at Ain Shams University Maternity Hospital (infertility clinic) in a period of 6 months from June 2019 to November 2019. The population of this study was women with unexplained infertility in childbearing period, normal husband semen analysis, regular menstrual cycle, normal transvaginal ultrasonography, normal HSG and/or laparoscopy with chromotubation and normal hormonal profile for FSH, LH, Prolactin and thyroid gland.

Results: The vitamin D level was significantly higher in pregnant women than non pregnant. After adjustment for the effect of other variables, there was no statistically significant relation between vitamin D level and chemical pregnancy. On the other hand, secondary infertility was an independent predictor of chemical pregnancy. Our results showed that vitamin D level has limited predictive value with an area under the ROC curve (AUC) of 0.621. The best cutoff is a value of >32.5 ng/mL, which has a sensitivity of 28% and specificity of 95%. There is weak negative correlation between serum vitamin D and LH level. Lastly, after adjustment for the effect of other variables, adequate vitamin D and secondary infertility were an independent predictors of chemical pregnancy. There was significantly high rate of positive chemical pregnancy test among women had sufficient serum vitamin D when compared to those had deficient/ insufficient serum vitamin D.

Conclusion: There was no statistically significant relation between vitamin D level and pregnancy outcome in women with unexplained infertility undergoing induction of ovulation. Moreover, our results showed that vitamin D level has limited predictive value in women with unexplained infertility undergoing induction of ovulation, the best cutoff value was >32.5 ng/mL. After adjustment for the effect of other variables, adequate vitamin D was an independent predictors of chemical pregnancy.

Key Words: Induction of ovulation, unexplained infertility, vitamin D

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INTRODUCTION

Infertility is a unique medical condition because it involves a couple, rather than a single individual. It is defined as inability of a couple to conceive after 12 months of regular intercourse without use of contraception in women less than 35 years of age; and after six months of regular intercourse without use of contraception in women 35 years and older. For health young couples, the probability of getting pregnancy per a reproductive cycle is about 20-25%. Their cumulative probabilities of conception are 60% within the first 6 month, 84% within the first year, and 92% within the second year of regular fertility focused sexual activity[10].

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Primary infertility in couple is defined as the inability to conceive after one year of regular sexual intercourse without the use of any contraceptive method[14].

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. 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].

Vitamin D has profound effects on many biological systems, including the reproductive system[13]. The spectrum of Vitamin D target organs has expanded and the reproductive role of Vitamin D is highlighted by expression of the Vitamin D receptor (VDR) and enzymes that metabolise Vitamin D in testis, the male reproductive tract and human spermatozoa. The presence of Vitamin D receptor (VDR) in glandular epithelial cells of endometrium, granulosa cells, fallopian epithelial cells and cumulus oophorus cells of ovary has been confirmed[2].

Different studies have indicated that Vitamin D deficiency is directly responsible for a reduced fertility and reproduction capacity in female rat[13].

Some studies on the Vitamin D status of women undergoing controlled ovarian hyper-stimulation (COH) for Assisted Reproduction Technologies have been done. Results showed that Vitamin D deficiency is highly prevalent amonZg women undergoing COH, ranging from 21% to 31% across studies conducted in Western countries and reaching 75-99% in Iranian studies and showed that Vitamin D supplementation increase fertility and reproduction capacity. Women with higher level of Vitamin D in serum and follicular fluid are more likely to develop a pregnancy following in vitro fertilisation[11].

Literature review shows that Vitamin D is involved in many functions of the human reproductive system in both genders, but no complete analysis of the potential relationship between Vitamin D status and pregnancy rate is currently available.

AIM OF THE STUDY

This cohort study will assess the association between serum level of Vitamin D and pregnancy outcome in women with unexplained infertility undergoing an induction of ovulation.

PATIENTS AND METHODS

This is a cohort study was conducted at Ain-Shams University Maternity Hospital (infertility clinic) in a period of 6 months from June 2019 to November 2019.

One hundred and forty-three women with unexplained infertility were included in the study according to the following criteria: Women with unexplained infertility in childbearing period, normal husband semen analysis, regular menstrual cycle, normal transvaginal ultrasonography, normal HSG and/or laparoscopy with chromotubation and normal hormonal profile for FSH, LH, Prolactin and thyroid gland.

Women with (diminished ovarian reserve as denoted by a serum FSH > 10 mIU/ml or a serum AMH < 1 ng/ml, body mass index 35 kg/m2 or more, have clomiphene citrate (CC) resistance, had prior history of serious adverse effects with clomiphene citrate (CC), with expected decreased endometrial receptivity, with endocrinal disorders as hyperprolactinemia and thyroid dysfunction or chronic illness as active liver diseases and renal disease, with documented pelvic diseases as endometriosis, any ovarian pathology, hydro- or pyo-salpinx and uterine fibroids, previous history of ovarian drilling, consuming drugs interfering with Vitamin D metabolism and/or refused to participate were excluded from our study.

Ethical considerations: This study was done after approval of the ethical committee of the department of obstetrics and gynaecology, faculty of medicine, Ain Shams University. Informed consent was taken from all participants before recruitment in the study, and after explaining the purpose and procedures of the study. The investigator obtained the written, signed informed consent of each subject prior to performing any study specific procedures on the subject. The investigator retained the original signed informed consent form. All laboratory specimens, evaluation forms, reports, video recordings and other records that leave the site did not include unique personal to maintain subject confidentiality. The study was based on the investigator self-funding.

Methodology: This was cohort study of women with unexplained infertility who were underwent induction of ovulation at Ain-Shams University Maternity Hospital (infertility clinic). For All selected women, the following was done; detailed history, physical examination including general, abdominal and local examination, baseline pelvic ultrasound and baseline serum FSH, LH, E2, free testosterone, estradiol, TSH, free T3 and T4 and prolactin, HSG, Semen analysis to rule out male factor.

Sample collection and preparation : The Blood sample (5ml) were collected on the third of the 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. Levels of 25(OH)D will be determined by using 25-OH Vitamin D ELISA kit according to manufacturer's instructions. The serum 25(OH)D will be categorized according to clinically accepted ranges for Vitamin D deficiency (<20 ng/ml), insufficiency (20-30 ng/ml) and replete (>30 ng/ml). The kit name: (The Calbiotech, Inc. 25-hydroxy (25-OH) Vitamin D ELISA.

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). SA-HRP conjugate immunologically bound to the well progressively decrease as the concentration of vitamin D in the specimen increases. Unbound SA-HRP conjugate is then removed and the wells are washed. Next a solution of transient monocular blindness (TMB) reagent is added and incubated at room temperature for 30 minutes, resulting in the development of blue colour. The colour development is stopped with the addition of stop solution and the absorbance is measured spectrophotometrically at 450nm. A standard curve is obtained by plotting the concentration of the standard versus the absorbance. The colour intensity was inversely proportional to the amount of 25-OH vitamin D in the sample. The assay measures both the 25-OH vitamin D2 and vitamin D3. The total assay procedure run time is 2.5 hours.

Study procedure: On day 3 of spontaneous cycle, all patients had basal hormone profile, vitamin D level and transvaginal ultrasound. Clomiphene Citrate 50mg tablet was taken orally for 5 days, from cycle day 3 to cycle day 7. Transvaginal ultrasound folliculometry was done at ultrasound unit by expert doctor using (Samsung H 60) at 9th day of menstrual cycle, then follow up every other day until the mean diameter of the largest follicle reaches18mm, Triggering ovulation was made by intramuscular injection of 10,000IU human chorionic gonadotropin when the leading follicle reached ≥18mm diameter. Free sexual intercourse was encouraged from the day of hCG administration only in cases when no more than three follicles bigger than 17mm were observed; then, hCG injection did not administered and protected sexual intercourse was recommended in order to avoid high order multiple conception. The number and size of follicles were calculated, the follicle was identified and displayed in its maximum diameter, two dimensional measurements were made, and their mean was taken as the true follicle diameter, then the endometrial thickness in mm was measured in the sagittal view as the maximum thickness between the highly reflective interface of the endometrial-myometrial junction and the mean endometrial thickness was taken in day of hCG injection. When 2-3 follicles with 18-30 mm diameter were visible, an injection of hCG was performed. If no dominant follicle detected till day 15 of the cycle, transvaginal ultrasound was continued every other day till day 20 and the endometrial thickness was measured, if still no dominant follicle till that time, patient was considered as failed induction. Diagnosis of pregnancy was done by performing qualitative serum β hCG one week after missed period.

STATISTICAL ANALYSIS

Data were analysed using IBM© SPSS© Statistics version 23 (IBM© Corp., Armonk, NY) and MedCalc© version 18.2.1 (MedCalc© Software bvba, Ostend, Belgium). Continuous numerical variables were presented as mean and SD and inter-group differences were compared using the unpaired t-test. Categorical variables were presented as number and percentage and differences were compared using the Pearson chi-squared test. Ordinal data were compared using the chi-squared test for trend. Multivariable binary logistic regression analysis was used to examine the relation between vitamin D level (or status) and biochemical pregnancy as adjusted for age and type and duration of infertility. Two-sided *p-values <0.05* were considered statistically significant.

RESULTS

The mean age among study women was 33±7 years, the mean BMI 23.1±2.3 kg/m2, mean duration of infertility was 3±0.8 years and the largest proportion 62.2% of our study women had primary infertility (Table 1).

The means of FSH, LH level among study women were 6.3±3.3, 5.3±1.8 IU/ml respectively (Table 2). The mean duration of induction was 12±3 days, the mean diameter of largest follicle was 19±3 mm, the mean number of follicles was 3±1 and the mean endometrial thickness was 9.7±1.6 mm (Table 3).

This table shows that mean of vitamin D level was (19.6±10.2 ng/ml). 80 cases (55.9%) of women had deficient serum vitamin D. 30.8% had insufficient vitamin D level and 13.3% had adequate vitamin D level (Table 4). Tables 5 and 6 showed that positive chemical pregnancy test among study women was less than a third of cases, exactly 27.3%.

This table shows that vitamin D level was significantly higher in pregnant women than non-pregnant (Table 7). This table shows there was no statistically difference according vitamin D deficiency. On the other hand, there was statistical significant difference according to vitamin D status. There was significantly high rate of positive chemical pregnancy test among women had sufficient serum vitamin D when compared to those had deficient/ insufficient serum vitamin D.

Table and figure show that women with secondary infertility had significantly higher pregnancy rate (Table 8).

This table shows Vitamin D level has limited predictive value with an area under the ROC curve (AUC) of 0.621. The best cutoff is a value of >32.5 ng/mL, which has a sensitivity of 28% and specificity of 95% (Table 9).

This table shows there was no significant correlation between vitamin D level and age, BMI, duration of infertility, FSH, duration of induction, size of largest follicle, number of follicles and endometrial thickness while there was weak negative correlation regarding serum LH (Table 10).

Table 11A showed the results of multivariable binary logistic regression analysis for the relation between Vitamin D level and biochemical pregnancy as adjusted for age and type and duration of infertility. After adjustment for the effect of other variables, there was no statistically significant relation between vitamin D level and chemical pregnancy (*P-value = 0.140*). On the other hand, secondary infertility was an independent predictor of chemical pregnancy (odds ratio = 12.457, 95% CI = 4.942 to 31.399, *P-value <0.0001*).

Table 11B showed the results of multivariable binary logistic regression analysis for the relation between Vitamin D status and biochemical pregnancy as adjusted for age and type and duration of infertility. After adjustment for the effect of other variables, adequate vitamin D (odds ratio = 3.840, 95% CI = 1.068 to 13.810, *P-value = 0.039*) and secondary infertility (odds ratio = 13.339, 95% CI = 5.123 to 34.734, *P-value <0.0001*) were an independent predictors of chemical pregnancy.

Table 1: Demographic characteristics whole study women

Variable	Mean ± SD (range) / N (%)
Age (years)	33 ± 7 (19 - 46)
BMI (kg/m^2)	23.1 ± 2.3 (18.2 - 35.4)
Duration of infertility (years)	3 ± 0.8 (1 - 7)
Type of infertility	
1ry	89 (62.2%)
2ry	54 (37.8%)

Data are mean ± standard deviation (SD) and range or number (N) and percentage (%)

Table 2: Hormonal profile for study women

Variable	Mean ± SD (range)
FSH (IU/ml)	6.3 ± 3.3 (3.1 - 18.2)
LH (IU/ml)	5.3 ± 1.8 (1.3 - 9.7)

Data are mean ± standard deviation (SD) and range

Table 3: Ultrasound workup for study women and duration of induction

Variable	Mean ± SD (range)
Duration of induction (days)	12 ± 3 (8 - 14)
Size of largest follicle (mm)	19 ± 3 (2 - 24)
Number of follicles	3 ± 1 (1 - 7)
Endometrial thickness (mm)	9.7 ± 1.6 (8.0 - 18.0)

Data are mean ± standard deviation (SD) and range

Table 4: Vitamin D level and status among study women

Variable	Mean ± SD (range) / N (%)
Vitamin D level (ng/ml)	19.6 ± 10.2 (0.0 - 47.5)
Vitamin D status	
<20 ng/ml	80 (55.9%)
20-30 ng/ml	44 (30.8%)
>30 ng/ml	19 (13.3%)
Vitamin D deficiency	
No vitamin D deficiency (≥20 ng/ml)	63 (44.1%)
Vitamin D deficiency (<20 ng/ml)	80 (55.9%)

Data are mean ± standard deviation (SD) and range or number (N) and percentage (%)

Table 5: Pregnancy rate among study women

Variable	N (%)
Pregnancy test	
Negative	104 (72.7%)
Positive	39 (27.3%)

Data are number (N) and percentage (%)

Table 6: Comparison of women with positive or negative pregnancy test

	Negative pregnancy test (n=104)		Positive pregnancy test (n=39)		95% CI			
					Mean Difference	Lower	Upper	*P-value**
Variable	Mean	SD	Mean	SD				
Age (years)	32.9	7.0	31.9	6.6	0.9	-1.7	3.5	0.482
BMI (kg/m^2)	22.9	2.1	23.6	2.7	-0.8	-1.7	0.1	0.070
Duration of infertility (years)	8.3	5.2	8.4	4.7	0.0	-1.9	1.8	0.968
FSH (IU/ml)	6.2	3.1	6.7	2.7	-0.5	-1.7	0.6	0.336
LH (IU/ml)	5.4	1.8	5.0	1.5	0.4	-0.2	1.1	0.210
Duration of induction (days)	13.4	3.2	12.8	2.3	0.6	-0.5	1.7	0.303
Size of largest follicle (mm)	19.3	2.3	18.3	3.7	1.0	-0.3	2.3	0.117
Number of follicles	2.9	0.9	3.2	0.9	-0.2	-0.6	0.1	0.140
Endometrial thickness (mm)	9.7	1.3	9.6	2.3	0.1	-0.7	0.9	0.814
Vitamin D level (ng/ml)	18.3	9.5	23.0	11.2	-4.7	-8.4	-1.0	**0.014**

Data are mean and standard deviation (SD).
95% CI = 95% confidence interval.
*Unpaired t-test.

Table 7: Vitamin D status in women with positive or negative pregnancy test

	Negative pregnancy test (n=104)		Positive pregnancy test (n=39)			
Variable	n	%	n	%	χ^2, (df,1)	*P-value*
Vitamin D deficiency					3.321	0.068§
No vitamin D deficiency (≥20 ng/ml)	41	39.4%	22	56.4%		
Vitamin D deficiency (<20 ng/ml)	63	60.6%	17	43.6%		
Vitamin D status						
Deficiency (<20 ng/ml)	63	60.6%	17	43.6%	7.761	0.005*
Insufficiency (20-30 ng/ml)	33	31.7%	11	28.2%		
Adequate (>30 ng/ml)	8	7.7%	11	28.2%		

Data are number (n) and percentage (%). χ^2 = chi-squared statistic, df = degrees of freedom.*. Chi-squared test for trend
§. Pearson chi-squares test.

Table 8: Type of infertility in women with positive or negative pregnancy test

	Negative pregnancy test (n=104)		Positive pregnancy test (n=39)			
Variable	n	%	n	%	χ^2, (df,1)	*P-value*
Type of infertility					39.723	<0.001*
1ry	81	77.9%	8	20.5%		
2ry	23	22.1%	31	79.5%		

Data are number (n) and percentage (%). χ^2 = chi-squared statistic, df = degrees of freedom. *. Chi-squared test for trend
§. Pearson chi-squares test.

Table 9: Receiver-operating characteristic (ROC) curve analysis for prediction of positive chemical pregnancy

ROC metric	Value
AUC	0.621
SE	0.057
95% CI	0.536 to 0.700
Z	2.122
P-value	0.034
J-index	0.234
Cutoff criterion	>32.5
Sensitivity	28.21
95% CI	15.0 - 44.9
Specificity	95.19
95% CI	89.1 - 98.4
+LR	5.87
95% CI	2.2 - 15.8
-LR	0.75
95% CI	0.6 - 0.9
+PV	68.7
95% CI	45.0 - 85.6
-PV	78
95% CI	74.3 - 81.2

ROC = receiver-operating characteristic curve, AUC = area under ROC curve, SE = standard error, 95% CI = 95% confidence interval, Z = Z-statistic, J-index = ([sensitivity + specificity] - 1), +LR = positive likelihood ratio, -LR = negative likelihood ratio, +PV = positive predictive value, -PV = negative predictive value.

Table 10: Correlation between Vitamin D level and other numerical variables

	Vitamin D level	
Variable	Pearson r	*P-value*
Age	0.059	0.480
BMI	0.104	0.216
Duration of infertility	0.127	0.130

FSH	-0.091	0.278
LH	-.211*	0.011
Duration of induction	0.037	0.662
Size of largest follicle	0.060	0.475
Number of follicles	-0.046	0.589
Endometrial thickness	-0.021	0.802

*. Correlation is significant at the 0.05 level (2-tailed)

Table 11A: Multivariable binary logistic regression analysis for the relation between Vitamin D level and biochemical pregnancy as adjusted for age and type and duration of infertility

Variable	B	SE	Wald	*P-value*	Odds ratio	95% CI
Vitamin D level (ng/ml)	0.032	0.022	2.180	0.140	1.033	0.990 to 1.078
Age (yr)	-0.010	0.060	0.026	0.873	0.990	0.880 to 1.114
Duration of infertility (yr)	0.006	0.081	0.006	0.936	1.007	0.859 to 1.179
Secondary Infertility *	2.522	0.472	28.597	<0.0001	12.457	4.942 to 31.399
Constant	-2.675	1.568	2.910	0.088		

*. Reference category primary infertility

Table 11B: Multivariable binary logistic regression analysis for the relation between Vitamin D status and biochemical pregnancy as adjusted for age and type and duration of infertility

Variable	B	SE	Wald	*P-value*	Odds ratio	95% CI
Vitamin D status *						
Vitamin D insufficiency (20-30 ng/ml)	-0.297	0.525	0.319	0.572	0.743	0.266 to 2.080
Adequate Vitamin D (>30 ng/ml)	1.345	0.653	4.244	0.039	3.840	1.068 to 13.810
Age (yr)	-0.028	0.061	0.207	0.650	0.973	0.863 to 1.096
Duration of infertility (yr)	0.034	0.081	0.183	0.669	1.035	0.884 to 1.212
Secondary Infertility **	2.591	0.488	28.154	<0.0001	13.339	5.123 to 34.734
Constant	-1.822	1.527	1.424	0.233		

*. Reference category Vitamin D deficiency (<20 ng/ml).
**. Reference category primary infertility.

DISCUSSION

This prospective cohort study assessed the association between serum level of Vitamin D and pregnancy outcome in women with unexplained infertility undergoing an induction of ovulation. i.e. prediction of response to clomiphene citrate induction of ovulation for women with unexplained infertility.

For All selected women (143), the following was done. The serum level of 25 (OH) D3 and baseline hormone including (FSH, LH) were measured on the third day of the menstrual cycle when ovulation induction was started. Then Levels of 25(OH) D was determined by using 25-OH Vitamin D ELISA kit (Euroimmun, Luebeck, Germany) according to manufacturer's instructions. The serum 25(OH)D was categorized according to clinically accepted ranges for Vitamin D deficiency (<20 ng/ml), insufficiency (20–30 ng/ml) and replete (>30 ng/ml).

In the present study, we found that the mean age of our study women was 33±7 years as shown in Table 1 and there was no significant correlation between level of serum vitamin D and age as shown in Table 10.

This is consistent with[7], who found that mean age was 33 years, and disagree with[2] where the mean age was 29.8 also showed there was significant linear correlation between level of serum vitamin D.

In this study, the mean for BMI was 23±1 Kg/m2 as shown in Table 1, and there was no signification correlation between BMI and Vitamin D level as shown in Table 10.

This is supported by[3,2] who found that BMI did not have any correlation to vitamin D level. Against our result[5] evidenced a significant negative correlation between BMI and vitamin D level.

The mean duration of infertility in present study was 3±0.8 years as shown in Table 1, we found there was no significant correlation between duration of infertility and vitamin D level as shown in Table 10.

This was inconsistent with[6] who found that duration of infertility longer in vitamin D deficient group when compared to vitamin D insufficient and replete groups.

Our study stated that, 89 cases (62.2%) had primary infertility while 54 cases (37.8%) had secondary infertility as shown in Table 1.

Among studied women the mean of FSH and LH level were 6.3± 3.3, 5.3±1.8 IU/ML respectively as shown in Table 2, regarding FSH Level there was no significant correlation to vitamin D level, whereas LH Level had weak negative correlation as shown in Table 10.

This goes with[16] where it was no significant correlation between serum vitamin D level and FSH level was observed in study women, and doesn't go with[7] who showed that there was negative correlation between serum level of vitamin D and FSH level.

It was important to study ovarian stimulation parameters and endometrial thickness. The mean size of largest mature follicle was 19±3mm, the mean number of follicle was 3±1 and the mean endometrial thickness was 9.7±1.6mm as shown in Table 3. There were no significant correlation between ovarian stimulation parameters, endometrial thickness and vitamin D level as shown in Table 10.

The study of[13] corresponded with our results and stated that vitamin D deficiency was not correlated with ovarian stimulation parameters. On the other hand, suggesting its effect may be mediated through the endometrium.

However, other studies found different results[7] vitamin D has been associated with uterine respectively and embryonic implantation, which might also be relevant for the observed association with fecundability.

A randomized trial of vitamin D reported improved endometrial thickness in women who received vitamin D supplementation[1].

Vitamin D levels may predict ovarian response to ovarian stimulation. This suggestion is consistent with another study showing that vitamin D receptors exists in human ovaries and is important for sex steroid synthesis[15].

Our study showed that, 55.9% of women had deficient serum vitamin D, 30.8% had insufficient vitamin D level and 13.3% had adequate vitamin D level as shown in Table 4.

The mean of vitamin D level was 19±10.2 ng/ml, and pregnancy rate was 27.3% among studied women. The vitamin D level was significantly higher in pregnant women than non pregnant as shown in Table 6.

After adjustment for the effect of other variables, there was no statistically significant relation between vitamin D level and chemical pregnancy. On the other hand, secondary infertility was an independent predictor of chemical pregnancy, as shown in Table 11A.

In agreement with our study, [6]study stated that Vitamin D deficiency and insufficiency are common in women undergoing assisted reproductive treatments. The crude live birth rate achieved in women undergoing assisted reproductive treatments are associated with serum vitamin D, although statistical significance is lost when adjusting for important prognostic variables. Vitamin D deficiency could be an important condition to treat in women considering fertility treatment. A research trial to investigate the benefits of vitamin D deficiency treatment would test this hypothesis. The primary outcome was live birth. Secondary outcomes included biochemical pregnancy, clinical pregnancy and pregnancy loss rates. There is insignificant relationship between vitamin D level and biochemical pregnancy, clinical pregnancy and pregnancy loss rates and disagree with[11], the women with higher vitamin D level in the serum and follicular fluid are significantly more likely to achieve clinically pregnancy following IVF.

Regarding receiver-operating characteristic (ROC) curve analysis for prediction of positive chemical pregnancy using serum Vitamin D level. Vitamin D level has limited predictive value with an area under the ROC curve (AUC) of 0.621. The best cutoff is a value of >32.5 ng/mL, which has a sensitivity of 28% and specificity of 95%, as shown in Table 9.

Lastly, after adjustment for the effect of variables as shown in Table 11B adequate vitamin D and secondary infertility were an independent predictors of chemical pregnancy. There was significantly high rate of positive chemical pregnancy test among women had sufficient serum vitamin D when compared to those had deficient/ insufficient serum vitamin D, as shown in Table 7.

Nearly to the aim of our study, [12]study, which assessed relationship of vitamin D status with ovulation induction (OI) outcomes in women with polycystic ovary syndrome (PCOS), accepted with our study and stated that in women with PCOS, serum 25OHD was an independent predictor of measures of reproductive success following OI. On adjusted analyses, vitamin D status was an independent predictor of live birth and ovulation following OI.

[5]study, which evaluated the relationship between vitamin D deficiency and reproductive outcomes after ovarian stimulation in women with either polycystic ovary syndrome (PCOS) or unexplained infertility was opposite to our results and proved that given the generally modest success of ovarian stimulation, addressing vitamin D deficiency may prove an important treatment adjunct for many infertile women. In PPCOS II, subjects with vitamin D deficiency than those not deficient. In assessment of multiple intrauterine gestations from ovarian stimulation, no significant association between vitamin D deficiency and live birth was noted. In pregnant subjects from both studies, vitamin D deficiency was associated with elevated risk of early pregnancy loss.

A study by[13] found that a vitamin D deficiency and insufficiency is associated with lower pregnancy rates which is in concordance with this study.

Lastly, [3]study, which assessed the effect of serum vitamin D, level on endometrial thickness and parameters of follicle growth in infertile women undergoing induction of ovulation partially accepted with our study and stated that there was no correlation between the serum level of vitamin D and pregnancy rate. Vitamin D status was associated with endometrial thickness and number of antral follicles, but this study did not find a pivotal effect of serum vitamin D level on pregnancy rate. In this paper the results showed that a correlation exists between endometrial thickness as well as the number of antral follicles and replete level of vitamin D. Interestingly, the median level of these two parameters was of upmost level in the most replete tertile of serum Vitamin D level.

It appears that skin complexion, poor sun exposure, dietary habits (e.g. vegetarian), air pollution, clothing habits (especially among women), socio-economic factors such as lifestyle, older age, institutionalized or hospitalized persons and lack of the Vitamin D food fortification programme can explain the high prevalence of Vitamin D deficiency in the Middle East, despite their sunny climate[3].

Due to limited human data about the role of Vitamin D in reproductive physiology, and small sample sizes in existing research[8,9], including the present study, more powerful studies with a much larger sample size are needed to compare pregnancy and live birth rates with more realistic results.

CONCOLUSION

In present study, there was no statistically significant relation between vitamin D level and pregnancy outcome in women with unexplained infertility undergoing induction of ovulation. Moreover, our results showed that vitamin D level has limited predictive value in women with unexplained infertility undergoing induction of ovulation, the best cutoff value was >32.5 ng/mL. After adjustment for the effect of other variables, adequate vitamin D was independent predictors of chemical pregnancy.

CONFLICT OF INTEREST

There are no conflicts of interests.

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