Assessment of Vitamin D3 Levels in Cases of Unexplained Recurrent Pregnancy Loss in Assiut

Original Article

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ABSTRACT

Background: Pregnancy loss (PL) is a common adverse outcome of pregnancy that may be caused by multiple risk factors. Vitamin D deficiency (VDD) may be a possible risk factor for pregnancy loss.

Aim of the Work: To evaluate serum vitamin D status in women with unexplained recurrent pregnancy loss and to determine the potential risk factors inducing vitamin D deficiency among women in the child bearing period.

Methods: We conducted a matched case control study including 60 women with history of RPL, (with the last abortion of 6 months) and 60 women with normal pregnancy outcomes between August 2019 and August 2020. The data were collected by an interview questionnaire including baseline characteristics in addition to ultrasonography and blood samples for assessment of vitamin D level.

Results: There was no statistically significant difference in 25-OH vitamin D levels between cases and controls (11.61 ± 4.82 in cases versus 12.73 ± 6.68 in controls, P=0.297) or in 25-OH vitamin D status as (48.3% in both study groups had severe deficiency, 43.3% & 35.0 had moderate deficiency and only 8.3% & 16.7% had normal levels in cases and controls respectively). VDD was associated with breastfeeding, insufficient sun exposure, darker skin, and insufficient nutritional intake. There was no association between vitamin D levels and BMI.

Conclusion: The association between Vitamin D level and recurrent pregnancy loss could not be confirmed. Risk factors for vitamin D deficiency should be assessed in all pregnant women with RPL.

Key Words: Recurrent abortion, unexplained recurrent pregnancy loss, vitamin D deficiency.

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INTRODUCTION

Recurrent pregnancy loss (RPL) is known as the loss of two or more pregnancies, and it affects around 1-5% of reproductive women. Many factors may lead to RPL such as anatomical abnormalities, genetic factors, endocrine disorders, and/or infections[1]. It is suggested that vitamin D deficiency (VDD) may be a possible risk factor for pregnancy los[2,3]; this may be attributed to the presence of vitamin D nuclear receptors (VDR) in organs responsible for reproduction and infant growth such as the ovary, testis, mammary gland and placenta[4]. Vitamin D also has important effects in the regulation of cell proliferation and differentiation, and innate and adaptive immune responses modulation[5,6]. A normal immune response is essential to preserve the maternal–fetal association for a successful pregnancy. In contrast, autoimmunity and unorganized cellular immune reactions may be considered the immunological bases promoting recurrent pregnancy loss[7].

Maternal VDD is associated with several complications, including preterm labor, low birth weight, sporadic spontaneous abortion, gestational diabetes, pre-eclampsia and caesarean section[8,9]. However, the relationship between VDD and insufficiency in the first-trimester pregnancy with PL or a history of PL in non-gravid childbearing aged women with spontaneous pregnancy loss is less clear. Some studies found a positive association between VDD and RPL as reported by Andersen *et al* in a prospective cohort of 1683 pregnant woman. Another study by Hou **et al** reported that women with early pregnancy loss had significantly lower 25(OH)D levels (m=34.49 µg/l) than women with normal pregnancy (m=49.32 µg/l)[2].

AIM OF THE WORK

To evaluate serum vitamin D status in women with unexplained recurrent pregnancy loss and to determine the potential risk factors inducing vitamin D deficiency among women in the child bearing period.

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CASES AND METHODS

Type of study: Matched case control study.

Study population

60 women with history of RPL in last 2 pregnancies and last abortion of 6 months at El-mabara and Assuit university hospitals and another 60 with normal pregnancy outcome as control group.

Inclusion criteria

1. Age of women 20:35 years old, with history of regular menstrual cycles and without history of hormonal or vitamin D supplementation in last 3 months.
2. History of unexplained recurrent pregnancy loss (defined as two or more consecutive missed miscarriage before 14 weeks of gestation).
3. The inclusion criteria For selection controls include women within the same age, during the same gestational age in pregnant cases and without current or even previous history of abortion.

Exclusion criteria

1. History of uterine anomalies.
2. History of DM on treatment.
3. History of SLE and Rheumatoid arthritis.
4. Thyroid dysfunction (hyper thyroid and hypothyroid patient).
5. Antiphospholipid antibody syndrome.

All enrolled patients were subjected to:

1=Full history and clinical examination.

Obstetrics and Gynecological history as gestational age parity and history of previous abortion and possible risk factors for recurrent abortion, and factors affecting vitamin D level as sun exposure, skin color, sun protection use, dietary intake and physical activity.

Assessment of vitamin D intake: ask about intake of milk (fresh and fortified) cups/day, eggs numbers /day, canned food (salmon, tuna, and sardines), liver, beef, chicken.

Assessment of Sun Exposure: Ask Participants about time, exposed part of skin (both limbs) to sun/day in the last three months.

Assessment of skin color: The Fitzpatrick skin type (or photo-type) depends on the amount of melanin pigment in the skin. This is determined by constitutional color (white, brown or black skin). We examined skin of face and ask about reaction to sun.

2=Anthropometric measurements

Body weight, height and body mass Index (BMI) were calculated

3=Laboratory tests

Including complete blood picture, Rhesus factor , kidney functions liver functions, thyroid function, HbA1c, Lupus anticoagulant, Anticardiolipin antibodies.

Measuring serum 25(OH)D3 (using VIDAS by ELFA method).

It is an automated quantitative enzyme immunoassay for use on the instrument of VIDAS family, using the ELFA (Enzyme Linked Fluorescent Assay) ,using enzyme immunoassay (EIA kit).KT815 with ELISA SystemAS1851Das; Italy (reader) and 16041412BioTek; USA (washer). Vitamin D was considered to be deficient if serum level was ≤20 ng/dl.

4= Ultrasonography assessment was performed to detect uterine anomalies or masses, ovarian masses or cyst, or any other pelvic masses

Outcome measure: primary outcome is assessment of Vit D3 level in cases of unexplained recurrent pregnancy loss.

Ethical consideration

The study was approved by the ethical committee of the Faculty of Medicine, Assiut University. Privacy and confidentiality of all data were assured and informed consent was obtained from all patients.

Statistical analysis

Statistical analyses were done using IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY. The qualitative data were presented as frequencies and percentages. The quantitative numeric variables were presented as mean, standard deviations and ranges. Pearson Chi square- test (X^2) for independence was used for qualitative data, and Fishers Exact test was used for cells less than 5. The

Student's independent t-test was used for the differences between means of two continuous variables of unpaired groups. The significance level was taken at 0.05 with a 5% confidence limit. The results were deemed to be statistically significant if the *p-value* (two tallied) was ≤ 0.05. Odds ratios (OR) was the preferred measure of association.

RESULTS

The baseline characteristics of the two groups showed no significant differences as shown in (Table 1).

Using independent samples t-test : There was no significant difference between the two groups regarding 25-OH Vitamin D levels (11.61 ± 4.82 in cases versus 12.73 ± 6.68 in controls, P=0.297). Similarly, there was no significant difference between the two groups regarding 25-OH Vitamin D status (severe deficiency: 48.3% in both groups; moderate deficiency: 43.3% in cases and 35% in controls; normal levels: 8.3% in cases and 6.7% in controls) as shown in (Table 2).

25-OH vitamin D was significantly higher in the RPL group participants who had daily sun exposure (13.11 ± 4.94) than those who weren't exposed to sun light (10.91 ± 4.66) as p=0.014. As general serum level of 25-OH vitamin D significantly related to sun light exposure as shown in (Table 3).

In the RPL group, 80% of the participants had medium skin color and 10% had either light or dark skin. However, in the control group, 85% had dark skin, 8.3% had medium colored skin and 6.7% had light skin without significant difference between the RPL and the control groups. In the RPL group, 25-OH vitamin D level was significantly higher in patients with light skin (15.77 ± 6.41) than medium skin color (11.38 ± 4.22) or dark skin (9.33 ± 7.70) as p=0.021. Generally, the serum level of 25-OH vitamin D was significantly more common with light skin as shown in (Table 4).

Patients with sufficient nutritional intake (16.85 ± 5.45) had significantly higher levels of 25-OH vitamin D than patients with insufficient intake (11.04 ± 4.45) p=0.017 (Table 5).

Table 1: Demographic data of the studied groups:-

Personal data	Study group (n= 60)	Control group (n= 60)	*P-value*
Age: (years)			
Mean ± SD	30.08 ± 3.78	28.77 ± 3.81	0.060
Range	19.0-35.0	18.0-34.0	NS
Level of education:			
Bachelor	27 (45.0%)	30 (50.0%)	0.583
Technical Secondary School	33 (55.0%)	30 (50.0%)	NS
Occupation:			
Housewife	11 (18.3%)	19 (31.7%)	0.092
Employee	49 (81.7%)	41 (68.3%)	NS
Residence:			
Rural	34 (56.7%)	37 (61.7%)	0.577
Urban	26 (43.3%)	23 (38.3%)	NS
BMI:(Kg/m2)			
Mean ± SD	21.28 ± 1.59	21.93 ± 2.43	0.084
Range	18.5-24.0	19.0-32.0	NS

Independent samples t-test(age ,BMI) Chi-square test SD=stander deviation NS: not significant

Table 2: 25-OH Vitamin D of the studied groups

25-OH Vitamin D (ng/dl)	Study group(n= 60)		Control group (n= 60)		*P-value*
	No.	%	No.	%	
Severe deficiency	29	48.3	29	48.3	
Moderate deficiency	26	43.3	21	35.0	0.333
Normal	5	8.3	10	16.7	
Mean ± SD	11.61 ± 4.82		12.73 ± 6.68		0.297
Range	4.2-25.0		7.0-33.7		

Table 3: Relation between 25-OH Vitamin D and Exposure to sun:

	Exposure to sun	25-OH Vitamin D		*P-value*
		Mean ± SD	Range	
Study	Yes	13.11 ± 4.94	8.2 - 25.0	0.014* S
	No	10.91 ± 4.66	4.2 - 20.0	
Control	Yes	13.76 ± 6.41	10.0 - 33.7	0.043* S
	No	10.65 ± 3.39	7.0 - 23.7	
Total	Yes	13.46 ± 5.75	8.2 - 33.7	0.010* S
	No	10.86 ± 5.98	4.2 - 23.7	

Table 4: Relation between 25-OH Vitamin D and Skin color

	Skin color	25-OH Vitamin D		*P-value*
		Mean ± SD	Range	
Study	Light	15.77 ± 6.41	8.1 - 25.0	0.021*
	Medium	11.38 ± 4.22	4.2 - 22.0	
	Dark	9.33 ± 7.70	4.2 - 20.0	
Control	Light	13.22 ± 7.04	7.0 - 33.7	0.027*
	Medium	10.12 ± 3.52	7.0 - 15.3	
	Dark	8.75 ± 2.56	8.1 - 13.5	
Total	Light	14.16 ± 5.41	7.0 - 33.7	0.030*
	Medium	10.82 ± 5.89	4.2 - 22.0	
	Dark	9.03 ± 5.91	4.2 - 20.0	

Table 5: Relation between 25-OH Vitamin D and Nutritional status:

	Nutritional status	25-OH Vitamin D		*P-value*
		Mean ± SD	Range	
Study	Sufficient	16.85 ± 5.45	8.1 - 22.0	0.017* S
	In-sufficient	11.04 ± 4.45	4.2 - 20.0	
Control	Sufficient	19.16 ± 8.53	8.1 - 33.7	0.000* S
	In-sufficient	10.39 ± 3.84	7.0 - 23.8	
Total	Sufficient	15.74 ± 7.62	8.1 - 33.7	0.000* S
	In-sufficient	10.70 ± 4.13	4.2 - 25.0	

DISCUSSION

In our study, we found no statistically significant difference in 25-OH vitamin D level (11.61 ± 4.82 in the RPL group versus12.73 ± 6.68 in controls, P=0.297). Similarly, there was no significant difference between the two groups regarding 25-OH vitamin D status (severe deficiency: 48.3% in both groups; moderate deficiency: 43.3% in cases and 35% in controls; normal vitamin D levels: 8.3% in cases and 6.7% in controls). Thus the association between vitamin D level and recurrent pregnancy loss could not be confirmed. This may be partially attributed to the prevalence of vitamin D deficiency in Egypt regardless of RPL[13,14]. Thus a lower cutoff value for vitamin D levels may yield different results.

Several researchers have reported similar results[10,11]. In a 2017 systematic review and meta-analysis, Amegah *et al*., found that vitamin D deficiency was not related to increased risk of spontaneous abortion despite its association with preterm birth[12]. Furthermore, in an Australian nested case-control study by Schneuer *et al*. that involved 5109 women in the first trimester of pregnancy, the researchers did not observe a relation between 25(OH) D concentrations and low 25(OH)D levels[15].

In disagreement with the current results, Andersen *et al*. reported an association between low maternal serum concentrations of serum 25(OH)D and the risk of consequent miscarriage in a prospective cohort of 1683 pregnant woman (<22 weeks) (3). Moreover, in a crossectional study, Hou *et al*. found that women with early pregnancy loss had significantly lower 25(OH)D levels (m=34.49 µg/l) than women with normal pregnancy (m=49.32 µg/l)[2].

This inconsistency in results may be caused by differences between our study and others regarding the

study population and methodology, including the difference in maternal age and gestational age of the participants.

In current study, more women in the control group had daily sun exposure than women in the RPL group. However, the difference was not statistically significant. Vitamin D levels were also significantly greater in women in the RPL group who had daily sun exposure than those who did not have sufficient daily sunlight exposure. Moreover, the serum level of 25-OH vitamin D was significantly associated with sun light exposure. Therefore, ensuring sufficient sunlight exposure is essential to avoid VDD[16].

Our results were consistent with other studies as in the cross-sectional study by Song *et al*., in which deficient Vitamin D levels were more common with insufficient sun exposure ($r = 0.332$, $P < 0{\cdot}001$). The percentage of severe vitamin D deficiency was much higher in women with shorter duration of sun exposure (≤ 0.5 h/day; 58.3%) than that of women with longer duration of sun exposure (> 0.5 h/day; 36.4%.[17]. Another Australian crossectional study reported that skin exposure was a significant predictor of vitamin D[18].

In the current study, in the RPL group, 25-OH Vitamin D levels were significantly higher in women with light skin (15.77 ± 6.41) than medium skin (11.38 ± 4.22) and dark skin (9.33 ± 7.70) as p=0.021. The serum level of 25-OH Vitamin D significantly related to light skin.

Similarly, Libon *et al*., found that skin pigmentation negatively affects vitamin D production as Vitamin D levels were significantly higher in fair skinned individuals compared to black-skinned individuals after ultraviolet B exposure in the two groups[19].

Moreover, according to many studies, circulating vitamin D concentrations differ by skin color: Individuals with darker skin produce less vitamin D with the same amount of sunlight exposure than individuals with lighter skin color. Dark-skinned individuals produce less 25(OH) D than individuals with light skin with the same sunlight exposure (UVB)[20].

In addition, Richard *et al*., found that vitamin D deficiency was more common in women with dark skin color with a prevalence almost double the normal population[21].

In our study, 25-OH Vitamin D was significantly higher among women with sufficient nutritional intake than women with insufficient intake. This is attributed to the importance of adequate nutrition as one of the two main sources of vitamin D in addition to endogenous skin synthesis through ultraviolet radiation. However, food sources of vitamin D are limited (i.e., seafood, eggs, milk and dairy products, meats, and mushrooms), and thus, vitamin D is synthesized in the body primarily through ultraviolet exposure[22].

Several studies have reported the importance of dietary 25(OH)D intake in food for adequate serum levels of vitamin D[23,24]. Black *et al*, described how an intake of 1 µg/day of vitamin D-rich foods could increase serum 25(OH)D levels by 1.2 nmol/L[25].Thus, it is essential to increase the consumption of vitamin D-rich foods in order to sustain optimum serum 25(OH)D concentrations[26].

CONCLUSION

In conclusion, we found no statistically significant difference in 25-OH vitamin D level or status (normal or deficient) between women with RPL compared to controls. Thus the association between vitamin D level and recurrent pregnancy loss could not be confirmed. With regards to risk factors in pregnant women with RPL, VDD was associated with breastfeeding, insufficient sun exposure, darker skin, and insufficient nutritional intake. There was no association between vitamin D levels and BMI. We advise clinicians to assess these risk factors in all pregnant females with RPL.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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