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Association of arginine vasopressin (AVP) promoter polymorphisms with preeclampsia

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ABSTRACT

Objectives: Preeclampsia (PE) is a disease of pregnancy characterized by early onset of maternal hypertension and proteinuria. New findings indicate that arginine vasopressin (AVP) may be a contributing factor to ignite PE. The aim of this study was to identify if there is any correlation between arginine vasopressin promoter polymorphisms and PE.

Study design: Venous blood samples of 100 PE and 100 normal pregnant women were obtained for DNA extraction to identify the polymorphisms of AVP promoter by RFLP and nested-PCR techniques.

Main outcome: rs3729965 polymorphism of PE women was detected to have significant correlation with body mass index (BMI) ($P = 0.028$).

Results: Statistical analysis of three polymorphisms namely rs3729965, rs61138008 and rs3761249 of preeclamptic women (PEW) and none preeclamptic pregnant women (NPEW) revealed that rs3729965 genotypic distribution was significantly different between both groups ($P = 0.04$). Further analysis revealed that rs3729965 CT genotype of PEW had significant correlation to their BMI ($P = 0.028$).

Conclusion: Polymorphic variants located on the promoter region of AVP are associated with PE. Thus we hypothesize that allelic variation may have a role in increasing the risk of developing PE.

1. Introduction

One of the hypertensive disorders of pregnancy is preeclampsia (PE) [1]. It is associated with maternal and neonatal mortality and morbidity. PE is a disease of pregnant women, characterized by an early onset of hypertension (> 140/90 mmHg), proteinuria greater than 300 mg/day, preterm delivery, and low birth weight of the babies [2,3]. The incidence of PE is 1.5 to 2-fold higher in first compared with subsequent pregnancies. In this disease complication may involve a number of organs including kidney, liver, lung and even the brain [4]. Various pathways have been proposed for the evolution of PE [4]. Based on the epidemiological and basic science data experts in this field indicate that initial factors in the cadence of PE are ignited from the placenta. It is thought that an immune reaction like graft rejection involves the fetal placental unit [5]. Additionally, there are conditions that increase the risk of developing PE such as obesity and overweight during pregnancy when associated with hyperinsulinism, insulin resistance and maternal systemic inflammation [6]. This link of the obesity and development of PE is supported by growing epidemiological evidence [7]. Obesity is defined as increased body mass index (BMI) of greater than or equal to 30 kg/m^2 [8]. Even after extensive research worldwide, the mechanism(s) leading to PE is not completely understood. Hence for the management of patients, diagnosis and identification of potential biomarkers for early prediction of PE is important.

Arginine vasopressin (AVP), also known as anti-diuretic hormone or vasopressin, is one of the hormones involved in the regulation of blood pressure. The role of this hormone during pregnancy in PE has attracted attention of researchers [9]. AVP is made up of nine amino acid residues. The gene that encodes AVP is a member of the vasopressin/oxytocin family and a preproprotein that is proteolytically processed to generate multiple protein products. These proteins include the neuropeptide hormone AVP, and two other peptides, neurophysin 2 and copeptin. AVP is a posterior pituitary hormone that is synthesized as pre-

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provasopressin, containing 164 amino acids, in the supraoptic and paraventricular nuclei of the hypothalamus [10], and stored together with neurophysin II and copeptin. Role of AVP is essential in homeostasis of blood pressure through regulation of body fluid osmolarity and volume [11]. Multiple studies elucidated possible relationship between AVP and pregnancy and hence its role in pathophysiology of PE [5]. AVP exerts its effect through three distinct receptors, V1a, V1b, and V2.

AVP exhibits a short biological half-life (5–20 min in blood), so measurement of mature AVP is difficult and subject to pre-analytical errors. It is secreted in 1:1 stoichiometric ratio with a 39-amino acid glycopeptide that comprises the C-terminal part of the AVP precursor (CT-proAVP), the inactive pro-segment copeptin [12]. Therefore, for measurement of AVP, copeptin which is a stable peptide is used as a sensitive surrogate marker for AVP release, analogous to C-peptide for insulin measurement [13]. Some reports have shown the allelic effects of AVP rs3761249A in childhood-onset aggressive behavior [14] and association of TT genotype of rs3761249 with higher plasma concentration of copeptin [15]. AVP haplotype (rs3761249) may contribute to individual differences in childhood externalizing problems [16]. To the best of our knowledge no study has examined the association of AVP gene polymorphisms with preeclampsia.

AVP gene (Gene ID: 551) is located on the short arm of chromosome 20 (20p13). Up to 1284 polymorphisms (SNPs) for the AVP gene have been submitted in dbSNP until Feb 10, 2019 [17]. Thus we were intrigued to investigate whether the AVP promoter polymorphisms including rs3729965, rs3761249 and rs61138008 are associated with the criteria of PE.

2. Materials and methods

2.1. Subjects

Among pregnant women referred to hospitals of Jahrom University of Medical Sciences, two hundred age-matched subjects including 100 preeclamptic women (PEW) and 100 none preeclamptic pregnant women (NPEW) were recruited between February 2016 and March 2017 on the basis of study protocol. In the PEW group the women who had criteria of twin pregnancy, chronic disorders including kidney and liver disease, diabetes, cancer, and urinary tract infections were excluded from the study. NPEW group composed of women who were followed during pregnancy to assure that none develop the criteria of PE. The recorded clinical data and filled out questionnaire by each participant were collected. The criteria for this study were maternal age, gravida, birth weight, gestation at delivery, familial history of hypertension, weight, height, level of education, familial history of PE, previous pregnancy with PE, fetal sex and gender, and blood group. A physician and a nurse approved the criteria for diagnosing PE which included familial history of hypertension, preeclampsia in previous pregnancies, high systolic and diastolic blood pressure (SBP & DBP) of ≥140/90), increased calculated BMI of ≥30 kg/m^2 and proteinuria (≥300 mg/24 h or ≥ +1 on dipstick). Dipstick was used to test the urine sample for proteinuria. The study protocol was approved by research ethics committee of vise chancellery of research affairs of Jahrom University of Medical Sciences.

SNPs within regulatory regions with a minor allele frequency (MAF) > 10% were selected within arginine vasopressin promoter region.

2.2. DNA extraction

Whole blood samples from fasted subjects were collected in EDTA-containing tubes and rapidly stored at −80 °C. The genomic DNA was extracted from WBC using salting out method and stored at −20 °C [18].

The alleles at three positions were identified using Nested- and RFLP-PCR techniques. Reactions were performed in a micro-tube at a final volume of 25 μl containing $MgCl_2$ (1.5 mM), Hot start Taq DNA polymerase (0.8 U) and genomic DNA (0.2 μg). The temperature cycles for rs3761249 were 94 °C for 45 s, 65 °C for 45 s and 72 °C for 45 s; all for 30 cycles, and for rs61138008 were 94 °C for 45 s, 60 °C for 30 s and 72 °C for 45 s (n = 30), and for rs3729965 were 94 °C for 45 s, 64 °C for 45 s and 72 °C for 45 s (n = 35). Initial incubation period was 5 min at 94 °C and a final extension incubation step was set at 72 °C for 10 min for all reactions. Sequences of primers are summarized in Table 1.

Table 1
Sequence of primers.

Primers	Sequences
AVP8008F	5′- CAGTCTCGGCAACATAGCAAGAC- 3′
AVP8008R	5′- GAGCTGAGATCACGCCACTGC - 3′
AVP9965F	5′- GTATGCAGCACTGCTTGGTGG - 3′
AVP9965R	5′- CCTCGGTTCCTCTTACCCTCT - 3′
AVP1249F	5′- CCAGGACCTTAGTATGTGGCAACC- 3′
AVP1249R	5′- CTCACTGCCCTTGCTGGAGATG- 3′
1249.Nes.R	5′- GGCATTCTGGTGGCCCAGGGAGAG- 3′
1249.Nes.F	5′- ATAATTATATTA ATACCTAAATGGACCCAGCCTGAGAAAGCCACGT- 3′

Then, the PCR products of rs61138008 and rs3729965 were subjected to overnight digestion with BsaJI (Newengland Biolab; 10U, overnight) and FatI (Newengland Biolab; 8U) enzymes. Since there was no restriction enzyme site to cut the nested-PCR product containing the rs3761249 position thus, special primers were designed for overnight digestion and detection with AfI III enzyme (Newengland Biolab; 10U). The digested PCR products by BsaJI, FatI and AfI III enzymes were run on 2% agarose gel and visualized by UV transillumination after DNA Green Viewer staining [19].

2.3. Statistical analysis

Statistical software package (SPSS 18.0, Chicago) was used for statistical analyses. The numeric parameters were reported as Mean ± SD. Normality of the data was determined by Kolmogorov-Smirnov test. Hardy-Weinberg equilibrium was performed to evaluate allele distribution. Student-t and χ2tests were used to evaluate the differences between groups. The association of variables of preeclampsia and polymorphisms was tested by logistic regression. P value less than 0.05 was considered to be significant.

3. Results

3.1. Characteristic of the study population

This study recruited 100 PEW (single-ton) and 100 NPEW (single-ton). A description of clinical and demographic characteristics of the study population is provided in Table 2. All subjects were close to 26–29 years of age (NPEW; range from 18 to 44 years and PEW; range from 17 to 42 years). Our analysis revealed no significant differences of age between the both groups (P > 0.05). As expected, a significant statistical difference in systolic blood pressure (P < 0.001), diastolic blood pressure (P < 0.001), gestation week (P < 0.001) and birth weight (P < 0.001) was observed between the groups. The magnitude of BMI of PEW group was significantly higher than the NPEW group (P < 0.001). In addition, NPEW were followed over the pregnancy period and none of the subjects showed preeclampsia manifestations. Education level, fetal sex (data not shown) and blood groups showed no significant difference between both groups (P > 0.05). Familial history of preeclampsia was investigated in the PEW and NPEW groups. Frequency of preeclampsia in PEW group families (mother and sister) was 3 times more than the NPEW group (P = 0.025). PE in previous pregnancy in PEW group was more frequent than the NPEW group albeit not significant (P = 0.06). In addition, familial history of hypertension in

Table 2
Clinical and demographic characteristics of study population.

Parameters	NPEW	PEW	*P*- Value
Maternal age (years)	26 ± 5	29 ± 6	0.29
Gestation at delivery (week)	38.01 ± 5.62	31.93 ± 14.1	P < 0.001
Systolic blood pressure (mmHg)	112.35 ± 8.39	139.25 ± 19.8	P < 0.001
Diastolic blood pressure (mmHg)	68.4 ± 7.34	87.64 ± 11.06	P < 0.001
Birth weight(g)	3133.7 ± 544.95	2414.3 ± 1286.23	P < 0.001
Proteinuria (on dipstick)			
+1	NA	67 (67%)	
+2	NA	16 (16%)	
+3	NA	17 (17%)	
Gravida			P > 0.05
Primigravida	51 (51%)	51 (51%)	
Multigravida	49 (49%)	49 (49%)	
BMI (Kg/m^2)	32.84 ± 9.4	43.45 ± 17.78	0.001
Education			0.44
Under diploma	44	40	
Diploma-BSc	53	60	
Postgraduate	1	0	
Familial history of preeclampsia	4	13	0.25
Preeclampsia in previous pregnancies	1	9	0.06
Fetus sex			0.57
Male	49	54	
Female	49	46	
Blood group			0.134
A	23	30	
B	20	25	
AB	4	9	
O	51	36	
Familial history of hypertension	15	73	0.04

NPEW: None preeclamptic pregnant women, PEW: Preeclamptic women, NA: Not Applicable.

the PEW group was statistically significantly higher than the NPEW group (P = 0.04).

3.2. Genotype distributions

3.2.1. rs3729965 (C/T) genotype

As seen in Table 3 the C and T allele frequencies and the CC and TT genotype distributions had not significant differences between PEW and NPEW groups (P > 0.05). However, CT genotype distribution was significantly different between the groups (P = 0.04). Multinomial logistic regression analysis showed that the CT genotypes (containing T allele) are correlated to BMI in PEW group (P = 0.028).

3.2.2. rs3761249 (G/T) genotype

The GG, GT and TT genotype distributions and the T and G allele frequencies were not significantly different between NPEW and PEW groups (P > 0.05) (Table 3). Furthermore, the GT + TT versus GG distribution showed no significant difference between the groups. Multinomial logistic regression analysis showed that the genotypes containing T allele had no correlation with the other study variables in the groups (P > 0.05).

3.2.3. rs61138008 (G/A) genotype

No significant differences were found between the GG, GA and AA genotypes and the G and A allele frequencies of NPEW group compared to PEW group (P > 0.05) . The GA + AA distribution versus GG was not significantly different between the groups (Table3). Furthermore, Multinomial logistic regression analysis showed that the rs61138008 genotypes (containing A allele) had no correlation with other parameters in both groups (P < 0.05).

Table 3
Genotype and allele distribution of arginine vasopressin promoter polymorphisms.

Allele/Genotype		NPEW (n = 100)	PEW (n = 100)	P value
rs3729965				
Allele	C	146 (76%)	157 (84.4%)	NS
	T	46 (24%)	29 (15.6%)	NS
Genotype	CC	50 (52%)	66 (71%)	
	CT	46 (48%)	25 (26.9%)	0.04
	TT	0 (0%)	2 (2.1%)	–
	TT + CT	46	27	0.008
rs3761249				
Allele	G	118 (84.3%)	135 (89.4%)	NS
	T	22 (15.7%)	16 (10.6%)	NS
Genotype	GG	55 (78.6%)	65 (87.8%)	
	GT	8 (11.4%)	5 (6.8%)	NS
	TT	7 (10%)	4 (5.4%)	NS
	GT + TT	15	9	NS
rs61138008				
Allele	G	126 (69.3%)	126 (68.5%)	NS
	A	56 (30.7%)	58 (31.5%)	NS
Genotype	GG	40 (44%)	35 (38%)	NS
	GA	46 (50.5%	56 (61%)	NS
	AA	5 (5.5%)	1 (1%)	NS
	GA + AA	51	5	NS

NS: Not significant, NPEW: None preeclamptic pregnant women, PEW: Preeclamptic women.

4. Discussion

Preeclampsia is a multisystem and gestational kidney disease, characterized by sudden hypertension and proteinuria, which develops after 20 weeks of gestation in previously normotensive women. PE is one of the leading causes of maternal and neonatal morbidity and mortality. This disorder can cause hepatic and neurologic dysfunction [4]. Some diseases such as PE, metabolic syndrome, and cardiovascular diseases have common risk factors, including obesity, hypertension, dyslipidemia, hypercoagulability, and insulin resistance, and these conditions are characterized by endothelial dysfunction [2].

Some studies claimed that excess of weight gain during pregnancy or a pre-pregnancy state of obesity and overweight have been proposed as mechanisms that contribute to endothelial dysfunction, hypertension, proteinuria, thrombotic responses, multi-organ damage, and high maternal mortality and morbidity [6]. Our result showed greater BMI in PEW group compared to NPEW group. Familial history of preeclampsia in PEW families (Mother and sister) was 3 times more than the NPEW group. Phipps et al. declared that risk factors for the disease include maternal comorbidities, such as hypertension and obesity, a family history of PE as well as previous preeclampsia [20]. We found that Familial history of hypertension in the PEW group was significantly greater than the NPEW group. Ayorinde et al. surveyed 17,302 nulliparous women to assess the magnitude of familial risk of preeclampsia and gestational hypertension. They found that 424 (2.5%) and 2940 (17.0%) had maternal history of PE and gestational hypertension, respectively [21].

AVP increases arterial blood pressure through the reabsorbing of water back into the circulation from the filtrate in the kidney tubules of the nephrons and constricting the arterioles, which increases peripheral vascular resistance. AVP role(s) in normal and abnormal pregnancies has been investigated since 1950 s. Some studies demonstrated that chronic low-dose infusion of AVP into wild-type mice throughout gestation is sufficient to induce all of the cardinal maternal and fetal symptoms of preeclampsia [12].

Although the role of AVP gene and receptor polymorphism were investigated in some other diseases like diabetes mellitus, social and psychiatric traits, cognitive disorders, etc. however, our study is the first that examined the relation of AVP promoter polymorphisms with preeclampsia.

rs3729965 CT genotype distribution had a significant differences between the groups. To the best of our knowledge, this polymorphism was not investigated in previous studies. Because this polymorphism is located on the regulatory region of AVP gene, so we hypothesized that genetic variation may change the plasma concentration of AVP hormone in affected subjects. Previous studies have shown that, copeptin, a glycopeptide that comprises the C-terminal part of the AVP precursor, could be a stable marker for AVP release, analogous to C-peptide for insulin. Copeptin measurement has been shown to be useful in various clinical indications including PE [5,12,13]. We attempted to measure plasma concentration of copeptin. Unfortunately, this critical biomarker was destroyed in the processes of preservation. Therefore, future studies are needed to test whether this genetic variation of AVP gene could alter plasma concentration of copeptin. Anderson et al examined comprehensively the association between Hypothalamic pituitary adrenal-related (HPA) gene SNPs including rs3761249 and basal HPA-activity. They claimed that rs3761249 TT genotype is associated with saliva and plasma HPA outcomes [22]. In our study, allelic and genotype distributions of rs3761249 were not significantly different between the PEW and NPEW groups. Our finding also showed no association between rs3761249 and preeclampsia. Ayesha et al. studied the role of genetic variants in genes regulating the oxytocin-vasopressin neurohumoral system in childhood-onset aggregation [14]. rs3761249 C haplotype was more frequent in male cases as compared with the controls. They concluded that genetic variation in oxytocin-vasopressin system may be associated with childhood-onset aggression.

It should be noted that this is the first study that examine the rs61138008 allelic and genotype distribution in association with preeclampsia. We found no association between rs61138008 and PE.

Some investigators have considered the link between obesity and hypertensive disorder of pregnancy [23]. Therefore, we assessed the possible correlation of the variables within each group. We detected a positive correlation between BMI and rs3729965 CT genotype in the PEW group. Taken together our findings suggest that the rs3729965 polymorphism has correlation to PE through increased BMI. However, further studies are required to determine whether the presence of this genotype in overweight women leads to PE or the presence of this genotype in PE background results in increased BMI.

5. Conclusion

We hypothesized that at least one genetic variation in AVP promoter region may be associated with PE and contribute to preeclampsia morbidity. rs3729965 showed an association with PE but further studies are needed to show whether this polymorphism affect the phenotype. rs3729965 CT genotype in PE women with high BMI may be more prone to develop preeclampsia. It should be noted that our sample size was relatively small so the results should be interpreted with caution.

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Declaration of Competing Interest

None of the authors had any personal or financial conflict of interest.

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