ORIGINAL ARTICLE

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The Relationship Between the First Trimester Pregnancy Loss and Maternal Serum 25-Hydroxyvitamin D Level. A Case-control Study

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Abstract

Objective: Following the discovery of 25-hydroxyvitamin D [25(OH)D] receptor expression in the reproductive system organs and placenta, many studies exploring the relationship between pregnancy complications and 25(OH)D is being performed. In this study, we examined serum 25(OH)D levels in early pregnancy loss.

Methods: Patients who were in 20-30 years old age range whose body mass index >25 and <30, between April 2019 and January 2020 were included in the study at the Clinic of Obstetrics and Gynecology of University of Health Sciences Turkey, Prof. Dr. Cemil Tascioglu City Hospital in Istanbul/Turkey. Those who had miscarriage within the first 10 weeks (as a study group) and those who were diagnosed within the first 10 weeks and continued their pregnancies as live pregnancies after the 10th week (as a control group) were included in the study as two groups. Cases where the pregnancy was conceived through artificial reproductive technology, cases with a history of recurrent pregnancy losses, cases with chronic diseases and cases treated for imminent abortion were excluded. First prenatal visit 25(OH)D levels were retrospectively extracted from the hospital medical records.

Results: A statistically significant difference was observed between the 25(OH)D levels in women with an early pregnancy loss and 25(OH)D levels in women with an ongoing healthy pregnancy ($p<0.01$). The mean 25(OH)D level was found to be 8.61 ng/mL in the spontaneous pregnancy loss group; whereas, the mean level was found to be 16.61 ng/mL in the control group. As of this significance, a cut-off value for 25(OH)D levels was calculated using receiver operator characteristic curve analysis (sensitivity 94%, specificity 74%). Additionally, among other factors, older paternal age and vaginitis were also correlated with early pregnancy loss.

Conclusion: Our results show that 25(OH)D deficiency may play a role in early pregnancy loss. 25(OH)D may be a useful marker in predicting and preventing early pregnancy loss.

Keywords: Pregnancy loss, early, pregnancy trimester, first, 25(OH)D, miscarriage, spontaneous abortion

INTRODUCTION

Early pregnancy loss is the most common early pregnancy complication and is observed in approximately 30% of all pregnancies. This incidence decreases to 10% when evaluated as clinically recognized pregnancies (1). It is difficult to measure the full burden of miscarriage and is related to how early women realize their pregnancy. However, it is difficult to distinguish between abortion and stillbirth and the concept of early pregnancy is not fully clarified, and differences between studies make it difficult to give a clear incidence of miscarriage. Early pregnancy loss (miscarriage) is defined as a non-viable empty intrauterine gestational sac or a gestational sac containing an embryo or fetus without fetal heart activity within the first 10 0/7 weeks of gestation (2). In recent studies, modifiable factors like advanced maternal age, prepregnancy body mass index (BMI) and alcohol consumption have been emphasized (3-5). Although the cause of most miscarriages is unknown, it is likely due to

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the complex interplay between parental age, genetic, hormonal, immunological and environmental factors (6).

The fetus is characterized as a semi-allograft which is incapable of survival if there is no maternal immune tolerance. 25-hydroxyvitamin D [25(OH)D] is a fat-soluble steroid prohormone mainly synthesized in the lower layers of the skin epidermis through a chemical reaction that depends on ultraviolet B radiation by sun exposure, and it can also be ingested from the diet and from supplements (7). Although the main function of 25(OH)D is on calcium metabolism and bone mineralization, recent studies have noted the expression of 25(OH)D receptors in peripheral and central organs that regulate the female reproductive system such as the uterus, ovary, placenta, pituitary gland and hypothalamus (8). Correlations between low levels of 25(OH)D and adverse outcomes of pregnancy such as preeclampsia, gestational diabetes mellitus, fetal growth restriction and preterm labor have been shown (9-11). This study aimed to compare the 25(OH)D levels in women having a first trimester pregnancy loss with the 25(OH)D in women ongoing a healthy first trimester pregnancy.

METHODS

Forty women with early pregnancy loss and 62 women with a healthy ongoing first trimester pregnancy seen between April 2019 and January 2020 at the Clinic of Obstetrics and Gynecology of University of Health Sciences Turkey, Prof. Dr. Cemil Tascioglu City Hospital (formerly known as Okmeydani Education and Research Hospital) in Istanbul/Turkey were included in this retrospective case-control study. Women aged 20-30 years and having a single pregnancy less than 10 weeks of gestation according to transvaginal ultrasound (TVUSG) or the last menstrual period and admitted to the hospital for antenatal care were included in the study population.

Women without any chronic disease like diabetes, hypertension, hypo or hyperthyroidism, a history of 2 or more previous miscarriages, consanguineous marriage, multiple pregnancies, ectopic pregnancies, pregnancies conceived through artificial reproductive technology, patients with a BMI less than 25 or greater than 30, Rh incompatibility, any confirmed chromosomal abnormality in either one of the couples, vitamin D supplement use and patients using any medications other than only folic acid were excluded from the study.

Comprehensive gynecology and obstetric history was conducted. Patient ID number, hospital registry number, age, height, weight, education level, gravidity, parity, smoking, and alcohol intake history, any vaginal bleeding or vaginitis episodes during the current pregnancy, a history of pelvic surgery, sexual intercourse history in the current pregnancy, paternal age, blood group and Rh status and consanguine marriage if present, were recorded.

Miscarriage was defined as confirmation of a pregnancy with a crown-rump length (CRL) of 7 mm or greater without cardiac activityor an empty gestational sac measuring 25 mm or greater in mean gestational sac diameter according to the guidelines of the Royal College of Obstetricians and Gynecologists (12). When these ultrasonographic criteria were not met but when there were findings suspicious for pregnancy failure, additional criteria from the Society of Radiologists in Ultrasound Multispecialty Panel on Early First Trimester Diagnosis of Miscarriage and Exclusion of a Viable Intrauterine Pregnancy (13) were considered:

- Absence of embryo with heartbeat 2 weeks or more after a scan that showed a gestational sac without a yolk sac.

- Absence of embryo with heartbeat 11 days or more after a scan that showed a gestational sac with a yolk sac.

Gestational age was calculated from the date of the last menstrual period and verified by ultrasound findings. If the patient could not recall or was not sure of the first day of her last menstrual period, or had irregular cycles, CRL was used to determine the gestational age. Speculum was examined in patients with vaginal bleeding to check the cervical status and identify the bleeding. Ultrasonographic evaluation of the pregnancy allowed us to document intrauterine pregnancy, measure CRL, evaluate gestational sac, yolk sac, fetal cardiac activity, and visualize any subchorionic hematoma. The control group consisted of patients with a live pregnancy whose gestational week was less than 10 weeks according to the last menstrual period and confirmed by TVUSG.

The medical records and laboratory results of the pregnant women who met the inclusion criteria were checked and extracted from the hospital database. Pregnant women who had routine first prenatal care visit laboratory workup results (complete blood count, biochemistry, 25(OH)D, vitamin B12, folate, blood group, and Rh type) were included in the study.

The study was approved by the University of Health Sciences Turkey, Okmeydani Training and Research Hospital Ethics Committee in April 2019 (no: 4867071-514.10, approval number: 1216). All participants provided informed written consent if they agreed to participate after oral description of the study.

Statistical Analysis

Descriptive statistical methods (mean, standard deviation, median, frequency, ratio) as well as the Shapiro-Wilk test for

normally distributed data and box plot graphs for visualization were used. Mann-Whitney U test was used for comparing of non-normally distributed data between groups. Student's t-test was used for comparison of normally distributed data, Chi-square test, Fisher's Exact test and Fisher-Freeman-Halton test were used for comparing categorical data. Cut-off value for 25(OH)D levels was determined by receiver operator characteristic (ROC) curve analysis as for determination of the most appropriate cut-off value in a diagnostic test. P<0.05 was considered statistically significant.

RESULTS

A total of 102 patients, 62 in the control group and 40 in the study group, were included in the study. No significant difference was observed between the mean ages of the control group and the study groups (26.21±3.01 vs. 26.33±3.2, respectively; p=0.750). When the ages of the husbands were analyzed, it was realized that the mean husband age of the group with early pregnancy loss was significantly higher than the mean husband age of the control group (32.3±5.71 and 30.18±4.59, respectively; p=0.041). BMI, gravidity, parity in the two groups did not have a statistically significant differences (p=0.278, p=0.099, p=0.517; respectively) (Table 1). Vaginitis was significantly higher among women with early pregnancy loss (p=0.003). Sexual intercourse during pregnancy, folic acid usage, Rh incompatibility and consanguineous marriage status did not show statistically significant differences between the two groups (p>0.05) (Table 1).

A statistically significant difference was observed between vitamin D levels of the two groups; 25(OH)D levels of the early pregnancy loss group were significantly lower than those of the control group [miscarriage mean 8.61 mcg/L (21.4 nmol/L) vs. non-miscarriage mean 16.61 mcg/L (41.43 nmol/L); p=0.001, Studen's t-test]. Folate, PTH and vitamin B12 levels were not significantly different between the two groups (p>0.05) (Table

Table 1. Demographic characteristics of patients

	Total (n=102)		**Abort group (n=40)**	**Alive group (n=62)**	**p value**
Age*	26.21±3.01		26.33±3.2	26.13±2.91	0.750[a]
BMI*	22.86±1.99		23.13±2.00	22.68±1.99	0.278[a]
Gravida*	2.55±1.26		2.83±1.38	2.37±1.16	0.099[b]
Parity*	1.13±1.35		1.86±1.02	1.78±1.43	0.087[b]
Educational status n (%)	Illiterate	7 (6.9)	4 (10)	3 (4.8)	0.517[c]
	Primary school	34 (33.3)	16 (40)	18 (29)	-
	Middle school	27 (26.5)	8 (20)	19 (30.6)	
	High school	19 (18.6)	6 (15)	13 (21)	
	Graduate	15 (14.7)	6 (15)	9 (14.5)	
Partner age*	31.01±5.14		32.3±5.71	30.18±4.59	**0.041**[a]
Alchol status; n (%)	No		40 (100)	62 (100)	-
Smoking status; n (%)	No		30 (75.0)	54 (87.1)	0.118[c]
	Yes		10 (25.0)	8 (12.9)	
Vaginitis; n (%)	No		30 (75.0)	59 (95.2)	**0.003**[c]
	Yes		10 (25.0)	3 (4.8)	
Sexual intercourse during pregnancy n (%)	No		7 (17.5)	11 (17.7)	0.975[c]
	Yes		33 (82.5)	51 (82.3)	
Folic acid usage n (%)	No		21 (52.5)	39 (62.9)	0.297[c]
	Yes		19 (47.5)	23 (37.1)	
Rh incompatibility n (%)	No		40 (100)	60 (96.8)	0.519[d]
	Yes		0 (0)	2 (3.2)	
Consanguineous marriage status n (%)	No		40 (100)	61 (98.4)	1.000[d]
	Yes		0 (0)	1 (1.6)	

[a]Student's t-test,[b] Mann-Whitney U test,[c] Chi-square test, [d] Fishers Exact test. Bold text p value is statistically significant, *Data are given as mean ± standard deviation. BMI: Body mass index

2). However, phosphorous levels showed significant differences between the groups, where levels in the early pregnancy loss group were significantly higher than the levels in the control group (miscarriage median 4.18 mg/dL vs. non-miscarriage median 3.46 mg/dL; p=0.043, Mann-Whitney rank-sum test). A cut-off value of 12.5 mcg/L for 25(OH)D levels in women with early pregnancy loss and 25(OH)D levels in women with a healthy pregnancy was determined. For this 12.5 mcg/L cut-off value, sensitivity and specificity were 95% and 74.19%, respectively, with a positive predictive value of 70.37% and a negative predictive value of 95.83%. The area under the curve was calculated as 90.1% in the ROC curve used to determine the cut-off value (Tables 3, 4, Figure 1). The difference observed between the two groups at this cut-off value of 12.5 g/L for vitamin D levels was statistically significant (p=0.001) (Figure 1).

DISCUSSION

In this study, we attempted to reveal any relationship between early pregnancy loss and low levels of 25(OH)D, which is a predictable, preventable and treatable condition. Our study results demonstrate a significant difference between the 25(OH)D levels of women with an early pregnancy loss and the 25(OH)D levels of women without an early pregnancy loss.

Andersen et al. (12) found an association with low maternal serum 25(OH)D levels and the first trimester pregnancy loss in their cohort study on 1,683 patients. The authors attribute the beneficiary effects of 25(OH)D to its regulation of the innate and adaptive immune system. In a small Danish controlled cohort study (13). Three women who had miscarriage during the second trimester of pregnancy had a lower 25(OH)D than 84 controls who completed their pregnancy.

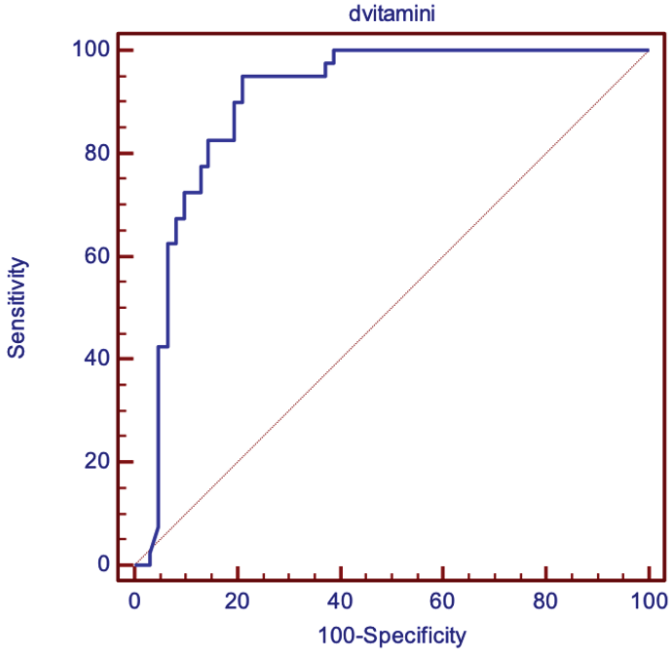

Figure 1. ROC curve for 25(OH)D2
ROC: Receiver operator characteristic, 25(OH)D: 25-hydroxyvitamin D

Conversely, in a randomized controlled trial of pregnant women taking different doses of vitamin D supplements (400, 2000, or 4000 IU of vitamin D 3 per day until delivery), 25(OH)D levels at the 12th week of pregnancy. There was no significant difference in l25(OH)D concentrations [circulating 25(OH)D level mean ± standard deviation; 61.2±27.1, 57.5±22.4, 59.8±25.4, respectively) at 12 weeks gestation when women who experienced pregnancy loss were compared with women who gave birth to a live baby (14). In a cohort study from Australia, when vitamin D levels at 10-14 weeks of pregnancy were compared among 3,714 women with healthy pregnancies and 39 women with pregnancy losses no significant difference was observed with mean 25(OH)D concentrations of 56.9 nmol/L [95% confidence interval (CI): 43.9, 70.8] vs. 53.5 nmol/L (95% CI: 42.4, 61.7) (15). In our study, we found that 25(OH)D was related to miscarriage in the first trimester. It is possible that 25(OH)D plays a protective role against miscarriage.

Discussions on vitamin D deficiency as a global public health problem continue to be relevant due to low levels of serum 25(OH)D in a vast number of studies conducted worldwide. Prevailing low 25(OH)D levels, especially in pregnant women and neonates is keeping drawing attention (16).

Nevertheless, Dietary Reference Intake (DRI) recommendations for daily intake of vitamin D by the US Institute of Medicine (IOM) and Health Canada is 600 IU/day; these DRIs being based on maintaining bone health, do not provide recommendations on health issues besides maintaining skeletal health (17). Although a 2016 update of a Cochrane Systematic Review and some other current reviews propose that vitamin D supplement

Table 2. Evaluations of blood biochemical measurements

	Abort group (n=40)*	Alive group (n=62)*	p value
25(OH)D (µg/L)	8.61±2.42	16.61±6.12	**0.001**[a]
Hemoglobin (g/L)	12.71±1.04	12.07±1.38	0.014[a]
Folate (mcg/L)	16.69±5.33	14.55±5.11	0.077[b]
PTH (pcg/mL)	36.17±17.29	36.62±17.03	0.997[b]
P (mg/dL)	4.18±2.37	3.46±0.54	**0.043**[b]
B12 (ng/L)	178.33±60.53	198.98±66.76	0.257[b]

[a]Student's t-test, [b]Mann-Whitney U test, *Data are given as mean ± standard deviation. PTH: Parathormone, P: Phosphorus, B12: Vitamin B12, 25(OH)D: 25-hydroxyvitamin D

Table 3. Diagnostic screening tests and ROC curve results for vitamin D measurement							
Diagnostic scan					**ROC curve**		
Cutt-off	**Sensitivity**	**Specifity**	**PPV**	**NPV**	**AUC**	**95% CI**	**p value**
≤12.5	95.00	74.19	70.37	95.83	0.901	0.838- 0.963	0.001
PPV: Positive predictive value, NPV: Negative predictive value, CI: Confidence interval, AUC: Area under curve, ROC: Receiver operator characteristic							

Table 4. Relationship between groups and 25(OH)D2 measurement					
	25(OH)D2 (cut-off value 12.5)				p value*
	>12.5		≤12.5		
	n	%	n	%	
Alive group	46	95.8	16	29.6	**0.001**
Abort group	2	4.2	38	70.4	
*Pearson's chi-squared test, 25(OH)D: 25-hydroxyvitamin D					

during pregnancy may lower the incidence of pre-eclampsia and fetal growth restriction, no high-quality evidence for significant effects on other maternal and fetal outcomes could be found (18-20).

Ergocalciferol (vitamin D2) absorbed through diet and cholecalciferol (vitamin D3), mainly synthesized in the skin by UV rays, are converted to 25(OH)D in the liver, 25(OH) D is later transformed into the most active form 1,25-OH 2D, primarily in the kidneys. 25(OH)D is the major circulating form of vitamin D, but its activity is less than 1% of 1,25-OH 2D, the most active form of vitamin D. Serum concentration of 25(OH)D is currently the main indicator of vitamin D status. It reflects vitamin D produced endogenously through sun exposure and that obtained from foods and supplements (21).

ACOG (22) has emphasized that measuring serum 25(OH)D levels would be an acceptable indicator of the vitamin D status of pregnant women. However, there is no consensus on what a healthy serum level should be. Generally, for optimal skeletal health, a minimum serum level is considered as 20 ng/mL (50 nmol/L) (23). However, some researchers have stressed in a serum level of at least 32 ng/mL (80 nmol/L) (24). In 2010, the Food and Nutrition Board of the National Academies' IOM stated that a daily intake of 600 IU of vitamin D would be sufficient during pregnancy and lactation (25).

Over the past decades, there has been growing research on the potential links between 25(OH)D and major human diseases and clinical conditions. Dark skin pigmentation, insufficient sun exposure, inadequate dietary intake are major reasons for 25(OH)D deficiency (26). 25(OH)D metabolism during pregnancy is still less clear than in the non-pregnant state and is under ongoing research.

The immunomodulator effect of vitamin D on the endometrium that secures implantation, may imply that in 25(OH)D deficiency or insufficiency, dysfunction through the implantation process may be responsible for early pregnancy losses and late placental dysfunctions (27). Additionally, fetal rejection that may occur during this period because the conceptus may be considered a semi-allograft, will not occur due to the effects of 25(OH)D on the immune system. Consequently, the immunomodulator and the anti-inflammatory properties of 25(OH)D play an important role in preventing early pregnancy losses (28).

In a study of 133 women with 3 or more consecutive pregnancy losses, those with 25(OH)D levels less than 30 ng/mL (75 nmol/L) had a significantly higher prevalence of auto- and cellular immune abnormalities (29). Similarly, various *in vitro* studies have illustrated that in the endometrial cells of women with spontaneous recurrent abortions, 1,25(OH)2D3 plays an essential role in cytokine regulation (30).

Vitamin D receptor is expressed in many cell types and controls antigen-receptor signalization and T-cell activation (31). It has also been demonstrated that the active form of 25(OH)D inhibits the secretion of proinflammatory cytokines such as tumor necrosis factor-alpha, interleukin-6, and interferon-gamma in the placenta (32).

1,25(OH)2D3, the active form of 5(OH)D, has potent anti-inflammatory effects at the maternal-fetal interface, by decreasing the T helper cell type 1 (Th1)/Th2 ratio, suppressing Th17 cell activity, and increasing T regulatory cell production (33). Animal studies have shown that treatment with vitamin D regulates endometrial decidualization (34). *In vitro* studies on trophoblasts cultured from human placenta revealed that 1,25(OH)2D3 stimulated estradiol and progesterone secretion in trophoblasts (35). It has also been demonstrated that 1,25(OH)2D3 is involved in HOXA10 expression, a key target gene in the implantation process (36).

Some study findings suggest that women with sufficient levels of 25(OH)D are more likely to achieve clinical pregnancy following in vitro fertilisation (8). Bacterial vaginosis, which has been shown to be associated with adverse pregnancy outcomes, mostly with preterm birth, has also been shown to be associated with low vitamin D levels (37). Many studies have demonstrated

that vitamin D deficiency may play a role in recurrent pregnancy losses. Still, it may seem compulsive to presume an active role for vitamin D at the maternal-fetal immunologic interface leading to pregnancy loss.

We found that vitamin D levels were low both in the study group and in the control group of our study. We assume that these low levels are due to the low economic profile of our patient group, leading to nutritional deficiencies and most of the patient population choosing to dress according to religious restrictions leading to limited sun exposure.

Study Limitations

The limitations of our study are the retrospective design of the study, the small number of participants, the likely confounding factors resulting from the low socioeconomic profile of the participants, and the serum vitamin D measurements not being cleared of seasonal variations.

CONCLUSION

As a result, we found that women with the first trimester pregnancy losses had much lower serum 25(OH)D levels of <12.5 ng/mL. These findings suggest that vitamin D plays a protective role in preventing pregnancy losses. Randomized controlled trials are needed to test the protective effect of vitamin D supplements for low levels of 25(OH)D during early pregnancy or even in the preconception period.

Ethics

Ethics Committee Approval: The study was approved by the University of Health Sciences Turkey, Okmeydani Training and Research Hospital Ethics Committee in April 2019 (no: 4867071-514.10, approval number: 1216).

Informed Consent: All participants provided informed written consent if they agreed to participate after oral description of the study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: B.K., O.Ş., V.M., Concept: B.K., O.Ş., V.M., Design: B.K., O.Ş., V.M., Data Collection or Processing: B.K., O.Ş., V.M., Analysis or Interpretation: B.K., O.Ş., V.M., Literature Search: B.K., O.Ş., V.M., Writing: B.K., O.Ş., V.M.

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