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Plasma 25-Hydroxyvitamin D and Severe Pre-Eclampsia in a Population With Profound Vitamin D Deficiency

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Abstract

Background: There is biologic and clinical evidence that links vitamin D deficiency to pre-eclampsia. The Vitamin D receptor is present in the placenta, cardiovascular system, and lymphocytes. It has anti-inflammatory, immune regulatory, and antihypertensive properties and facilitates placental implantation. Each of these processes is involved in the pathogenesis of pre-eclampsia.
Objectives: The main purpose of this study was to study the relationship between vitamin D deficiency and severe pre-eclampsia in a population residing in southern Iran that is generally known to have a high prevalence of vitamin deficiency. As a second objective, the prevalence of vitamin D deficiency in pregnant women was investigated.
Patients and Methods: We conducted a case-control study of 59 patients with severe preeclampsia and 217 controls, all of whom were from southern Iran. Cases and controls were matched for age, body mass index, and gestational age. The study was carried out in autumn and winter. Plasma 25-hydroxyvitamin D was measured using high performance liquid chromatography, and the results were compared between the two groups.
Results: Almost all controls and patients had 25-hydroxyvitamin D levels below normal, and 69% had levels below 10 ng/mL. The mean 25-hydroxyvitamin D levels in the patient and control groups were 8.4 (6.2) and 8.5 (6.9) ng/ml (P = 0.80), respectively. The level of 25-hydroxyvitamin D had no significant association with subjects' body mass index or age.
Conclusions: Pregnant women in our region have a high prevalence of vitamin D deficiency, and in a population with severe vitamin D deficiency, there is no significant correlation between 25-hydroxyvitamin D levels and preeclampsia. Severe deficiency masks any possible association in a case-control study. Controlled trials with vitamin D supplementation are recommended for further studies.

Keywords: 25-Hydroxyvitamin D, Preeclampsia, Vitamin D, Iran

1. Background

Vitamin D is a steroid vitamin-hormone that is mainly produced in the skin by exposure to ultraviolet radiation. It is converted to 25-hydroxyvitamin D (25-OH D) in the liver and then to 1, 25-dihydroxyvitamin D in the kidneys. The active form of the vitamin is 1, 25 dihydroxyvitamin D, and it has a short half-life of only several minutes, so the assessment of vitamin D status is determined by measuring 25-OH D, which has a half-life of about three weeks (1). The main role of vitamin D in humans is the maintenance of normal levels of calcium and phosphate by facilitating the absorption of calcium from the intestines and enhancing the mobilization of calcium from the bone (2).

Recent studies have shown a broader effect of vitamin D in humans. Vitamin D receptors have been found in many tissues, including lymphocytes, the cardiovascular system and placenta. It has immune regulatory, anti-inflammatory, and antihypertensive effects (3). Pre-eclampsia is a major health problem affecting 2% - 8% of pregnancies (4) and the pathogenesis of pre-eclampsia includes a number of processes that may be affected by vitamin D, including inflammation, immune dysfunction, hypertension, and abnormal placenta implantation (5-8). Vitamin D deficiency has been hypothesized to be linked to the development of pre-eclampsia, but the results of studies on the matter have been inconsistent.

Vitamin D deficiencies have been reported to as prevalent in pregnant Iranian women, affecting up to 80% of all cases (9, 10).

2. Objectives

The primary purpose of this study was to investigate the relationship between vitamin D deficiency and severe preeclampsia. As a secondary goal, the frequency of vitamin D deficiency in pregnancy in our population was also studied.

3. Patients and Methods

This was a case-control study conducted in autumn and winter of 2011 - 2012. The ethical board of Shiraz University of Medical Sciences approved the study design (approval No.: ec-P-90-2988). Patient and control consent was obtained for the collection of data and blood samples. Cases were recruited using the sequential method from the inpatient obstetric units at Shiraz University of Medical Sciences after confirmation of a diagnosis of severe pre-eclampsia, according to the American college of obstetrics and gynecology criteria (11). Patients with pre-gestational diabetes, renal disease, chronic hypertension, lupus, and multiple gestations were excluded. The hospitals where this study was conducted are tertiary referral centers. For each patient, about three controls with singleton gestation were recruited from ambulatory prenatal care units. Controls were matched according to age $\pm$ 5 years, gestational age $\pm$ 1 week, and body mass index (BMI). The use of vitamin D supplements was also recorded for each patient and control. The exclusion criteria for controls were the same as the patients. Blood samples were obtained, and the serum was separated and frozen within 30 minutes of collection. Levels of 25-OH D were measured by high performance liquid chromatography (HPLC, Agilent 1100, USA), and Vitamin D status was reported according to the Endocrine Society criteria: normal > 32 ng/mL, insufficient $\geq$ 20 and $\leq$ 32 ng/mL, deficient < 20 ng/mL, and severe deficiency < 10 ng/mL (12).

Statistical analyses were completed using SPSS 19. Comparison of means of continuous variables in the case and control groups was done with a t-test. The chi-square test was used to compare proportions in case and control groups. We used a multiple linear regression analysis to investigate the association of 25-OH D with maternal age, body mass index, gestational age, and season of year in the case and control groups. Binary logistic regression was used to evaluate the effect of each 5 ng/mL change in 25-OH D levels on the odds of severe pre-eclampsia in the whole study group. P-values less than 0.05 were considered significant.

4. Results

Fifty-nine patients and 217 controls were recruited for this study. The mean maternal age was 28.1 (5) years in patients and 26.3 (4.3) years in the control group. The mean BMI was 29.1 (3.9) kg/m^2 among patients and 27.6 (2.9) kg/m^2 in the control group. The patients had a mean gestational age of 33 weeks 4 days, and in the control group it was 33 weeks 5 days.

In their follow up examinations, none of the controls had developed preeclampsia. An analysis of 25-OH D showed that almost all patients and controls had a vitamin D deficiency or insufficiency, and only one individual in the control group had a normal level of 25-OH D. Sixty nine percent of all the study group had severe vitamin D deficiency (Table 1). The distribution of different degrees of vitamin D deficiency in the two groups is shown in Table 2. The mean 25-OH D level in the patient group was 8.4 (6.2) ng/mL, and it was 8.5 (6.9) ng/mL among the controls (P = 0.80). In a multiple linear regression analysis, BMI (P = 0.06), gestational age (P = 0.14), time of the year, winter versus autumn (P = 0.22), and maternal age (P = 0.12) in either group had no significant relationship with the 25-OH D level. In binary logistic regression analysis, which included the whole study population, the odds of developing pre-eclampsia for each 5-ng/mL rise in 25-OH D level was 0.9 (95% confidence interval = 0.7 - 2.2, P = 0.34). Fourteen patients and 49 controls were taking calcium-vitamin D supplements containing 200 units of vitamin D for at least one month. The proportions of subjects taking vitamin D supplement were not statistically different between the patient and control groups (23% versus 27%, P = 0.66). The level of 25-OH D among those taking vitamin D supplement was higher than others, 11.3 (5.8) versus 7.8 (6.9) ng/mL, but the difference was not significant (P = 0.14).

Table 1. Frequency of Different Levels of 25-OH D for the Entire Study Population (n = 276).

25-OH D level, ng/mL	No. (%)
< 10	190 (69)
10.1 - 20	64 (23)
20.1 - 32	22 (8)

Table 2. Comparison of Frequency of Different Levels of 25-OH D in Patients and Controls[a,b]

25OH D level, ng/mL	Case (n = 59)	Control (n = 217)
< 5	21 (36)	93 (43)
5.1 - 10	19 (32)	59 (27)
10.1 - 20	17 (28)	46 (21)
20.1 - 32	2 (4)	19 (9)

[a]Values are expressed as No. (%).
[b]The differences were not significant (P = 0.27).

5. Discussion

In our study, there was no association found between 25-OH D levels and the occurrence of pre-eclampsia in a population with severe vitamin D deficiency. In recent years, there has been much attention paid to studying this issue.

In a study by Baker and colleagues on 51 women with pre-eclampsia and 204 controls, 25-OH D levels of less than 20 ng/mL, compared with levels above 31 ng/mL, were associated with 4-times increase in the odds of developing pre-eclampsia (13). In Robinson and colleagues' study on 50 cases of pre-eclampsia and 100 controls, the level of 25-OH D in the patient group was significantly lower than among the controls (18 versus 32 ng/mL) and for each 10 ng/mL increase in 25-OH D level there was a 63% decrease in the odds of pre-eclampsia (14). In a study by Bodnar and colleagues, 25-OH D levels before 16 week of pregnancy were lower in those who later developed pre-eclampsia (18.9 versus 22 ng/mL) (15). In a controlled trial, vitamin D supplementation reduced the odds of pre-eclampsia by 27% (16). In contrast to previous reports, four case-control studies (17-20) and one controlled trial (21) failed to show any relationship between vitamin D and pre-eclampsia. Although the results of the studies have been discordant, a recent meta-analysis has pointed to an association between vitamin D deficiency and pre-eclampsia (22).

There are also biological mechanisms that link vitamin D deficiency to pre-eclampsia. The pathogenesis of pre-eclampsia has two stages (23). In the initial stage, there is decreased placental perfusion secondary to abnormal implantation. The hypo-perfused placenta then produces substances that initiate generalized endothelial dysfunction and inflammatory activation (23). Active vitamin D regulates genes that are involved in normal placental implantation and angiogenesis (8). It also regulates the immune response of the mother to the placenta, which is an important factor in normal implantation (6) and reduces the production of pro-inflammatory cytokines, which are involved in the pathogenesis of pre-eclampsia (24). Vitamin D also decreases blood pressure by suppressing the renin angiotensin system (7).

It is clear that there is both biological and clinical evidence to support the association between vitamin D deficiency and pre-eclampsia. The lack of such an association in our study may be due to the severity of our subjects' vitamin D deficiency. In most studies that showed an association, the protective effect of vitamin D was evident in those with 25-OH D levels above 30 ng/mL, and there is a threshold effect for vitamin D in the prevention of pre-eclampsia. In our sample study, there was only one individual in the control group with a 25-OH D above 30 ng/mL, so the severity of vitamin D deficiency may have masked any protective effect of the vitamin. In this situation, case-control studies lack adequate power to detect possible associations. Future randomized clinical trials in our population with vitamin D supplementation may be more conclusive. Sixty-three individuals in our study population were taking 200 IUs of vitamin D per day, but their average 25-OH D level remained in the deficient level, at 11.3 (5.8) ng/mL. This implies that small maintenance doses of 200 - 400 IUs of vitamin D cannot cure vitamin D deficiency in pregnancy. Larger daily doses of 1000 - 2000 IUs are recommended by the American college of gynecology and obstetrics (25), and some authors have suggested an even higher daily dose of 4,000 IUs (26).

An important finding of our study was that almost all of our sample study members had some degree of vitamin D deficit. Reports from other parts of Iran have had similar results (9,10). Vitamin D deficiency is also prevalent in non-pregnant women. In a recent study on women in Tehran, 69% of the study population had some degree of vitamin D deficiency (27). Our study population had a more severe degree of vitamin D deficiency than reported in other studies, and this may be due to the seasons of sampling (27). Our sampling was performed during the autumn and winter, when blood levels of 25-OH D are lowest due to the decrease in sunlight and excess clothing, which prevents the exposure of skin to ultra-violate light. Fars is a sunny area, but the tradition of avoiding sunlight may be a major factor in the development of women's vitamin D deficiencies. In a recent report from Shiraz, 95% of female university students had some degree of vitamin D deficiency, and sun protection was a major factor (28).

5.1. Conclusions

Pregnant women in our region have severe vitamin D deficiencies and, in such a population, there is no significant association between levels of 25-OH D and severe pre-eclampsia. Future randomized clinical trials with vitamin D supplements are needed to further investigate this issue.

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Footnotes

Authors' Contribution: Study concept and design: Mahmood Soveid; acquisition of data: Nasrin Asadi, Mahnoosh Sianati; data analysis: Mahmood Soveid, Nasrin Asadi, Mahnoosh Sianati; drafting of manuscript: Mahmood Soveid, Mahnoosh Sianati; critical revision of manuscript: Nasrin Asadi; statistical analysis: Mahnoosh Sianati; study supervision: Mahmood Soveid, Nasrin Asadi.

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References

1. Hollis BW, Wagner CL, Drezner MK, Binkley NC. Circulating vitamin D3 and 25-hydroxyvitamin D in humans: An important tool to define adequate nutritional vitamin D status. *J Steroid Biochem Mol Biol.* 2007;**103**(3-5):631–4. doi: 10.1016/j.jsbmb.2006.12.066. [PubMed: 17218096].
2. Ponsonby AL, Lucas RM, Lewis S, Halliday J. Vitamin D status during pregnancy and aspects of offspring health. *Nutrients.* 2010;**2**(3):389–407. doi: 10.3390/nu2030389. [PubMed: 22254029].
3. Bikle D. Nonclassic actions of vitamin D. *J Clin Endocrinol Metab.* 2009;**94**(1):26–34. doi: 10.1210/jc.2008-1454. [PubMed: 18854395].
4. Duley L. The global impact of pre-eclampsia and eclampsia. *Semin Perinatol.* 2009;**33**(3):130–7. doi: 10.1053/j.semperi.2009.02.010. [PubMed: 19464502].
5. Thota C, Farmer T, Garfield RE, Menon R, Al-Hendy A. Vitamin D elicits anti-inflammatory response, inhibits contractile-associated proteins, and modulates Toll-like receptors in human myometrial cells. *Reprod Sci.* 2013;**20**(4):463–75. doi: 10.1177/1933719112459225. [PubMed: 23012315].
6. Hewison M. An update on vitamin D and human immunity. *Clin Endocrinol (Oxf).* 2012;**76**(3):315–25. doi: 10.1111/j.1365-2265.2011.04261.x. [PubMed: 21995874].
7. Li YC, Kong J, Wei M, Chen ZF, Liu SQ, Cao LP. 1,25-Dihydroxyvitamin D(3) is a negative endocrine regulator of the renin-angiotensin system. *J Clin Invest.* 2002;**110**(2):229–38. doi: 10.1172/JCI15219. [PubMed: 12122115].
8. Evans KN, Bulmer JN, Kilby MD, Hewison M. Vitamin D and placental-decidual function. *J Soc Gynecol Investig.* 2004;**11**(5):263–71. doi: 10.1016/j.jsgi.2004.02.002. [PubMed: 15219879].
9. Kazemi A, Sharifi F, Jafari N, Mousavinasab N. High prevalence of vitamin D deficiency among pregnant women and their newborns in an Iranian population. *J Womens Health (Larchmt).* 2009;**18**(6):835–9. doi: 10.1089/jwh.2008.0954. [PubMed: 19514825].
10. Bassir M, Laborie S, Lapillonne A, Claris O, Chappuis MC, Salle BL. Vitamin D deficiency in Iranian mothers and their neonates: a pilot study. *Acta Paediatr.* 2001;**90**(5):577–9. [PubMed: 11430721].
11. Acog Committee on Practice Bulletins–Obstetrics . ACOG practice bulletin. Diagnosis and management of preeclampsia and eclampsia. Number 33, January 2002. *Obstet Gynecol.* 2002;**99**(1):159–67. [PubMed: 16175681].
12. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab.* 2011;**96**(7):1911–30. doi: 10.1210/jc.2011-0385. [PubMed: 21646368].
13. Baker AM, Haeri S, Camargo CJ, Espinola JA, Stuebe AM. A nested case-control study of midgestation vitamin D deficiency and risk of severe preeclampsia. *J Clin Endocrinol Metab.* 2010;**95**(11):5105–9. doi: 10.1210/jc.2010-0996. [PubMed: 20719829].
14. Robinson CJ, Alanis MC, Wagner CL, Hollis BW, Johnson DD. Plasma 25-hydroxyvitamin D levels in early-onset severe preeclampsia. *Am J Obstet Gynecol.* 2010;**203**(4):366 e1–6. doi: 10.1016/j.ajog.2010.06.036. [PubMed: 20692641].
15. Bodnar LM, Catov JM, Simhan HN, Holick MF, Powers RW, Roberts JM. Maternal vitamin D deficiency increases the risk of preeclampsia. *J Clin Endocrinol Metab.* 2007;**92**(9):3517–22. doi: 10.1210/jc.2007-0718. [PubMed: 17535985].
16. Haugen M, Brantsaeter AL, Trogstad L, Alexander J, Roth C, Magnus P, et al. Vitamin D supplementation and reduced risk of preeclampsia in nulliparous women. *Epidemiology.* 2009;**20**(5):720–6. doi: 10.1097/EDE.0b013e3181a70f08. [PubMed: 19451820].
17. Shand AW, Nassar N, Von Dadelszen P, Innis SM, Green TJ. Maternal vitamin D status in pregnancy and adverse pregnancy outcomes in a group at high risk for pre-eclampsia. *BJOG.* 2010;**117**(13):1593–8. doi: 10.1111/j.1471-0528.2010.02742.x. [PubMed: 21040394].
18. Yu CK, Ertl R, Skyfta E, Akolekar R, Nicolaides KH. Maternal serum vitamin D levels at 11-13 weeks of gestation in preeclampsia. *J Hum Hypertens.* 2013;**27**(2):115–8. doi: 10.1038/jhh.2012.1. [PubMed: 22336906].
19. Powe CE, Seely EW, Rana S, Bhan I, Ecker J, Karumanchi SA, et al. First trimester vitamin D, vitamin D binding protein, and subsequent preeclampsia. *Hypertension.* 2010;**56**(4):758–63. doi: 10.1161/HYPERTENSIONAHA.110.158238. [PubMed: 20733087].
20. Halhali A, Villa AR, Madrazo E, Soria MC, Mercado E, Diaz L, et al. Longitudinal changes in maternal serum 1,25-dihydroxyvitamin D and insulin like growth factor I levels in pregnant women who developed preeclampsia: comparison with normotensive pregnant women. *J Steroid Biochem Mol Biol.* 2004;**89-90**(1-5):553–6. doi: 10.1016/j.jsbmb.2004.03.069. [PubMed: 15225837].
21. Marya RK, Rathee S, Manrow M. Effect of calcium and vitamin D supplementation on toxaemia of pregnancy. *Gynecol Obstet Invest.* 1987;**24**(1):38–42. [PubMed: 3623260].
22. 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):889–99. doi: 10.3109/14767058.2013.765849. [PubMed: 23311886].
23. Roberts JM, Gammill HS. Preeclampsia: recent insights. *Hypertension.* 2005;**46**(6):1243–9. doi: 10.1161/01.HYP.0000188408.49896.c5. [PubMed: 16230510].
24. Zhang Y, Leung DY, Richers BN, Liu Y, Remigio LK, Riches DW, et al. Vitamin D inhibits monocyte/macrophage proinflammatory cytokine production by targeting MAPK phosphatase-1. *J Immunol.* 2012;**188**(5):2127–35. doi: 10.4049/jimmunol.1102412. [PubMed: 22301548].
25. Acog Committee on Obstetric Practice . ACOG Committee Opinion No. 495: Vitamin D: Screening and supplementation during pregnancy. *Obstet Gynecol.* 2011;**118**(1):197–8. doi: 10.1097/AOG.0b013e318227f06b. [PubMed: 21691184].
26. Hollis BW, Johnson D, Hulsey TC, Ebeling M, Wagner CL. Vitamin D supplementation during pregnancy: double-blind, randomized clinical trial of safety and effectiveness. *J Bone Miner Res.* 2011;**26**(10):2341–57. doi: 10.1002/jbmr.463. [PubMed: 21706518].
27. Alipour S, Saberi A, Seifollahi A, Shirzad N, Hosseini L. Risk factors and prevalence of vitamin d deficiency among Iranian women attending two university hospitals. *Iran Red Crescent Med J.* 2014;**16**(10):eee15461. doi: 10.5812/ircmj.15461. [PubMed: 25763193].
28. Faghih S, Abdolahzadeh M, Mohammadi M, Hasanzadeh J. Prevalence of vitamin d deficiency and its related factors among university students in shiraz, iran. *Int J Prev Med.* 2014;**5**(6):796–9. [PubMed: 25013702].