Research Reports in Clinical Cardiology

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ORIGINAL RESEARCH

Association Between *ACE* I/D Gene Polymorphism and Dyslipidemia in Hypertensive Patients with Ischemic Heart Disease Complication Among Ethiopian Population

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Background: Ischemic heart disease (IHD) is characterized by lesions in major coronary arteries produced by the atherosclerotic phenomenon. IHD is currently thought to be a complicated disorder, and studies have revealed that, in addition to the usual traditional risk factors, genetic factors also play major roles in its occurrence. Due to the intricate interactions between genetic and environmental risk factors, the link between *ACE* polymorphisms and other risk variables in IHD is not fully characterized. The purpose of this study was to look at how ACE gene I/D polymorphism and dyslipidemia affect the risk of developing IHD complications in hypertensive patients.

Methods: A hospital-based case–control study of 70 hypertensive IHD patients and 70 age- and sex-matched healthy controls was conducted. Clinical parameters were measured to assess the associated risk factors. Deoxyribonucleic acid (DNA) was isolated from blood samples, and the *ACE* I/D genotypes were identified using polymerase chain reaction (PCR) and analyzed by agarose gel electrophoresis.

Results: Our analysis showed that the *ACE*-DD genotype (OR = 2.72, 95% CL = 1.11–6.64; P < 0.05) and D allele (OR = 1.93, 95% CL = 1.18–3.13; P < 0.05) are considerably higher in patients than controls. Our study also identified dyslipidemia, which was found to be considerably greater in patients than controls (OR = 4.69, 95% CL = 1.86–11.82; *P* < 0.001), indicating that it is a major risk factor for the onset and progression of IHD.

Conclusion: The *ACE* I/D gene of the DD genotype and the D allele have been linked to an increased risk of developing hypertensive IHD complications. Moreover, dyslipidemia is a risk factor for the onset of ischemic heart disease.

Keywords: angiotensin-converting enzyme, blood pressure, coronary artery disease, genotype, hyperlipidemia

Introduction

Ischemic heart disease (IHD) is one of the leading causes of death globally and is characterized by fat accumulation in the blood vessels, a reduction of the artery cavity, slowed blood flow, and ultimately ischemic heart failure.[1] IHD is currently regarded as a complex illness, and new research has demonstrated that genetic and environmental variables play a significant role in the development of the disease in addition to established risk factors such as age, sex, smoking, dyslipidemia, hypertension (HTN), and type 2 diabetes mellitus T2DM.[2] Hypertension, a significant risk factor for IHD, is estimated to cause 7.5 million deaths worldwide. It has been demonstrated that blood pressure levels are gradually and favorably associated with the onset of ischemic heart disease.[3]

Lipids and lipoproteins are becoming increasingly important in clinical practice, mainly because they are associated with IHD in cases of abnormalities known as dyslipidemia.[4] Increased blood levels of triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), or total cholesterol (TC) and decreasing blood level of high-density lipoprotein cholesterol (HDL-C) are the hallmarks of dyslipidemia.[5] Although estimates suggest that over 50% of adults globally have dyslipidemia, the prevalence of the condition varies depending on the population.[6]

Received: 12 November 2022
Accepted: 4 February 2023
Published: 12 February 2023

The *ACE*, a key element of RAAS, catalyzes the formation of the vasoactive peptide angiotensin II from its substrate, angiotensin I. Angiotensin II plays a critical role in the control of blood pressure, sodium homeostasis, fluid volume, and electrolyte balance.[7] Apart from this role, recent evidence suggests that RAAS and its component *ACE* are involved in the pathogenesis of IHD by promoting the development of HTN and dyslipidemia. The I/D polymorphism of the *ACE* gene is due to an insertion (I) or deletion (D) of a 287-base pair (bp) Alu sequence in intron 16, giving rise to three genotypes: II, ID, and DD.[8] Previous research has indicated that the *ACE* DD genotype is linked with higher plasma *ACE* concentrations and increased vasoconstriction, which raises the risk of HTN-related IHD complications. However, this is controversial, as researchers have produced contradictory findings with no link between the DD genotype and IHD.[9] Thus, this study aimed to identify the link between the *ACE* gene I/D polymorphism and hypertensive IHD complications and, further, to examine the effect of dyslipidemia on the onset of IHD in the Ethiopian population.

Materials and Methods

Study Participants

From May to August 2022, a hospital-based matched case-control study was conducted at Debre Tabor Referral Hospital. It has a follow-up medical referral clinic (MRC) for serious chronic conditions like IHD and HTN, where treatment and patient follow-up are provided. All patients who visit MRC were the source population, and patients who are under follow-up for HTN with IHD complications were study subjects. The controls for this investigation were any normotensive healthy volunteers who were available during the study period and matched for age and sex. A total of 140 participants of both sexes were enrolled in the study, including 70 IHD patients with hypertension and 70 healthy control subjects. Patients who are diagnosed with kidney disease, secondary HTN, or a chronic bacterial or viral infection, or who are unable to respond or are not willing to sign informed consent were excluded.

Data Collection Methods

The socio-demographic characteristics of both patients and healthy control subjects were taken through a semi-structured questionnaire. Portable digital scales and portable stadiometers were used to determine body weight and height, respectively. Weight in kilograms divided by the square of height in meters is how body mass index (BMI) is determined. Participants were classified 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 (≥30 kg/m^2) based on their BMI.[10] A digital instrument was used to measure blood pressure in the sitting stance after 5 minutes of rest, and the mean of three readings was used to compute SBP and DBP. Participants were categorized as hypertensive, if mean SBP ≥140mmHg and mean DBP ≥90mmHg or if they used antihypertensive medication; pre-hypertension, SBP 120–139 mmHg or DBP 80–89 mmHg; normal blood pressure, SBP <120 mmHg and DBP <80 mmHg.[11]

Sample Collection and Laboratory Methods

All participants, including patients and healthy controls, had a blood sample of 5 mL taken from the median cubital vein by laboratory staff under quality control and safety procedures. From the 5 mL sample, 3 mL was retained in the test tube without anticoagulants to allow the blood to clot. The tubes were then spun at 7602 ×g for 5 minutes to extract the serum, which was then collected into new tubes for biochemical tests. Enzymatic analyses of TC, TG, LDL, HDL, creatinine, and glucose were performed on each test in the Debre Tabor Referral Hospital diagnostic laboratory using the Dimension EXL 200 fully automated analyzer. If the fasting plasma glucose concentration is greater than 110 mg/dL, diabetes mellitus has been identified.[12] Dyslipidemia can be defined if TC, TG, and LDL levels are above 200 mg/dL, 150 mg/dL, and 1300 mg/dL, respectively, and the HDL level is below 60 mg/dL.[13] Kidney disease is diagnosed if the blood creatinine concentration is greater than 1.3 mg/dL.[14]

Genomic DNA was extracted from the remaining 2 mL samples collected in ethylenediaminetetraacetic acid (EDTA) (anticoagulant) containing tubes of each participant in the University of Gondar molecular biology laboratory. The non-enzymatic salting-out approach[15] was used to isolate DNA from EDTA anticoagulated blood from both patients and controls. This blood was then transferred to a sterile 1.5-mL Eppendorf tube. Red blood cells (RBCs) were lysed and eliminated using a buffer solution. Similarly, nuclear lysis buffer solution was used to lyse white blood cells. Then, to precipitate and remove proteins, 6M NaCl of a highly concentrated salt was applied. After freezing with isopropanol and washing with 70% ice-cold

ethanol, the DNA was precipitated. Genomic DNA was then dissolved in Tris-EDTA buffer (TE). The purity of extracted genomic DNA was verified utilizing 1% agarose gel electrophoresis (Figure 1), and the sample was stored at −20 °C till used.[16]

Direct PCR was used to identify the I/D alleles of the *ACE* gene polymorphism using specific primers (5′- CTG GAG ACC ACT CCC ATC CTT TCT-3′ and 5′- GAT GTG GCC ATC ACA TTC GTC AGA T-3′, respectively).[17] A final volume (25 µL) of PCR mixture was prepared by combining 12.5 µL of master mix (MgCl2, dNTPs, PCR buffer, and Taq polymerase), 1 µL of forward primer, 1µL of reverse primer, 2 µL of each sample, and 8.5 µL of PCR-grade water. The first denaturation step of the PCR amplification was set at 95°C for 5 minutes. The DNA was then amplified for 35 cycles with denaturation at 94°C for 30s, annealing at 58°C for 30s, extension at 72°C for 1 minute, and a final extension at 72°C for 5 minutes.[18]

ACE I/D genotypes 490 bp band (II), 190 bp band (DD), and both 490 and 190 bp band (ID) PCR products were electrophoretically separated for 50 minutes at 120 V on a 2% agarose gel (Figure 2). The PCR-amplified products (12 µ l) were mixed with 3 µ l of loading dye before being injected into the agarose gel wells. DNA ladders, which are molecular weight markers, were electrophoresed along with the DNA fragments to be able to estimate the sizes of fragments of interest, and 3 µ l of 2% Ethidium Bromide was also used for staining. In 1X tris acetate EDTA (TAE) buffer, electrophoresis was performed, and a UV transilluminator was used to see the gel.[19]

Statistical Analysis

The data were analyzed using STATA version 14.1. The means and standard deviations (x±s) were the measures used to describe quantitative data. A *t*-test for independent samples was used to compare continuous variables between hypertensive IHD patients and healthy controls. Using the chi-square test, the genotype and allele frequency distributions were compared. The risk associations of *ACE* gene I/D polymorphisms with hypertensive IHD were assessed using logistic regression at a 95% confidence level (CL). A one-way analysis of variance (ANOVA) was used to compare the association between the *ACE* genotypes and clinical variables. P values of less than 0.05 were considered statistically significant.

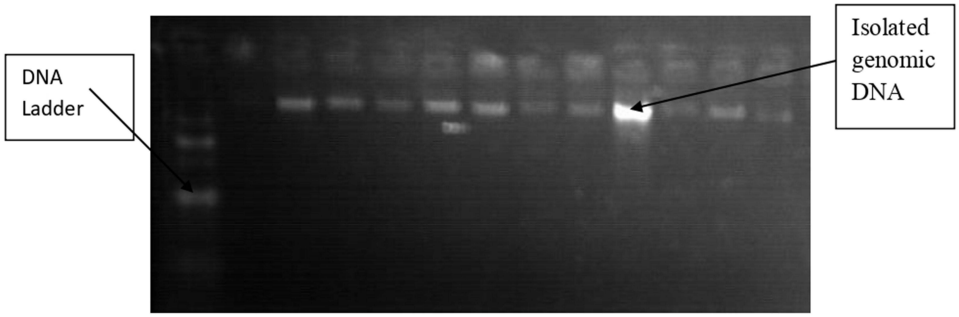

Figure 1 1% agarose gel electrophoresis showing the quality of isolated genomic DNA.

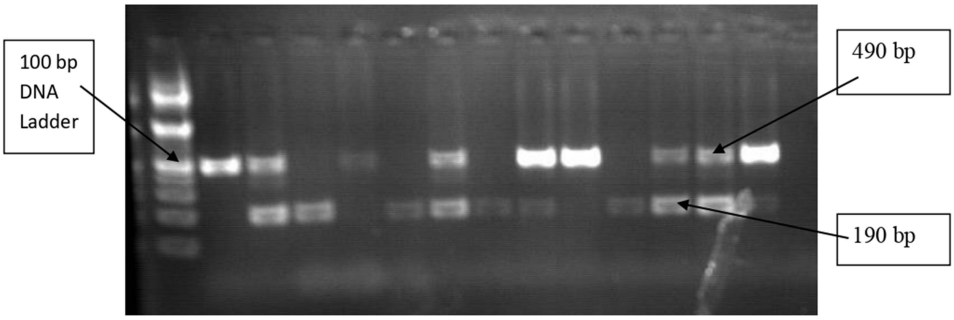

Figure 2 Agarose gel (2 %) electrophoresis showing PCR products of the *ACE* I/D gene.

Results

Socio-Demographic and Clinical Characteristics

Distribution by sex and age was similar between hypertensive IHD patient cases and normotensive healthy control groups. Of the total 70 hypertensive IHD participants, 37 (52.86%) were male and 33 (47.14%) were female. Similarly, among the 70 healthy control groups, 36 (51.43%) were males and 34 (48.57%) were females. The mean ages of the cases and control study groups were 59.84 ±14.25 and 57.21 ± 6.66, respectively. SBP, DBP, TC, TG, and LDL-C levels were considerably higher on average in patients compared to controls, but HDL-C levels were lower ($p < 0.001$). Patients and controls did not differ in terms of family history of HTN and IHD ($p > 0.05$), nor did they differ in terms of body mass index (BMI), fasting blood glucose (FBG), or blood creatinine (CR) level ($p > 0.05$) (Table 1).

Distribution of *ACE* Genotypes and Allele Frequencies

The genotype distribution of *ACE* I/D gene polymorphisms is given in Table 2. In IHD patients, the frequencies of the DD, ID, and II genotypes were respectively 51.43%, 31.43%, and 17.14%; in controls, the rates were 31.43%, 40.00%, and 28.57% (Figure 3). The homozygous *ACE* DD genotype was observed to be substantially more common in IHD cases than in controls (odds ratio [OR] = 2.72, 95% CL = 1.11–6.64; P = 0.007).

Association Between *ACE* Genotypes and Clinical Parameters

Table 3 lists the clinical parameters of hypertensive IHD patients and normotensive healthy controls in relation to *ACE* ID genotypes. The *ACE* genotypes (DD, ID, and II) in the study groups were assessed with fasting blood glucose, blood pressure, and lipid profiles. Blood pressure was more strongly correlated with the *ACE*-DD genotype than the ID and II genotypes for SBP (136.65±15.68 Vs 129.24±16.02 and 125.81±15.55; $P < 0.01$) and DBP (85.10±11.91 Vs 82.40±8.57 and 80.91±8.14; $P < 0.05$), respectively. The other clinical parameters were not found to be significant with the genotypes in the study groups ($P > 0.05$).

Table 1 Demographic, Clinical and Behavioural Characteristics of the Study Participants in Debre Tabor Referral Hospital, Northwest Ethiopia, 2022

Variables	IHD (n=70)	Control (n=70)	P-value
Sex (M/F)	37/33	36/34	0.7344
Age (years)	59.84 ± 14.25	57.21 ± 6.66	0.1645
BMI (kg/m^2)	23.20 ± 4.22	22.44 ± 4.22	0.2893
SBP (mmHg)	146.62±7.04	116.18±4.04	<0.001*
DBP (mmHg)	91.08±4.05	75.17±7.75	<0.001*
FBG (mg/dL)	94.14±18.47	91.02±8.97	0.2066
Creatinine (mg/dL)	0.82±0.15	0.79±0.13	0.3434
Total cholesterol (mg/dL)	195.90±64.78	149.48±53.34	<0.001*
Triglyceride (mg/dL)	145.77±74.77	108.40±37.77	<0.001*
LDL-cholesterol (mg/dL)	96.97±37.34	75.61±26.82	<0.001*
HDL-cholesterol (mg/dL)	43.94±11.31	50.22±9.04	<0.001*
Dyslipidemia (%)	34.28	10.00	<0.001*
Family history of HTN (%)	47.14	58.57	0.1756
Family history of IHD (%)	14.29	21.14	0.1423
Smoking habit (yes/no)	12/58	7/63	0.2173
Alcohol intake (yes/no)	44/26	40/30	0.4902
Salt intake (yes/no)	64/6	67/3	0.3012
Physical exercise (yes/no)	5/65	11/59	0.1110
Stress (yes/no)	41/29	34/36	0.2355

Note: *P-value <0.05 is considered statistically significant.

Abbreviations: HTN, hypertension; IHD, ischemic heart disease; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; FBG, fasting blood glucose; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

Table 2 Distribution of *ACE* Genotypes and Allele Frequencies of the Study Participants in Debre Tabor Referral Hospital, Northwest Ethiopia, 2022

Genotype	IHD (n=70)	Control (n=70)	OR (95% CL)	p-value
DD	36 (51.43%)	22 (31.43%)	2.72 (1.11–6.64)	0.027*
ID	22 (31.43%)	28 (40.00%)	1.30 (0.52–3.24)	0.560
II	12 (17.14%)	20 (28.57%)	Ref	
Allele Frequency				
D	94 (67.14%)	72 (51.43%)	1.93 (1.18–3.13)	0.007*
I	46 (32.86%)	68 (48.57%)	Ref	

Note: *P-value <0.05 is considered statistically significant.
Abbreviations: Ref, reference; CL, confidence level; OR, odds ratio.

Association Between Ischemic Heart Disease and Dyslipidemia

The association between IHD and dyslipidemia was analyzed in our study. A comparison of lipid profiles between patients and controls revealed that patients with hypertensive IHD had lower levels of HDL-C and higher levels of TC, TG, and LDL-C than apparently healthy control groups ($P < 0.001$), and patients had a higher percentage of dyslipidemia 34.28% (n=24) as compared with controls 10% (n=7) (Table 4).

Discussion

Ischemic heart disease is a polygenic illness that is characterized by complicated interactions across several pathophysiological processes involving numerous genes and environmental risk factors. Considerable research has looked at how the D allele and IHD are related, and the *ACE* DD genotype is linked to greater blood *ACE* activity.[20] In our study, the *ACE*-DD genotype (odds ratio [OR] = 2.72, 95% CL = 1.11–6.64; P = 0.027) and D allele (odds ratio [OR] = 1.93, 95% CL = 1.18–3.13; P = 0.007) showed a stronger association with IHD patients compared to ID/II genotype and I allele, respectively. Compared with other studies, our results are in agreement with a meta-analysis carried out in the Chinese population that included 44 studies and found that patients with the DD genotype were found to be nearly twice as likely to develop IHD compared to the ID and II genotypes (odds ratio [OR] = 1.95, 95% CL = 1.66–2.29; $P < 0.001$).[21] The findings of other investigations conducted in Iran[22] and Pakistan[23] did not, however, uncover any links between IHD and the *ACE* I/D polymorphisms.

The etiology by which the *ACE* I/D genotype may influence individuals to develop IHD remains incomplete. According to a number of findings, coronary endothelial dysfunction is linked to significant coronary risk factors.[24]

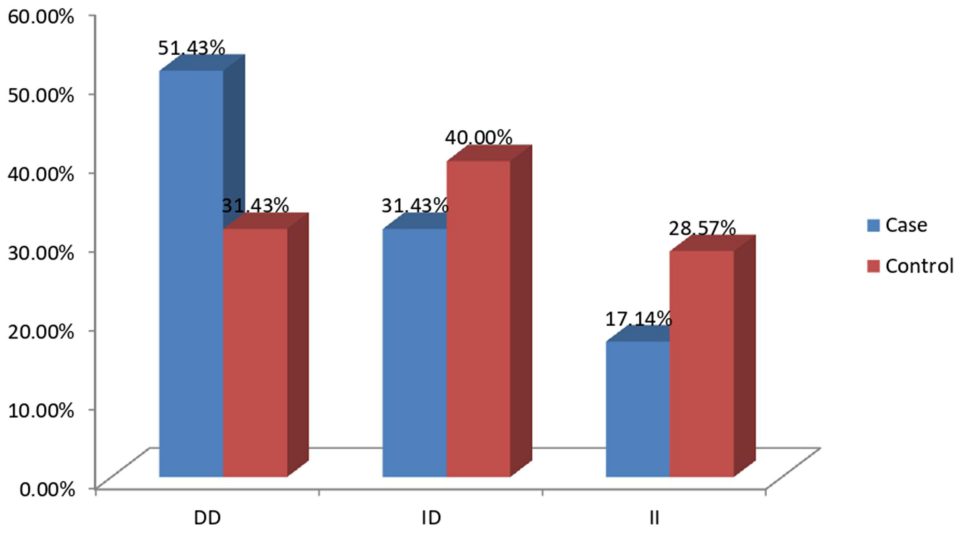

Figure 3 Distribution of the *ACE* I/D genotypes in cases and controls.

Table 3 Association of *ACE* I/D Genotype with Clinical Characteristics in Debre Tabor Referral Hospital, Northwest Ethiopia, 2022

Variables	Genotypes			
	DD (N=58)	ID (N=45)	II (N=37)	p-value
Sex (M/F)	30/28	30/15	16/21	0.5892
Age (years)	57.72±12.31	59.88±10.69	58.13±9.89	0.4595
Family history HTN (%)	58.62	42.22	56.75	0.6938
Family history IHD (%)	22.41	13.33	8.10	0.0562
BMI (kg/m^2)	22.90±4.32	22.40±3.99	23.21±4.41	0.3184
SBP (mmHg)	136.65±15.68	129.24±16.02	125.81±15.55	<0.01*
DBP (mmHg)	85.10±11.91	82.40±8.57	80.91±8.14	<0.05*
FBG (mg/dL)	93.63±15.02	92.46±13.14	91.08±15.64	0.2446
TC (mg/dL)	176.98±70.12	179.86±62.55	157.24±51.82	0.1011
TG (mg/dL)	136.62±72.20	130.26±52.90	108.27±51.08	0.1769
LDL-C (mg/dL)	85.27±36.29	90.22±35.46	83.10±29.01	0.1836
HDL-C (mg/dL)	85.27±36.29	47.28±12.10	49.54±10.00	0.0737
Creatinine (mg/dL)	0.81±0.14	0.81±0.12	0.86±0.14	0.9985

Note: *P-value <0.05 is considered statistically significant.

Table 4 Association Between Ischemic Heart Disease and Dyslipidemia in Debre Tabor Referral Hospital, Northwest Ethiopia, 2022

Genotype	IHD (n=70)	Control (n=70)	OR (95% CL)	p-value
Dyslipidemia	24 (34.28%)	7 (10.00%)	4.69 (1.86–11.82)	<0.001*
Non-dyslipidemia	46 (65.71%)	63 (90.00%)	Ref	

Note: *P-value <0.05 is considered statistically significant.
Abbreviations: Ref, reference; CL, confidence level; OR, odds ratio.

The pathophysiology of IHD and endothelial dysfunction are both affected by the *ACE* I/D polymorphism. Increased *ACE* activity raises angiotensin II, which influences cell development and proliferation by triggering a number of cytokines and growth factors that reduce nitric oxide bioavailability and result in endothelial dysfunction.[25] It was previously reported that increased *ACE* expression in macrophages and smooth muscle cells from coronary artery plaques suggests that *ACE* activity in lesions is a major cause of IHD progression.[26]

We also analyzed a number of conventional cardiovascular risk variables in patients with IHD and found that HTN and dyslipidemia significantly influence the occurrence of IHD. These findings agreed with research conducted in Pakistan,[23] Egypt,[27] and Iran.[28] Hypertension and dyslipidemia contribute to IHD through a number of different mechanisms. An increased risk of developing dyslipidemia, atherosclerosis, and IHD may result from abnormal processes such as arterial wall damage, metabolic abnormalities, oxidative stress, and endothelial dysfunction.[29]

Our study indicated that IHD patients had significantly higher levels of dyslipidemia than did controls (odds ratio [OR] = 4.69, 95% CL = 1.86–11.82; $P< 0.001$). It is increasingly documented that dyslipidemia has been identified as a major risk factor for HTN-correlated IHD complications. However, it is still uncertain whether there is a complex interplay between genetic predisposition and environmental variables in the emergence of dyslipidemia.[30] So, we explored the link between *ACE* gene I/D polymorphism and dyslipidemia based on TC, TG, LDL-C, and HDL-C levels. According to our findings, the relationship between *ACE* gene I/D polymorphisms and dyslipidemia in our study group was not significant (P > 0.05) (Table 3).

Conclusion

In summary, this study shows an association between the DD genotype and D allele of the *ACE* gene I/D polymorphisms and the occurrence of hypertensive IHD complications. In order to diagnose HTN early, identify it, and avoid its IHD

consequence, the *ACE* gene I/D polymorphism may be employed as a biomarker. Additionally, IHD was linked to higher SBP, DBP, TC, TG, LDL-C, and lower HDL-C values. These findings further support the key significance of the relationship between HTN and dyslipidemia in the onset of ischemic heart disease.

Abbreviations

ACE, angiotensin-converting enzyme; BMI, body mass index; IHD, ischemic heart disease; DNA, deoxyribonucleic acid; DBP, diastolic blood pressure; EDTA, ethylenediaminetetraacetic acid; FBG, fasting blood glucose; HDL, high-density lipoprotein; HTN, hypertension; I/D, insertion/deletion; LDL, low-density lipoprotein; PCR, polymerase chain reaction; RAAS, renin–angiotensin–aldosterone system; SBP, systolic blood pressure; TC, total cholesterol; TG, triglyceride; T2DM, type 2 diabetes mellitus.

Data Sharing Statement

The corresponding author can provide the data used and/or analyzed during the current study upon request.

Ethics Approval and Consent to Participate

The study protocol was approved by the University of Gondar Institutional Review Board (IRB) for Ethics in Human Research (Ref. VP/RTT/05/1016/2022). Study participants were recruited only after informed written consent was obtained from each of them. All the data were obtained anonymously and treated confidentially. All the procedures for data collection were conducted according to the principles of the Helsinki Declaration.

Acknowledgments

We would like to give our heartfelt thanks to the Debre Tabor Referral Hospital diagnostic laboratory and the University of Gondar molecular biology laboratory staff for the support we needed to do this study.

Author Contributions

Both authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable or all aspects of the work.

Disclosure

The authors report no conflicts of interest.

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