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Association between angiotensin converting enzyme gene insertion/deletion polymorphism and risk of hypertension: a case-control study

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Abstract

Background: Hypertension (HTN) is a major global public health problem associated with high morbidity and mortality. Both genetic and environmental factors contribute to its development. The ACE I/D polymorphism of the ACE gene has been widely investigated in relation to HTN susceptibility. This study sought to ascertain if ACE I/D polymorphism of the ACE gene and the risk of HTN were related at Debre Tabor Comprehensive Specialized Hospital, Northwest Ethiopia.

Methods: A hospital-based age- and sex-matched case-control study was conducted involving 118 HTN patients and 118 healthy controls at Debre Tabor Comprehensive Specialized Hospital, Northwest Ethiopia. Clinical and biochemical data were collected using standard procedures. Genomic DNA was extracted from peripheral blood samples, and ACE I/D polymorphism genotypes were determined using polymerase chain reaction and agarose gel electrophoresis.

Results: Both the ACE-DD genotype (OR: 2.72; 95% CI: 1.29–5.71; $P = 0.008$) and D allele (OR: 1.73; 95% CI: 1.19–2.51; $P = 0.003$) were substantially more prevalent in patients than in controls, suggesting they may be risk factors for the development of hypertension.

Conclusion: The findings point to a potential link between the ACE I/D polymorphism of the ACE gene and the occurrence of HTN in the Ethiopian population under study.

Keywords: Angiotensin Converting Enzyme; Genotypes; Hypertension; Risk Factors

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1 Introduction

Hypertension (HTN), a prevalent illness marked by persistently high blood pressure (BP) in the systemic arteries, continues to be one of the primary causes of mortality from cardiovascular disease [1]. Globally, an estimated one billion adults have HTN, with projections reaching 1.7 billion by 2025 [2, 3]. In Sub-Saharan Africa, the prevalence is approximately 30%, with disproportionately low rates of awareness, treatment, and control [4]. A meta-analysis from Ethiopia reported an overall HTN prevalence of 19.6% [5]. The disease is influenced by both genomic and environmental factors, as well as their interactions [6]. Although the genetic susceptibility to HTN is non-modifiable and conveys lifelong cardiovascular disease risk, the environmental risks for HTN are changeable and largely avoidable due to the considerable influence of major behavioral and lifestyle factors such as excess salt intake, smoking, alcohol intake, insufficient physical exercise, and unhealthy feeding habits [7]. HTN is thought to be affected by age, sex, and ethnicity, as with the majority of challenging illnesses [8]. In addition to demographic concerns, obesity, insulin resistance, and dyslipidemia are clinical and biomedical factors that are hypothesized to play a substantial role in the development of HTN and its associated complications variability. Genetic mechanisms, particularly the renin–angiotensin–aldosterone system (RAAS) pathway, also play a central role in blood pressure regulation and HTN pathogenesis [9].

The RAAS is one of the most crucial routes in the pathogenesis and management of hypertension. Angiotensin-converting enzyme (ACE) is among the proteins involved in this pathway, which affects salt retention, water balance, and blood vessels; hence, it controls blood pressure [10]. It is located in the endothelium lining of blood vessels, where it is used to promote the growth of vascular smooth muscle cells by converting angiotensin-I into angiotensin II [11]. The human ACE gene is situated on the long arm (q) of chromosome 17 (17q23.3) and is 21 kb in size with 26 exons and 25 introns [12]. Three genotypes—II homozygote, ID heterozygote, and DD homozygote—are created when a 287-base pair (bp) Alu sequence in ACE gene's intron 16 is inserted (I) or deleted (D) [13]. The DD genotype was associated with increased ACE activity and levels, which therefore increased angiotensin II production and raised blood pressure [14].

The chromosomal region encoding the ACE I/D polymorphism of the ACE gene and blood pressure have been linked, according to studies in hypertensive and normotensive individuals. Despite numerous studies investigating the ACE I/D polymorphism and HTN risk, findings remain inconsistent across different ethnic and geographic populations [15]. Some studies report a strong association, while others find no significant relationship [16]. Furthermore, no published data exist on this association specifically in the South Gondar population of Northwest Ethiopia. Therefore, this study aimed to determine the association between the ACE I/D polymorphism of the ACE gene and the risk of HTN, and to assess the role of clinical variables in predicting HTN development in this understudied population.

2 Subjects and Methods

2.1 Study Design

A hospital-based age- and sex-matched case-control study was conducted at Debre Tabor Comprehensive Specialized Hospital (DTCSH). The study's source population consisted of all clients who visited the DTCSH medical referral clinic (MRC). The study's participants were those who were receiving follow-up care at MRC for hypertension.

2.2 Study Area

This research was conducted in DTCSH, found in Debre Tabor Town, located in the Amhara Region, 103 km east of Bahir Dar City and 667 km north-west of Addis Ababa, the capital of Ethiopia. DTCSH is the only referral hospital found in the South Gondar Zone that provides different inpatient and outpatient services for over 2.7 million inhabitants in South Gondar. The hospital provides

treatment and follow-up care for significant chronic diseases, including HTN.

2.3 Inclusion and Exclusion Criteria

Patients identified with HTN who had been monitored at the MRC for at least a year were included in this study. Healthy volunteers with normal blood pressure who were available during the research period served as the controls. They were matched for age and sex and were from the same geographic region and socioeconomic status. Patients with hepatic and renal illness, cardiac issues, stroke, secondary HTN (with a history of Cushing's syndrome, aldosteronism, thyroid problems, and so on), or a persistent bacterial or viral infection were not permitted to take part. This study also excluded patients who were unable to sign informed consent or who refused to do so.

2.4 Sample Size Determination and Sampling Technique

The sample size was determined using an analytical study sample size calculation with a confidence level of 95%, a power of 80%, and a double population proportion formula. Based on the previous case-control study carried out at the University of Gondar Comprehensive Specialized Hospital, Gondar, Ethiopia, the sample size was determined by considering the 48.4% association of ACE DD genotype among hypertensive patients and 29.7% among normotensive control groups [17]. The final sample size after adding a 10% non-response rate was 236 participants of both sexes, consisting of 118 HTN patients and 118 healthy controls. Participants were selected using a simple random sampling technique based on a table of random numbers, without replacement. Healthy normotensive controls were recruited from the same geographic area and socioeconomic background using a 1:1 frequency-matching approach according to age and sex distribution.

2.5 Data Collection Methods

Similar to our earlier research [18, 19, 20, 21], a semi-structured questionnaire was used to collect information on the socio-demographic backgrounds, HTN risk factors, and family history of both patients and healthy control participants. We measured body weight and height using portable digital scales and portable stadiometers, respectively. Body mass index (BMI) was calculated by dividing the weight in kilograms by the square of the height in metres. Participants were categorised as underweight (BMI $<$18.5 kg/m^2), healthy (18.5–25 kg/m^2), overweight (25.0–29.9 kg/m^2), or obese ($\geq$30 kg/m^2) [22]. Blood pressure was taken using a digital device in the sitting position after five minutes of rest; SBP and DBP were calculated as the mean of three measurements. Participants were categorised as normal blood pressure (SBP $<$120 mmHg and DBP $<$80 mmHg), pre-hypertension (SBP 120–139 mmHg or DBP 80–89 mmHg), or hypertensive (mean SBP $\geq$140 mmHg and mean DBP $\geq$90 mmHg, or if they used antihypertensive medication) [23].

2.6 Sample Collection and Laboratory Methods

Five millilitres of blood were drawn from the median cubital vein of each participant under strict safety guidelines [18, 19, 20, 21]. Three ml were kept in tubes without anticoagulants for serum biochemistry; serum was separated by centrifugation and analysed on the Dimension EXL 200 automated analyser for total cholesterol (TC), triglycerides (TG), LDL, HDL, creatinine, and glucose. Diabetes mellitus was diagnosed when fasting blood glucose was $\geq$126 mg/dl [24]. Dyslipidaemia was defined according to NCEP ATP III guidelines: TC $>$200 mg/dl, TG $>$150 mg/dl, LDL-C $>$130 mg/dl, or HDL-C $<$40 mg/dl (men) or $<$50 mg/dl (women) [22]. Kidney disease was identified when blood creatinine was $>$1.3 mg/dl [25].

2.7 Genomic DNA Isolation and Genotyping

The remaining 2 ml collected in EDTA-containing tubes were sent to the University of Gondar Molecular Biology Laboratory for extraction of genomic DNA using a non-enzymatic salting-out method [18, 19, 20, 21]. Red blood cells were lysed and removed with a buffer solution; white blood cells were lysed

with a nuclear lysis buffer. Proteins were precipitated with 6 M NaCl, DNA was precipitated with isopropanol, washed with 70% ice-cold ethanol, and dissolved in Tris-EDTA (TE) buffer [26]. DNA quality was assessed by 1% agarose gel electrophoresis and samples were stored at −20°C (Figure 1) [27].

ACE I/D genotypes were determined by direct PCR using primers 5′-CTG GAG ACC ACT CCC ATC CTT TCT-3′ (forward) and 5′-GAT GTG GCC ATC ACA TTC GTC AGA T-3′ (reverse) [28]. Each 25 μl PCR reaction contained 2 μl sample, 1 μl forward primer, 1 μl reverse primer, 12.5 μl master mix ($MgCl_2$, dNTPs, PCR buffer, Taq polymerase), and 8.5 μl PCR-grade water. Thermocycler conditions: initial denaturation at 95°C for 5 min; 35 cycles of 94°C for 30 s (denaturation), 58°C for 30 s (annealing), 72°C for 1 min (extension); and final extension at 72°C for 5 min [29].

PCR products were resolved on a 2% agarose gel at 120 V for 50 min; 490 bp (II), 190 bp (DD), and both bands (ID) were visualised under UV after ethidium bromide staining (Figure 2) [30].

2.8 Statistical Analysis

STATA version 17 was utilised to analyse the data. Quantitative data are expressed as mean ± standard deviation. Continuous variables were compared between patients and controls using independent-samples t-test. Genotype and allele frequency distributions were compared using the chi-square test. Logistic regression was used to assess the risk correlations of ACE I/D polymorphism with HTN at the 95% confidence level. ANOVA was used to compare ACE genotypes with clinical risk variables. A p-value of <0.05 indicated statistical significance.

3 Results

The distribution of sex and age between the hypertensive case and healthy control groups was comparable. In the HTN group ($n = 118$), 62 (52.5%) were male and 56 (47.5%) female; in the control group ($n = 118$), 61 (51.7%) were male and 57 (48.3%) female. Mean ages were 59.3 ± 12.9 years for cases and 58.6 ± 9.6 years for controls. Systolic blood pressure, diastolic blood pressure, total cholesterol, triglyceride, and LDL-cholesterol were all significantly higher in patients than controls, but HDL-cholesterol was lower ($p < 0.001$). Blood creatinine, fasting blood glucose, and BMI did not differ significantly between groups (Table 1).

The distribution of ACE I/D genotypes differed significantly between cases and controls ($p < 0.05$). As presented in Table 2, the DD genotype was more frequent among hypertensive patients than controls, reaching statistical significance. The D allele frequency was also significantly higher in the patient group. The II genotype and I allele were more common in the control group. Both groups were in Hardy–Weinberg equilibrium ($p > 0.05$).

$$\text{Sample size} = \frac{r+1}{r}\frac{(p^*)(1-p^*)(Z_\beta + Z_{\alpha/2})^2}{(p_1 - p_2)^2}$$

Figure 1. Representative 1% agarose gel electrophoresis showing the quality of isolated genomic DNA. Lane 1: 100 bp DNA ladder (Ready to Load, Solis Bio Dyne, Tartu, Estonia); Lanes 2–15: isolated genomic DNA.

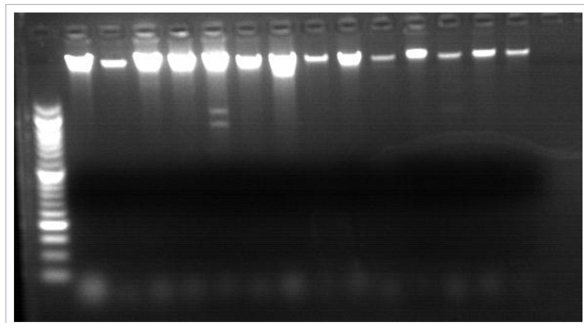

Figure 2. Representative 2% agarose gel electrophoresis showing PCR products of the ACE I/D polymorphism. Lane 1: 100 bp DNA ladder; Lanes 2, 5, 10, 14: homozygous II; Lanes 3, 7, 9, 12, 13: heterozygous ID; Lanes 4, 6, 8, 11: homozygous DD.

Table 3 presents the association between ACE I/D genotypes and clinical characteristics among the pooled study population ($n = 236$). Although participants with the DD genotype showed relatively higher mean values for blood pressure and lipid profile parameters compared with ID and II genotypes, these differences did not reach statistical significance ($p > 0.05$).

4 Discussion

Table 1. Demographic, clinical and behavioural characteristics of the study participants

Variables	HTN ($n = 118$)	Control ($n = 118$)	P-value
Sex (Male), n (%)	62 (52.5)	61 (51.7)	0.8963
Age (years), mean ± SD	59.3 ± 12.9	58.6 ± 9.6	0.5276
Family history of HTN, n (%)	52 (44.1)	56 (47.5)	0.6012
BMI (kg/m^2), mean ± SD	23.1 ± 4.3	22.5 ± 4.1	0.2433
SBP (mmHg), mean ± SD	146.7 ± 6.8	116.2 ± 3.9	<0.001*
DBP (mmHg), mean ± SD	90.9 ± 4.0	75.0 ± 8.2	<0.001*
FBG (mg/dl), mean ± SD	93.3 ± 19.5	91.5 ± 8.8	0.3529
Total cholesterol (mg/dl), mean ± SD	189.7 ± 57.4	151.6 ± 49.5	<0.001*
Triglyceride (mg/dl), mean ± SD	134.4 ± 53.7	108.1 ± 35.5	<0.001*
LDL-cholesterol (mg/dl), mean ± SD	94.2 ± 34.4	76.2 ± 27.0	<0.001*
HDL-cholesterol (mg/dl), mean ± SD	45.1 ± 10.1	51.1 ± 10.0	<0.001*
Creatinine (mg/dl), mean ± SD	0.81 ± 0.14	0.78 ± 0.13	0.2177
Smoking habit (Yes), n (%)	14 (11.9)	7 (5.9)	0.1095
Alcohol intake (Yes), n (%)	66 (55.9)	56 (47.5)	0.1927
High salt intake (Yes), n (%)	115 (97.5)	112 (94.9)	0.3079
Physical exercise (Yes), n (%)	10 (8.5)	16 (13.6)	0.2122
Stress (Yes), n (%)	72 (61.0)	62 (52.5)	0.1888

Note: * Statistically significant differences at $p < 0.05$.

Abbreviations: BMI, Body Mass Index; SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure; FBG, Fasting Blood Glucose; LDL, Low Density Lipoprotein; HDL, High Density Lipoprotein; HTN, Hypertension.

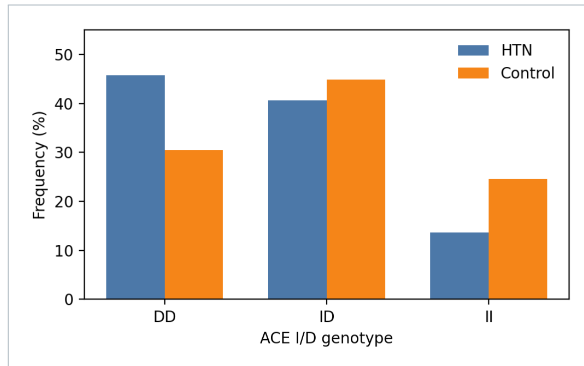

Figure 3. Distribution of ACE I/D polymorphism genotypes in hypertensive cases ($n = 118$) and healthy controls ($n = 118$). The overall genotype distribution differed significantly between cases and controls ($\chi^2 = 9.84$, $p = 0.007$). DD: homozygous deletion; ID: heterozygous; II: homozygous insertion.

The ACE I/D polymorphism of the ACE gene is potentially associated with HTN and can support the progression of hypertensive complications. However, there is inconsistent data on the relationship between HTN risk and ACE gene polymorphisms [15]. The ACE gene polymorphisms and related risk variables in HTN patients and healthy controls were studied in this investigation. It was demonstrated that individuals with HTN had significantly higher frequencies of the DD genotype and D allele (45.7% and 66.1%; $P < 0.05$) when compared to healthy controls (30.5% and 52.9%; $P < 0.05$) (Table 2; Figure 3). In contrast to the II genotype and I allele carriers, the DD genotype and D allele carriers demonstrated a higher risk of developing HTN.

This finding is in agreement with a case-control study at the University of Gondar Comprehensive Specialized Hospital, in which DD, ID, and II genotypes were 48.4%, 29.7%, and 21.9% in hypertensive patients, and 29.7%, 25.0%, and 45.3% in normotensive controls, respectively. That study found that HTN patients had significantly higher rates of the DD genotype (OR 3.38, 95% CL: 1.44–7.96; $P < 0.05$) and D allele (OR 2.36, 95% CL: 1.43–3.90; $P < 0.001$) [17]. A meta-analysis conducted in Africa, including eight studies, found that carriers of the D allele were 1.49 times more likely to develop HTN compared to I allele carriers (OR: 1.49; CL: 1.07–2.07), with greater susceptibility in

Table 2. Distribution of ACE genotypes and allele frequencies of the study participants

Genotype	HTN ($n = 118$)	Control ($n = 118$)	OR (95% CL)	p-value
DD	54 (45.7%)	36 (30.5%)	2.72 (1.29–5.71)	0.008*
ID	48 (40.6%)	53 (44.9%)	1.64 (0.79–3.39)	0.180
II	16 (13.7%)	29 (24.6%)	Ref.	
Allele Frequency				
D	156 (66.1%)	125 (52.9%)	1.73 (1.19–2.51)	0.003*
I	80 (33.9%)	111 (47.1%)	Ref.	

Note: * Statistically significant at $p < 0.05$. Abbreviations: Ref., Reference; CL, Confidence Level; OR, Odds Ratio.

Table 3. Association of ACE I/D polymorphism genotypes with clinical characteristics

Variables	DD ($n = 90$)	ID ($n = 101$)	II ($n = 45$)	p-value
BMI (kg/m^2)	22.9 ± 4.1	22.6 ± 4.2	22.9 ± 4.3	0.2739
SBP (mmHg)	135.5 ± 15.6	130.3 ± 16.2	125.8 ± 15.6	0.2299
DBP (mmHg)	84.9 ± 10.4	82.3 ± 10.6	80.5 ± 8.0	0.1158
FBS (mg/dl)	91.9 ± 14.4	93.2 ± 15.1	93.2 ± 16.5	0.8160
TC (mg/dl)	172.2 ± 61.9	175.9 ± 55.5	161.4 ± 48.6	0.5360
TG (mg/dl)	127.4 ± 54.2	121.4 ± 40.3	110.8 ± 41.3	0.2295
LDL-C (mg/dl)	86.3 ± 34.3	86.4 ± 31.1	82.1 ± 29.7	0.7345
HDL-C (mg/dl)	46.5 ± 9.7	48.7 ± 11.8	50.1 ± 9.7	0.1538
Creatinine (mg/dl)	0.79 ± 0.13	0.78 ± 0.13	0.82 ± 0.15	0.9729

Note: * Statistically significant at $p < 0.05$.

sub-Saharan Africa than North Africa [31]. Supporting evidence from Burkina Faso [32], Nigeria [33], Pakistan [34], Brazil [35], South India [36], and China [37] also showed an association between ACE-DD and elevated HTN risk.

A possible explanation for the ACE-DD–HTN link lies in circulating ACE levels and enzyme structure. Biochemical investigations show that individuals with the DD genotype have greater circulating ACE levels, which decrease progressively from DD to ID to II [38]. Structural analyses have also revealed that the D allele contains an additional C domain that more effectively binds angiotensin I and produces angiotensin II [39]. Consequently, increased plasma ACE levels raise bioactive angiotensin II, decrease arterial elasticity, and cause vasoconstriction, resulting in HTN [40].

In contrast, studies in Afro-Brazilian and Caucasian [41], Peruvian [42], Gabonese [43], Emirati [44], North Indian [45], and Tunisian populations [46] found no link between ACE I/D polymorphism and HTN. Ethnic disparities, demographic heterogeneity, regional variances, sample biases, and ecological variables all contribute to these inconsistencies [36]. Behavioral factors such as diet and exercise are also linked to changes in epigenetic state [47]; therefore, interactions between polymorphism and epigenetic alteration may further explain the complexity [48].

Although DD genotype participants exhibited

relatively higher mean values for blood pressure and lipid parameters compared with ID and II groups, these differences were not statistically significant (Table 3). This suggests that the ACE I/D polymorphism alone may not strongly influence clinical and biochemical characteristics in this study population. The absence of significant associations may reflect a relatively small sample size, inter-individual variability, ongoing antihypertensive treatment, and the multifactorial nature of HTN.

This study has limitations. First, the relatively limited sample size may introduce bias in detecting ACE I/D genotype–HTN associations. Second, there is no evidence that the examined genetic variants directly correlate with plasma ACE levels or other RAAS genes. The strength of the study is its combined evaluation of genetic, clinical, and biochemical risk factors for HTN in an understudied Ethiopian population.

5 Conclusion

This study demonstrated an association between the ACE I/D polymorphism of the ACE gene and HTN risk in the studied Ethiopian population. The DD genotype and D allele were more frequent among hypertensive patients than healthy controls. Therefore, the ACE I/D polymorphism may be a potential genetic marker associated with HTN risk, warranting further validation through larger multicenter and population-based studies. A population-based investigation should be required in future research to clarify the association between ACE and hypertension.

Abbreviations

ACE: Angiotensin Converting Enzyme; BMI: Body Mass Index; DNA: Deoxyribonucleic Acid; DBP: Diastolic Blood Pressure; FBG: Fasting Blood Glucose; HDL: High Density Lipoprotein; HTN: Hypertension; LDL: Low Density Lipoprotein; PCR: Polymerase Chain Reaction; RAAS: Renin-Angiotensin-Aldosterone System; SBP: Systolic Blood Pressure; TC: Total Cholesterol; TG: Triglycerol.

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Declarations

Authors' Contributions

All authors made a significant contribution to the work reported, whether in conception, study design, execution, acquisition of data, analysis, or interpretation. A.M. prepared the final draft of the manuscript. M.J. critically reviewed the article and gave final approval. All authors agreed on the journal to which the article has been submitted.

Ethics Approval and Consent to Participate

Ethical approval was obtained from the Institutional Review Board of the University of Gondar (VP/RTT/05/1016/2022) on July 13, 2022. Written informed consent was obtained from all participants before enrolment. Participants were informed of their right to withdraw at any time without affecting their medical care. Participant information was anonymised using coded identifiers. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki.

Availability of Data

The anonymised data used and/or analysed in this study are available from the corresponding author upon reasonable request.

Competing Interests

The authors declare no conflicts of interest.

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