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Original Article

The relationship of serum vitamin D with pre-eclampsia in the Iranian women

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Abstract

Vitamin D deficiency may be a risk factor for negative outcome in pregnancy, such as pre-term labour, low birthweight, intrauterine growth retardation and gestational diabetes. This study aimed to evaluate the relationship between vitamin D and pre-eclampsia. This was a case–control study of 59 pre-eclamptic women and 59 healthy pregnant women selected in two hospitals in Ahvaz, Iran. Women with term singleton pregnancy, nulliparous and of reproductive age were selected. Venous blood samples (2 mL) were taken and the level of 25-dihydroxy vitamin D (25-OH-D) was measured. If the levels of 25-OH-D were less than 10 ng mL^{-1}, between 10 ng mL^{-1} and 29 ng mL^{-1} and more than 30 ng mL^{-1}, they were considered as indicating deficient, insufficient and normal 25-OH-D concentrations, respectively. The independent t-test, Mann–Whitney *U*-test, chi-square and logistic regression were used for analysing the data. Vitamin D deficiency was significantly higher in the pre-eclampsia group [odds ratio (OR) = 24.04, confidence interval (CI) = 2.10–274.8, $P = 0.01$]. Older women (30–35 years) were more likely to develop pre-eclampsia compared with the control group (OR = 10.36, CI = 2.18–49.09, $P = 0.003$). The results showed that women with body mass index (BMI) <20 were more likely to develop pre-eclampsia. The ages between 20 years and 30 years and normal BMI were not the risk factors for pre-eclampsia. Vitamin D deficiency has a statistically significant relationship with pre-eclampsia. It seems that the serum vitamin D levels are low in Iranian women because of their particular lifestyle and they may need more than 400 IU day^{-1} vitamin D supplement during pregnancy.

Keywords: pre-eclampsia, vitamin D, pregnancy, 25-OH-D.

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Introduction

Pre-eclampsia is a serious complication in pregnancy and is the second leading cause of maternal mortality and morbidity after haemorrhage (ACOG Committee on Obstetric Practice 2002). Pre-eclampsia is defined as blood pressure ≥140/90 mmHg and the presence of 0.3 g or more protein in a 24-h urine specimen arising after the 20th week of gestation in a previously normotensive woman (Report of the National High Blood Pressure Education Program 2000). The World Health Organization (WHO) announced that 35 197 maternal deaths occurred in 2006; of this number, 16.7% in developing countries, 9.1% in Africa and 25.7% in Latin America were due to hypertension disorders (Khan *et al*. 2006). In Iran, research in 2005 showed that the cause of 14% of maternal mortality was

pre-eclampsia (EmamiAfshar & Jalilvand 2006). Furthermore maternal and neonatal morbidities due to pre-eclampsia are high. Disseminated intravascular coagulation (DIC), intracranial haemorrhage, renal failure, retinal detachment, lung oedema, liver rupture and low birthweight are examples of such morbidity in mothers and neonates (Gibbs *et al*. 2008). The aetiology of pre-eclampsia is not known. Inadequate nutrients, especially protein, calcium, magnesium, selenium, vitamin A and C, are proposed as predisposing factors for pre-eclampsia (Homfmyer *et al*. 2007; Wallis & Saftlas 2008; and Rayman *et al*. 2003).

Some research has shown that vitamin D deficiency might be a risk factor for pre-eclampsia, pre-term labour, low birthweight, intrauterine growth retardation and gestational diabetes (Lapillonne 2010). Due to the increased demand for calcium, vitamin D has an essential role in the calcium metabolism, especially in the third trimester of pregnancy. Vitamin D is critical for maternal health, fetal skeletal growth, and favourable maternal and fetal outcomes. It is estimated that 5–50% of pregnant women have vitamin D insufficiency (Mulligan *et al*. 2010). The increase in the cytokines, e.g. tumour necrotizing factor-α, in pregnancies with vitamin D deficiency has been reported. Furthermore, the active metabolite of vitamin D (25-dihydroxy vitamin D) acts as a stimulating factor for T-cells, which, in turn, has a critical role in egg implantation (Lapillonne 2010). The placenta is responsible for regulating vitamin D during pregnancy. It is possible that in pre-eclampsia, due to the insufficiency in the supply of vitamin D to the placenta, the gastrointestinal absorption of calcium is also reduced. With a decrease in the level of calcium, parathyroid hormone is also increased and this may cause hypertension (Hulter *et al*. 1986).

Some researchers have reported that the serum level of vitamin D is significantly lower in women with pre-eclampsia compared with the healthy pregnant women (Bodnar *et al*. 2007; Lapillonne 2010; Robinson *et al*. 2010). It has also been suggested that using a vitamin D supplement could reduce the risk of pre-eclampsia and hypertension (Marya *et al*. 1987; Haugen *et al*. 2009). However, other researchers have not found any significant relationship between pregnancy outcome and serum level of vitamin D3 (Shand *et al*. 2010).

Because pre-eclampsia is one of the major causes of maternal mortality, the timely diagnosis and treatment could help to reduce maternal mortality (Sobande *et al*. 2007), particularly as Iran is included among those developing countries with a special culture and lifestyle; and reducing maternal mortality is one of the goals of the WHO. Furthermore, to the best of our knowledge, there is lack of information about the relationship of vitamin D and pre-eclampsia in Iran. This study aimed to explore the relationship between serum vitamin D level and pre-eclampsia.

Material and method

Study design and population

This case–control study was conducted on 59 pre-eclamptic women, who were selected from two educational hospitals (Razi and Imam Khomaini) in Ahvaz, Iran, from July to November 2012. Fifty-nine healthy pregnant women were selected among women who attended two hospitals with labour pain as the control group (the control group were not matched with the case group). This study was approved by the Ethics Committee in Ahvaz Jundishapur University of Medical Sciences and written informed consent was obtained from all participants prior to the study. The inclusion criteria were singleton pregnancy, nulliparous women of reproductive age and term pregnancy.

Key messages

- Pre-eclampsia is a leading cause of maternal mortality and morbidity.
- Vitamin D deficiency has a significant relationship with pre-eclampsia.
- It seems that serum vitamin D in Iranian pregnant women is low because of particular lifestyle and they may need more than 400 IU day vitamin D supplementation.

Women who had essential hypertension, diabetes, other medical problems (renal, liver, heart diseases and lupus), gestational hypertension and whose body mass index (BMI) is >30 were excluded. The BMI during the first trimester of pregnancy was calculated using the antenatal records. The criteria for diagnosis of pre-eclampsia are considered to be met if women had blood pressure ≥140/90 Hg and proteinuria ≥300 mg in the 24-h urine after the 20th week of gestation (Report of the National High Blood Pressure Education Program 2000). All participants were recruited if they were admitted in hospital for delivery or due to having signs and symptoms of pre-eclampsia. The diagnosis of pre-eclampsia was confirmed by a gynaecologist. A questionnaire was used for collecting socio-demographic data and a checklist was utilized for the results of the blood tests. To assess vitamin D supplementation, we asked participants about the regularity of their intake. Vitamin D3 is a regular supplement in the public health clinics of Iran. Before labour onset, 2-mL venous blood samples were drawn and banked within 30 min after collection. In the laboratory, blood samples were centrifuged and banked in –25° until evaluation. At the end of data collection, all the blood samples were sent to one reference laboratory (where they were kept in a cold box).

Laboratory method description

We measured the level of 25-dihydroxy vitamin D (25-OH-D) using an ELISA kit from Immunodiagnostic System Limited and validated against an HPLC method and results were reported in nanogram. The IDS 25-OH-D EIA kit is an enzyme immunoassay for quantitation of 25-OH-D. The measurement method consisted of enzymatic immunity for which the control was 3 (provided separately: IS-2730). Units in the quality control (QC) report are in nanomole per litre and nanogram per millilitre, while in Ahvaz's laboratory nanogram was used. The ranges for the controls are stated on the QC report for each kit LOT. IDS are part of the DEQAS scheme for vitamin D. The intra-assay mean according to the guideline of the kit for high and low human standards was 39 nmol L^{-1} with CV = 5.3, 67.1 nmol L^{-1} with CV = 5.6 and 165 nmol L^{-1} with CV = 6.7). The inter-assay mean was 40.3 nmol L^{-1} with CV = 4.6, 72 nmol L^{-1} with CV = 6.4 and132 nmol L^{-1} with CV = 8.7.

If the level of 25-OH-D was less than 10 ng mL^{-1}, it was considered as a deficiency; between 10 ng mL^{-1} and 29 ng mL^{-1}, it was considered as an insufficiency and >30 ng mL^{-1} was considered normal. All participants were followed until 24 h after completion of delivery. Pregnancy outcomes, i.e. pre-eclampsia in the healthy women group and newborn weight in the two groups were measured after delivery.

Statistical analyses

The Statistical Package for the Social Sciences (SPSS) version 19 (SPSS Inc., Chicago, IL, USA) was used for data entry and analysing. The Kolmogorov–Smirnov test was used for testing normality. The vitamin D level and BMI did not have normal distribution and the Mann–Whitney *U*-test was used for comparing two groups, while for the data with normal distribution, the independent *t*-test was used. The chi-square test was used for comparing categorical data between two groups. Logistic regression was used to estimate the effect of vitamin D levels on pre-eclampsia risk after adjusting for potential confounders (age and BMI).

Results

Compared with the control group, women in the pre-eclampsia group had a lower BMI, gestational age and newborn weight. Consequently, blood pressure of the cases (systolic and diastolic) was higher than that in the control group. The basic characteristics of the participants are listed in Table 1. None of the women in the healthy group demonstrated pre-eclampsia after delivery. Of 59 pre-eclamptic women, 46 (77.97%) had mild pre-eclampsia and 13 (22.03%) had severe pre-eclampsia. The mean serum 25-OH-D of women with severe pre-eclampsia tended to be lower compared with the women with mild pre-eclampsia (14.33 ± 9.8 ng mL^{-1} vs. 18.37 ± 14.4 ng mL^{-1}, $P = 0.34$) (results not shown in table). The mean serum 25-OH-D of women with

Table 1. Basic characteristics of participants in women with pre-eclampsia and healthy women

Characteristics	Pre-eclampsia	Healthy women	*P*-value
	n = 59	*n* = 59	
	Mean ± SD or N (%)		
Age	26.58 ± 5.3	23.31 ± 4.04	0.001
Gestational age (week)	37.5 ± 0.66	38.8 ± 1.23	0.001
BMI (kg m^{-2})	22.39 ± 3.6	23.6 ± 3.5	0.049
Systolic blood pressure (mmHg)	148 ± 10.5	109 ± 7.5	0.001
Diastolic blood pressure (mmHg)	93.9 ± 7.8	69.07 ± 8.1	0.001
Newborn weight (g)	2922 ± 525	3128 ± 353	0.014
Education			
Illiterate	10 (16.9)	5 (8.5)	0.28
Primary	19 (32.2)	23 (39)	
Secondary	15 (25.4)	21 (35.6)	
Diploma	15 (5.6)	11 (17)	

BMI, body mass index; SD, standard deviation.

pre-eclampsia was significantly lower compared with the control group (17.48 ng mL^{-1} = 43.4 nmo L^{-1} vs. 22.93 ng mL^{-1} = 57.23 nmo L^{-1}, $P = 0.001$). The two groups did not show any significant difference regarding taking vitamin D supplements and exposure to sunlight ($P > 0.05$, Table 2). We used multiple logistic regressions to investigate the relationship of maternal plasma 25-OH-D on the odds of getting pre-eclampsia. Vitamin D deficiency was significantly higher in the pre-eclampsia group [odds ratio (OR) = 24.04, confidence interval (CI) = 2.10–274.8, $P = 0.01$]. Older women (30–35 years) were more likely to get pre-eclampsia compared with the control group (OR = 10.36, CI = 2.18–49.09, $P = 0.003$).

In this study, we excluded women with BMI > 30; the results showed that women with BMI < 20 were more likely to get pre-eclampsia (Table 3).The ages between 20 years and 30 years and normal BMI were not risk factors for pre-eclampsia.

Discussion

Considering that the amount of vitamin D is dependent on sunlight exposure, diet and vitamin D supplementation, the vitamin D level remains unchanged or with a small change during pregnancy (Fernández-Alonso *et al*. 2012). Multivitamin supplementation, according to the protocol of the Ministry of Health in Iran, is distributed in the public health clinics from the beginning of the 13th week of gestation until 1 month after completion of delivery. These supplements contain 400 IU vitamin D3. Most of the women in the two groups received their multivitamin supplements regularly. In a study by Shand *et al*. (2010), the results showed that, despite taking vitamin D supplements (400 IU day^{-1}), 80% of pregnant women had an insufficiency or deficiency of vitamin D. These results are in line with our study. Less than half of the women in the two groups had adequate exposure to sunlight. It has been estimated that 10–15-min exposure to sunlight two or three times a week is sufficient to prevent vitamin D deficiency (Mahan *et al*. 2012). Seasons, geographic latitude, smog, cloud cover and using sun block creams are factors that affect the ultraviolet radiation and the vitamin D synthesis in the skin (Holick 2002).

A study in Iran showed that 86% of pregnant women during winter and 46% of them during summer had hypovitaminosis D (Kazemi *et al*. 2009). Ahvaz is located in the Southwest of Iran and lies on the latitude 31°′N; and the weather is very hot and sometimes humid during the summer. Having such a climate can provide sufficient ultraviolet radiation. The present study was conducted in the summer and fall. During fall, the temperature is around 30–35°C. Women in Iran (especially in urban areas) have to wear long black clothing, which can block the sunlight. Most women in the present study, who received enough sunlight exposure, lived in rural areas (where they do not cover their face and arms).

Mean newborn weight was lower in women with pre-eclampsia compared with the healthy pregnant women. Low birthweight in newborns can result from the reduction of placenta perfusion (Halhali *et al*. 2000). Other studies confirm our findings relating to newborn weight (Halhali *et al*. 2000; Pérez-López 2007). The mean newborn weight in the study by Robinson *et al*. (2010) was lower than the present study, which might be due to the fact that in their study, they only recruited cases of severe pre-eclampsia whereas we recruited cases of mild and severe pre-eclampsia.

Table 2. Characteristics related to Vitamin D level in women with pre-eclampsia and healthy women

Characteristics	Pre-eclampsia	Healthy women	*P*-value
	n = 59	*n* = 59	
	Mean ± SD or N (%)		
Regular taking of vitamin D supplement	37 (78.7)	44 (88)	0.21
Sufficient exposure to sunlight >15 min day^{-1}	23 (39)	21 (35.5)	0.47
25-OH-D ng mL^{-1}	17.48 ± 13.58	22.93 ± 11.36	0.001
Deficiency of 25-OH-D <10 ng mL^{-1}	17 (28.8)	1 (1.7)	0.001
Insufficient 25-OH-D 10–29 ng mL^{-1}	36 (61)	52 (88.1)	
Normal level of 25-OH-D	6 (10.2)	6 (10.2)	

25-OH-D, 25-dihydroxy vitamin D; SD, standard deviation.

Table 3. The unadjusted and adjusted odds ratios for variables related to pre-eclampsia

Variables	Unadjusted analysis	*P*-value*	Adjusted analysis	*P*-value*
	Odds ratio (95% CI)		Odds ratio (95% CI)[†]	
Deficiency of 25-OH-D	24 (2.10–274.8)	0.01	24.7 (2.14–285.4)	0.01
Insufficiency 25-OH-D	0.71 (0.18–2.69)	0.62	0.73 (0.19–2.79)	0.64
Age 20–30	2.42 (0.73–8.04)	0.14	2.51 (0.74–8.45)	0.13
Age 30–35	10.5 (2.21–49.85)	0.003	10.36 (2.18–49.09)	0.003
BMI <20 kg m^{-2}	4.26 (1.20–15.1)	0.02	5.05 (1.35–18.92)	0.01
Normal BMI kg m^{-2}	2.20 (0.75–6.19)	0.13	2.16 (0.76–6.22)	0.14

BMI, body mass index; CI, confidence interval. *Back Wald method. [†]Adjusted for age and BMI.

During pregnancy deciduas, placenta and maternal kidney production of 25-OH-D is increased, which in turn can increase the level of vitamin D of mothers. This function is necessary to increase the calcium absorption in the body of the pregnant women in order to increase the calcium requirements of the baby (Pérez-López 2007). In the present study, the mean 25-OH-D in women with pre-eclampsia was significantly lower compared with the control group. The results of some studies are in line with this study (Baker *et al*. 2010; Mulligan *et al*. 2010). Bodnar *et al*. (2007) found that the mean 25-OH-D in early pregnancy in women with pre-eclampsia was 45.4 nmol L^{-1} compared with the control group (53.1 nmol L^{-1}, $P = 0.01$). The average 25-OH-D in Bodnar *et al*.'s study was slightly higher compared with this study. They conducted the study on African–American and Caucasian women who had a vitamin D deficiency, mostly dark-skinned, African–American; and most of them (>90%) reported regular multivitamin use in the last trimester of pregnancy at a geographic latitude of 40°′N. The present study is almost in agreement with Bodnar *et al*.'s study, except that the women in our study were totally white and most of them took their vitamin D supplement regularly (78.7% in the pre-eclampsia and 88% in the healthy women group). Studies that have been conducted in Middle Eastern countries have shown that vitamin D deficiency is common among pregnant women (Taha *et al*., 1984; Bassir *et al*., 2001; Pehlivan *et al*. 2003); moreover, comparable studies in European countries have shown similar results (Brunvand & Haug 1993; Henriksen *et al*. 1995). It seems that in Bodnar *et al*.'s (2007) study, dark skin and high latitude impaired the coetaneous synthesis of vitamin D.

Some studies have reported that a vitamin D supplement of more than 400 IU can reduce the risk of pre-eclampsia. In a randomized controlled trial, researchers prescribed 1200 IU vitamin D plus

375 mg calcium in early pregnancy (20–24 weeks). The results showed that mean blood pressure was reduced significantly in the intervention group while the risk of pre-eclampsia remained unchanged in two groups (Marya *et al*. 1987). The current prenatal supplement in Iran contains 400 IU of vitamin D3 daily; however, some studies have shown that a dose of 4000 IU day^{-1} is sufficient to maintain a pregnant woman's serum level and also raise vitamin D in breast milk during infancy (Hollis & Wagner 2004). In this study, our results revealed that vitamin D deficiency is a significant risk (OR = 24.04, CI = 2.10–274.8, $P = 0.01$) for pre-eclampsia. In a nested case–control study, vitamin D deficiency <50 nmol L^{-1} in pregnancy was associated with almost fourfold odds of severe pre-eclampsia (Baker *et al*. 2010). The discrepancy between our study and Baker *et al*'s study may result from our measurements being taken in late pregnancy while those in Baker's study were taken in the middle of the pregnancy.

Strength and limitations of the study

This is the first time that the relationship between 25-OH-D and pre-eclampsia has been explored in Iran. Moreover the confounding factors – e.g. age, twin pregnancy and obesity – that have a strong relationship with pre-eclampsia have been controlled. However, variables, such as the use of vitamin D supplements and sunlight exposure, may be affected by recall bias. Furthermore there was no apparent relationship between dose–response and vitamin D in this study. This could be related to the limited sample size, which may be related to the choice of the fixed cut offs to categorize the vitamin D status. We were not able to measure the parathyroid hormone level, which is an important biomarker of vitamin D deficiency and this measurement should be considered in future studies.

Conclusion

Vitamin D deficiency has a statistically significant relationship with pre-eclampsia. It seems that serum vitamin D level is low in Iranian women because of the particular lifestyle and there may be a need for more than 400 IU day^{-1} vitamin D supplements during pregnancy.

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Conflicts of interest

The authors declare that they have no conflicts of interest. This paper is part of ZM's Masters Thesis.

Contributions

PA was supervisor of this thesis and contributed in design, interpretation, writing and finalising article in English. ZM was responsible for design, data collection and writing thesis in Persian. PA was co-supervisor of thesis and contributed in design and interpretation of data. ML was responsible in data analysing and interpretation.

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