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# THE IMPACT OF ANGIOTENSIN CONVERTING ENZYME GENE (I/D) POLYMORPHISM ON PREDISPOSITION TO PREECLAMPSIA

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## ABSTRACT
Preeclampsia is a multifactorial condition that affects pregnant women and, subsequently, their fetuses. The etiology is still debatable, but a combination of genetic and environmental factors can increase the risk of developing preeclampsia. Angiotensin-converting enzyme (ACE) is involved in blood pressure homeostasis. Insertion/deletion (I/D) is one of the most important variations in the *ACE* gene. The study included A total 188 pregnant women (102 healthy controls and 86 women with preeclampsia). The genotypes of *ACE* (I/D) polymorphism were determined using PCR technique. Results: The frequencies of DD, ID, and II genotypes in the control group were 42.2%, 46.1%, and 11.8%, and in the preeclampsia were 38.4%, 52.3%, and 9.3% respectively. The distribution of genotypes did not differ between the control group and the preeclampsia group ($P>0.05$). On the other hand, there was a significant difference in (BMI), baby weight at delivery, WBC count, hemoglobin, (RDW), neutrophils, eosinophils, and platelets between the two groups ($P<0.05$). In addition, a significant higher basophil count was found in individuals with the DD genotype ($P<0.05$) compared to other genotypes. In conclusion, the *ACE* (I/D) polymorphism did not contribute to preeclampsia risk in Jordan. The impact of *ACE* (I/D) polymorphism on basophil count should be confirmed in future investigations.

## INTRODUCTION
Preeclampsia is a hypertensive disorder that affects some pregnant women and poses a risk to the life of both the mother and fetus (Rana et al. 2019). Although the causes of preeclampsia are not fully clear, it is suggested that improper placental development and function is a key contributing factor (Shaheen et al. 2019). Preeclampsia is diagnosed after 20 weeks of gestation and is characterized by high blood pressure (BP) ≥(140/90mmHg) and the presence of protein in the urine (Young et al. 2010; Makhlouf et al. 2024). It usually accompanied by other symptoms, such as edema, thrombocytopenia, abdominal pain, headache, and vision problems (Abedin Do et al. 2018) Preeclampsia can lead to multiple complications including stroke, venous occlusion, ischemic heart diseases, and liver/kidney dysfunction (Shaheen et al. 2019; Makhlouf et al. 2024). The etiology of preeclampsia is complex and includes both genetic and environmental factors. Environmental risk factors include chronic hypertension, diabetes, multifetal pregnancies, older maternal age, the use of assisted reproductive technologies, obesity and others (Young et al. 2010; Rana et al. 2019). Among the genetic risk factors are genes that code for products involved in blood pressure regulation (González-Garrido et al. 2017; Abedin Do et al. 2018; Dmitrenko et al. 2020). The angiotensin converting enzyme (ACE) controls the conversion of angiotensin I to angiotensin II, an active component of the renin angiotensin system (RAS), which is involved in blood pressure homeostasis (Saleh et al. 2025). In addition to systemic expression of ACE and other RAS components, ACE is also expressed in the uteroplacental region (Rodriguez et al. 2012). In a normal pregnancy, RAS system is tightly controlled, enabling it to meet the needs of increased blood volume without causing high blood pressure. However, in preeclampsia, the RAS system becomes dysregulated, leading to the development of high blood pressure (Leal et al. 2022).
The insertion/deletion (I/D) polymorphism occupies intron 16 with the presence or absence of 264 bps Alu-repeats. This polymorphism has been shown to modulate ACE expression, with the highest enzyme activity detected in individuals with the DD genotype (Dmitrenko et al. 2020). Therefore, this study aimed to investigate the association of *ACE* (I/D) polymorphism and preeclampsia risk in Jordan. In addition, the study examined the relationships between blood indices, environmental factors, and the risk of preeclampsia.

## MATERIALS AND METHODS
### Study design
One hundred and eighty-eight pregnant women with a gestational age more than 20 weeks were recruited from Jordan. The study used a case-control approach and included 102 health pregnant women (control group) and 86 pregnant women with preeclampsia (cases group). The diagnosis of preeclampsia was based on blood pressure (>140/90 mmHg) and proteinuria

(>± one) on a urine strip test. Exclusion criteria involved pregnant women with heart disease, diabetes and kidney disease.

**Sample size calculation**

The sample size was calculated using G power 3.1 software (University of Kiel/Germany) with a moderate effect size, alpha 0.05, and power 0.85. The total sample size required for the study was 152.

**Sample collection**

Blood samples were collected in EDTA containing tubes by a licensed lab technician in a health facility. Samples were used for subsequent molecular and hematological analysis.

**Hematological indices and Anthropometric measurements**

Hematological indices were analyzed in the diagnostic laboratories of King Abdullah University Hospital, Irbid, Jordan using a Roche diagnostics analyzer system. Anthropometric measurements, such as height, weight, and body mass index (BMI), were measured as previously described (Khabour et al. 2018).

**DNA extraction**

DNA was extracted from whole blood using a column-based Zymo research kit as described by the manufacturer (Irvine, CA, USA). DNA quality and quantity were assessed using a NanoDrop device (ThermoFisher, 20000/2000c). DNA samples were stored at -20˚C until used.

**Molecular analysis**

*ACE* (I/D) polymorphism was genotyped using a Bio-Rad (USA) thermocycler. The PCR primers were forward: 5′CTGGAGACCACTCCCATCCTTTCT 3′, and reverse: 5′GATGTGGCCATCACATTCGTCAGAT 3′ (Salem and Batzer 2009). The final PCR reaction volume was 20uL and consisted of 0.1ng of DNA, 10µL of Promega (2x) master mix, 1µMol of each primer, and nuclease free water. PCR reaction conditions included initial denaturation at 94˚C/3mins, followed by 28 cycles of denaturation at 94˚C/ one min, annealing 60˚C/ one min, elongation 72˚C/ one min, and final extension at 72˚C/ four min. Because PCR may selectively amplify D in heterozygous samples (ID), samples identified as DD were amplified in a new reaction using the forward: 5'TGGGACCACAGCGCCCGCCACTAC3′, and reverse: 5'TCGCCAGCCCTCCCATGCCCATAA3′ primers. The reaction volume and conditions of PCR were as described above except that the annealing temperature was carried out at 67˚C instead of 60˚C (Elbasan et al. 2023).

**Statistics**

Categorical variables were expressed as frequency, while continuous variables were expressed as mean ± standard deviation. Allele and genotype frequencies and Hardy-Weinberg equilibrium were analyzed using SNPstat software. Continuous variables were analyzed using analysis of variance (ANOVA) (Graph pad software). $P<0.05$ was determined to be statistically significant.

## RESULTS

A total of 188 pregnant women were enrolled in the study. Participants were categorized into women with preeclampsia (cases group, n=86), and healthy pregnant women (control group, n=102). Table 1 shows the demographics of participants. BMI was significantly higher in the cases group than in the control group ($P<0.05$). In addition, birth weight was lower in the cases group than in the control group ($P<0.05$). On the other hand, there was no statistical significance between the number of births per pregnancy (singleton vs multiple), mode of conception (natural vs using assisted reproductive technology), delivery method (vaginal vs cesarean) or blood type between the two groups ($P>0.05$).

**Table 1: Demographic and clinical distribution of the participants**

| Parameter | Control (n=102) | Patients (n=86) | P-value |
|---|---|---|---|
| Age (years) | 29.94±4.704 | 30.11±5.957 | 0.822 |
| BMI (kg/m²) | 27.35± 2.983 | 29.51±4.501 | 0.0009 |
| Singleton: multiple (n) | 94:8 | 81:5 | 0.298 |
| natural conception: ART* (n) | 100: 2 | 81: 5 | 0.164 |
| Baby weight at delivery (kg) | 2.91± 0.560 | 2.43± 0.86 | 0.0002 |
| Mode of delivery, Vaginal: CS* (n) | 21: 81 | 19: 67 | 0.801 |
| Blood types; A: B: O: AB (n) | 29: 17: 51: 4 | 36: 11: 32: 8 | 0.081 |

*ART: Assisted reproductive technology, CS: Caesarean section.

With respect to the blood profile (Table 2), hemoglobin, platelet, and eosinophil count were significantly lower in the cases group than in the control group ($P<0.05$). In contrast, white blood cells, red cell distribution width, and neutrophils count were higher in the cases group than in the control group ($P<0.05$). However, there was no significant difference in red blood cell count, hematocrit, lymphocytes, monocytes, and basophil counts, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and mean platelet volume between the two groups ($P> 0.05$).

**Table 2: The hematological parameters of the participants**

| Parameter (mean±SD) | Control | Patient | P-value |
|---|---|---|---|
| Red blood cells (×10^12/L) | 4.30 ±0.54 | 4.19± 0.56 | 0.161 |
| Hemoglobin (g/dL) | 11.72±1.52 | 11.28±1.54 | 0.049 |
| Hematocrits (%) | 34.52±5.31 | 33.69±4.11 | 0.073 |
| White blood cells (×10^9 /L) | 9.07±3.18 | 10.25±2.90 | 0.005 |
| Platelets (×10^9 /L) | 252.8±105.0 | 222.8±75.8 | 0.028 |
| Neutrophils (×10^9 /L) | 6.271±3.05 | 7.618±2.65 | 0.040 |

| | | | |
|---|---|---|---|
| Lymphocytes (×10^9 /L) | 2.096±0.72 | 1.94± 0.66 | 0.112 |
| Monocytes (×10^9 /L) | 0.56±0.20 | 0.60±0.24 | 0.327 |
| Eosinophils (×10^9 /L) | 0.11±0.115 | 0.073±0.1 | 0.0255 |
| Basophils (×10^8 /L) | 0.036±0.029 | 0.036±0.03 | 0.888 |
| mean corpuscular volume (fL) | 81.34±7.21 | 80.98±8.66 | 0.692 |
| mean corpuscular hemoglobin (MCH, g/dL) | 27.36±2.92 | 27.17±3.68 | 0.923 |
| MCH concentration (pg/cell) | 33.59±1.05 | 33.44±1.35 | 0.325 |
| Red cell distribution width (fL) | 14.96±2.35 | 15.6±2.6 | 0.021 |
| Mean platelet volume (fL) | 9.57±3.03 | 9.63±1.38 | 0.172 |

The frequencies of alleles and genotypes of *ACE* (I/D) were consistent with the Hardy Weinberg equation (P>0.05). The frequencies of D and I alleles were 65% and 35%, respectively, in all participants. Table 3 shows the frequencies of ACE (I/D) genotypes between the two groups. There were no significant differences in the distribution of the different genotypes between the cases group and control groups in any inheritance model (P>0.05).

**Table 3: The distribution of *ACE* (I/D) polymorphism according to different inheritance models among participants**

| Model | Genotype | Control | Patient | OR (95 CI) | P-value |
|---|---|---|---|---|---|
| **Codominant** | D/D | 43 (42.2) | 33 (38.4) | 1.00 | 0.67 |
| | I/D | 47 (46.1) | 45 (52.3) | 1.25 (0.68-2.30) | |
| | I/I | 12 (11.8) | 8 (9.3) | 0.87 (0.32-2.37) | |
| **Dominant** | D/D | 43 (42.2) | 33 (38.4) | 1.00 | 0.60 |
| | I/D-I/I | 59 (57.8) | 53 (61.6) | 1.17 (0.65-2.10) | |
| **Recessive** | D/D-I/D | 90 (88.2) | 78 (90.7) | 1.00 | 0.58 |
| | I/I | 12 (11.8) | 8 (9.3) | 0.77 (0.30-1.98) | |
| **Overdominant** | D/D-I/I | 55 (53.9) | 41 (47.7) | 1.00 | 0.39 |
| | I/D | 47 (46.1) | 45 (52.3) | 1.28 (0.72-2.28) | |

Table 4 illustrates the impact of ACE(I/D) polymorphism on clinical parameters in the study participants. No significant difference was observed in all studied parameters, except for basophil count, which was higher in the DD genotype compared to the other genotypes (P<0.05).

A multivariable logistic regression was performed to investigate risk factors associated with preeclampsia.

**Table 4: Association of *ACE (I/D)* polymorphism with hematological indices among the whole sample.**

| Genotype | DD | ID | II | p-value |
|---|---|---|---|---|
| BMI (kg/m²) | 27.9± 3.6 | 28.4± 4.0 | 29.4±4.4 | 0.442 |
| RBC (×10^12/L) | 4.32±0.56 | 4.20±0.54 | 4.19±0.46 | 0 .341 |
| HB (g/dL) | 11.5± 1.6 | 11.4±1.5 | 11.7±1.0 | 0.665 |
| HCT (%) | 34.3±4.3 | 33.8±5.4 | 34.8±3.3 | 0.739 |
| WBC (×10^9 /L) | 9.61±3.02 | 9.63±3.1 | 9.49±3.47 | 0.982 |
| PLT (×10^9 /L) | 245±111 | 235±82 | 235±75 | 0.916 |
| Neutrophils (×10^9 /L) | 6.9±2.9 | 6.87±2.92 | 6.77±3.47 | 0.975 |
| Lymphocytes (×10^9 /L) | 1.98±0.66 | 2.06±0.74 | 2.02±0.64 | 0.787 |
| Monocytes (×10^9 /L) | 0.55±0.19 | 0.61±0.24 | 0.56±0.20 | 0.277 |
| Eosinophils (×10^9 /L) | 0.095±0.12 | 0.087±0.08 | 0.094±0.08 | 0.845 |
| Basophils (×10^8 /L) | 0.042±0.03 | 0.03±0.02 | 0.035±0.02 | 0.040 |
| mean corpuscular volume (fL) | 79.9±8.5 | 81.7±7.5 | 83.2±5.7 | 0.274 |
| mean corpuscular hemoglobin (MCH, g/dL) | 26.8±3.5 | 27.3±3.1 | 28.2±2.5 | 0.230 |
| MCH concentration (pg/cell) | 33.5±1.2 | 33.4±1.2 | 33.9±1.0 | 0.303 |
| Red cell distribution width (fL) | 15.3± 2.3 | 15.1±2.4 | 15.6± 3.1 | 0.567 |
| Mean platelet volume (fL) | 9.7±3.2 | 9.6±1.5 | 9.13±1.02 | 0.460 |

Table 5 illustrates that age, multifetal pregnancy, mode of conception, mode of delivery, and blood group were not associated with preeclampsia. On the other hand, BMI was significantly associated with preeclampsia (OR: 1.178, 95% CI: 1.074-1.293, P<0.05).

**Table 5: Association of several independent factors with preeclampsia**

| Variables | P-value | OR | CI interval 95% |
|---|---|---|---|
| **Age** | 0.411 | 0.975 | (0.919- 1.035) |
| **BMI** | P < 0.001 | 1.178 | (1.074- 1.293) |
| **Singleton vs multiple** | 0.351 | 0.559 | (0.147- 2.132) |
| **Natural vs ART** | 0.121 | 4.227 | (0.683-26.166) |
| **Blood group** | - | - | - |
| **Blood group (B Vs A)** | 0.114 | 0.469 | (0.183 – 1.199) |

| | | | |
|---|---|---|---|
| **Blood group (O Vs A)** | 0.102 | 0.556 | (0.275 – 1.124) |
| **Blood group (AB Vs A)** | 0.825 | 1.164 | (0.303 – 4.469) |
| **Genotype** | - | - | - |
| **Genotype I/D Vs D/D** | 0.644 | 1.169 | (0.603-2.264) |
| **Genotype I/I Vs D/D** | 0.504 | 0.692 | (0.235-2.037) |

## DISCUSSION

Preeclampsia is a serious pregnancy related condition and one of the five leading causes of maternal mortality and morbidity (Dmitrenko et al. 2020). Angiotensin-converting enzyme (ACE) is involved in the production of angiotensin II, a key player in the renin angiotensin system (RAS) that regulates blood pressure in the body (Abedin Do et al. 2018). In addition, abnormal levels of angiotensin II bind to the angiotensin II receptor 1, and the subsequent interference with trophoblast invasion and placenta hypoxia development (Young et al. 2010; Saito and Nakashima 2014). Previous literature indicates dysregulation of the RAS system in preeclampsia (Leal et al. 2022). Therefore, genetic variations in the *ACE* gene are candidates for examining genetic associations with the risk of preeclampsia. Genome wild association studies have indicated that the *ACE* gene is among the candidate genes that might be linked to preeclampsia (Williams and Morgan 2012; Parada-Niño et al. 2022).

In the current study, this assumption was tested by examining the association between the *ACE* (I/D) polymorphism and the risk of preeclampsia. The D allele of this polymorphism has been shown to be associated with elevated ACE enzyme activity, which may affect blood pressure balance (Dmitrenko et al. 2020). The results showed no association between *ACE* (I/D) polymorphism and preeclampsia risk. Previous studies that examined the association between *ACE* (I/D) polymorphism and the risk of preeclampsia have been controversial (Choi H et al. 2004; Li et al. 2007; Naghavi et al. 2011; Alkanli 2014; González-Garrido et al. 2017; Dmitrenko et al. 2020). For example, lack of association between *ACE* (I/D) polymorphism and the risk of preeclampsia has been reported among studies of pregnant women of Turkish, North Indian, Columbian, African American, and Brazilian populations (TAMURA et al. 1996; Serrano et al. 2006; Aggarwal et al. 2011; Alkanli 2014; Cristina dos Santos Lopes et al. 2019). On the other hand, an association between *ACE* (I/D) polymorphism and the risk of preeclampsia has been reported in pregnant women in Egypt, Mexica, China and Iran (Li et al. 2007; Naghavi et al. 2011; Kamha et al. 2013; González-Garrido et al. 2017). A study from Pakistan showed an impact for *ACE* (I/D) polymorphism on placental villi and umbilical cord development in pregnant women with preeclampsia (Shaheen et al. 2019) The variability in findings regarding the association between *ACE* (I/D) polymorphism and preeclampsia risk suggests a complex genetic contribution to this condition that is modified by the genetic backgrounds of human populations.

The study results showed that *ACE* (I/D) polymorphism is common among Jordanian population, with the frequency of D allele reaching 65%. A similar distribution of the D allele was reported in studies from Turkey (61%) and Brazil (68.5%) (Alkanli 2014; Cristina dos Santos Lopes et al. 2019). However, relatively low frequency was reported in studies from Russia (43.5%), China (42.2%), Korea (52.9%), Columbia (50.1%), and North India (45.6%) (Choi H et al. 2004; Serrano et al. 2006; Li et al. 2007; Aggarwal et al. 2011; Dmitrenko et al. 2020). On other hand, relatively, high frequency of D allele was reported in studies from Egypt (74.4%), and Saudi Arabia (73.7%) (Kamha et al. 2013; Gull et al. 2020).

Obesity is a risk factor for preeclampsia. Adipokines secreted by adipose tissue can contribute to inflammation and oxidative stress, which may lead to improper placentation and eventually preeclampsia (Roberts et al. 2011). Moreover, cholesterol levels have been suggested to be a predictor of preeclampsia in the first and second trimesters of pregnancy (Dey et al. 2013). In the current study, we found that BMI was a strong risk factor of preeclampsia (OR= 1.178, CI (1.074- 1.293), P<0.001). This agrees with the findings of studies conducted in Mexico and Pakistan (González-Garrido et al. 2017; Shaheen et al. 2019) and in contrast others (Yılmaz et al. 2016; Abedin Do et al. 2018). The discrepancies in the results may be attributed to different study designs, ethnicity, sample size, environmental factors and genetic heterogenicity as preeclampsia is known to be a multifactorial condition (Abedin Do et al. 2018; Shaheen et al. 2019). Finally, we found no association between CBC indices and *ACE* (I/D) polymorphism except for basophil count where the DD genotype showed an elevation in the count. This may be explained by the fact that angiotensin II has a proinflammatory effect that can stimulate the production of MCP-1 which in turn activates basophils (Bischoff et al. [no date]; Cheng [no date]). However, more investigations are needed to confirm such findings.

Among the study limitations is the small sample size. In addition, only one polymorphism in *ACE* gene was investigated. Including more polymorphisms and a larger sample are recommended in future studies.

In conclusion, the *ACE* (I/D) polymorphism might not contribute to the risk of preeclampsia in Jordan. The *ACE* DD might be associated with elevated basophils count. BMI may be associated with preeclampsia risk.

## Declarations

### Ethics approval and consent to participate

The study was approved by "Institutional Review Board of Jordan University of Science and Technology (JUST) and King Abdullah University Hospital (KAUH)" (JUST/94/136/2020) in accordance with Declaration of Helsinki and institutional regulations. Written informed consent was obtained from the participants who agreed to enrol in the study as required by the IRB.

### Consent for publication

Not applicable

### Availability of data and material

Data will be available upon reasonable request from the corresponding author.

**Competing interests**
Authors have no conflict of interest to declare.
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**Authors' Contributions**
All authors (RS, OK, HR, and SM) have contributed to have contributed to concept, study design, experimental work, data acquisition, manuscript drafting/review. RS performed statistical analysis and involved in project management.
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