

Is There an Association Between Early Pregnancy Losses and Low 25-Hydroxy Vitamin D Levels?

Lebriz Hale Aktun^{1*}, Yeliz Aykanat², Oktay Olmuscelik³

¹Assistant Professor, Department of Obstetrics and Gynaecology, Istanbul Medipol University Hospital, Bagcilar, Istanbul, Turkey

²Department of Obstetrics and Gynaecology, Istanbul Medipol University Hospital, Bagcilar, Istanbul, Turkey

³Assistant Professor, Department of Internal Medicine, Istanbul Medipol University Hospital, Bagcilar, Istanbul, Turkey

***Corresponding Author:** Lebriz Hale Aktun, Assistant Professor, Department of Obstetrics and Gynaecology, Istanbul Medipol University Hospital, Tem cikisi Goztepe mah. Bagcilar, Istanbul, Turkey, Tel: +905322919196, Email: lebrizhale@gmail.com

Received Date: August 17, 2017 **Accepted Date:** September 10, 2017 **Published Date:** September 20, 2017

Citation: Lebriz Hale Aktun, Yeliz Aykanat, Oktay Olmuscelik (2017). Is There an Association Between Early Pregnancy Losses and Low 25-Hydroxy Vitamin D Levels?. POJ Gyn Obst Res. 1(1):1-5.

Abstract

Objective: Since the etiology of pregnancy losses during first trimester has not still been clear, we aim to analyze the relationship between vitamin D deficiency and early pregnancy losses.

Patients and Methods: The study was conducted on 200 women Plasma was collected from 100 nulliparous women with singleton at 7-10 weeks of gestation (50 with viable gestation and 50 with pregnancy loss) and 100 non-gravid reproductive age women (50 with a successful pregnancy history and 50 with one or more spontaneous first-trimester pregnancy loss history). Serum 25 (OH) D and calcium levels were compared between groups.

Results: The serum 25(OH)D levels for the groups turned out to be 47.64 ± 3.2 (95% CI: 44.4-50.8 ng/ml) for normal pregnancy group, 27.3 ± 1.2 (95% CI: 26.1-28.5 ng/ml) for the group of early pregnancy loss, 38.5 ± 5.1 (95%CI: 33.4-43.6 ng/ml) for the non gravid women with healthy pregnancy history and 11.6 ± 4.2 (95% CI: 7.9 - 15.6 ng/ml) for the non-gravid women with history of 1 or more first trimester pregnancy loss. There was a strong correlation between low 25(OH) D levels and early pregnancy loss (odds ratio (OR): 1.70, 95% CI: 1.2-2.3, $p < 0.001$). The calcium levels were significantly lower in pregnancy loss group than normal pregnancy and non-gravid groups ($p=0.005$, $p=0.033$ respectively).

Conclusions: Although our study is emphasized on role of vitamin D in early pregnancy it is not possible to recommend screening and supplementation of vitamin D in early pregnancy, as prognosis of pregnancies receiving supplementation and the incidence of pregnancy related complications in follow-up are not known. Well designed studies with long term follow up results needed.

Keywords: 25 hydroxyvitamin D, Pregnancy loss, 25 (OH) D vitamin

Introduction

25(OH) D levels have been found below the effective levels in most of the reproductive age women and newborns worldwide [1]. Based on this information, replacement programs started to be implemented in many countries. Moreover, the need for replacement is due to the multiple functions of this hormone. Vitamin D deficiency is associated with many clinical entities. Increasing evidence suggests that this hormone is linked to many diseases. In this respect, its effects have been continuously emphasized outside the skeletal system including also its critical role in pregnancy loss. Studies indicate that the effects of the vitamin D on the immune system play an important role in the sustainability of the pregnancy [2]. According to these studies, vitamin D acts as an immunomodulator and has been shown to contribute to successful decidualization by exerting significant local anti-inflammatory activity [3].

Vitamin D effects cellular and humoral immunity in different ways. One of the important factors in keeping the pregnancy health is the T-helper (Th) 2 response. In this context, Vitamin D has been shown to trigger Th2 mediated cytokines (IL-4, IL-5, IL-6, IL-9, IL-10 and IL-13) and inhibit Th1 cells and the production of related cytokines [4]. This is in line with studies showing that Th1 mediated cytokines decrease during the healthy pregnancy and Th2 mediated cytokines increase [5,6]. Since the pregnancy losses during first-trimester are still not clear, we aimed to evaluate the role of vitamin D deficiency during pregnancy.

Material and Method

The study was conducted on pregnant women who admitted to Medipol University Hospital Department of Gynecology and Obstetrics between January 2014 and December 2016. Before the study was undertaken, permission was obtained from the University's board of ethics along with the consents of all the patients.

The study was built on 4 groups. The week of pregnancy was calculated according to the last menstrual period and confirmed by ultrasonography examination. Group 1 (n=50) consisted of patients with 7-10 weeks of pregnancy with vaginal bleeding and diagnosed as early pregnancy loss after ultrasound examination. Group 2 (n=50) consisted of patients with viable pregnancy at 7-10 gestational weeks, no abnormal gynecological history and maintained normal pregnancy with follow-up until the week 12 of pregnancy. Group 3 (n=50) consisted of non-pregnant patients examined for pre-pregnancy along with a healthy pregnancy history. Group 4 (n=50) consisted of non-gravid women with history 1 or more first trimester pregnancy loss.

The women between 18-35 years old were involved in the study. The nulliparous pregnant women were included in the study out of the group of pregnancy. The women with a known metabolic disease (thyroid dysfunction, Diabetes etc.), uterine malformation, a severe liver or kidney disease, infection with rubella, toxoplasma, cytomegalovirus and herpes virus, and history of pregnancy loss with chromosomal abnormality excluded. The group 1 and the group 2 were classified as the groups of early pregnancy while the group 3 and the group 4 were considered to be the group of non-gravid.

The clinical parameters comprised of age, body mass index (BMI), socio-economic status and gestational age. Blood samples were obtained in an effort to measure 25 (OH) D and calcium (ca) levels of all the women. The demographic and laboratory data of all the patients were compared to one another. The range between 30 and 80 ng/ml was considered optimal, 20 to 29 ng/ml as insufficiency and 20 ng/ml and less as deficiency.

Statistical analysis: The data were analyzed using Statistical Package Social Sciences (SPSS) Version 15.0 (Inc., Chicago, IL, USA). The descriptive statistics were expressed as standard deviations and mean, categorical data (%) for numerical variables. The Kolmogorov-Smirnov test was performed to analyze the normal distribution of the variables, and the Mann-Whitney U test was performed for subgroup comparisons. P value <0.05 was considered statistically significant. The abnormal distribution was analyzed through nonparametric

tests. For multiple group comparisons, One-Way ANOVA (Robust Test: Brown-Forsythe) and Kruskal-Wallis H test post hoc analyzes [Nonparametric post-hoc test (Miller, 1966)] were put to use.

Results

Comparisons of sociodemographic and clinical data of the groups are presented in (Table 1). There was no statistically significant difference among the four groups in terms of age, BMI and socio-economic status ($p>0.05$). There was no significant difference in gestational ages between the groups of early pregnancy.

(Table 2) offers a comparison of laboratory data for the groups. The serum 25(OH)D levels for the groups turned out to be 47.64 ± 3.2 (95% CI: 44.4-50.8 ng/ml) for the normal group of pregnancy, 27.3 ± 1.2 (95% CI: 26.1 - 28.5 ng/ml) for the group of early pregnancy loss, group 1, 38.5 ± 5.1 (95%CI: 33.4 - 43.6 ng/ml) for the group 3 and 11.6 ± 4.2 (95% CI: 7.9 -15.6 ng/ml) for the group 4 respectively.

The ANOVA test associated with 25(OH) D revealed significant differences among the 4 groups. 25(OH) D concentrations were highest in the group of normal pregnancy (group 2), and the lowest in group 4. The low serum 25(OH) D levels amounted to 42% (n=21) for the group 1 and 98% (n=49) for the group 4. However, this ratio accounted for 2% (n=1) in the non-gravid group (group 2) and 4% (n=2) in the group 3.

There was a statistically significant difference between serum 25(OH) D levels in the group 1 and the group 2, and between the group 3 and the group 4 ($p = 0.000$). There was a strong correlation between low 25(OH) D levels and the pregnancy loss (odds ratio (OR): 1.70, 95% CI: 1.2-2.3, $p<0.001$). When the calcium levels were compared (table 2), serum ca levels were found significantly different ($p = 0.021$, $p = 0.000$, respectively). The calcium levels were significantly lower for the group 1 of pregnancy loss group than for the group of normal pregnancy (group 2) and non-gravid (group 3-4) ($p=0.005$, $p=0.033$ respectively).

	Group 1(n=50) Mean	Group 2(n=50) mean	Group 3(n=50) mean	Group 4(n=50) Mean
Age^a (year)	27.8 ± 2.4	28.5 ± 2.1	28.2 ± 2.4	27.9 ± 2.3
BMI^b, kg/m²	22.8 ± 3.1	22.7 ± 3.5	23.1 ± 2.6	22.8 ± 3.3
Socio-economic status^c				
Low	19%	18%	19%	19%
Medium	63%	65%	64%	64%
High	18%	17%	17%	18%

Gestational age (day^d)	52.4	53.2		
Season^e				
Winter	45.3%	46.7%	44.9%	46.1%
Fall /Spring	54.7%	53.3%	55.1%	53.9%

Table 1: Comparisons of demographic and clinical data among the groups

n= number (%), mean +/- SD, mean (min-max)

BMI= body mass index

^a p=0.908

^b p=0.916

^c p=0.216

^d p=0.478

^e p=0.341

	Group 1(n=50)	Group 2(n=50)	Group 3(n=50)	Group 4(n=50)
25(OH)D (ng/ml)	27.3 ± 1.2	47.64 ± 3.2 ^a	38.5 ± 5.1	11.6 ± 4.2 ^b
Ca (mg/dl)	8.6 ^d ± 0.12	10 ^c ± 0.13	9.8 ± 0.16	8.7 ± 0.20

Table 2: Comparison of laboratory data among the groups

a Significantly higher than the group 1, the group 3 and the group 4 (p = 0.001, p = 0.003, p = 0.000, respectively)

b Significantly lower when compared to the group 1 and the group 3 (p = 0.001 and p = 0.000, respectively)

c Significantly higher when compared to the group 1 and the group 4 (p = 0.005 and p = 0.042, respectively)

d Significantly lower when compared to the group 2 and the group 3 (p = 0.005 and p = 0.033, respectively)

Discussion

The effects of vitamin D on bone and calcium metabolism are well understood, while the roles on the cardiovascular system, central nervous system, immune system and reproductive system are still subject to researches. Vitamin D is considered to be a systemic steroid hormone [7] and basically strengthens innate immunity and regulates adaptive immunity [8]. Immune system cells; macrophages, dendritic cells, T cells and B cells has vitamin D receptor (VDR) and can produce 1,25-dihydroxyvitamin D; the active form of vitamin D [9].

The effects on the immune system are mainly through the balance between Th1 / Th2 cells. 1, 25-dihydroxyvitamin D is an inhibitor of Th1 cell proliferation and cytokines; it activates and upregulates Th2 cells and its functions [10]. Studies have shown that vitamin D deficiency plays a role in natural killer cell (NK) cytotoxicity, and it has been shown that the peripheral effects and cytotoxicity of CD19 + B, CD 56 + NK cells in women

with vitamin D deficiency are higher [3]. With these immune modulatory effects of vitamin D, it has been speculated that vitamin D could act as an immune regulator during implantation and play an important role in regulation of reproductive function [3].

Pregnancy is basically a process that's carried out on an immunological basis where the host-antigen relationship between mother and fetus is regulated [11]. Maternal immunosuppression is essential for a healthy pregnancy and humoral immunity will dominate during pregnancy. Maternal immunosuppression is provided by suppression of Th1 and NK cells and altering the balance of Th1/Th2 cells; thus rejection of the pregnancy is prevented³. While overexpression of Th1 cytokines may induce abortion, excessive Th2 activity is also one of the causes [12]. Lee et al. showed dominant Th1 immune response and increased NK cell levels and cytotoxicity in women with idiopathic recurrent pregnancy loss and multiple implantation failures (MIF) after in vitro fertilization

and embryo transfer cycles [13].

In early pregnancy, trophoblasts produce and respond to vitamin D, and some investigators have demonstrated that vitamin D triggers local anti-inflammatory responses and induces decasualization for healthy pregnancy [14].

A. Halhali et al. injected 1, 25-dihydroxyvitamin D₃ into uterine cavity of female rats at Day 5 of pseudopregnancy, at the time when endometrial sensitivity is maximal for the decidual reaction [15], and showed that it significantly increased uterine weight and induced decidual reaction [16] Yayla et al. evaluated placental volume and placental volumetric mean gray values in patients with vitamin D deficiency in first trimester of pregnancy. Vitamin D concentration was significantly associated with the placental volume. They recommend vitamin D screening and supplementation in first trimester of pregnancy [17].

Studies showed that maternal vitamin D deficiency is also associated with poor obstetric outcomes and pregnancy complications like preeclampsia, gestational diabetes (GDM), abortus, preterm birth, small for gestational age (SGA) [18].

Our study showed that low vitamin D and calcium levels are associated with early pregnancy loss. Women with history of abortion had lower vitamin D levels than healthy pregnant and non-pregnant controls. This can be due to immunomodulator and anti-inflammatory effects of vitamin D [19]. Hou et al. found low maternal serum vitamin D and 1 alpha hydroxylase (CYP27B1) levels in early pregnancy losses [20]. This can be due to low vitamin D and CYP27B1 levels in chorionic villi and decidua [21].

Anti-phospholipid syndrome is one of the well established cause of recurrent miscarriage. Several studies showed that low vitamin D levels are related with positive APA, anti-nuclear antigen antibody, anti-ss DNA and TPO antibody in women with recurrent pregnancy loss [22-23].

As a steroid hormone vitamin D has a significant role during pregnancy from implantation and decidualization phases of pregnancy. Although the current literature relates vitamin D deficiency to poor obstetric outcomes, conflicting results have been reported that vitamin D supplementation improves outcomes. There is insufficient data to recommend screening all pregnant women for vitamin D deficiency [24].

It is not possible to recommend routine screening and supplementation of vitamin D in early pregnancy, as prognosis of pregnancies receiving supplementation and the incidence of pregnancy related complications in follow-up are not known and well designed studies with long term follow up results needed. However it is advisable to consider prepregnancy

vitamin D screening in women having first trimester pregnancy loss history.

References

1. Saraf R1, Morton SM, Camargo CA Jr2, Grant CC. Global summary of maternal and newborn vitamin D status - a systematic review. *Matern Child Nutr.* 2016;12(4):647-668.
2. Stephanou A, Ross R, Handwerger S. Regulation of human placental lactogen expression by 1, 25-dihydroxyvitamin D₃. *Endocrinology.* 1994;135(6):2651-2656.
3. Ota K, Dambaeva S, Han AR, Beaman K, Gilman-Sachs A, Kwak-Kim J. Vitamin D deficiency may be a risk factor for recurrent pregnancy losses by increasing cellular immunity and autoimmunity. *Hum Reprod.* 2014;29(2):208-219.
4. Adams JS, Hewison M. Unexpected actions of vitamin D: new perspectives on the regulation of innate and adaptive immunity. *Nat Clin Pract Endocrinol Metab.* 2008; 4(2):80-90.
5. Marzi M, Vigano A, Trabattoni D, Villa ML, Salvaggio A, Clerici E, Clerici M. Characterization of type 1 and type 2 cytokine production profile in physiologic and pathologic human pregnancy. *Clin Exp Immunol.* 1996;106(1):127-133.
6. Matthiesen L, Ekerfelt C, Berg G, Ernerudh J. Increased numbers of circulating interferon-gamma- and interleukin-4-secreting cells during normal pregnancy. *Am J Reprod Immunol.* 1998;39(6):362-367.
7. Norman AW. Mini review: Vitamin D receptor: new assignments for an already busy receptor. *Endocrinology.* 2006;147(12):5542-5548.
8. Hou W, Yan XT, Bai CM, Zhang XW, Hui LY, Yu XW. Decreased serum vitamin D levels in early spontaneous pregnancy loss. *Eur J Clin Nutr.* 2016;70(9):1004-1008.
9. Adorini L, Penna G. Control of autoimmune diseases by the vitamin D endocrine system. *Nat Clin Pract Rheumatol.* 2008;4(8):404-412.
10. Lemire JM, Adams JS, Sakai R, Jordan SC. 1 Alpha, 25-dihydroxyvitamin D₃ suppresses proliferation and immunoglobulin production by normal human peripheral blood mononuclear cells. *J Clin Invest.* 1984;74(2):657-661.
11. Szekeres-Bartho J. Immunological relationship between the mother and the fetus. *Int Rev Immunol.* 2002;21(6):471-495.

12. Zarnani AH, Moazzeni SM, Shokri F, et al. Microenvironment of the feto-maternal interface protects the semiallogenic fetus through its immunomodulatory activity on dendritic cells. *Fertil Steril*. 2008;90(3):781-788.
13. Lee SK, Na BJ, Kim JY, et al. Determination of clinical cellular immune markers in women with recurrent pregnancy loss. *Am J Reprod Immunol*. 2013;70(5):398-411.
14. Barrera D, Avila E, Hernández G, et al. Estradiol and progesterone synthesis in human placenta is stimulated by calcitriol. *J Steroid Biochem Mol Biol*. 2007;103(3-5):529-532.
15. De Feo VJ. Temporal aspect of uterine sensitivity in the pseudopregnant or pregnant rat. *Endocrinology*. 1963;72:305-316.
16. Halhali A, Acker GM, Garabedian M. 1,25-Dihydroxyvitamin D3 induces in vivo the decidualization of rat endometrial cells. *J Reprod Fertil*. 1991;91(1):59-64.
17. Holick MF, Binkley NC, Bischoff-Ferrari HA, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab*. 2011; 96:1911-1930.
18. Wei SQ, Qi HP, Luo ZC, Fraser WD. Maternal vitamin D status and adverse pregnancy outcomes: a systematic review and meta-analysis. *J Matern Fetal Neonatal Med*. 2013; 26(9):889-899.
19. Twig G, Shina A, Amital H, Shoenfeld Y. Pathogenesis of infertility and recurrent pregnancy loss in thyroid autoimmunity. *J Autoimmun*. 2012;38(2-3):275-281.
20. Hou W, Yan XT, Bai CM, Zhang XW, Hui LY, Yu XW. Decreased serum vitamin D levels in early spontaneous pregnancy loss. *Eur J Clin Nutr*. 2016; 70:1004-1008.
21. Wang LQ, Yan XT, Yan CF, et al. Women with Recurrent Miscarriage Have Decreased Expression of 25-Hydroxyvitamin D3-1 α -Hydroxylase by the Fetal-Maternal Interface. *PLoS One*. 2016;11(12).
22. Kaider BD, Price DE, Roussev RG, Coulam CB. Antiphospholipid antibody prevalence in patients with IVF failure. *Am J Reprod Immunol*. 1996;35(4):388-393.
23. Agmon-Levin N, Blank M, Zandman-Goddard G, et al. Vitamin D: an instrumental factor in the anti-phospholipid syndrome by inhibition of tissue factor expression. *Ann Rheum Dis*. 2011;70(1):145-150.
24. ACOG Committee Opinion No. 495: Vitamin D: Screening and supplementation during pregnancy. *Obstet Gynecol*. 2011;118(1):197-198.