

SERUM LEVEL OF 25-HYDROXYVITAMIN D IN PREGNANCY, AND ITS CORRELATION WITH SPONTANEOUS ABORTION

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ABSTRACT

Background: Vaginal bleeding before 20 weeks of gestation occurs in up to 20% of pregnancies, and half of these cases will have a spontaneous abortion. Vitamin D deficiency is common in women of reproductive-age and pregnant women. Aim of study to Measurement of serum level of 25-hydroxyvitamin D (25OHVD) in women spontaneously aborted during early pregnancy and women with successful ongoing pregnancy to identify its association with spontaneous abortion and to assess the correlation of 25OHVD level with maternal ages, their body mass index and gestational ages. **Method:** This case-control study included 82 women; 41 pregnant women suffered from abortion as patient group and 41 pregnant women presented with ongoing pregnancy as control group. Aborted women were sub classified according to number of their abortions into; first and recurrent abortion women. Women with uterine diseases or abnormalities, immunological diseases, endocrine disorders such as diabetes and thyroid diseases, history of infections, consanguinity, family history of genetic disorders, and smoker patients were excluded from this study. Both groups' participants were aged between 18 and 35 years old, and none of them had taken vitamin D supplements for the last three months prior to pregnancy. Body mass index (BMI) was calculated from weight and height and serum total 25OHVD was measured by using enzyme linked immunosorbent assay (ELISA) technique. **Results:** The highest proportion of study participants in patient and control groups was aged ≥ 25 years (68.3% and 53.7% respectively). The results of study revealed that 36.6% of patient women were of normal weight, 39% were overweighted, and 24.4% of them were obese. The results also showed that mean \pm SD value of 25-hydroxyvitamin D (25OHVD) levels was significantly lower in aborted women group than in control group ($p < 0.05$). In addition, the mean value of 25OHVD levels of patient women with recurrent abortions was significantly lower than that of first time abortion ($p < 0.05$). There was no significant correlation between 25-hydroxyvitamin D level and each of age, body mass index, and gestational age in both patients group and control group. **Conclusion:** Spontaneously aborted women are suffered from significant decrease of serum total 25-hydroxyvitamin D level and that vitamin deficiency was more overt and significant in women with recurrences of abortion suggesting that vitamin D deficiency might be a modifiable risk factor for spontaneous abortion.

KEYWORDS: 25-hydroxyvitamin D, pregnancy, correlation, spontaneous abortion.

INTRODUCTION

Spontaneous abortion refers to spontaneous loss of a pregnancy before 12 weeks (early miscarriage) or from 12 to 24 weeks (late miscarriage) of gestation in the absence of elective medical or surgical measures to terminate the pregnancy.^[1] There is a spectrum of terms including threatened abortion, inevitable or missed abortion that describe different states of pregnancy loss. The American College of Obstetricians and

Gynecologists (ACOG) estimates it is the most common form of pregnancy loss. It is estimated that as many as 26% of all pregnancies end in miscarriage and up to 10% of clinically recognized pregnancies.^[2] The most common cause of spontaneous pregnancy loss in 1st trimester is chromosomal abnormalities. In most cases, it is too early to determine the exact cause of the abnormality. Risk of early pregnancy loss decreases with increasing gestational age and is relatively low after 15-weeks' gestation in a genetically normal fetus.^[3]

Maternal comorbidities like thrombophilia, antiphospholipid antibody syndrome, and hypertension also increase the risk of spontaneous abortion. Additional risk factors as smoking, large amounts of caffeine use, trauma and malnutrition, have been identified.^[4] Evaluation of abortion depends on the type suspected based on history and physical exam. For example, a missed abortion has no reliable symptoms or signs; it can only be diagnosed by measurement of beta-human chorionic gonadotropin (beta-hCG) levels and pelvic ultrasound.^[5] Vitamin D (Vit.D) in its active form 1,25-dihydroxyvitamin D is a lipid-soluble hormone that has well-established classic function in maintaining calcium homeostasis and promoting bone mineralization. In addition, Vit.D has an essential role in regulating cell proliferation and differentiation and modulating innate and adaptive immune responses. Vit.D status during pregnancy has been drawing great attention. There is some evidence suggesting that Vit.D modulates human reproductive processes.^[6] Immunological mechanisms have been proposed to underlie the pathogenesis of spontaneous abortion. Vit.D has a potent immunomodulatory effect, which may affect pregnancy outcome.^[7] The human reproductive process is regulated by the immune system. Vit.D has been associated with infertility, polycystic ovary syndrome, in vitro fertilization outcomes, obstetrical outcomes.^[8] Normal pregnancies depend on synchronized immune- endocrine crosstalk at the maternal-fetal interface.^[9] Aim of study to Measurement of serum level of 25-hydroxyvitamin D (25OHVD) in women spontaneously aborted during early pregnancy and women with successful ongoing pregnancy to identify its association with spontaneous abortion and to assess the correlation of 25OHVD level with maternal ages, their body mass index and gestational ages.

METHOD

This case-control study was carried out at Biochemistry Department, College of Medicine, University of Baghdad, and at Obstetrics and Gynecology outpatients' clinic in Karbala City, during period from August 2020 to December 2020. It comprised of 82 women; 41 pregnant women suffered from abortion as a patient group and 41 women presented with ongoing pregnancy as a control group. Both groups' participants were aged between 18 and 35 years old, and none of them had taken vitamin D supplements for the last three months prior to pregnancy. cases enrolled 41 pregnant women presented with abortion; the diagnosis of abortion was achieved by specialist Gynecologist. The gestational age of this group of women was less than 20 weeks, which was calculated from the first day of last menstrual period. The enrolled aborted women were sub classified according to number

of their abortions into; first time abortion and recurrent abortion.

Exclusion criteria included any patient woman with: Uterine diseases or abnormalities, Immunological diseases, Endocrine disorders such as diabetes and thyroid diseases, History of TORCH infections (toxoplasmosis, rubella, cytomegalovirus and herpes simplex virus), Consanguinity, Family history of genetic disorders. 7-Smoker patients. Control patients involved 41 pregnant women presented with normal ongoing pregnancy with a gestational age greater than 20 weeks calculated from the first day of last menstrual period and had no previous history of abortion. History of the study participants the data that taken from each participant woman in this study involved questions about age, gestational age, gravidity, presence of previous abortions with their numbers, obstetrical history, history of medical diseases and chronic diseases as shown in the study's questionnaire. Anthropometrics measurement of the study participants after obtaining a vocal informed consent, body weight and height of the study participants were measured. The weight of pregnancy was subtracted from the body weight for each participant.^[10] and then body mass index (BMI) was calculated by dividing the weight in kilogram (kg) by the height in squared meter (m²). The BMI results were classified according to WHO classification.^[11] Two milliliters of blood were collected from antecubital fossa vein of each study participant using (5ml) disposable syringes. The blood samples then transferred into gel tubes (serum separator tubes) and left for 15-30 minutes until the blood clotted, then centrifuged at 3000 round per minute for 5 minutes to obtain the serum. The serum samples were kept into disposable Eppendorf tubes and stored at (-20 °C) until the time of measurement of 25-hydroxyvitamin D (25OHVD) level by using ELISA technique. The data were analyzed using Statistical Package for Social Sciences (SPSS) version 26. The data were presented as mean, standard deviation and ranges. Categorical data were presented by frequencies and percentages. Independent t-test was used to compare the continuous variables accordingly. Pearson's correlation test (r) was used to assess correlation between vitamin D level with age, body mass index, and gestational age. A level of P-value less than 0.05 was considered significant.

RESULTS

Figure 1 shows that participants' age was ranging from 18 to 35 year with a mean \pm SD value of (26.48 \pm 4.8 year). The highest proportion of study participants in patient and control groups was aged \geq 25 years (68.3% and 53.7% respectively), while the percentage of those aged <25 years in patient group was (31.7%), and in control group was (46.3%).

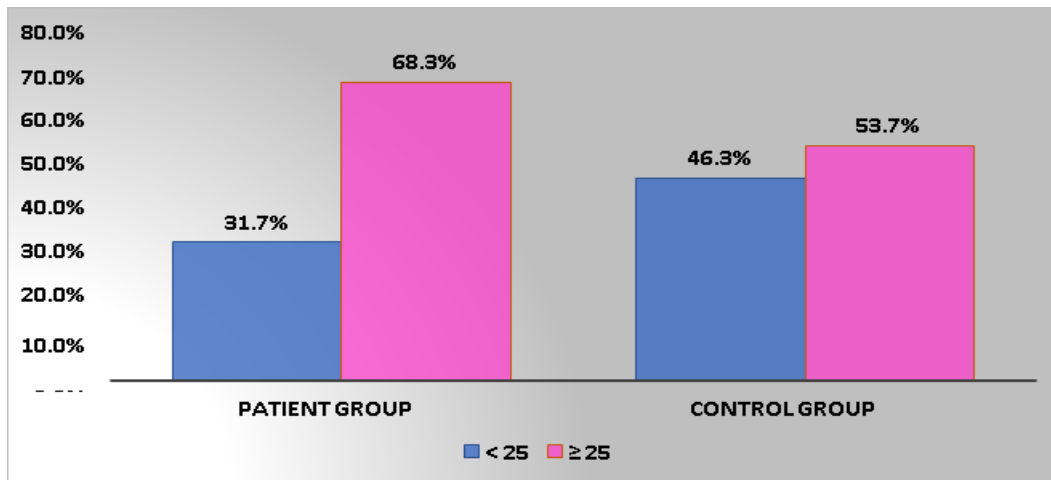


Figure 1: Distribution of study participants by age.

The results of study revealed that 36.6% of women in patient group were of normal weight, 39% were overweighted, and 24.4% of them were obese. In control group, 14.6% of them were of normal weight, 43.9% were overweighted, and 41.5% were obese. In addition,

the study found that 48.8% of patient women were primigravida, and 51.2% were multigravida, while 51.2% of control women were primigravida, and 48.8% were multigravida, as shown in table (1).

Table 1: Distribution of study groups by certain clinical information.

Parameter	Study group		Total Number (%) n= 82
	Patient women Number (%) n= 41	Control women Number (%) n= 41	
BMI Level (Kg/m²)			
Normal weight	15 (36.6)	6 (14.6)	21 (25.6)
Overweight	16 (39.0)	18 (43.9)	34 (41.5)
Obese	10 (24.4)	17 (41.5)	27 (32.9)
Gravidity			
Prim gravida	20 (48.8)	21 (51.2)	41 (50.0)
Multigravida	21 (51.2)	20 (48.8)	41 (50.0)

Table 2 shows the mean ± SD values of age, body mass index, and gestational age of patient women and control ones. The mean values of age (27.48 ± 5.1 year) and body mass index (27.56 ± 4.0 kg/m²) of patient women did not differ significantly from those of control women

(25.48 ± 4.3 years, 29.05 ± 4.1kg/m², respectively). While, the gestational age of patient women (10.07 ± 4.2 week) was significantly lower than that of controls (29.73 ± 5.3 week; P< 0.05).

Table 3: Mean ± SD values of age, body mass index and gestational age of patient and control women.

Parameter	Study group		P - Value
	Patient women (n=41)	Control women (n=41)	
Age (Year)	27.48 ± 5.1	25.48 ± 4.3	0.058
BMI (kg/m ²)	27.56 ± 4.0	29.05 ± 4.1	0.085
GA (Week)	10.07 ± 4.2	29.73 ± 5.3	0.001*
* Significant, P< 0.05			

Figure 2 shows the distribution of patients group by number of abortions. In the current study, 51.2% of patients had recurrent abortions; while 48.8% of them presented with first time abortion.

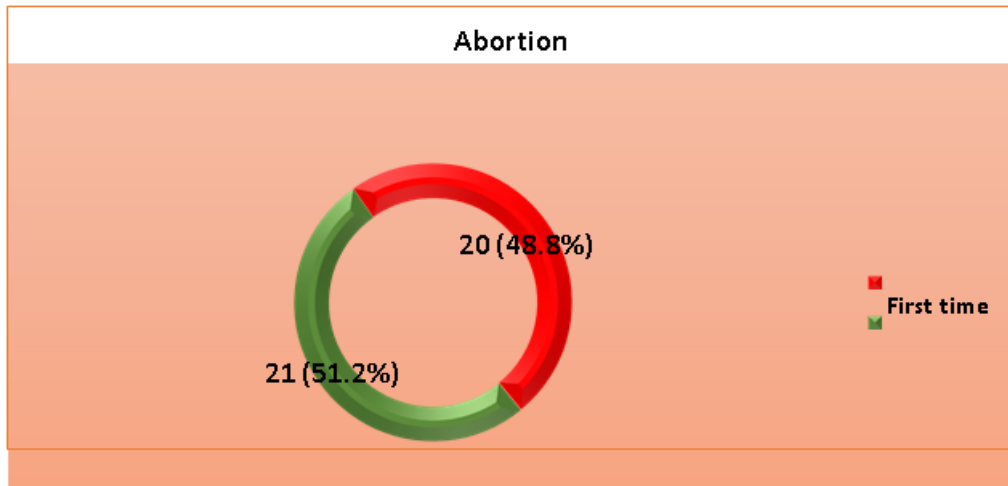


Figure 2: Distribution of patient group by number of abortions.

Table 3 reveal that the mean ± SD value of 25-hydroxyvitamin D (25OHD) level of aborted women (15.15 ± 6.9ng/ml) was significantly lower than that of control group (21.52 ± 8.2 ng/ml, P< 0.05).

Table 3: Mean ± SD value of 25-hydroxyvitamin D levels of patient and control women.

25-hydroxyvitamin D level (ng/ml)	Study group		P - Value
	Patient women (n=41)	Control women (n=41)	
	15.15 ± 6.9	21.52 ± 8.2	0.001*
* Significant, P< 0.05			

Table 4 the comparison in 25-hydroxyvitamin D level according to number of abortions (first and recurrent abortions). The results found that mean ±SD value of 25OHD levels of patient women with recurrent abortions (13.02 ± 6.3 ng/ml) was significantly lower than that of first time abortion (17.38 ± 6.9 ng/ml, P< 0.05).

Table 4: Mean ±SD values of 25-hydroxyvitamin D levels in patient women with first time and recurrent abortions.

25-hydroxyvitamin D level (ng/ml)	Abortion		*P - Value
	First time (n=20)	Recurrent (n=21)	
	17.38 ± 6.9	13.02 ± 6.3	0.041*
* Significant, P < 0.05			

The results of the present study observed that there was no significant correlation between 25-hydroxyvitamin D level and each of woman age, body mass index, and gestational age in both patient group and control group (p>0.05) as shown in table 5 and 6.

Table 5: Correlation between 25-hydroxyvitamin D level and certain parameters in patient group.

Parameter	25-hydroxyvitamin D level (ng/ml)	
	r	P - Value
Age (Year)	- 0.155	0.333
BMI (kg/m ²)	- 0.273	0.085
GA (Week)	0.227	0.145
r = Pearson's correlation, significant when P < 0.05		

Table 6: Correlation between 25-hydroxyvitamin D level and certain parameters in control group.

Parameter	25-hydroxyvitamin D level (ng/ml)	
	r	P - Value
Age (Year)	- 0.162	0.312
BMI (kg/m ²)	- 0.249	0.116
GA (Week)	0.103	0.52
r = Pearson's correlation, significant when P < 0.05		

DISCUSSION

The present study found that the major proportion of study aborted women was aged ≥ 25 years (68.3%), and their mean value was 26.48 year. This result agrees with the result that was noticed in Fan *et al.* study in 2020, in which the highest proportion of study patients was aged in second decade (67.5%).^[12] Also agrees with Hou *et al.* (2016), who observed that the mean age of their aborted women was 28.56 year.^[6] And disagrees with result of Albahlol *et al.* (2020), in which the majority of their enrolled aborted women were older ones with mean age of 31.2 years.^[13] In fact, risk of abortion was slightly elevated in the youngest mothers and then rose sharply in older mothers. The association between young maternal age and abortion could reflect biological mechanisms as well as an effect of reproductive immaturity.^[14] The present study also revealed that majority of aborted women were normal weighed and overweighed with small percentage were obese, while majority of control women were overweighed and obese. In addition, 48.8% of patient women were prim gravida; while in controls, they were 51.2%. Also, 51.2% of aborted women had recurrent abortions, while 48.8% had first time abortion. These results agree with Flood-Nichols *et al.* (2015), who found that 47% of their enrolled aborted women were normal weighed, 35% were overweight and 18% obese.^[15] but disagree with results reported by Fan *et al.* (2020), who found that highest proportion of their participant women were of normal weight (71.4%), while the overweighed and obese patients represented 17.3% of study population. In addition, they noticed that prim gravida women constitute about 80.4%.^[12] The vast majority of the study sample in Fadhil *et al.* (2014) study was within normal weight group and they were accounted for 48.0%.^[16] while in Fadhil *et al.* study in 2015, 33% of study sample were primigravida, and the highest percentage (52.5%) of the study sample had abortion.^[17] Turner *et al.* (2010) concluded that abortion rate was 2.3% in the obese category compared with 3.3% in the overweight category and 2.3% in the normal weight one.^[18] These differences in weight indices could be attributed to life style and dietary habit of different population and suggested that obesity by itself has no direct significant effect on gestation success. In this study, women age and BMI values did not differ significantly between aborted women and control ones, while gestational age was significantly increased in gestation success women than in aborted ones, these findings disagree with Hou *et al.* (2016), who observed that gestational age value was higher in normal pregnant women than in abortion group, but without significant difference.^[6] The results of the present study revealed that mean of serum 25OHVD level in aborted group was significantly lower than in control group. In addition, the present study revealed that mean of 25OHVD level in aborted women with recurrent abortions was significantly lower than that in women who presented with first abortion. These results agree with Li *et al.* (2017) who reported that serum Vitamin-D level was significantly decreased in recurrent spontaneous abortion

group compared with the control group^[7]. In the same accordance, Hou *et al.* (2016) reported that serum Vitamin-D concentration was significantly higher in women with normal pregnancies than in those with first trimester miscarriages, suggesting that deficiency of Vitamin-D is associated with spontaneous abortion.^[6] Andersen *et al.* (2015) found significant association between Vitamin-D insufficiency and the first-trimester abortion and suggested that serum concentration of Vitamin-D of less than 20 ng/ml was associated with more than two-fold increased hazard ratio (HR) for abortion.^[19] Similarly, Al-Shaikh *et al.* (2014) found higher prevalence of abortion in women with low level of Vitamin-D.^[20] However, these mentioned results disagree with Albahlol *et al.* (2020), who noticed that serum vitamin-D level was insignificantly lower in abortion group than control one (23.9 ± 19.5 vs. 28.46 ± 22.77 ng/mL respectively, $P > 0.05$).^[13] Also Schneuer *et al.* (2014) found that low level of Vitamin-D in the first trimester of pregnancy was not associated with adverse pregnancy outcomes and did not predict complications any better than routinely assessed clinical and maternal risk-factor information.^[21] Similarly, Zhou *et al.* study in 2014, reported that there was no significant difference in most adverse pregnancy outcomes (including abortion) among pregnant women with different levels of Vitamin-D at 16-20-week gestation except for higher prevalence of gestational diabetes and preterm delivery in women with decreased level of Vitamin-D.^[22] Finally, Møller *et al.* (2012) observed that Vitamin-D level was not associated with overall risk of abortion. However, miscarriage in the second trimester was observed in women who had lower serum Vitamin-D level than measurement performed in the first trimester compared with women without abortion.^[23] There was no significant correlation between Vitamin-D level and each of women age, BMI, and gestational age in both patient group and control group. These results agree with Fan and colleagues in their study in 2020, who reported that there was no significant relation between gravidity, number of abortions, and BMI of their enrolled women and serum Vitamin-D level.^[12] In contrary, disagree with results observed in Albahlol *et al.* study in 2020, in which there was significant negative correlation between age and vitamin-D level, also age was significantly positively related to gravidity, parity, BMI, and pregnancy duration.^[13] Also disagree with Li *et al.* (2017) who reported that abortion was significantly negatively correlated with vitamin-D level ($P=0.019$), indicating that lower concentrations of vitamin-D level are associated with a higher risk of spontaneous abortion.^[7] Similarly, Hou *et al.* (2016) concluded that recurrent abortion was significantly negatively correlated with Vitamin-D levels ($P=0.006$), as concentration of Vitamin-D decreased, the risk of early pregnancy loss increased.^[6]

CONCLUSION

Spontaneously aborted women are suffered from significant decrease of serum total 25-hydroxyvitamin D

level and that vitamin deficiency was more overt and significant in women with recurrences of abortion suggesting that vitamin D deficiency might be a modifiable risk factor for spontaneous abortion.

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