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Gene variants and haplotypes of Vitamin D biosynthesis, transport, and function in preeclampsia

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ABSTRACT

Objective: To find whether the gene variants and haplotypes of cytochrome (CYP) 27B1 (1α-hydroxylase), group-specific component (GC) that is a vitamin D binding protein, vitamin D receptor (VDR), peroxisome proliferator-activated receptor γ (PPARγ) and retinoid-X receptor (RXR) affect the risk of preeclampsia.

Methods: In a case–control study 100 women with preeclampsia and 100 healthy pregnant women were investigated for gene variants and haplotypes of vitamin D biosynthesis, transport, and function using the polymerase chain reaction-restriction fragment length polymorphism method.

Results: The frequency of gene variants of PPARγ Pro12Ala and RXR -α (A/G, rs749759) were not significantly different comparing patients and controls. The TT genotype of CYP 27B1 (G > T) was associated with 2.2-fold (95% CI 1.04–4.7, p = 0.039) increased risk of early-onset preeclampsia. Also, the TT genotype of GC rs7041 (T > G) increased the risk of preeclampsia [OR = 2.13 (95% CI 1.09–4.17, p = 0.027)]. The VDR ApaI GT genotype elevated susceptibility to preeclampsia (OR = 2.55, p = 0.04). Further, the presence of VDR ApaI GT+TT genotype was associated with higher levels of body mass index, and systolic blood pressure, and lower level of 25 (OH)-D3. In the presence of haplotype CYP T, VDR T, and RXR A (TTA) compared to haplotype GTG the risk of preeclampsia was 6.71-fold (p = 0.044).

Conclusions: The present study indicated an association between the CYP 27B1, GC, and VDR ApaI variants with the risk of preeclampsia. Also, the variants of the latter polymorphism influenced BMI, blood pressure, and vitamin D levels.

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Introduction

Preeclampsia that is known as a serious complication of pregnancy is a multifactorial disorder defines as the occurrence of hypertension and proteinuria after 20 weeks of gestation in a previously normotensive woman. Abnormal placentation in the first trimester and the presence of maternal syndrome in the late second and third trimesters are known to be involved in the pathogenesis of preeclampsia (1). This disorder results from complex interactions between genetic and environmental factors. The prevalence of preeclampsia among Iranian pregnant women has been reported to be 7% (2).

There is a balance between vasoconstriction and vasodilation that is controlled by the genetic and epigenetic interaction. Vitamin D deficiency as an epigenetic factor shifts this balance to the vasoconstriction. An inverse correlation between blood pressure with 25 (OH)-D levels has been demonstrated (3). Vitamin D might play an important role in the pathology of preeclampsia through affecting blood pressure.

The metabolic pathway of vitamin D includes 1) hydroxylation of vitamin D by cytochrome (CYP) 2R1 hydroxylase in liver at the 25-C position that produces 25 (OH)-D3 (calcidiol) (4) and 2) hydroxylation of 25 (OH)-D by 1α-hydroxylase (CYP2R1 and CYP27B1) that convert it to 1,25 (OH)2-D3, the active form of vitamin D (5). In circulation, vitamin D and its metabolites bind to vitamin D binding protein that is encoded by a highly polymorphic gene named group-specific component (GC) (6). The active form of

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vitamin D, 1,25 (OH)2-D3, exerts its function through binding to the nuclear vitamin D receptor (VDR) and influences gene transcription in target tissues. Also, for effective interaction of VDR with DNA, the VDR performs a heterodimer with the retinoid-X receptor (RXR) (7). The RXR as a member of the nuclear hormone receptor superfamily binds to many nuclear receptors such as the peroxisome proliferator-activated receptor (PPAR) (8). Also, the VDR regulates the expression of thousands of genes through binding to DNA as complex of VDR/VDR or VDR/RXR heterodimers (9). Since polymorphism in some candidate genes in the metabolic pathway and function of vitamin D could affect the level of vitamin D and blood pressure, the aim of present study was to answer the question of whether polymorphism/haplotype of the genes coding the biosynthesis of active form of vitamin D, and the transporter of this vitamin in blood, along with the receptor complexes involved in the action of vitamin D, are involved in the risk of preeclampsia.

Materials and methods

Sample

The present case–control study consisted of 100 women with preeclampsia, the mean age of 31.4 ± 6.4 years, and 100 healthy women with normal pregnancy (the mean age of 29 ± 6 years). Among preeclamptic patients, there were 36 patients with severe preeclampsia. The samples were provided by women who referred to Imam Reza Hospital of Kermanshah University of Medical Sciences during March to September 2017. Women with multiple-birth pregnancy, known hypertension, diabetes, renal and cardiac diseases were excluded from the study. Patients with preeclampsia were parity-matched with healthy pregnant women. Both preeclamptic patients and controls did not receive vitamin D supplementation. Although the type of clothing in Iranian women is the highly covered type of dressing but to prevent the seasonal effect of sunlight exposure on the level of vitamin D, sampling of controls and patients was performed at the same season. The ethnic background of individuals was Kurds.

The preeclampsia was defined as systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg, the excretion of protein >300 mg in 24 hours, a urine protein: creatinine ratio of >0.3 and ≥30 mg/dl protein in a random urine sample (1+ reaction on a standard urine dipstick). Severe preeclampsia was defined as a blood pressure >160/110 mmHg, proteinuria >3+, headache, visual disturbances, upper abdominal pain, serum creatinine and transaminase elevation, thrombocytopenia, and fetal-growth restriction (10). Preeclampsia before 34 weeks of gestation that was defined as early-onset preeclampsia was detected in 23 patients.

Vitamin D

The status of vitamin D status was detected by the measurement of serum level of 25 (OH)-D3 using the immunodiagnostic systems limited (IDS) EIA kit as previously described (11).

Genotyping

The EDTA treated whole blood was used for DNA extraction by phenol-chloroform method (12). The integrity of DNA was evaluated by 1% agarose gel electrophoresis and the concentration and purity of extracted DNA were checked using a Nanodrop spectrophotometer (Thermo) through measurement of absorbance at the wavelength of 260 nm and calculating the ratio of absorbance at wavelength of 260 to 280 nm, respectively.

The CYP 27B1 rs10877012 G > T was detected by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) using the HinfI restriction enzyme (13). The GC rs7041 was determined by the PCR-RFLP method using the enzyme of HaeIII (6). For the detection of VDR-Apa I, the PCR-RFLP method by the enzyme Apa I was used (14). In all PCR experiments a sample without DNA, negative control, was used to indicate if there is contamination of the PCR experiment with foreign DNA. A reference DNA ladder contains a mixture of DNA pieces was used to indicate the presence of PCR product with known size.

The RXR -α (A/G, rs749759) were detected by PCR-RFLP using the BstXI restriction enzyme (15). The PPARγ Pro12Ala (C/G) gene variants were detected by PCR-RFLP using BstUI restriction enzyme (16).

An informed written consent was obtained from each individual before participation in the study. The Ethics Committee of Kermanshah University of Medical Sciences approved the study and the study was in accordance with the principles of the Declaration of Helsinki II.

Statistical analysis

The chromosome counting method was used to calculate the allelic frequencies (10). The significance of differences in genotype and allele frequencies of CYP27 B1, GC, VDR Apa I, RXR, and PPARγ polymorphisms between patients and controls were

calculated using the χ^2 test. The Odds ratios (OR) were calculated as the estimates of relative risk for disease, and 95% confidence intervals (CI) were obtained by SPSS logistic regression software. A two-tailed student's t-test was used to compare quantitative data. The SPSS (SPSS Inc., Chicago, IL, USA) statistical software package version 22.0 was used for statistical analysis. Haplotype analysis was performed by using SNPSTATS software (http://bioinfo.iconcologia.net/snpstats/start.htm).

Results

Table 1 indicates the characteristics of patients and controls. The mean gestational age in patients was 35.3 ± 2.5 weeks and in controls was 37.4 ± 2.3 weeks ($p < 0.001$). The mean levels of 25 (OH)-D3 was 16.6 ± 4.2 ng/ml (41.5 ± 10.5 nmol/L) in all preeclamptic patients that was significantly lower than controls (19.6 ± 3.8 ng/ml, $p < 0.001$) (Table 1).

CYP 27B1 (G > T) genotyping

Distribution of CYP 27B1 (G > T) genotypes was in Hardy–Weinberg equilibrium in both patients ($\chi^2 = 0.51$, $p > 0.1$) and controls ($\chi^2 = 1.18$, $p > 0.1$). The frequencies of CYP27B1 T allele were 32.5% and 34% in patients and controls, respectively ($p = 0.68$), that is depicted in Table 2. Comparing early-onset preeclampsia with late-onset preeclampsia indicated a significantly higher frequency of TT genotype (21.7% vs. 5.2%, $p = 0.03$) in the first group that was associated with 2.2-fold (95% CI 1.04–4.7, $p = 0.039$) risk of early-onset preeclampsia. However, the level of 25 (OH)-D3 was not significantly difference comparing early-onset (16.5 ± 3.6 ng/ml) with late-onset (16.7 ± 4.4 ng/ml) preeclampsia ($p = 0.084$).

Table 1. Characteristics of patients and controls.

Variables	All Patients n = 100	Severe Preeclampsia n = 36	Controls (n = 100)
Age (years)	31.4 ± 6.4 P = 0.01	29.8 ± 6.9 P = 0.57	29 ± 6
Gestational age (weeks)	35.3 ± 2.5 P < 0.001	35.1 ± 2.3 P < 0.001	37.4 ± 2.3
Before pregnancy weight (Kg)	71.5 ± 10.9 P = 0.042	70.7 ± 12.9 P = 0.3	68.1 ± 11.3
After pregnancy weight (Kg)	82.4 ± 13 P = 0.1	81.1 ± 14.5 P = 0.51	79.2 ± 12.1
Before pregnancy BMI (Kg/m^2)	26.6 ± 5.9 P < 0.001	26.1 ± 7.1 P < 0.032	22.1 ± 9.9
After pregnancy BMI (Kg/m^2)	30.5 ± 7.6 P < 0.001	30.5 ± 8 P = 0.012	24.7 ± 12.3
Systolic blood pressure (mmHg)	152.5 ± 16.5 P < 0.001	165.8 ± 18.3 P < 0.001	113.6 ± 7.6
Diastolic blood pressure (mmHg)	92.5 ± 14.3 P < 0.001	102.2 ± 11.4 P < 0.001	73.3 ± 6.6
25 (OH)-D (ng/ml)	16.60 ± 4.2 P < 0.001	17.3 ± 4.5 P = 0.012	19.6 ± 3.8
AST (U/L)	36.6 ± 41.7 P = 0.002	39.1 ± 45.6 P = 0.039	22.7 ± 8.3
ALT (U/L)	31.7 ± 62.9 P = 0.047	43.8 ± 98.2 P = 0.13	18.8 ± 9.7
Alkaline phosphatase (U/L)	331.5 ± 133 P = 0.001	330 ± 134.9 P = 0.016	260.4 ± 116.4

Table 2. Distribution of CYP 27B1 rs10877012 G > T genotypes and alleles in preeclamptic patients and controls.

Parameters	All preeclamptic patients n = 100 n (%)	Severe preeclampsia n = 36 n (%)	Controls n = 100 n (%)
CYP genotypes			
GG	44 (44)	10 (27.8)	46 (46)
GT	47 (47)	22 (61.1)	40 (40)
TT	9 (9)	4 (11.1)	14 (14)
Alleles			
G	135 (67.5)	46 (63.9)	132 (66)
T	65 (32.5)	26 (3.1)	68 (34)

*Overall χ^2 comparing three genotypes between all preeclamptic patients and controls was 1.69, $p = 0.42$

** Overall χ^2 comparing three genotypes between severe preeclamptic patients and controls was 0.24, $p = 0.88$

*** Overall χ^2 comparing alleles between patients and controls is 0.76, $p = 0.68$

GC (T > G) genotyping

Distribution of GC rs7041 (T > G) genotypes was in Hardy–Weinberg equilibrium in all preeclamptic patients ($\chi^2 = 1.31$, $p > 0.1$) and also in controls ($\chi^2 = 0.65$, $p > 0.1$). Comparing TT with TG genotype between preeclamptic patients with controls indicated a higher risk of preeclampsia in the presence of TT genotype compared to the TG genotype [OR = 2.13 (1.09–4.17, $p = 0.027$)]. In dominant genetic model of TT vs. TG+GG, the first genotype increased the risk of preeclampsia [OR = 2.08 (95% CI 1.12–4.0, $p = 0.021$)] (Table 3). In early-onset preeclampsia the frequencies of TT, TG and GG genotypes were 30.4%, 34.8%, and 34.8%, respectively, and in late-onset preeclampsia were 39%, 45.5%, and 15.6%, respectively ($p = 0.13$). No significant difference was found comparing the level of 25 (OH)-D3 between three genotypes of GC in patients ($p = 0.9$) and in controls ($p = 0.45$).

Genotyping of receptors (VDR, RXR)

In controls, distribution of VDR ApaI genotypes (G > T) was in Hardy–Weinberg equilibrium ($\chi^2 = 0.17$, $p > 0.1$). The frequency of VDR ApaI genotypes and alleles among preeclamptic patients and healthy individuals is demonstrated in Table 4.

Table 3. Comparing the distribution of GC rs7041 genotypes and alleles between preeclamptic patients and controls.

Parameters	All preeclamptic patients n = 100 n (%)	Severe preeclampsia n = 36 n (%)	Controls n = 100 n (%)
Codominant			
TT	37 (37) # χ^2 = 4.96 P = 0.026 OR = 2.13, 95%CI (1.09–4.17, P = 0.027)	13 (36.1)	22 (22)
TG	43 (43)	18 (50)	54 (54)
GG	20 (20)	5 (13.9)	24 (24)
Dominant	37 (37)	13 (36.1)	22 (22)
TT	63 (63)	23 (63.9)	78 (22)
TG+GG	χ^2 = 5.4	χ^2 = 2.76	24 (24)
Recessive	P = 0.02	P = 0.097	76 (76)
GG	OR = 2.08 (1.12–4.0, P = 0.021)	5 (13.9)	54 (54)
TG+TT	20 (20)	31(86.1)	46 (46)
Over	80 (80)	χ^2 = 1.61,	
dominant	χ^2 = 0.46, P = 0.49	P = 0.2	
TG	43 (43)	18 (50)	
TT+GG	57 (57)	18 (50)	
	χ^2 = 2.42, P = 0.12	χ^2 = 0.17, P = 0.68	
Alleles			
T	117 (58.5)	44 (61.1)	98 (49)
G	83 (41.5)	28 (38.9)	102 (51)

*Overall χ^2 comparing three genotypes between all preeclamptic patients and controls was 5.42, p = 0.066

** Overall χ^2 comparing three genotypes between severe preeclamptic patients and controls was 3.5, p = 0.18

***Overall χ^2 comparing alleles between all preeclamptic patients and controls was 3.63, p = 0.057

Table 4. Frequencies of VDR ApaI genotypes and alleles in preeclamptic patients and controls.

Parameters	All preeclamptic patients n = 100 n (%)	Severe preeclampsia n = 36 n (%)	Controls n = 100 n (%)
Codominant			
GG	9 (9)	4 (11.1)	17 (17)
GT	62 (62)	22 (61.1)	46 (46)
	χ^2 = 4.37, P = 0.037	χ^2 = 1.37,	
	#OR = 2.55, 95%CI (1.042–6.22, p = 0.04)	P = 0.24	
TT	29 (29)	10 (27.8)	37 (37)
Dominant	9 (9)	4 (11.1)	17 (17)
GG	91 (91)	32 (88.9)	37 (37)
GT+TT	χ^2 = 2.8, P = 0.09	χ^2 = 0.7,	46 (46)
Recessive	29 (29)	P = 0.4	
TT	71 (71)	10 (27.8)	
GT+GG	χ^2 = 1.4, P = 0.2	26 (72.2)	
Over	62 (62)	χ^2 = 0.99,	
dominant	38 (38)	P = 0.31	
GT	χ^2 = 5.15, P = 0.023	22 (61.1)	
GG+TT	OR = 1.92, 95%CI 1.09–3.66,	14 (38.9)	
	P = 0.024)	χ^2 = 2.4,	
		P = 0.12	
Alleles			
T	80 (40)	30 (41.7)	80 (40)
C	120 (60)	42 (58.3)	120 (60)

*Overall χ^2 comparing three genotypes between all preeclamptic patients and controls was 5.8, p = 0.055

** Overall χ^2 comparing three genotypes between severe preeclamptic patients and controls was 2.45, p = 0.29

*** Overall χ^2 comparing alleles between all preeclamptic and controls is 0.0, p = 0.1

The frequency of GT genotype was significantly higher among preeclamptic patients (62%, p = 0.04) compared to healthy individuals (46%) that was associated with the 2.55-fold increased risk of preeclampsia (p = 0.04) (Table 4). No significant difference was found comparing three genotypes between early-onset with late-onset preeclampsia (0.93).

In all individuals examining the dominant model of VDR ApaI indicated in the presence of GG genotype, the before and after pregnancy body mass index (BMI) was 20 ± 11.8 kg/m^2 and 22 ± 14.6 kg/m^2, respectively, compared to 25.4 ± 7.1 kg/m^2 (p = 0.049) and 28.8 ± 9 kg/m^2 (p = 0.043) in the presence of GT+TT, respectively. Also, in subjects, carrier of GT+TT genotype the systolic blood pressure was significantly higher (135.5 ± 23.8 mmHg, p = 0.026) compared to the GG genotype (124.8 ± 19 mmHg). Further, in the recessive model, the level of 25 (OH)-D3 was significantly lower (17.5 ± 4.2 ng/ml) in the presence of VDR ApaI GT +GG than the TT genotype (18.9 ± 4.4 ng/ml, p = 0.049).

Distribution of RXR -α (A/G) genotypes was in Hardy–Weinberg equilibrium only in preeclamptic patients (χ^2 = 3.71, p > 0.1). The absence of a significant difference was detected in the frequency of G allele comparing patients (60.6%, p = 0.69) with controls (62.5%) (Table 5). No significant difference was

Table 5. Distribution of PPARγ Pro12Ala (C/G) and RXR -α (A/G, rs749759) genotypes and alleles in preeclamptic patients and controls.

Parameters	All patients n = 100 n (%) χ^2 P	Severe preeclampsia n = 36 n (%) χ^2 P	Controls n = 100 n (%)
PPAR genotypes			
CC	75 (75)	26 (72.2)	77 (77)
CG	25 (25)	10 (27.8)	23 (23)
Alleles			
C	175 (87.5)	62 (86.1)	177 (88.5)
G	25 (12.5)	10 (13.9)	23 (11.5)
PXR genotypes			
AA	11 (11)	2 (5.6)	7 (7)
AG	57 (57)	21 (58.3)	61 (61)
GG	32 (32)	13 (36.1)	32 (32)
Alleles			
A	78 (39.4)	24 (34.3)	75 (37.5)
G	120 (60.6)	46 (65.7)	125 (62.5)
	0.15 0.69		

*Overall χ^2 comparing two genotypes of PPAR between all preeclamptic patients and controls was 0.11, p = 0.74

** Overall χ^2 comparing two genotypes of PPAR between severe preeclamptic patients and controls was 0.24, p = 0.88

**** Overall χ^2 comparing alleles of PPAR between patients and controls was 0.095, p = 0.75

#Overall χ^2 comparing three genotypes of RXR between all preeclamptic patients and controls was 1.024, p = 0.59

Overall χ^2 comparing three genotypes of RXR between severe preeclamptic patients and controls was 0.25, p = 0.88

Overall χ^2 comparing alleles of RXR between patients and controls was 0.15, p = 0.69

Table 6. Haplotype analysis of CYP 27B1, VDR ApaI, and RXR polymorphisms in patients and controls.

CYP	ApaI	RXR	Haplotype frequency Patients	Haplotype frequency Controls	OR (95%CI, p)
G	T	G	0.2326	0.3152	1.00
G	G	G	0.1435	0.1227	0.67 (0.24–1.89,45)
T	T	G	0.1709	0.0914	**4 (1.02–16.66,0.048)**
G	T	A	0.1863	0.0788	0.32 (0.10–1.02, 0.055)
G	G	A	0.1059	0.1398	1.33 (0.52–3.38, 0.56)
T	T	A	0.029	0.1156	**6.71 (1.07–42.16, 0.044)**
T	G	G	0.0569	0.0969	4.27 (0.69–26.41, 0.12)
T	G	A	0.0749	0.0395	0.25 (0.05–1.32, 0.1)

observed comparing three genotypes of RXR between early-onset with late-onset preeclampsia (0.89).

PPAR (C > G) genotyping

The PPAR (C > G) genotype's distribution was in Hardy–Weinberg equilibrium among preeclamptic patients (χ^2 = 2.04, p > 0.1) and also in controls (χ^2 = 1.69, p > 0.1). The frequency of PPAR CC genotype was 75% in patients that was not significantly different compared to controls (77%, p = 0.74) (Table 5). The frequency of CC genotype was 65.2% in early-onset preeclampsia compared to 77.9% in late-onset preeclampsia (OR = 1.88, 95%CI 0.68–5.2, p = 0.22).

Haplotype analysis

Haplotype analysis of three CYP 27B1, VDR ApaI and RXR polymorphisms is demonstrated in Table 6. The presence of haplotype CYP T, VDR T, and RXR G (TTG) compared to haplotype GTG was associated with 4 times enhanced risk of preeclampsia (p = 0.048). Also, in the presence of haplotype CYP T, VDR T, and RXR A (TTA) compared to haplotype GTG the risk of preeclampsia was 6.71-fold (p = 0.044).

Discussion

The active form of vitamin D is 1,25(OH)2-D3, but its precursor, 25 (OH)-D, is considered as an indicator of vitamin D status (17). The present study detected a significantly lower level of 25 (OH)-D3 in preeclamptic patients compared to controls. Regarding the suppression of the renin angiotensin aldosterone system by 1,25(OH)2-D3 to maintain a normotensive blood pressure (18), the low level of vitamin D through increasing blood pressure enhances the risk of preeclampsia.

Genetic background plays an important role in the individual variation of the circulating levels of 25 (OH)-D. In the present study, no significant differences were detected in the frequency of the CYP27B1 (G > T) genotypes and alleles between patients and controls. However, the presence of TT genotype of CYP27B1 was associated with 2.2 times the risk of early-onset preeclampsia. This polymorphism is known to be associated with the higher levels of 25 (OH)-D3 in the variant genotype of TT in gestational diabetes but its role on the level of 25 (OH)-D3 is unknown since the function of CYP27B1, conversion of 25 (OH)-D to the 1,25 (OH)2-D3, is downstream of circulating 25(OH)-D3 (19). In spite of the higher frequency of CYP27B1 TT genotype in early-onset preeclampsia compared to late-onset preeclampsia, the level of 25 (OH)-D3 was not significantly different comparing both groups. So, the role of this polymorphism in the risk of preeclampsia needs to be elucidated.

Regarding the gene of GC, which encodes vitamin D transporter, we detected that TT genotype of GC rs7041 (T > G) compared to TG genotype increased the risk of preeclampsia 2.13 times (P = 0.027). It has been reported that the GC (rs7041) polymorphism is related to different binding affinities for 25 (OH)-D (6). Reduced binding of 25 (OH)-D to GC protein might decrease the 25(OH)-D and other vitamin D metabolite levels. So, GC genetic variation might enhance the risk of preeclampsia through increased risk of vitamin D deficiency (20). In some studies, the GC polymorphism was significantly associated with lower serum levels of 25(OH)-D but the other reports did not confirm such association. We did not detect an association between 25(OH)-D level and GC polymorphism. Association of GC (rs7041) polymorphism with 25 (OH)-D is ethnic dependent as has been observed in some specific ethnic populations such as Arab and South Asian populations, but not in South East Asians. Also, it has been suggested that the polymorphism in the GC may influence the GC-bound fraction of 25(OH)-D, but did not affect on total 25(OH)-D levels (4). Further, discrepancies might in part be due to the effect of regular vitamin D supplementation in some studied populations and the season of sample collection (21).

The active form of vitamin D binds to nuclear VDR and affects the various genes and metabolic pathways. Previously, we suggested an association between VDR FokI polymorphism with the risk of preeclampsia (11). However, in the study of Rezende et al. none of the VDR polymorphisms including VDR ApaI was associated with the risk of hypertensive disorder of pregnancy (14). In the present study, the VDR GT compared to the wild genotype of GG increased the

risk of preeclampsia by 2.55-fold (P = 0.04). The GT +TT genotype of VDR compared to GG genotype was associated with a significantly higher level of BMI and systolic blood pressure. Further, the GT+GG genotype was related to a significantly lower level of 25 (OH)-D3 compared to TT genotype. Since 1,25 (OH)2-D3 through binding to VDR exerts its effect on the regulation of blood pressure (15) and the presence of this polymorphism (VDR Apa I) modulates the VDR expression through regulation of mRNA stability and efficiency of protein translation (22), the presence of this polymorphism through alteration in VDR expression affects on the systolic blood pressure and increased risk of preeclampsia. This hypothesis needs to be confirmed.

In our study, the RXR -α (A/G) was not associated with the risk of preeclampsia. To our best knowledge, there is no available report about the role of RXR variants in the development of preeclampsia but it has been reported that the variants of RXR have been associated with hyperlipidemia and renal cell carcinoma (23).

The PPAR (C > G) genotypes did not correlate with susceptibility to preeclampsia. However, the frequency of CC (Pro/Pro) genotype in early-onset preeclampsia was non significantly lower than late-onset preeclampsia. There are controversial reports related to the role of PPAR polymorphism in the risk of hypertension. Among the population of Qatar an association between the PPARγ2 Ala allele and hypertension has been reported (24). However, in postmenopausal women, Pro12Pro genotype was associated with the highest blood pressure compared with Pro12Ala genotype (25). In a meta-analysis by Cai et al. consisted of 4,151cases and 4,997 controls the PPARγ Pro12Ala polymorphism was associated with essential hypertension among Asians and the Ala allele decreased essential hypertension risk (26). Also, in a recent meta-analysis the Ala allele had a protective role against hypertension (27). The substitution of alanine for proline results in a significant change in protein structure and the function of PPARγ2 with the lower affinity of PPARγ2 for binding to the PPAR response elements in the target genes (24).

Haplotype analysis indicated that the haplotype CYP T, VDR T, and RXR A (TTA) compared to haplotype GTG significantly increased the risk of preeclampsia by 6.71-fold. It has been indicated that the heterodimer complex of VDR/RXR is a potent negative regulator of the renin gene transcription (28). The VDR and RXR agonists synergistically attenuate atherosclerosis progression through oxidative stress and inflammation inhibition (29). Also, the ablation of the CYP27B1 in knockout mice has been associated with increased tone in RAAS and hypertension (28). Since both CYP27B1, through synthesis of 1,25 (OH)2-D3, and receptors involved in the function of vitamin D (VDR and RXR) have a regulatory effect on the blood pressure the haplotype CYP T, VDR T, and RXR A might be involved in the development of hypertension and preeclampsia.

Conclusion

The present study indicated the low level of 25 (OH)-D was associated with the risk of preeclampsia. Also, we found an association between CYP27B1 polymorphism with the risk of early-onset preeclampsia. However, this polymorphism did not affect the level of 25 (OH)-D3 in these patients that might be due to low sample of patients with early-onset preeclampsia. The GC variant of vitamin D transporter was associated with the risk of preeclampsia but not with the 25 (OH)-D3 level. Since the variants of these genes have been studied for the first time in preeclamptic patients the mechanism of their effect on susceptibility to preeclampsia needs to be elucidated. Further, the VDR ApaI polymorphism was associated with higher BMI and systolic blood pressure and lower 25 (OH)-D3 level and also was correlated with susceptibility to preeclampsia Our study indicated the absence of association between the gene variants of PPARγ Pro12Ala and RXR -α with the risk of preeclampsia. Further, the haplotype CYP T, VDR T, and RXR A (TTA) compared to haplotype GTG was associated with the risk of preeclampsia. The clinical importance of our findings is considering the metabolism and the level of vitamin D during pregnancy in relation to genetic factors in women with vitamin D deficiency and/or insufficient level of this vitamin along with its supplementation to prevent increased risk of hypertension and preeclampsia.

Main findings

*The 25 (OH)-D deficiency was associated with the risk of preeclampsia.

* Polymorphism in the receptor of active form of vitamin D, VDR, increased blood pressure, and the risk of preeclampsia.

* Variant of CYP27B1 gene (involved in the biosynthesis of vitamin D) was correlated with the risk of early-onset preeclampsia.

*The variant of vitamin D transporter, GC, increased susceptibility to preeclampsia.

*The haplotype CYP T, VDR T, and RXR A (TTA) increased the risk of preeclampsia.

Disclosure statement

The authors declare that they have no conflict of interest.

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