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Association between angiotensin converting enzyme gene polymorphism (rs4343) and susceptibility to gestational hypertension and preeclampsia in pregnant women

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Abstract

Hypertension is the most frequent medical complication of pregnancy, and the most severe clinical presentation of hypertensive disorders of pregnancy (HDP) is preeclampsia (PE). PE is a condition significantly associated with maternal and perinatal morbidity and mortality. The etiology of PE remains unknown. However, it has been found that genetic factors cause a defective immune adaptation, which in turn leads to inadequate trophoblast invasion and inappropriate placenta development. This study involved 30 patients with gestational hypertension (GH), 30 patients with PE and 30 normotensive pregnant women as controls. We aimed to evaluate the association between the angiotensin-converting enzyme (ACE) gene polymorphism (rs4343) and susceptibility to GH and PE. Genotyping for rs4343 polymorphism was performed by real-time polymerase chain reaction. The differences of genotypes and allele frequencies were analyzed as well as the relationship between *ACE* polymorphism and susceptibility to PE. The GG genotype of ACE gene rs4343 and G allele frequency were significantly associated with increased risk of PE [OR (95% CI) 10.3125 (2.1043 to 50.5388), p=0.004 and OR (95% CI) 3.4714 (1.6352 to 7.3697), p = 0.001, respectively]. Also, G allele frequency was significantly associated with severity of PE [OR (95% CI) 15.5455 (1.8938 to 127.6075), p = 0.011]. However, the GG genotype and G allele frequency were not associated with GH. In conclusion, ACE rs4343 polymorphism may be associated with PE susceptibility and severity but not with GH.

Keywords: ACE, Gestational hypertension, preeclampsia, polymorphism

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Introduction

Hypertensive disorders of pregnancy constitute one of the leading causes of maternal and perinatal mortality worldwide.[1] In Egypt, Ameen et al., 2023[2] reported that preeclampsia and eclampsia complicate about 6%-8% of all pregnancies and can reach up to 15% in referral centers like university hospitals and became

increasingly common during the COVID-19 pandemic, with preeclampsia and eclampsia prevalence rates of 19% and 1%, respectively.

Gestational hypertension (GH) is defined as a systolic blood pressure (SBP) 140 mm Hg or more or a diastolic blood pressure (DBP) of 90 mm Hg or more, or both, on two occasions at least 4 hours apart after 20 weeks of gestation, in a woman with a previously normal blood pressure.[3] GH occurs when hypertension without proteinuria or severe features develops after 20 weeks of gestation and blood pressure levels return to normal in the postpartum period.[4] Up to 50% of women with GH will eventually develop proteinuria or other end-organ dysfunction consistent with the diagnosis of preeclampsia (PE), and this progression is more likely when the hypertension is diagnosed before 32 weeks of gestation.[5]

Several studies have implicated the role of the renin-angiotensin system (RAS) in the development of obstetric complications since it is crucial for the uteroplacental function.[6,7,8] A major reticular activating system (RAS) component is the angiotensin-converting enzyme (ACE), which hydrolyses angiotensin I to angiotensin II, and not only regulates arterial pressure, but also fibrinolytic activity, indirectly, through the expression of plasminogen activator inhibitor.[9]

Little is known about the exact cause for RAS dysfunction occurring in preeclampsia, although many pathophysiological mechanisms have been suggested including autoimmunity, oxidative stress, and endothelial damage.[10] Several genetic studies have found gene polymorphisms in RAS components that explain variance in activity of this system.[11] Studying these polymorphisms showed both positive [12]and negative linkage with PE.[13]

Single nucleotide polymorphism (SNP), which accounts for more than 90% of human gene polymorphism, is the most common and stable gene variation in the human DNA chain [14]. It is generally used to determine the genetic variables that are associated with the disease. Their association with the disease is established when the occurrence is significantly different between cases and controls.[15]

The ACE gene is located in chromosome 17q23 and comprises several polymorphisms, including (I/D) and rs4343, that alter the enzyme activity.[8] ACE gene rs4343 (A2350G) polymorphism is associated with the increased ACE activity among normal individuals as well as in various pathological conditions.[8] An increased level of ACE activity is considered a key factor in blood pressure alteration because of the increase in the potent vasoconstrictor angiotensin II and inactivation of bradykinin as a vasodilator factor.[13]

As studies on the etiology of PE have suggested an inherited susceptibility, any relation between PE and genetic polymorphisms of the components of the RAS related genes will be of interest.[14] Therefore, the present research explored the relationship of ACE rs4343 polymorphism with the susceptibility to GH and PE. The primary aim of this study was to determine whether ACE gene rs4343 is associated with susceptibility to GH and PE. The secondary aim was to determine whether the association, if present, related to the degree of severity of GH and PE or not.

Subjects and Methods

In this case–control study, 90 pregnant females were recruited from Alzahraa University Hospital. Their ages ranged from 19-42 years. The study protocol was reviewed and approved by the Research Ethics Committee of the Faculty of Medicine (for Girls), Al-Azhar University (Approval number: 2022121644, dated March 2019). An informed consent was obtained from each study participant. Participants were divided into 3 groups: 30 patients with PE, 30 patients with GH and 30 age and parity-matched normotensive pregnant females. PE women were further subdivided into two subgroups 20 females with mild preeclampsia and 10 females with severe preeclampsia.

The defined criteria for GH were as SBP 140 mm Hg or more or a DBP of 90 mm Hg or more, or both, on two occasions at least 4 hours apart after 20 weeks of gestation, in a woman with a previously normal blood pressure. The criteria for PE were as SBP ≥ 140 mm Hg or DBP ≥ 90 mm Hg measured at least two times after 20

weeks of pregnancy, and the existence of proteinuria ≥ 300 mg/24 h or ≥ 1 + reading on dipstick in a random urine sample at least twice with no evidence of a urinary tract infection. Severe PE patients had SBP ≥ 160 mm Hg, DBP ≥ 110 mm Hg, and a proteinuria level of > 5 g/24 h or > 3 +. Patients who had history of hypertension before pregnancy, chronic kidney diseases, autoimmune and endocrine diseases were excluded from our study.

Assessment and Procedures

All participants of the study underwent the following laboratory investigations. Venous blood samples (6 ml) were collected from each subject after 8 hours fasting. Of these, 2 ml were placed into one EDTA (K_3EDTA) vacutainer for complete blood count (CBC) assay and 2 ml placed into a second EDTA vacutainer for the polymerase chain reaction (PCR) test. CBC was done immediately using fully automated cell counter (Sysmex KX21N, Kobe, Japan), according to the manufacturer's instructions. The second EDTA sample was kept in deep freezer (-80) for PCR test. The remaining blood was placed in a sterile vacutainer with a clot activator and left to clot for 30 minutes. Samples were centrifuged at 2000-3000 rpm for 20 minutes. The separated serum was used for immediate assay of fasting blood sugar (FBS), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and serum creatinine using fully automated chemistry analyzer (Cobas c 311, Germany), according to the manufacturer's instructions.

Also, 10 ml of morning urine samples were collected from each participant for urinary protein estimation by the dipstick method which is based on the change in the colour of the dye bromophenol blue, in the presence of a binding protein as albumin. The intensity of the blue colour is proportional to the concentration of protein in the specimen. This assay is semiquantitative as results of +1 corresponds to protein concentration of 30 mg/dL, +2 corresponds to protein concentration of 100 mg/dL, +3 corresponds to protein concentration of 300 mg/dL and+4 corresponds to protein concentration of 1000 mg/dL.

Estimated Glomerular Filtration Rate (eGFR) was calculated using Cockcroft- Gault equation.

= (140 − age (years) × weight (kg) × constant/serum creatinine (mg/dl)

Molecular analysis of blood samples

- *Genomic DNA extraction*

Genomic DNA was extracted from blood samples using commercially available kits (Lot. No.00672154, ThermoFisher Scientific Gene JET Whole Blood Genomic DNA Purification Mini Kit, Baltics UAB V. A. Graiciuno 8, LT-02241 Vilnius, Lithuania), according to the manufacturer's instructions.

- *Assessment of genomic DNA concentration and purity*

DNA concentration was assessed using the QIA Expert spectrophotometer (Qiagen, Hilden, Germany). The concentrations of DNA were determined by measuring the absorbance at 260 nm (A_{260}) and at 280 nm (A_{280}), respectively. The ratio of absorbance at 260 and 280 nm was used to assess DNA purity. A ratio of ∼1.8 was accepted as "pure" DNA. A lower ratio (≤1.6) may indicate the presence of proteins, phenol, or other contaminants. DNA concentration was estimated by measuring the absorbance at 260 nm, adjusting the A_{260} measurement for turbidity (measured by absorbance at 320 nm), multiplying by the dilution factor, and using the relationship that an A_{260} of 1.0 = 50 µg/ml pure dsDNA (DNA concentration µg/ml = (A260 reading –A320 reading) × dilution factor × 50 µg/ml).

- *Genotyping of ACE (rs4343) by Real-time PCR using TaqMan® SNP Genotyping Assay*

TaqMan® SNP genotyping Assay of ACE rs4343 was used (Applied Biosystems, Thermo Fisher Scientific, USA) The assay contained sequence-specific forward and reverse primers to amplify the polymorphic sequence of interest, two TaqMan® minor groove binder (MGB) probes with non-fluorescent quenchers (NFQ): one VIC™-labelled probe to detect the allele 1 sequence and one FAM™-labelled probe to detect allele 2 sequence (the context sequence

was [VIC/FAM]: CAGATCTGACGAATGTGATGG CCAC[A/G]TCCCGGAAATATGAAGACCTGTTAT).

The genotyping PCR reaction was conducted in 100 µl PCR tubes. Each reaction total volume was 20 µl, contained 10 µl of TaqMan Genotyping Master Mix (Lot. No. 00728613), 0.5 µl of TaqMan® SNP Genotyping Assay, and 9.5 u µl of diluted DNA template (5.5 µl nuclease free water, Lot No. 01069419, and 4 µl template). The PCR process consisted of 40 cycles, each of an initial denaturation at 95°C to separate the nucleic acid double chain, annealing at 58°C for binding of primers, and extension by DNA polymerase at 72°C. Data were collected and read based on fluorescence signals using a Rotor Gene real-time system (QIAGEN, Hilden, Germany). The allelic discrimination data were plotted as a comparison of allele 1 (VIC™ dye) and allele 2 (FAM™ dye) using real-time PCR instrument software (QIAGEN, Hilden, Germany). In Figures 1, 2 and 3, every specimen is represented as a separate curve that represents the amplified alleles and the specimen genotype.

Statistical analysis

The statistical analysis was performed using on the SAS software package (ver. 9.1.3; SAS Institute, Cary, NC, U.S.A.). The Hardy–Weinberg equilibrium (HWE) of the SNP genotypes was analyzed by the goodness-of-fit chi-square (χ^2) test to compare the observed and expected genotype frequencies amongst controls. For HWE, a p>0.05 was considered to be consistent with HWE. Association between ACE gene polymorphism (rs4343) and risk for PE and GH was assessed by logistic regression with odds ratio (OR) and 95% confidence interval (CI). Chi-square (χ^2) test was used to assess the difference in the demographic characteristics, variables, and the genotypes of the ACE gene (rs4343) polymorphism. Post Hoc test: Tukey's test was used for multiple comparisons between different variables. A $p<0.05$ was considered significant.

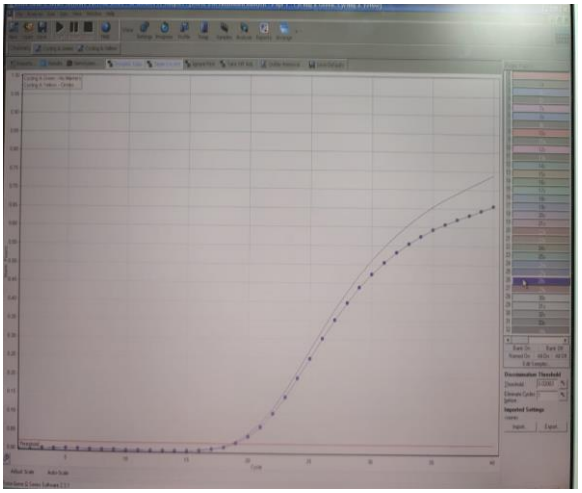

Figure 1. Amplification plot showing a case with heterozygous genotype (AG) for ACE gene rs4343. It shows amplification of both A allele and G allele. G allele is represented by curve with circles and A allele is represented by curve without circles.

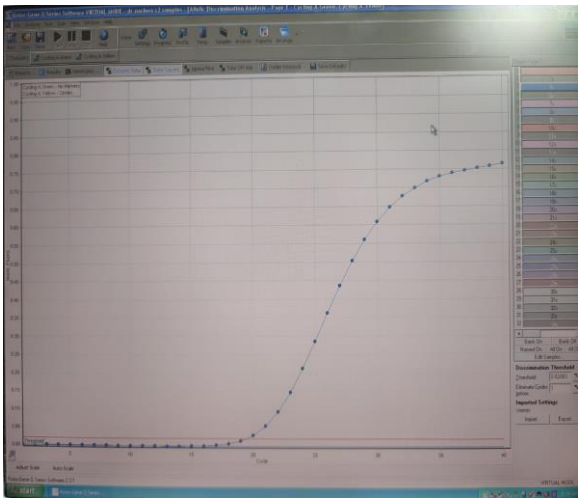

Figure 2. Amplification plot showing a case with homozygous genotype (GG) for ACE gene rs4343.
It shows amplification of G allele with no amplification of A allele.

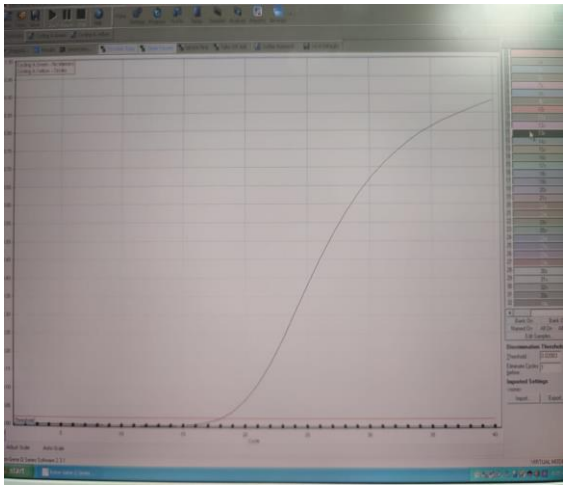

Figure 3. Amplification plot showing a case with homozygous genotype (AA) for ACE gene rs4343. It shows amplification of A allele with no amplification of G allele.

Results

The characteristics of the study patients and controls are demonstrated in Table 1. Blood pressure and urine protein were significantly higher in the PE group than the GH and the control groups (Table 1). Blood pressure and urine protein were also higher in the severe PE than in the mild PE (p<0.001, Table 2). The distribution of ACE rs4343 genotypes was consistent with the HWE equilibrium in the control, GH and PE groups (p=0.752, p=0.273, and p=0.273, respectively).

Table 1. Comparison between age, laboratory parameters, systolic and diastolic blood pressure in the studied groups.

Study parameters		Control group	GH group	PE group	*p*-value
		No. = 30	No. = 30	No. = 30	
Age (years)	Mean ± SD	28.63 ± 6.41	29.67 ± 3.70	31.67 ± 6.57	NS
	Range	19 – 42	24 – 36	20 – 42	
Hemoglobin (g/dl)	Mean ± SD	10.87 ± 0.93	11.21 ± 1.00	10.95 ± 0.88	NS
	Range	10 – 13	10 – 13.1	10 – 12.9	
Total leucocytic count (10^3/mm^3)	Mean ± SD	7.86 ± 1.33	8.17 ± 1.68	7.98 ± 1.94	NS
	Range	5.3 – 10.1	4.5 – 11	4 – 11	
Platelets (10^3/mm^3)	Mean ± SD	252.77 ± 87.93	274.47 ± 63.86	265.53 ± 57.74	NS
	Range	137 – 489	170 – 410	151 – 360	
ALT (U/L)	Mean ± SD	17.11 ± 5.19	15.53 ± 4.22	15.58 ± 5.43	NS
	Range	8.4 – 28	10 – 22	8 – 28	
AST (U/L)	Mean ± SD	19.76 ± 5.19	18.30 ± 4.47	18.67 ± 4.31	NS
	Range	13 – 30	12 – 28	13 – 30	
Creatinine (mg/dl)	Mean ± SD	0.55 ± 0.14	0.54 ± 0.09	0.50 ± 0.11	NS
	Range	0.3 – 0.8	0.4 – 0.7	0.3 – 0.7	
eGFR (ml/min/1.73 m^2)	Mean ± SD	109.07 ± 6.45	106.90 ± 6.60	106.10 ± 6.31	NS
	Range	95 – 116	95 – 116	95 – 116	
FBS (mg/dl)	Mean ± SD	82.80 ± 8.38	80.40 ± 8.48	82.30 ± 11.14	NS
	Range	70 – 99	69 – 99	47 – 96	
Protein in urine (mg/dl)	Mean ± SD	21.97 ± 2.16	20.67 ± 2.06	154.67 ± 115.90	<0.0001
	Range	19 – 26	17 – 25	30 – 300	
Systolic BP	Mean ± SD	111.83 ± 6.88	154.00 ± 16.99	160.33 ± 19.69	<0.0001
	Range	100 – 120	140 – 200	140 – 230	
Diastolic BP	Mean ± SD	71.83 ± 6.50	96.50 ± 9.02	100.83 ± 10.35	<0.0001
	Range	60 – 80	90 – 115	90 – 130	

PE (Preeclampsia), GH (Gestational Hypertension), FBS (Fasting blood sugar), ALT (Alanine Aminotransferase), AST (Aspartate Aminotransferase), eGFR (Estimated Glomerular Filtration Rate), SBP (Systolic Blood Pressure), DBP (Diastolic Blood Pressure). P > 0.05 is not significant (NS).

Table 2. Comparison between subtypes of PE as regard age, laboratory parameters, systolic and diastolic blood pressure.

Study parameters		Mild	Severe	*p*-value
		No. = 20	No. = 10	
Hemoglobin (g/dl)	Mean ± SD	10.93 ± 0.87	11.00 ± 0.94	NS
	Range	10 – 12.9	10 – 12.7	
Total leucocytic count (10^3/mm^3)	Mean ± SD	7.80 ± 1.99	8.34 ± 1.88	NS
	Range	4 – 11	5.5 – 11	
Platelets (10^3/mm^3)	Mean ± SD	258.10 ± 60.57	280.40 ± 51.28	NS
	Range	151 – 350	209 – 360	
ALT (U/L)	Mean ± SD	15.62 ± 5.05	15.50 ± 6.42	NS
	Range	8 – 23	9 – 28	
AST (U/L)	Mean ± SD	18.65 ± 3.68	18.70 ± 5.60	NS
	Range	13 – 27	13 – 30	
Creatinine (mg/dl)	Mean ± SD	0.52 ± 0.10	0.46 ± 0.12	NS
	Range	0.36 – 0.7	0.3 – 0.7	
Portion in urine	Mean ± SD	82.00 ± 61.87	300.00 ± 0.00	<0.0001
	Range	30 – 300	300 – 300	
eGFR (ml/min/1.73 m^2)	Mean ± SD	106.80 ± 6.53	104.70 ± 5.91	NS
	Range	99 – 116	95 – 114	
Systolic BP	Mean ± SD	150.50 ± 6.67	180.00 ± 22.61	<0.0001
	Range	140 – 170	160 – 230	
Diastolic BP	Mean ± SD	95.00 ± 6.07	112.50 ± 6.35	<0.0001
	Range	90 – 110	110 – 130	

ALT (Alanine Aminotransferase), AST (Aspartate Aminotransferase), eGFR (Estimated Glomerular Filtration Rate), SBP (Systolic Blood Pressure), DBP (Diastolic Blood Pressure), $P > 0.05$ is not significant (NS).

Table 3 displays the genotype and allele frequencies for rs4343 polymorphism in the studied groups. GG genotype was significantly higher in the PE group (50.0%) compared with the control group (13.3%) (OR = 10.3, 95% CI, 2.1-50.5; *p*=0.004). G allele frequency was significantly higher in PE group (68.3%) compared with the control group (38.3%) (OR = 3.4, 95% CI, 1.63-7.36; *p*=0.001). Moreover, the genotype frequencies were different in the PE and Control groups, being under dominant (OR = 3.7632, 95% CI, 1.0377-13.6468; *p*=0.043) and recessive (OR = 6.5000, 95% CI, 1.8201-23.2136; *p*=0.004) inheritance models. However, we did not observe any difference in the genotype and allele frequencies or in any of the inheritance (dominant and recessive) between the GH patients and controls.

Upon comparing the allelic distribution and genotypes of rs4343 between the PE and the GH groups, there was a significant increase in the G allele frequency in the PE group (68.3%) than the GH group (50.0%) (OR= 2.1579; 95% CI, 1.0265-4.5362; *p*=0.0425). However, there was no difference in genotypes between these two groups. Moreover, the genotype frequencies were not different in both groups under dominant model of inheritance (*p*=0.4909) while, the recessive inheritance model showed a significant difference (OR = 4.0, 95% CI, 1.2721-12.5781; *p* = 0.017).

Table 3. Comparison between genotypes and allelic distribution of ACE gene rs4343 in studied.

ACE gene rs4343	Control Group		PE Group		GH Group		Control vs. PE	Control vs. GH	PE vs. GH
	No.	%	No.	%	No.	%	OR (C.I. 95%)	OR (C.I. 95%)	OR (C.I. 95%)
Genotypes									
AA	11	36.7 %	4	13.3 %	6	20.0 %	Reference	Reference	Reference
GA	15	50.0 %	11	36.7 %	18	60.0 %	2.0167 (0.5054 - 8.0468), *p*= 0.320, (NS)	2.2 (0.6574 - 7.3622), *p*=0.201, (NS)	0.9167 (0.2106 - 3.9901), *p*=0.9077, (NS)
GG	4	13.3 %	15	50.0 %	6	20.0 %	10.3125 (2.1043 - 50.5388), *p*=0.004	2.75 (0.5500 - 13.7495), *p*=0.218, (NS)	3.7500 (0.7723 - 18.2098), *p*=0.1011, (NS)
Gene allele									
A	37	61.7 %	19	31.7 %	30	50.0 %	Reference	Reference	Reference
G	23	38.3 %	41	68.3 %	30	50.0 %	3.4714 (1.6352 - 7.3697), *p*=0.001	1.6087 (0.7784 - 3.3246), *p*=0.199, (NS)	2.1579 (1.0265 - 4.5362) *p*=0.0425
Dominant model									
AA	11	36.7 %	4	13.3 %	6	20.0 %	Reference	Reference	Reference
GG+ GA	19	63.3 %	26	86.7 %	24	80.0 %	3.7632 (1.0377 - 13.6468), *p*=0.043	2.3158 (0.7240 - 7.4068), *p*=0.156, (NS)	1.6250 (0.4082 - 6.4688) *p*=0.4909, (NS)
Recessive model									
AA+ GA	26	86.7 %	15	50.0 %	24	80.0 %	Reference	Reference	Reference
GG	4	13.3 %	15	50.0 %	6	20.0 %	6.5000 (1.8201 - 23.2136), *p*=0.004	1.6250 (0.4082 - 6.4688), *p*=0.490, (NS)	4.000 (1.2721 - 12.5781), *p*=0.0177

P > 0.05 is not significant (NS). ACE (Angiotensin Converting Enzyme), PE (Preeclampsia), GH (Gestational Hypertension), OR (Odd Ratio), CI (Confidence Interval).

Regarding PE subtypes, carriage of G allele was significantly associated with sever PE (OR 15.5, 95% CI, 1.8 - 127.6; *p* = 0.011). There was a significant difference in the recessive model of inheritance (*p* =0.009) but no difference was observed in the dominant model. Table (4)

Table 5 shows the relation of different genotypes of ACE gene (rs4343) with all studied parameters in the PE group. There was a significant relation between rs4343 genotypes and protein in urine, SBP ang DBP (*p*< 0.05). Otherwise, no relation was found between any of the studied parameters and genotypes of ACE rs4343 in the PE group (*p*>0.05). Post hoc analysis showed that the significant difference in the three parameters (urine protein, SBP and DBP), was due to the GG genotype.

Table 4. Comparison between genotypes and allelic distribution of ACE gene rs4343 in subtypes of PE.

Genotyping	Mild	Severe	Odds ratio (95% CI)	*p*-value
	No. = 20	No. = 10		
AA	4 (20.0%)	0 (0.0%)	Reference	-
GA	10 (50.0%)	1 (10.0%)	1.2857 (0.0435 to 37.9831)	NS
GG	6 (30.0%)	9 (90.0%)	13.1538 (0.6001 to 288.3439)	NS
Gene allele				
A	18 (45.0%)	1 (5.0%)	Reference	-
G	22 (55.0%)	19 (95.0%)	15.5455 (1.8938 to 127.6075)	0.011
Dominant model				
AA	4 (20.0%)	0 (0.0%)	Reference	-
GG+GA	16 (80.0%)	10 (100.0%)	5.7273 (0.2788 to 117.6544)	NS
Recessive model				
GA+AA	14 (70.0%)	1 (10.0%)	Reference	-
GG	6 (30.0%)	9 (90.0%)	21.000 (2.1552 to 204.6226)	0.009

P > 0.05 is not significant (NS). ; R (Odd Ratio), CI (Confidence Interval).

Table 5. Relation of different genotypes of ACE gene rs4343 with age, laboratory parameters, systolic and diastolic blood pressure in PE group.

Study parameters		Genotyping			*p*-value
		GG	GA	AA	
		No. = 15	No. = 11	No. = 4	
Age (years)	Mean ± SD	33.47 ± 6.20	30.64 ± 7.57	27.75 ± 2.63	NS
	Range	20 – 42	20 – 42	25 – 30	
Hemoglobin (g/dl)	Mean ± SD	10.84 ± 0.92	10.96 ± 0.94	11.35 ± 0.56	NS
	Range	10 – 12.7	10 – 12.9	10.8 – 12.1	
Total leucocytic count (10^3/ mm^3)	Mean ± SD	8.38 ± 1.95	7.43 ± 1.71	7.98 ± 2.67	NS
	Range	5.5 – 11	4 – 10.3	4.5 – 11	
Platelets (10^3/ mm^3)	Mean ± SD	266.87 ± 56.98	284.09 ± 52.58	209.50 ± 49.02	NS
	Range	151 – 360	215 – 350	151 – 250	
ALT (U/L)	Mean ± SD	15.51 ± 5.63	15.36 ± 5.52	16.43 ± 5.88	NS
	Range	8.7 – 28	8 – 23	8.7 – 21	
AST (U/L)	Mean ± SD	19.32 ± 4.42	18.49 ± 4.35	16.70 ± 4.30	NS
	Range	15 – 30	13 – 27	13 – 21	
	Range	16 – 36	16 – 30	22 – 32	
Creatinine (mg/dl)	Mean ± SD	0.51 ± 0.12	0.45 ± 0.09	0.58 ± 0.10	NS
	Range	0.3 – 0.7	0.36 – 0.6	0.5 – 0.7	
eGFR (ml/min/1.73 m^2)	Mean ± SD	105.20 ± 5.91	106.27 ± 7.44	109.00 ± 4.69	NS
	Range	95 – 116	99 – 116	104 – 115	
Protein in urine (mg/dl)	Mean ± SD	224.00 ± 113.12	80.00 ± 80.62	100.00 ± 0.00	0.002
	Range	30 – 300	30 – 300	100 – 100	
Systolic BP	Mean ± SD	170.33 ± 23.34	150.91 ± 7.01	148.75 ± 6.29	0.015
	Range	140 – 230	140 – 170	140 – 155	
Diastolic BP	Mean ± SD	107.00 ± 9.96	94.55 ± 6.88	95.00 ± 5.77	0.002
	Range	90 – 130	90 – 110	90 – 100	

Table 5. Continued.

Post hoc analysis			
	GG Vs GA	GG Vs AA	GA Vs AA
Protein in urine (mg/dl)	*P1*= 0.001	*P2*= 0.028	*P3*= 0.721(NS)
Systolic BP	*P1*= 0.009	*P2* =0.037	*P3*= 0.834(NS)
Diastolic BP	*P1*= 0.001	*P2*= 0.019	*P3* =0.928(NS)

ALT (Alanine Aminotransferase), AST (Aspartate Aminotransferase), eGFR (Estimated Glomerular Filtration Rate), SBP (Systolic Blood Pressure), DBP (Diastolic Blood Pressure). *P* > 0.05 is not significant (NS).

Table 6 shows the relation of different genotypes of ACE gene (rs4343) with all studied parameters in the GH group. No association was observed between the genotypes of ACE rs4343 and age, studied laboratory parameters, systolic and diastolic blood pressure in GH group (p>0.05).

Table 6. Relation of different genotypes of ACE gene rs4343 with age, laboratory parameters, systolic and diastolic blood pressure in GH group.

		Genotyping			*p*-value
		GG	GA	AA	
		No. = 6	No. = 18	No. = 6	
Age (years)	Mean ± SD	27.33 ± 3.39	30.22 ± 3.34	30.33 ± 4.68	NS
	Range	24 – 33	24 – 36	24 – 36	
Hemoglobin (g/dl)	Mean ± SD	11.35 ± 1.14	11.15 ± 1.04	11.23 ± 0.92	NS
	Range	10 – 12.5	10 – 13.1	10 – 12.5	
Total leucocytic count (10^3/mm^3)	Mean ± SD	9.32 ± 0.80	7.72 ± 1.76	8.40 ± 1.66	NS
	Range	8 – 10	4.5 – 11	6 – 11	
Platelets (10^3/mm^3)	Mean ± SD	262.50 ± 11.29	280.39± 72.59	268.67 ± 72.60	NS
	Range	250 – 280	170 – 410	170 – 390	
ALT (U/L)	Mean ± SD	15.00 ± 4.34	16.33 ± 4.52	13.67 ± 2.88	NS
	Range	11 – 21	10 – 22	10 – 18	
AST (U/L)	Mean ± SD	16.83 ± 4.54	18.39 ± 4.20	19.50 ± 5.54	NS
	Range	12 – 23	12 – 28	14 – 27	
Creatinine (mg/dl)	Mean ± SD	0.50 ± 0.06	0.55 ± 0.09	0.53 ± 0.10	NS
	Range	0.4 – 0.6	0.4 – 0.7	0.4 – 0.7	
eGFR (ml/min/1.73 m^2)	Mean ± SD	112.50 ± 4.32	105.56 ± 5.83	105.33 ± 8.43	NS
	Range	107 – 116	99 – 116	95 – 116	
Protein in urine (mg/dl)	Mean ± SD	19.33 ± 1.97	20.94 ± 2.01	21.17 ± 2.04	NS
	Range	17 – 22	19 – 25	20 – 25	
FBS (mg/dl)	Mean ± SD	75.83 ± 7.28	83.22 ± 8.79	76.50 ± 5.47	NS
	Range	69 – 85	70 – 99	70 – 85	
Systolic BP	Mean ± SD	161.67 ± 16.33	149.44 ± 15.89	160.00 ± 18.97	NS
	Range	150 – 185	140 – 200	140 – 180	
Diastolic BP	Mean ± SD	99.17 ± 10.68	94.72 ± 7.76	99.17 ± 11.14	NS
	Range	90 – 115	90 – 110	90 – 115	

ALT (Alanine Aminotransferase), AST (Aspartate Aminotransferase), eGFR (Estimated Glomerular Filtration Rate), FBS (Fasting blood sugar), SBP (Systolic Blood Pressure), DBP (Diastolic Blood Pressure). *P* > 0.05 is not significant (NS).

Discussion

The present study aimed to determine whether ACE gene rs4343 is associated with susceptibility to GH and PE and such association, if present, related to the degree of severity of GH and PE. The results of the present study showed that the G allele and GG genotype of ACE rs4343 were significantly related to an elevated risk of PE in our studied population and were represented as major risk factors for disease severity. Previous studies have revealed that rs4343 polymorphism is associated with left ventricular hypertrophy, blood pressure, coronary artery diseases and migraine.[16,17,18]

The ACE rs4343 polymorphism results in the Thr 776 Thr synonymous substitution in the ACE gene. Emerging evidence suggested that silent mutations could have functional consequences and therefore contribute to the human disease risk.[19] On the mRNA level, these types of mutations can affect stability, folding and translation rate. In addition, they can result in aberrant mRNA splicing. It is obvious that any alterations in the mRNA molecule will affect the related protein structure, expression, substrate specificity, secretion and/or enzymatic activity.[19,20]

The main reason that a silent mutation may change protein properties is the codon usage bias, which refers to the preferred use of particular codons instead of other synonymous codons during mRNA translation. The frequency of codon usage varies among species, tissues, and genes.[21] Abedin and his co-workers.,2018 determined the effect of rs4343 on mRNA folding and the kinetics of local translation and they found a slower local rate of translation elongation on the rare codon compared to the wild-type and therefore could affect the enzyme properties Moreover, it is suggested that rs4343 alters regulatory motifs for several transcription factors and therefore affects the ACE levels.[22]

Genotyping of the ACE gene rs4343 revealed three genotypes (GG, GA, AA). The current study indicated that the frequency of GG genotype and G allele in patients with PE was significantly higher than in the control group (p <0.01). Furthermore, ACE gene rs4343 in the presence of GG genotype was associated with a 10.3-fold increase in the risk of PE. Moreover, the genotype frequencies were significantly different in the studied groups under dominant (p= 0.043) and recessive (p = 0.004) inheritance models.

Our study findings agreed with those of a study by Abedin et al., 2018[22] who reported that, for the rs4343, the G allele frequency was significantly higher in the PE group compared to the control group and the frequency of AG and GG genotypes differed between PE patients and control subjects. They also found that genotype frequencies were different in the studied groups under dominant (OR=3.94, 95% CI, 2.05–7.56; p < 0.0001) and recessive (OR=2.21, 95% CI, 1.22–4.01; p=0.009) inheritance models.

Further agreement came with findings of a study by Zhu et al., 2001[23] who showed that the rs4343 had the greatest significant effect on the ACE function and the presence of the G allele increased both SBP and DBP. It seems that the rs4343 G allele increases activity of serum ACE and therefore produces higher levels of angiotensin II. Moreover, this allele was also associated with higher ACE serum levels in a sample of depressed patients.[24]

Based on our results, individuals carrying ACE rs4343 G allele are more susceptible to PE under both dominant and recessive inheritance models (p=0.043 and p=0.004, respectively). Therefore, we propose that if the G allele is associated with the pathogenesis of PE, it could function in both models of inheritance. In addition, carriage of G allele and GG genotype of ACE rs4343 might be independent risk factors influencing PE severity as they were significantly associated with severity markers of PE (urine protein and blood pressure) which could explain the higher susceptibility to develop severe forms of the disease in PE patients with the GG genotype.

A study by Íñiguez et al., 2021[25] reported that G allele of rs4343 was associated with severe COVID-19 in hypertensive patients, independently of gender and G-carrier genotypes of this polymorphism was also associated with higher mortality and higher severity of COVID-19 in dyslipidemia and type 2 diabetic patients.

To the best of our knowledge, the current study is the first report that studied the effect of

ACE rs4343 gene polymorphism on the susceptibility to GH. We found no evidence of association between ACE rs4343 gene polymorphism and GH. G allele frequency of the ACE gene was (38.3%) and (50%) in control subjects and GH women, respectively. Moreover, there was no difference between GH group and the control group regarding genotypes and inheritance models (p>0.05). However, confirmation of this hypothesis requires further studies with more participants and in diverse ethnic populations.

In conclusion, our results suggested an association between G allele of ACE (rs4343) and its related genotypes and susceptibility to PE but not to GH. Also, it had a significant relation between GG genotype and markers of severity of PE (protein in urine, increased systolic and diastolic blood pressure). These findings might be useful for the early detection of the disease's course and progression as well as for prioritizing patients who might need more care.

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Author Contributions

AAM; Study design, Design of Data analysis and Manuscript review. AAAN; Manuscript preparation and Manuscript review. NMA; Sample processing and Manuscript review. HWA; Sample collection. RMK; Manuscript preparation.

Declaration of Conflicting Interests

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Ethical approval

The study protocol was reviewed and approved by the Research Ethics Committee of the Faculty of Medicine (for Girls), Al-Azhar University (Approval number: 2022121644, dated March 2019).

Informed consent

An informed consent form was obtained from each study participant.

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