Maternal Vitamin D level and Early Pregnancy Loss A Nested Case Control Study

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Abstract

Objective: To assess the role of vitamin D deficiency and early pregnancy loss.

Patient and Method: A nested case control study conducted in outpatient antenatal care clinic under supervision of Ain Shams University Maternity hospital from the period of March 2020 to June 2020. Pregnant ladies in the first trimester were screened for eligibility criteria.Blood samples were taken from the participants at the time of presentation. All participants were followed till the end of the first trimester to report cases of miscarriage. Vitamin d was assessed for the 40 women who suffered from miscarriage (cases) and for 40 selected controls.The primary outcome was the relation between vitamin d level and early pregnancy loss. Secondary outcome was the relation between vitamin d deficiency and obesity.

Results: Our findings showed that the miscarriage groupwas significantly olderthan control group as $p<0.001$ and BMI was significantly higher as $p<0.007$.The mean value of 25(OH)D was significantly lower among miscarriage group (21.0±8.5) than control group (26.5±8.3) as $p=0.005$. And the majority of miscarriage group (42.5%) had 25(OH)D deficiency while (40.0%)&(17.5%) of cases had either 25(OH)D insufficiencyor sufficiency which significantly different than control group ($p=0.049$).25(OH)D ≤24.5 (ng/mL) was a significant factor that increased the likelihood of first-trimestric miscarriage with sensitivity 80%.No significant differences according to BMI grades regarding 25(OH)D grades.

Conclusion: Vitamin d deficiency is one of modifiable risk factors for first trimestric abortion. . Preconceptional vitamin D supplementation is an easy method for decreasing incidence of early pregnancy loss.

Keywords: vitamin D deficiency, early pregnancy loss, risk factors for miscarriage.

INTRODUCTION

Miscarriage is one of the most common pregnancy complications. Its prevalence ranges from 12 to 20%. Several risk factors are implemented in miscarriage including both genetic and acquired factors. Early detection and modification of these acquired risk factors is necessary. [1]

Pregnancy is considered as a challenge for the immune system. The human foetus is a semi-allograft, maternal immune tolerance is crucial for its survival. In the first trimester invasion of foetalcytotropho-

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blast into the maternal decidua and myometrium is crucial in placentation process and maintenance of pregnancy. [2]

Modification of maternal immune response in pregnancy is vital for normal pregnancy. It is believed that vitamin d has a significant role in its modification. Both vitamin D deficiency and insufficiency are very common among pregnant women. Several adverse pregnancy outcomes are associated with vitamin d deficiency such as Preeclampsia, gestational diabetes, and recurrent miscarriage, which are characterized by an exaggerated immune response. [3]

In addition to its well-known role in regulating calcium metabolism and maintain bone health, there is an increasing interest towards vitamin d immunomodulatory function. It has shown that vitamin d is synthesized by the tissues of the female reproductive system and to affect the reproductive events such as fertlisation and implantation by modulating the immune system function. Important constituents for vitamin d synthesis and activation are expressed in the endometrium, dicedua, placenta and ovaries. Low vitamin d level is associated with repeated failure of implantation, pregnancy loss and some obstetric complications such as preeclampsia and gestational diabetes.[4]

Up to 90% of daily requirement of vitamin D is absorbed through the skin via sunlight with minimal contribution from food. It is essentially required for everyone especially pregnant women to stay for 20 minutes in sunlight daily with more than 40 percent of skin exposed in order to avoid vitamin d deficiency.[5]

I t was suggested by some studies that obesity is accompanied with vitamin d defeceincy. As vitamin dis a fat soluble vitamin, it is accumulated in fat cells leading to decrease in its serum blood levels in obese persons.[6]

Although Egypt is a country with abundant sunshine all year round, majority of pregnant females had vitamin D deficiency and insufficiency. UnfortunatelyVitamin D deficiency is associated with many adverse effects in pregnancy.This nested case control study aimed to assess the role of vitamin D deficiency and early pregnancy loss.

PATIENTS AND METHODS

Study Design: A nested case control study.

Study setting: Outpatient clinics antenatal care clinicunder supervision of Ain Shams University Maternity hospital.

Study time: From March 2020 to June 2020

Study population: Pregnant ladies in the first trimester seeking antenatal care.

Inclusion criteria: Singleton pregnancy, spontaneous pregnancy and gestational age between (7-10 weeks).

Exclusion criteria: Ectopic pregnancy, molar pregnancy, thyroid disorders ,autoimmune disorders ,uterine malformation ,severe liver and kidney diseases ,diabetes mellitus,Pregnancy with assisted reproductive techniques,women consuming drugs interfering with Vitamin D metabolism and women who refused to participate.

Sample size: This study is conducted on 80 women.

Sample Justification: Sample size was calculated using the online PASS, setting the power at 0.80 and the type-1 error at 0.05. data from previous study byAndersen et al(7),showed that serum Vitamin D levels in women with miscarriage and women without miscarriage were 55.6 nmol/L, 66.0 nmol/L, respectively. Calculation according to these values produces a minimal sample size of 33 women.

Ethical Considerations: This study was done after approval of the ethical committee of the department of obstetrics and gynecology, faculty of medicine, Ain Shams University.

Study interventions and procedures:

All participating women were subjected to the following:

A) Detailed history (personal, present, obstetric, menstrual, family, medical and surgical).

B) Examination (general, abdominal and local).

C) Body mass index (BMI) calculation (weight/ height kg/ cm^2).

Gestational age was measured by first day of the last menstrual period and confirmed with ultrasound. Blood samples were taken from the participants. Venous samples were withdrawn from pregnant women at the time of presentation in 1st trimester and stored after centrifugation and serum separation.All participants were followed till the end of the first trimester to report the number of females who suffered miscarriage. After a number of 40 women with unexplained miscarriage have reached, recruitment was stopped. A control group of 40 women with ongoing pregnancy who passed the 13th week of pregnancy were selected by simple random method as a control group. Vitamin d was assessed for the 40 women who suffered miscarriage (cases) and for 40 selected controls.

Vitamin D status was determined by measuring serum levels of 25(OH) D (25 hydroxy vitamin d) using an enzyme immunoassay. Levels of 25(OH) D were measured by using 25-OH Vitamin D ELISA kit (Euroimmun, Luebeck, Germany) according to manufacturer's instructions. The serum vitamin d level is categorized depending on clinically accepted ranges for Vitamin D into : deficiency (<20 ng/ml), insufficiency (20–30 ng/ml) and replete (>30 ng/ml).

Outcomes: 1ry outcome: The relationship between vitamin d level and early pregnancy loss. **2ry outcome:** The relation between vitamin d deficiency and obesity.

Statistical Analysis:

The data was labelled, tabulated, and statistically analyzed using IBM SPSS statistics (Statistical Package for Social Sciences) software version 22.0, IBM Corp., Chicago, USA, 2013 and Microsoft Office Excel 2007. Descriptive statistics was done for quantitative data as minimum& maximum of the range as well as mean±SD (standard deviation) for quantitative normally distributed data, median and first& third inter-quartile range for quantitative non-normally distributed data, while it was done for qualitative data as number and percentage. Inferential analyses was done for quantitative variables using Shapiro-Wilk test for normality testing, independent t-test in cases of two independent groups with normally distributed data, Mann whiteny U in cases of two independent groups with non-normally distributed data, ANOVA test and Kruskal Wallis test. In qualitative data, inferential analyses for independent variables was done using Chi square test for differences between proportions and Fisher's Exact test for variables with small expected numbers. While correlations was done using Pearson correlation for numerical normally distributed data, and using spearman rho test for numerical non normally distributed and qualitative data. ROC curve was used to evaluate the performance of different tests differentiate between certain groups. Logistic regression model was used to find out independent factors affecting certain conditions. The level of significance was taken at P value < 0.050 is significant, otherwise is non-significant.

RESULTS

After excluding 30 ineligible cases, it was needed to recruit 298 cases to collect 40 (13.4%) cases with first-trimestric miscarriage. From the 258 cases that passed first trimester without miscarriage 40 cases were randomly included as a control group.

Table (1): Comparison between the studied groups regarding maternal age.

Variables		Miscarriage (N=40)	No Miscarriage (N=40)	P-value
Age (years)	Mean±SD	32.0±6.5	26.9±5.4	^<0.001*
	Range	20.0–46.0	17.0–37.0	
	Range	7.0–10.0	7.0–10.0	

^Independent t-test. *Significant

Table (1) shows that: Age was significantly higher among miscarriage group.

Table (2): Comparison between the studied groups regarding gravidity and parity.

Variables		Miscarriage (N=40)	No Miscarriage (N=40)	P-value
Gravidity	Median (1st–3rd IQ)	3.0 (2.0–4.0)	3.0 (2.0–3.8)	#0.363
	Range	1.0–5.0	1.0–6.0	
Parity	Median (1st–3rd IQ)	2.0 (1.0–2.0)	1.0 (1.0–2.0)	#0.541
	Range	0.0–4.0	0.0–3.0	
	Range	0.0–3.0	0.0–3.0	

IQ: Interquartiles. #Mann Whitney test

Table (2) shows that: No significant differences between the studied groups regarding parity and gravidity.

Table (3): Comparison between the studied groups regarding body mass index (BMI)

Variables		Abortion (N=40)	No abortion (N=40)	P-value
BMI (kg/m^2)	Mean±SD	28.0±4.5	25.4±3.8	^ 0.007*
	Range	21.9–39.8	18.0–33.6	
Grade	Normal	12 (30.0%)	23 (57.5%)	# 0.045*
	Over weight	16 (40.0%)	9 (22.5%)	
	Obese	12 (30.0%)	8 (20.0%)	

^Independent t-test. #Chi square test. *Significant

Table (3) shows that: BMI was significantly higher among miscarriage group. Overweight and obese grades were significantly more frequent amongmiscarriage group.

Table (4): Comparison between the studied groups regarding 25(OH)D

Variables		Miscarriage (N=40)	No Miscarriage (N=40)	P-value
Level (ng/mL)	Mean±SD	21.0±8.5	26.5±8.3	^ **0.005***
	Range	7.0–38.4	11.2–46.1	
Grade	Sufficiency	7 (17.5%)	16 (40.0%)	# **0.049***
	Insufficieny	16 (40.0%)	15 (37.5%)	
	Defficiency	17 (42.5%)	9 (22.5%)	

^Independent t-test. *Significant

Table (4) show that: 25(OH)D was significantly lower among miscarriage group

Table (5): Comparison according to 25(OH)D grades regarding BMI grades.

Variables		Normal	Over weight	Obese	p-value
Miscarriage group					
Number		12	16	12	
25(OH)D (ng/mL)		22.3±8.9	21.9±9.1	18.6±7.5	^0.513
25(OH)D grade	**Sufficiency**	3 (25.0%)	3 (18.8%)	1 (8.3%)	#0.874
	Insufficieny	4 (33.3%)	6 (37.5%)	6 (50.0%)	
	Deficiency	5 (41.7%)	7 (43.8%)	5 (41.7%)	
Control group					
Number		23	9	8	
25(OH)D (ng/mL)		27.2±8.2	26.4±10.6	24.2±6.0	^0.678
25(OH)D grade	**Sufficiency**	12 (52.2%)	3 (33.3%)	1 (12.5%)	#0.298
	Insufficieny	6 (26.1%)	4 (44.4%)	5 (62.5%)	
	Defficiency	5 (21.7%)	2 (22.2%)	2 (25.0%)	

^ANOVA test. #Fisher's Exact test.

Table (5) shows that: No significant differences according to BMI grades regarding 25(OH)D grades

Table (6): Diagnostic performance of age, BMI and 25(OH)D in predictingmiscarriage

Factors	AUC	SE	P-value	95% CI	Cut off
Age	0.712	0.057	0.001	0.600–0.823	≥32.0
BMI	0.647	0.062	0.024	0.525–0.768	≥26.0
25(OH)D	0.682	0.061	0.005	0.562–0.801	≤24.5

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

Table (6) and figure (1): Age, BMI and 25(OH)D had significant moderate diagnostic performance in predicting miscarriage

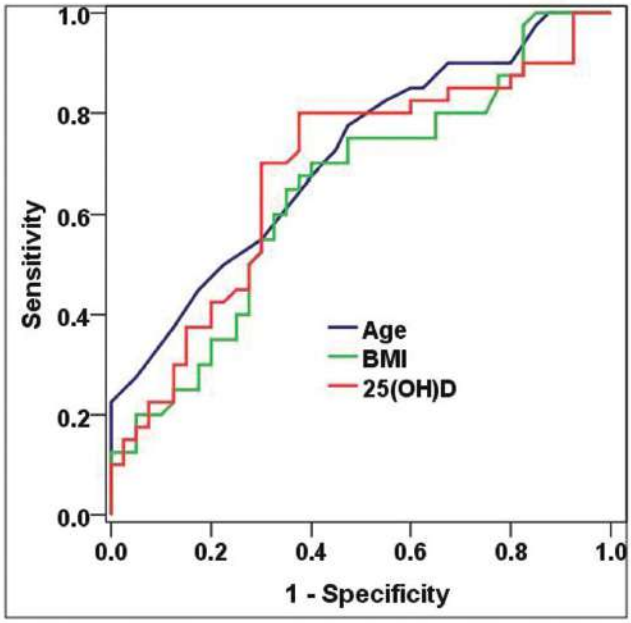

Figure (1): ROC curve for age, BMI and 25(OH)D in predicting miscarriage

Table (7): Diagnostic charactersitcis of age, BMI and 25(OH)D cutoff points in predicting miscarriage.

Characters	Age ≥32.0 (years)		BMI ≥26.0 (kg/m2)		25(OH)D ≤24.5 (ng/mL)	
	Value	95% CI	Value	95% CI	Value	95% CI
Sensitivity	50.0%	33.8%–66.2%	65.0%	48.3%–79.4%	80.0%	64.4%–90.9%
Specificity	77.5%	61.5%–89.2%	65.0%	48.3%–79.4%	60.0%	43.3%–75.1%
DA	63.8%	52.2%–74.2%	65.0%	53.5%–75.3%	70.0%	58.7%–79.7%
YI	27.5%	7.3%–47.7%	30.0%	9.1%–50.9%	40.0%	20.4%–59.6%
PPV	69.0%	49.2%–84.7%	65.0%	48.3%–79.4%	66.7%	51.6%–79.6%
NPV	60.8%	46.1%–74.2%	65.0%	48.3%–79.4%	75.0%	56.6%–88.5%
LR+	2.22	1.16–4.27	1.86	1.15–3.00	2.00	1.33–3.01
LR-	0.65	0.45–0.92	0.54	0.33–0.87	0.33	0.17–0.65
LR	3.44	1.31–9.06	3.45	1.38–8.64	6.00	2.21–16.31

CI: Confidence interval, DA: Diagnostic accuracy, YI: Youden's index, PPV: Positive Predictive value, NPV: Negative Predictive value, LR+: Positive likelihood ratio, LR-: Negative likelihood ratio, LR: Diagnostic odd ratio

Table (7) show that: Age ≥32.0 (years), BMI ≥26.0 (kg/m2) and 25(OH)D ≤24.5 (ng/mL) had moderate diagnostic charactersitcis in predicting miscarriage

Table (8): Logistic regression for factors affecting first-trimestric miscarriage

Factors	B	SE	P-value	OR (95% CI)
Age ≥32.0 (years)	1.25	0.56	**0.026***	3.49 (1.16–10.53)
BMI ≥26.0(kg/m2)	1.11	0.53	**0.035***	3.04 (1.08–8.54)
25(OH)D ≤24.5 (ng/mL)	1.80	0.56	**0.001***	6.05 (2.04–17.98)
Constant	-2.10	0.58	**<0.001***	

β: Regression coefficient. SE: Standard error. OR; Odds ratio. CI: Confidence interval. *significant

Table (8) shows that: Age ≥32.0 (years), BMI ≥26.0 (kg/m2) and 25(OH)D ≤24.5 (ng/mL) were significant factors that increased the likelihood of first-trimestricmiscarriage .

DISCUSSION

Most pregnancy loss cases occur early in pregnancy before the end of the first trimester and pathophysiology is therefore sufficient to be of great importance. Multiple etiologies, such as chromosomal anomalies, infections, hormonal abnormalities, uterine malformation, autoimmune diseases and coagulopathies, were identified for the pathogenesis of repeated pregnancy loss, while about half of pregnancy loss cases still have no identifiable etiology.[8]

Vitamin d is thought to make important immunological modifications that help maintaining successful pregnancy. Several studies found that vitamin d defeceincy is associated with unfavorable pregnancy outcomes.[7]

Despite the extensive use of vitamins prior to pregnancy, vitamin D deficiency is prevelant among pregnant women. Low serum Vitamin Dlevel during pregnancy has been associated with adverse pregnancy effects such as gestational diabetes, preeclampsia, and foetal growth restriction.[9]

This nested case control study aimed to assess the role of vitamin D deficiency and early pregnancy loss in the first trimester. From the 258 cases that passed first trimester without abortion, 40 cases were randomly included as a control group.

There was no significant differences between the studied groups regarding gestational age at enrollment, parity, gravidity (P= 0.245, 0.363, 0.541& respectively).

Our findings showed that the mean value of 25(OH) D was significantly lower among miscarriage group (21.0±8.5) than control group (26.5±8.3) as p=0.005. The majority of miscarriage group (42.5%) had 25(OH) D deficiency while (40.0%) & (17.5%) of cases had either 25(OH) D insufficiency or sufficiency which significantly different than control group (p=0.049).25(OH) D ≤24.5 (ng/mL) was a significant factor that increased the likelihood of first-trimestricmiscarriage with sensitivity 80%.No significant differences according to BMI grades regarding 25(OH)D grades.

The present study is in harmony with a cohort study byAndersen et al.,performed on 1683 pregnant women before 22 weeksto whom serum 25-hydroxyvitamin D [25(OH)D] withdrawn. They reported that the miscarriage group had lower maternal serum concentration of 25(OH)D with an increased risk of miscarriage in the first but not in the second.[7]

Our findings agreed with ***Gonçalves et al***., who systematically reviewed 11 articles investigating relationship between recurrent miscarriage and vitamin d. This systematic review showed high incidence of both vitamin D insufficiency (VDI) or vitamin D deficiency (VDD) in cases of recurrent pregnancy loss (RPL).[10]

Another cross-sectional study by, ***Hou et al.***, found that pregnancy loss was significantly association with low vitamin D levels (OR= 1.71; 95% C.I: 1.2–2.4, P0.001).[11]

Moreover, in acase control study by ***Hamad et al***.included250 pregnant females during the 1st twenty weeks of gestation, with ages varying from 20 to 35 years showed that vitamin D deficiency was more common in pregnant ladies and was considered as one of the as one modifiable risk factors for pregnancy loss mostly among recurrent losses.[12]

Another retrospective case control study by***Alya et al.***, in Iraq reported that vitamin D deficiency was associated with 1st trimester miscarriages.[13]

In contrary to our study results a recent meta-analysis and systematic review by ***Amegah et al***., found that low vitamin D levels were not accompanied with increased risk of spontaneous recurrent miscarriage.[14]

As regard relationship between obesity and vitamin d deficiency similarly to our results, ***Woon et al.***, revealed that, there were no associations between pre-pregnancy BMI with vitamin D deficiency.[15]

On the opposite side, a study by***Agarwal et al***., demonstrated a significant relation between increased body mass index (BMI) and low vitamin D serum levels. All women with BMI ≥30 were found to have low vitamin d levels (100% prevalence).[16]

In addition another study by ***Shen et al***., didnot support the current results as they found that, among pregnant women, maternal vitamin D values during first trimester of pregnancy have been positively associated with maternal BMI.[17]

And by comparing deficient and optimal groups, ***Hamad et al***., was found that obesity, was a possible risk factors for VDD.[12]

CONCLUSION

Although Egypt is a country with abundant sunshine all year round, majority of pregnant females had vitamin D deficiency and insufficiency. Vitamin D deficiency had a role in spontaneous abortion. Vitamin D level not related to the BMI.

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