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A Comparison of Apelin Rs56204867 and Apelin Receptor Rs11544374 Gene Polymorphisms and Their Association with Risk of Preeclampsia in Southeast Iran

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Abstract

Background: Pre-eclampsia (PE) is a severe pregnancy condition with genetic and environmental factors affecting the placental function and vascular changes. Genetic variants in the apelinergic system may influence preeclampsia risk and birth outcomes. Therefore, this study aimed to compare apelin (APLN) rs56204867 and apelin receptor (APLNR) rs11544374 gene polymorphisms and to investigate their association with mothers' body mass index and infant's birth weight among women with preeclampsia and control group in southeast Iran.

Methods: A total of 123 PE patients and 125 age- and gender-matched control subjects were enrolled in the study. The PCR–RFLP method was employed to genotype the APLN rs56204867 and APLNR rs11544374 gene polymorphisms.

Results: There was no significant association between the genotypes of the rs11544374 variant and the PE risk. The incidence of the AG genotype of the rs54204867 variant in the control group was considerably greater than in the PE group. Also, a significant relationship was found between the body mass profile of patients with PE and the APLN rs54204867 gene polymorphism.

Conclusions: It was observed that the APLN rs54204867 gene polymorphism could affect the PE risk. No significant difference was found between the PE group and the control group in terms of the genotypes of the APLNR rs11544374 variant. It was not statistically significant between mothers' BMI and rs11544374 of the APLNR gene, whereas an obvious link was observed between mothers' BMI and rs54204867 of the APLN gene.

Keywords: Apelin, Apelin receptor, Polymorphism, Preeclampsia.

Introduction

Preeclampsia (PE), as the most severe complication of gestation, is characterized by high blood pressure, proteinuria, edema, and often the IUGR (intrauterine growth restriction) (1, 2). It is associated with both genetic and environmental factors, contributing to the maladaptations of the placenta, inadequate remodeling of spiral

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arteries, the RUPP (reduced uteroplacental perfusion pressure), and the ischemic placenta. The ischemic placenta could promote the release of bioactive factors into the mother's circulation, resulting in imbalances among antiangiogenic factors such as sEng (soluble endoglin), proangiogenic vascular endothelial growth factor (VEGF), PlGF (placental growth factor), and TGF-β (transforming growth factor-β). These imbalances lead to a reduction in vasodilators like nitric oxide (NO) and an increase in vasoconstrictors including endothelin-1 (1). In addition to vascular dysfunction, PE is associated with hepatic complications. Hepatic involvement in preeclampsia can manifest as HELLP (hemolysis, elevated Liver enzymes, and low Platelets) syndrome or subcapsular hematoma. The pathophysiology involves *impaired invasion* of cytotrophoblast via the decidual spiral arteries, leading to deficient arterial remodeling (2). The incidence of PE is around 8% in first-time pregnancies, and it affects 8 million mother-infant pairs every year. It also contributes to at least 76,000 maternal deaths and 500,000 infant deaths globally (2, 3). Genetics ***plays a role*** in the the development of PE. Studies found that there are multiple gene variants related to an elevated risk of PE development (4-6).

The apelinergic system, which is composed of apelin (APLN) and its receptor (APLNR), has been implicated in various physiological and pathological conditions (7). Numerous studies have evaluated the effect of the apelinergic system on the complications of gestation like PE (8-12). The APLN, which is encoded by the APLN gene, is found on chromosome Xq25-q26 (13). A study has shown that APLN gene can have anti-inflammatory, antioxidant, and vasodilatory effects (14). In the non-human species, administration of exogenous apelin or its analogs has been found to improve symptoms associated with PE (15). APLN levels are reduced in women with PE compared to healthy healthy pregnant ones (16). In addition to APLN, other adipokines (including chemerin, visfatin, resistin, leptin, and adiponectin) have also been implicated in regulating reproductive functions and *the development of* reproductive disorders like PE. The expression of APLNR (APJ) has been shown in the human HPG (hypothalamic-pituitary-gonadal) axis. The secretion of GnRH (gonadotropin-releasing hormone), gonadotropins (FSH/LH), steroids (estradiol/progesterone/testosterone), and glucose transporters can be regulated by the APLN (8). APLN and its receptor have been shown to contribute to water homeostasis, the regulation of blood pressure of blood, balance of both sodium and water, high blood pressure, HF (heart failure), AKI (acute kidney injury), sepsis, diabetic nephropathy, and other renal disorders (9). This is the first study to evaluate the effect of APLN rs56204867 and APLNR rs11544374 gene polymorphisms on PE risk in southeast Iran.

Materials and Methods

Study design

A total of 123 PE patients and 125 age- and gender-matched controls were included in the study. Written informed consent was obtained from all participants. The Ethics Committee of Zahedan University of Medical Sciences, Zahedan, Iran approved the study protocol and PE was diagnosed by the guidelines recommended by the American College of Obstetricians and Gynecologists (ACOG) (17).

Inclusion and exclusion criteria

The study inclusion criteria were women who had given birth and suffered from PE, diagnosed by two obstetricians and gynecologists. Additionally, all women with normal pregnancy results were included in the control group Based on the report from the American College of Obstetricians and Gynecologists, a blood pressure reading equal to or higher than 140 mmHg systolic or 90 mmHg diastolic after the 20th week of pregnancy, along with proteinuria of at least 300 mg in 24 hours or a reading of +2 or higher (in a urine strip test) in a random urine sample, was used for the diagnosis of preeclampsia,

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which was confirmed by a gynecologist (17,18). The study's exclusion criteria were pregnant women with twin or multiple pregnancies and patients with gestational diabetes, polycystic ovary syndrome, kidney disease, liver disease, all systemic diseases, and lupus, or fetuses with hydatidiform mole and fetal hydrops (18).

DNA extraction and genotype analysis

The salting-out protocol was employed to extract the genomic DNA. To perform a polymerase chain reaction (PCR), primer pairs for a given sequence were designed for amplifying the polymorphic regions. PCR was performed using these specific primers (Table 1). The conditions were as follows: initial denaturation was carried out at 95 °C for 2 minutes, followed by 30 cycles of denaturation at 95 °C for 30 seconds, annealing temperature at 64 °C for 30 seconds for rs54204867, and 65 °C for 30 seconds for rs11544374, followed by extension at 72 °C for 30 seconds, and final extension at 72 °C for 3 minutes.

Table 1. Primers and PCR products of Apelin and apelin receptor gene variants.

SNP	Gene ID	Primer 5` → 3`		Product size (bp)	Restriction enzyme	Digest products (bp)
rs54204867	8862	F	GACCTAGAACAGTACCTGC	254	XhoI	A: 254 G: 156 & 90
		R	GAATGGTCTCCTGCTACCC			
rs11544374	187	F	CAGACTGGTTGTCTGCCCCA	215	Hpyf31	C: 215 T: 150 & 50
		R	GAGGCAGCTCCTCCTGAG			

The PCR product of rs5420487 was digested with XhoI, and **the product of** rs11544374 was digested **with DdeI,** incubated at 37°C **overnight.** Then, the safe-stained 2% agarose gel was used to perform the electrophoresis. The 100 bpDNA ladder was employed to determine the restriction fragment length polymorphism (RFLP) size. The UV light Gel Doc was applied to determine the types of polymorphic genotypes (Figs.1 & 2).

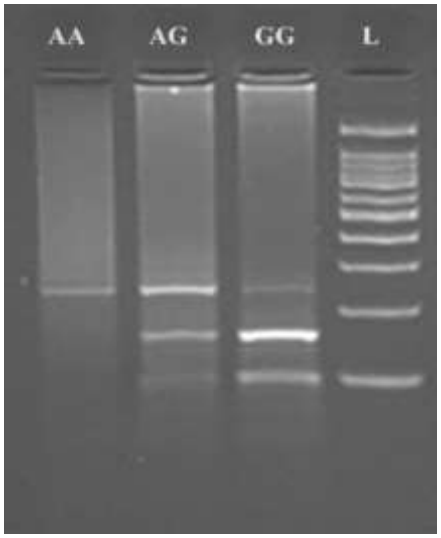

Fig. 1. Electrophoresis pattern after enzymatic digestion of rs54204867 polymorphism of Aplin gene. AA genotype (254 bp), AG genotype (90+156+254 bp), and GG genotype (90+156 bp) L: Ladder: 100 bp.

Fig. 2. Electrophoresis pattern after enzymatic digestion, rs11544374 polymorphism of Apelin receptor gene. Genotyp e CC (215 bp), Genotype CT (215+150+50 bp) and Genotype TT (150+50 bp) L: Ladder: 100 bp.

Statistical analysis

The data were analyzed using SPSS software version 26.0. Descriptive statistics, including frequency, percentage, mean, and standard deviation (SD) for both study groups (PE and control) were reported. Statistical differences between groups were examined using the

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Mann-Whitney U test or independent t-test. Furthermore, the effect of each polymorphism on the risk of PE was evaluated using the binary logistic regression model. The relative risk of disease was estimated using odds ratio (OR) with a 95% confidence interval (CI). The Chi-square, ANOVA, or Kruskal-Wallis tests were employed to investigate the relationship between clinical characteristics and different types of variants. P-values less than 0.05 were considered statistically significant.

Results

Considering that the two study groups were matched in terms of age, no significant difference was observed in their mean age. The mean gestational age of the PE group was substantially lower than the controls (36.61±2.94 weeks vs. 38.08±1.24 weeks) (P< 0.001). The average diastolic and systolic blood pressure in the PE group was considerably higher than the control group (P< 0.001). No significant difference was observed between the two groups in terms of the birth weights of the babies (Table 2).

The frequencies of CC, TC, and TT genotypes of APLNR rs11544374 gene polymorphism were found to be 6.5%, 42.3%, and 51.2% in the PE group, respectively, and 4.8%, 49.6%, and 45.6% in the control group. There was no significant difference between the groups in terms of the genotypes (P> 0.05). The frequencies of C and T alleles were 27.6% and 72.4% in the PE group, respectively, and 29.6% and 70.4% in the control group, respectively, showing no significant difference (P=0.63). Also, there was no correlation between this polymorphism and PE in dominant, recessive, and overdominant models (P> 0.05) (Table 3).

Table 2. Demographic and clinical characteristics of preeclampsia patients and controls. Results are reported as mean ±s SD or frequency (%).

Variable	Controls (N=125)	PE (N=123)	*P*-value
Maternal age (years)	28.58±5.49	27.28±6.37	0.116
Gestation age (weeks)	38.08±1.24	36.61±2.94	<0.001*
Birth weight (g)	3062.90±322.85	2884.37±646.05	.104
SBP (mmHg)	111.81±14.74	144.29±16.03	<0.001*
DBP (mmHg)	71.80±13.54	95.91±9.08	<0.001*
BMI (Kg/m^2)	24.71±3.03	25.71±3.07	0.010*
Family history			
Yes		41 (33.3)	
No		82 (66.7)	
Proteinuria Trace		10(8.1)	
1+		40(32.5)	
2+		34(27.6)	
3+		30(24.4)	
4+		9(7.3)	
History of hypertension in previous pregnancy			
Yes		24(19.5)	
No		99(80.5)	
Onset			
Early onset (N, %)		56(45.5)	
Late-onset (N, %)		67(54.5)	
Severity			
Mild (N, %)		101(82.1)	
Severe (N, %)		22(17.9)	

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Table 3. Genotypic and allelic distribution of Apelin receptor rs11544374 and apelin rs54204867 gene polymorphisms in preeclampsia (PE) and control groups.

	PE (N=123)	Control (N=125)	P-value	OR (95% CI)
Apelin receptor rs11544374				
TT, n (%)	63(51.2%)	57 (45.6%)	-	1
TC, n (%)	52(42.3%)	62 (49.6%)	0. 25	0.74(0.45-1.23)
CC, n (%)	8 (6.5%)	6 (4.8%)	0.56	1.38(0.46-4.10)
Dominant (TC+CC vs TT)			0.38	0.80 (0.46-1.32)
Recessive (CC vs TT+TC)			0.56	1.38 (0.46-4.10)
Overdominant (TC vs TT+CC)			0.52	0.74 (0.45-1.23)
Allele				
T, n (%)	178(72.4)	176(70.4)		1
C, n (%)	68 (27.6)	74 (29.6)	0.63	1.05 (0.87-1.27)
Apelin 54204867				
AA, n (%)	99 (80.5)	113 (90.4)	-	1
AG, n (%)	21 (17.1)	10 (8)	0.03*	.42(.18-.93)
GG, n (%)	3 (2.4)	2 (1.6)	0.64	1.54(0.25-9.36)
Dominant (AG+GG vs AA)			0.03*	2.28(1.09-4.80)
Recessive (GG vs AA+AG)			0.64	1.54(0.25-9.37)
Overdominant (AG vs AA+GG)			0.31	2.3(1.06-5.26)
Allele				
A, n (%)	208 (93.7)	247 (90.2)		1
G, n (%)	14 (6.3)	27 (9.8)	0.15	0.82 (0.65-1.04)

Table 4. Association between clinical characteristics of PE patients and Apelin and apelin receptor gene variants (n=123).

SNP	Genotype	BMI (Mean±SD)	Birth weight (Mean±SD)	Severity		Onset	
				Mild	Severe	Early	Late
rs11544374	TT	25.07±3.26	2968.01±496.21	52(82.5)	11(17.46)	31(49.2)	32(50.8)
	TC	25.31±2.94	2997.19±496.41	43(82.69)	9(17.3)	20(38.5)	32(61.5)
	CC	25.5756±2.80	2842.86±802.37	6(75.0)	2(25.0)	5(62.5)	3(37.5)
	P-value	0.793	0.545	0.749		0.313	
rs rs54204867	AA	25.28±3.11	2981.16±524.99	81(80.81)	18(81.18)	47(47/5)	52(52/5)
	AG	25.23±2.76	2897.87±489.60	17(81.95)	4(19.04)	8(38.1)	13(61/9)
	GG	21.66±1.52	3160±167.33	3(100)	0(0.0)	1(33/3)	2(66/7)
	P-value	0.028*	0.141	0.679		0.671	

The frequencies of GG, AG, and AA genotypes of the rs54204867 polymorphism of the APLN gene were found to be 2.4%,17.1%, 80.5%, obtained from the PE group and 1.6%, 8.0%, and 90.4% in the control group. The frequency of the AG genotype of the rs54204867 variant in the control group (17.1%) was considerably higher than the PE group (8%), which may act as a protective factor against PE development (OR=0.42; 95% CI=0.18-0.93; P= 0.03). The frequencies of G and A alleles were 6.3% and 93.7% in the PE group, respectively, and 90.2% and 9.8% in the control group, respectively, showing no significant difference (P=0.15). Also, there was a significant relationship between this polymorphism and PE in the dominant model (p=0.03), and no association was found in the recessive and overdominant models (P> 0.05) (Table 3).

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The average BMI for TT, TC, and CC genotypes was 25.07±3.26,25.31±2.94, and 25.57±2.80, respectively, which was not statistically significant (P=0.793). The average BMI (Kg/m2) for AA, AG, and GG genotypes was 25.28±3.11, 25.23±2.76, and 21.66±1.52, respectively. A significant relationship was found between the body mass profile of patients with PE and the APLNR rs54204867 gene polymorphism (P=0.028). The average birth weight (gram) for TT, TC, and CC genotypes was 2968.01±496.21, 2997.19±496.41, and 2842.86±802.37, respectively, which was not statistically significant (P=0.545). The average birth weight for AA, AG, and GG genotypes was 2981.16±524.99, 2897.87±489.60, and 3160±167.33, respectively, which was not statistically significant (P=0.141) (Table 4).

Discussion

The results of the present study indicated that the occurrence of the AG genotype of the rs54204867 APLN gene variant in the control group was considerably higher than that in the PE group, suggesting that this genotype may serve as a protective factor against preeclampsia. Moreover, there was no association between the APLNR rs11544374 gene polymorphisms and PE development. The observed relationship between mothers' BMI and rs54204867 suggests that this variant may influence BMI in mothers. This association could imply that the genetic influence of rs54204867 on BMI might also have implications for PE risk.

Various inherited, angiogenic, developmental, and metabolite deregulators have been linked to PE (19). rs56204867 (-1860 T > C) mapped in the promoter region of the APLN gene that encodes apelin. Apelin, as a vasodilator, affects blood pressure regulation (20,3). Therefore, changes in the coding regions or promoter may affect the susceptibility to disorders related to hypertension such as PE. Gandham et al. found that there was a significant difference between the groups in terms of the rs56204867 variant, so that the frequencies of the CC genotype and C allele in the PE group were higher than those in the controls (3). A meta-analysis did not show any association between rs3761581 and rs56204867 and systemic hypertension risk (21). Huang et al. in the Chinese population found positive associations of rs3115757 C allele, rs56204867 C allele, and rs3761581 A allele on an increased risk of high blood pressure (22). Yingxue et al. suggested a lower risk of myocardial infarction in carriers of AG and GG genotypes of rs56204867 (23). However, in Mexican patients, APLNR rs3761581 and rs56204867 gene polymorphisms had no significant association with blood pressure (24). Another study showed that carriers of rs3761581 were associated with high blood pressure, and men carrying rs3761581, and rs56204867 T-A haplotypes were associated with high blood pressure (25). Finally, the interaction effects of genetic variants on the apelin / APJ pathway may pose a potential risk in patients with chronic hypertension (26). Xiao-Dan et al. suggested a gender-dependent impact of apelin receptor gene polymorphism on hypertension risk, in which the rs10501367 had a significant association with hypertension risk in males and the rs11544374 in females (27). In a study conducted in northeastern China, significant differences were observed between patients with high blood pressure and the control group concerning for concerning genotypes and alleles of rs7119675 and rs11544374. Still, this association was independent of confounding factors including age, gender, and BMI (28). Moreover, apelin and leptin have potential physiological and pathological effects on cardiovascular homeostasis. A study showed that APLNR rs11544374 gene polymorphism is a predisposing factor contributing to CAD in the Iranian population (29). Another study performed in the Chinese population showed no significant difference between patients with metabolic syndrome and the control group regarding genotype or allele distribution of APLNR rs11544374 gene polymorphism (30).

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Conflict of interest
Every author state that they have no competing interests.

Ethical approval
Every procedure carried out in this study complied with the National Research

Committee ethical guidelines. Also, written informed consent was obtained from each participant. The ethics committee of ZUAMS confirmed the study protocol.

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