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Original Article

Association between angiotensinogen M235T gene polymorphism and risk of hypertension: A case control study among Ethiopian patients

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Abstract

Background*: Hypertension is a major public health problem in both developing and developed nations because it is highly prevalent and is associated with complications. Numerous environmental and genetic variables are linked to the occurrence of the disease. It may be influenced by the renin-angiotensin-aldosterone system, which preserves bodily homeostasis. The angiotensinogen gene M235T polymorphisms that has an effect on the activity of the renin-angiotensin-aldosterone system are related to the high hypertension risk. The aim of this study was to find out the association between angiotensinogen M235T gene polymorphism and the risk of developing hypertension.*

Methods: *A total of 306 samples - 153 patients with hypertension and 153 age- and sex-matched healthy controls were selected using a simple random sampling technique. Clinical and biochemical variables were measured to assess the associated risk factors. Blood samples from the patients and matched controls were used to isolate deoxyribonucleic acid. The AGT M235T genotypes were identified using polymerase chain reaction and analyzed by agarose gel electrophoresis. Logistic regression with a 95% confidence interval (CI) was employed to assess the risk correlations of AGT gene M235T polymorphisms with hypertension.*

Results*: Our analysis showed that the AGT-TT genotype (odds ratio [OR] = 3.11, 95% CL = 1.67–5.79, P< 0.001) and T allele (OR = 2.18, 95% CL = 1.56–3.04, P< 0.001) are considerably higher in hypertensive patients than in healthy controls. Our study also identified the clinical risk factors for hypertension, such as, total cholesterol, triglycerol, low density lipoprotein-cholesterol, and high density lipoprotein-cholesterol levels, which were significantly higher in patients compared to controls (P< 0.001).*

Conclusion*: The AGT M235T genes of the TT genotype and the T allele are associated with an increased risk of hypertension among the Ethiopian patients. A population-based epidemiological study is needed corroborate the association between AGT and HTN.*

Keywords : *Angiotensinogen; Blood Pressure; Genotypes; Renin-Angiotensin-Aldosterone System; Risk Factor*
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Introduction

Hypertension (HTN) is a common disease manifested primarily by elevated blood pressure and remains one of the leading causes of death from cardiovascular disease [1]. With accelerated population aging, the prevalence of HTN shows an increasing trend in both developed and developing countries [2]. It is a multifactorial and complex disorder that is influenced not only by several susceptible genes but also by environmental stimuli and lifestyle [3]. As with most difficult conditions, blood pressure fluctuations are thought to be influenced by age, gender, and ethnicity as well as clinical factors such as obesity, insulin resistance, and dyslipidemia [4]. There are more than 150 potential genes associated with the control of blood pressure that are connected to several pathways; the renin-angiotensin-aldosterone

system (RAAS) is one of these pathways that has received more attention. [5].

The natural substrate of RAAS, angiotensinogen (AGT), is produed in the liver and released into the bloodstream. It interacts with renin to form angiotensin I, a precursor to angiotensin II, and is crucial for maintaining fluid homeostasis and controlling blood pressure [6]. AGT is a 12 kb long gene on chromosome 1 (1q42–q43) that belongs to the serpin gene superfamily and has 5 exons and 4 introns. The AGT gene's M235T polymorphism refers to the substitution of the amino acid threonine (T) for methionine (M) at position 235, giving rise to three genotypes: MM, MT, and TT [7]. When compared to those with the MM genotype, those with the TT genotype have greater blood pressure and plasma AGT levels because of the AGT-M235T polymorphism [8].

Studies on hypertensive and normotensive individuals have shown an association between the chromosomal region containing the AGT M235T gene and blood pressure. This led to the hypothesis that AGT M235T may be a candidate gene for essential HTN in humans and that the TT genotype of the AGT gene is correlated with HTN in different ethnic populations [9]. However, there are conflicting results on the effect of the AGT M235T gene polymorphism on HTN. This discrepancy is emphasized in certain studies that established a link between these polymorphisms and HTN, while others did not [10]. Thus, the purpose of this study was to identify the association of AGT M235T gene polymorphisms with risk of HTN and to determine the effect of clinical parameters in predicting the occurrence of HTN among the Ethiopian population.

Patients and Methods

Study Design and Participants

From May to August 2022, a hospital-based matched case control study was conducted at Debre Tabor Referral Hospital. The hospital has a follow-up medical referral clinic (MRC) for chronic illnesses, including HTN where treatment and follow-up services are provided for patients with HTN take place. All patients who visited MRC were the source population, and patients who were under follow-up for HTN were the cases. Age and sex-matched normotensive patients managed at the facility during the study period served as controls.

Inclusion and Exclusion Criteria

The study included patients who were diagnosed to have hypertension and were receiving treatment and follow-up care at MRC for at least one year. The controls were age- and sex-matched healthy individuals with normal blood pressure results from the same geographical location and social status. Patients who were diagnosed to have renal disease, secondary HTN, or a chronic bacterial or viral infection were excluded. Patients who were unable to respond or are not willing to give informed consent were excluded from this study.

Sample Size Determination

The sample size was determined using analytical study sample size calculation by taking confidence level of 95%, a power of 80% with a double population proportion formula.

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

Since similar studies are not done in the Ethiopian population, the sample size was determined by assuming expected proportions of 0.35 associations among the hypertensive case group and 0.20 among the normotensive control group [11]. The final sample size after adding the 10% non-response rate was 306 (153 cases and 153 controls) of both sexes. Participants were selected by simple random sampling methods, using a table of random numbers (TRN), from all the registered patients.

Data Collection Methods

The socio-demographic characteristics of both patients and healthy control subjects were assessed using a semi-structured questionnaire. Portable digital scales and portable stadiometers were used to determine body weight and height, respectively. Body mass index (BMI) was computed by dividing weight (in kilograms) by height (in meters squared). 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 [12]. A digital instrument was used to measure blood pressure in the sitting stance after 5 minutes of rest, and the average of three readings was to determine and record the SBP and DBP. Participants were categorized as hypertensive if their 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 [13].

Sample Collection and Laboratory Methods

All participants, including patients and healthy controls had a blood sample of five milliliters taken from the median cubital vein by laboratory staff following 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 centrifuged 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. Results were then scored by an investigator blinded to the sample withdrawal condition

and experimental groups. diabetes mellitus has been identified if the fasting plasma glucose level is greater than 110 mg/dl [14]. Dyslipidemia can be defined if TC, TG, and LDL levels are above 200 mg/dl, 150 mg/dl, and 130 mg/dl, respectively, and the HDL level is below 60 mg/dl [12]. Kidney disease was diagnosed if the blood creatinine concentration was >1.3 mg/dl [15].

In the molecular biology laboratory at the University of Gondar, genomic deoxyribonucleic acid (DNA) was extracted from the remaining 2 ml of samples collected in EDTA-containing tubes from each participant. The non-enzymatic salting-out approach [16] was used to isolate DNA from ethylenediaminetetraacetic acid (EDTA) anticoagulated blood from both patients and controls. The blood was then put into a clean 1.5 ml Eppendorf tube. By lysing and eliminating them with a buffer solution, red blood cells were removed. To lyse white blood cells, a nuclear lysis buffer solution was used. Then, to precipitate and remove proteins, 6 M, highly concentrated sodium chloride (NaCl) was applied. After freezing with isopropanol and washing with 70% ice-cold ethanol, the DNA was precipitated. Then, Tris-EDTA (TE) buffer was used to dissolve genomic DNA. The quality of isolated genomic DNA was verified utilizing 1% agarose gel electrophoresis (Figure 1), and the sample was kept at -20 °C until it was needed [17].

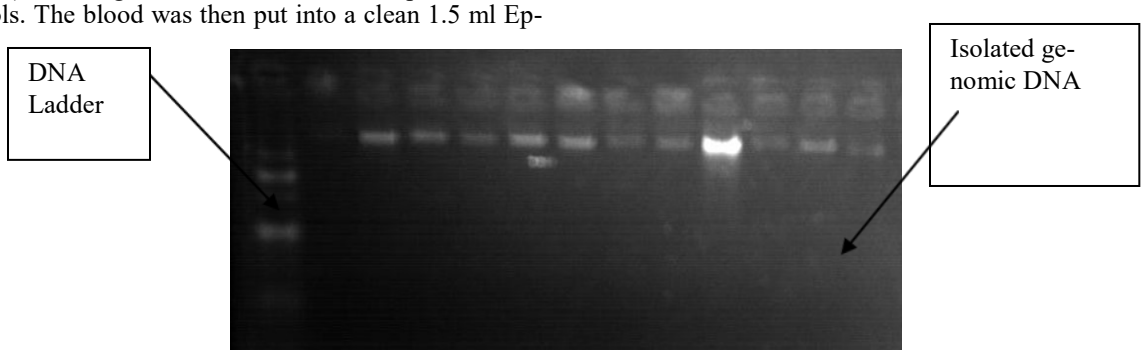

Figure 1: 1% agarose gel electrophoresis showing the quality of isolated genomic DNA

The AGT M235T genotypes were identified using the forward primer, 5'CAG GGT GCT GTC CAC ACT GGA CCC C-3' and the reverse primer, 5'-CCG TTT GTG CAG GGC CTG GCT CTC T-3'. Amplification was performed in a 25 µl reaction mixture using 12.5 µl of master mix (constituting of MgCl2, dNTPs, PCR buffer, and Taq polymerase), 1 µl forward primer, 1 µl reverse primer, 2 µl of each sample, and 8.5 µl PCR grade water added to complete the total volume . The initial denaturation stage of the amplification was set at 10 min at 95 °C, and it was then followed by 35 cycles of amplification with denaturation stages of 10 s at 94 °C, primer hybridization stages of 30 s at 64 ° C, elongation stages of 20 s at 72 °C, and a final elongation stage of 5 min at 72 °C. In a 10 µl mixture containing 5 U of the particular restriction enzyme Tth111I, the amplified fragment of 165 bp was exposed to enzymatic digestion for 3 h at 65 °C. The mutant T235 allele splits into two pieces that are 141 and 24 bp, but the normal M235 allele is not digested [18]. Finally, AGT M235T genotypes 165 bp band (MM), 141 bp band (TT), and both 165 and 141 bp band (MT) PCR products were separated electrophoretically for 50 minutes at 120 V on a 2% agarose gel (Figure 2).

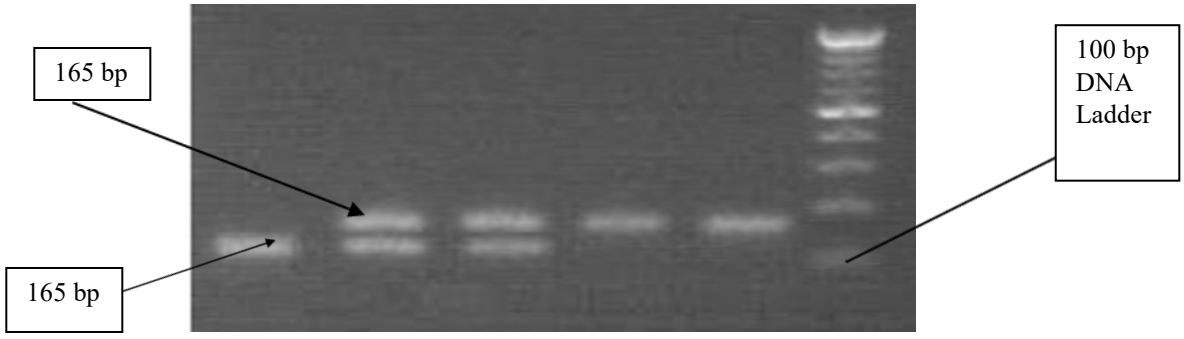

Figure 1: 1% agarose gel electrophoresis showing the quality of isolated genomic DNA

Statistical Analysis

The data were analyzed using STATA version 14. Mean and standard deviation (x+s) were used to summarize the quantitative data, and the t-test for independent samples was applied to test statistical differences in continuous variable measures among the cases and controls. The chi-square test was used to determine the level of significance the differences in genotype and allele frequencies in the two groups. Logistic regression with a 95% confidence interval (CI) was employed to assess the risk correlations of AGT gene M235T polymorphisms with HTN. A one-way analysis of variance (ANOVA) was used to compare the association between AGT genotypes and clinical explanatory factors. Statistical significance was defined as a p-value less than 0.05.

Results

Socio-Demographic and Clinical Characteristics

Of the total 153 patients with HTN, 80 (52.3%) were male and 73 (47.7%) were female. Among the 153 healthy control groups, 77 (50.3%) were male and 76 (49.7%) were female. The mean age of the study group was 58.7 ± 12.8 and 57.5 ± 6.9 for cases and controls, respectively. The clinical risk factors of HTN such as total cholesterol (TC), triglycerol (TG), LDL-cholesterol, and HDL-cholesterol levels are significantly higher in patients when compared to controls (P< 0.001). However, there were no significant differences in body mass index (BMI), fasting blood glucose (FBG), or blood creatinine level between the two

Table 1: Demographic and clinical characteristics of the study participants in Debre Tabor Referral Hospital, Northwest Ethiopia, 2022

Variables	HTN (n=153)	Control (n=153)	P-value
BMI (Kg/m^2)	23.9±3.9	23.2±3.5	0.0965
FBG (mg/dl)	93.8±19.1	91.1±8.6	0.1020
Creatinine (mg/dl)	0.82±0.14	0.80±0.12	0.1448
Total Cholesterol (mg/dl)	192.8±60.5	147.5±51.2	< 0.001*
Triglyceride (mg/dl)	142.6±67.5	104.9±37.0	< 0.001*
LDL-Cholesterol (mg/dl)	96.2±35.6	73.3±27.7	< 0.001*
HDL-Cholesterol (mg/dl)	43.4±10.2	51.6±10.0	< 0.001*
Family history of HTN (%)	54.2 %	55.5 %	0.8183

Note: *P-value <0.05 is considered statistically significant. **Abbreviations:** HTN, Hypertension; BMI, Body Mass Index;; FBG, Fasting Blood Glucose; LDL, Low Density Lipoprotein; HDL, High Density Lipoprotein.

Distribution of AGT Genotypes and Allele Frequencies

The frequencies of the TT, MT, and MM genotypes among the patient group were 56.2%, 28.1%, and 15.7%, respectively, whereas in the control group the same were found to be 30.1%, 43.8%, and 26.1%, respectively (Figure 3). A significant difference is observed in the distribution of AGT genotype polymorphism between the two groups. Furthermore, the frequency of homozygous TT genotype in patients was three times higher than in the control group (OR=3.11; 95% CI: 1.67-5.79; P< 0.001). The allelic frequencies showed high significance between the two groups, in which the T allele was two times higher than the M allele in hypertensive patients (OR:=2.18; 95% CI: 1.56-3.04; P< 0.001) compared to healthy controls. However, AGT genotypes MT and MM were less frequent in hypertensive patients in comparison to healthy controls (Table 2).

Table 2: Distribution of AGT genotypes and allele frequencies of the study participants in Debre Tabor Referral Hospital, Northwest Ethiopia, 2022

Genotype	HTN (n=153)	Control (n=153)	OR (95% CL)	p-value
TT	86 (56.2 %)	46 (30.1 %)	3.11 (1.67-5.79)	< 0.001*
MT	43 (28.1 %)	67 (43.8 %)	1.06 (0.56-2.01)	0.835
MM	24 (15.7 %)	40 (26.1 %)	Ref	
Allele Frequency				
T	215 (70.2 %)	159 (51.9 %)	2.18 (1.56-3.04)	< 0.001*
M	91 (29.8 %)	147 (48.1 %)	Ref	

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

Abbreviations: Ref, Reference; CL, Confidence Level; OR, Odds Ratio.

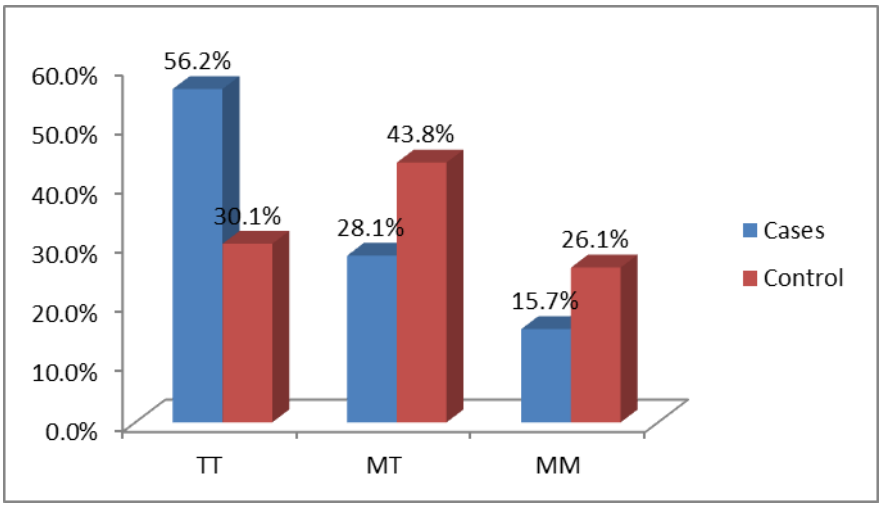

Figure 3: Distribution of the AGT M235T genotype in cases and controls

Association between AGT Genotypes and Clinical Parameters

Table 3 lists the clinical parameters of patients with HTN and normotensive controls in relation to the AGT M235T genotype. The AGT genotypes (TT, MT, and MM) in the study groups were assessed with fasting blood glucose, blood pressure, and lipid profiles. Blood pressure was more strongly correlated with the AGT-TT genotype than the MT and MM genotypes for SBP, Mean (SD) 138.1±17.2 Vs 127.0±13.7 and 125.3±13.4; *P*< 0.001), and DBP (86.8±9.2 Vs 81.4±7.2 and 80.5±7.2; *P*< 0.001), respectively. The other clinical variables were not found to be significant with the genotypes in the study groups (P> 0.05).

Table 3: Association of AGT M235T genotype with clinical characteristics in Debre Tabor Referral Hospital, Northwest Ethiopia, 2022

	Genotypes			
Variables	TT (N=132)	MT (N=110)	MM (N=37)	p-value
	Mean (SD)			
BMI (Kg/m^2)	23.5±3.8	23.6±3.1	23.5±3.4	0.1680
SBP (mmHg)	138.1±17.2	127.0±13.7	125.3±13.4	< 0.001*
DBP (mmHg)	86.8±9.2	81.4±7.2	80.5±7.2	<0.001*
FBG (mg/dl)	94.8±17.8	90.2±8.6	91.4±16.2	0.4157
TC (mg/dl)	167.8±56.6	170.3±58.4	174.8±71.0	0.1436
TG (mg/dl)	125.6±57.6	118.7±51.4	128.9±67.0	0.1578
LDL-C (mg/dl)	87.5±33.6	84.4±35.3	79.5±31.5	0.6981
HDL-C (mg/dl)	46.8±10.6	47.1±10.8	49.6±11.6	0.3027
Creatinine (mg/dl)	0.81±0.13	0.81±0.15	0.80±0.13	0.3530

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

Discussion

Although contradictory results have been reported, the AGT gene M235T polymorphism has been found to be highly related to a higher prevalence of HTN in populations from different ethnic groups [19]. In our investigation, patients with HTN had much greater rates of the T allele of the AGT M235T variation than did controls, and they also had significantly higher rates of AGT 235T homozygosity than did the healthy control subjects, as shown in Table 2. This finding is in agreement with a meta-analysis conducted in Indonesia, including 41 studies, which found that patients with the T allele were more likely to develop HTN compared to the carriers of the M allele (OR =1.15; 95%CI = 1.00–1.32; P <0.05) [20]. A case-control study with positive results was conducted in the Egyptian population, including 83 hypertensive cases and 60 age- and gender-matched normotensive controls. The study found that patients with the TT genotype (OR= 36.217, P <0.001) and T allele (OR= 7.267, P < 0.001) were significantly associated with a high risk of developing HTN [21]. Similarly, other studies conducted in populations from Greece [22] Malaysia [23], South India [19], and China [24] showed that the AGT gene TT genotype and 235T allele were associated with a high incidence of HTN.

The exact mechanism by which the M235T mutation in the AGT gene increases the risk of HTN is unknown. The AGT 235T variation features a guanine-to-adenosine transition at -6 bp upstream of the transcription initiation site and has been determined to be in full linkage disequilibrium. [25]. This nucleotide substitution affects the basal transcription rate of this gene in different cell lines, resulting in the AGT T235 variant and higher plasma AGT levels, which may contribute to the elevation of blood pressure [23]. AGT M235T-TT carriers have a higher level of angiotensin II than non-carriers, which affects the function of endothelial cells in a number of ways, including by promoting endothelial cell apoptosis, raising vascular endothelial growth factor, and impairing the production of nitric oxide. This results in a higher risk of HTN and its associated complications [26].

On the other hand, the findings of this study disagree with the case-control study conducted in Sudan, which included 96 patients with essential hypertension and 79 apparently healthy controls. The study showed no correlation between the AGT M235T gene polymorphism and hypertension [27]. In addition, other studies conducted in populations of Nigeria [25], Mongolia [28], and Thailand [29] contrasted with the findings of the current study, as they were unable to detect any significant association between 235T homozygosity and the risk of HTN. Conflicting results regarding the involvement of AGT gene M235T polymorphisms in HTN are likely due to ethnic differences, population heterogeneity, geographic differences, sampling biases, and possibly other ecological factors. Additionally, a number of environmental variables, including nutrition and exercise, are connected to alterations in the epigenetic state [30].

There are some limitations to this study. First, the relatively small sample size may lead to a

bias in identifying the AGT M235T genotype in hypertension patients. Second, there are no measurements of plasma angiotensinogen levels or other genes of RAAS that correlate directly with the genetic polymorphisms investigated in this study. Its strength is that this is the first investigation into the relationship between genetic variations of AGT and hypertension in the Ethiopian population. This study also examined the risk of hypertension associated with clinical and biomedical characteristics to underline the crucial importance of the link with the disease. The findings of this study will serve as a baseline for these locations, but further research must be done to find other gene polymorphisms that could be reliable indicators of hypertension in this population..

Conclusion

The present study indicated that the AGT M235T gene of the TT genotype and the T allele have been associated with a high risk of hypertension. As a result, the AGT gene M235T polymorphism may be used as a biomarker for early hypertension diagnosis as well as to manipulate antihypertensive medication therapeutic strategies. In future studies, a population-based study should be needed for further clarification of the association between AGT and hypertension.

Abbreviations: AGT: Angiotensinogen; BMI: Body Mass Index; DNA: Deoxyribonucleic Acid; DBP: Diastolic Blood Pressure; EDTA: Ethylenediaminetetraacetic Acid; 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.

Declarations

Ethics approval and consent to participate: The study protocol was approved by the University of Gondar institutional review board (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.

Consent to Publish: Not applicable

Availability of data: The data used and/or analyzed in the current study can be provided by the corresponding author upon request.

Competing interests: The authors claim to have no conflicts of interest.

Authors' contribution

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

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Reference

1 Amare F, Hagos B, Sisay M, Molla B, Uncontrolled hypertension in Ethiopia: A systematic review and meta-analysis of institution-based observational studies. BMC Cardiovasc Disord 2020;20 (1):1–9. doi: 10.1186/s12872-020-01414-3.

2 Carey RM, Muntner P, Bosworth HB, Whelton PK. revention and Control of Hypertension: JACC Health Promotion Series. J. Am. Coll Cardiol 2018;72 (11):1278–1293. doi: 10.1016/j.jacc.2018.07.008.

3 Sandar Oo K.et al., "Increased Plasma Angiotensinogen Level, BMI and Its Association with the Angiotensinogen Gene M235T Polymorphism and Essential Hypertension in Myanmar," Cardiol. Vasc. Res., 2017; 1 (1): 1–5, , doi: 10.33425/2639-8486.1006.

4 Saab Y. B., Gard P. R., and Overall A. D. J., "The association of hypertension with renin-angiotensin system gene polymorphisms in the Lebanese population," JRAAS - J. Renin-Angiotensin-Aldosterone Syst., 2011; 12 (4): 588–594, , doi: 10.1177/1470320311408465.

5 Charita B., Padma G., Sushma P., Deepak P., and Padma T., "Estimation of risk and interaction of single nucleotide polymorphisms at angiotensinogen locus causing susceptibility to essential hypertension: A case control study," JRAAS - J. Renin-Angiotensin-Aldosterone Syst., 2012; 13 (4): 461–471, , doi: 10.1177/1470320312444650.

6 Kusmierska-Urban K., "Influence of Selected Polymorphisms of the Renin-Angiotensin System on the Regulation of Blood Pressure during Pregnancy," Obstet. Gynecol. Int. J., 2015; 2(2): 52–56, , doi: 10.15406/ogij.2015.02.00030.

7 Alaee E., Mirahmadi M.,. Ghasemi M, Kashani E., Attar M., and Shahbazi M., "Association study of M235T and A-6G polymorphisms in angiotensinogen gene with risk of developing preeclampsia in

Iranian population," *Ann. Hum. Genet.*, 2019; 83(6): 418–425, , doi: 10.1111/ahg.12323.

8 Tsung Kao W., Lin Chang C., and Wey Lung F., "The Disparity of Angiotensinogen M235T Polymorphism in Patients with Major Depressive Disorder and Hypertension," *Neuropsychiatry (London).*, 2018; 8 (2): 669–676, , doi: 10.4172/neuropsychiatry.1000390.

9 Singh K. D., Jajodia A., Kaur H., Kukreti R., and Karthikeyan M., "Gender Specific Association of RAS Gene Polymorphism with Essential Hypertension : A Case-Control Study, 2014: , 2014 (1): 1-7 .

10 Wibowo A., Hastuti P., and Susanti V., "The Association of Angiotensin-converting Enzyme I/D and Angiotensinogen M235T Polymorphism Genes with Essential Hypertension: A Meta-analysis," *Open Access Maced. J. Med. Sci.*, 2021; 9(1): 739–746, , doi: 10.3889/oamjms.2021.7628.

11 Charan J.and Biswas T., "How to calculate sample size for different study designs in medical research?," *Indian J. Psychol. Med.*, 2013; 35(2): 121–126, , doi: 10.4103/0253-7176.116232.

12 Gadekar T., Dudeja P., Basu I., Vashisht S., and Mukherji S., "Correlation of visceral body fat with waist–hip ratio, waist circumference and body mass index in healthy adults: A cross sectional study," *Med. J. Armed Forces India*, 2020; 76(1): 41–46, , doi: 10.1016/j.mjafi.2017.12.001.

13 Flynn J. T.*et al.*, "Clinical practice guideline for screening and management of high blood pressure in children and adolescents," *Pediatrics*, 2017; 140(3): v , doi: 10.1542/peds.2017-1904.

14 Keutmann S. *et al.*, "Measurement Uncertainty Impacts Diagnosis of Diabetes Mellitus: Reliable Minimal Difference of Plasma Glucose Results," *Diabetes Ther.*, 2020; 11(1): 293–303, , doi: 10.1007/s13300-019-00740-w.

15 Helmersson-Karlqvist J., Ridefelt P., Boija E. E., and Nordin G., "Lower creatinine concentration values and lower inter-laboratory variation among Swedish hospital laboratories in 2014 compared to 1996: Results from the Equalis external quality assessment program," *Clin. Chem. Lab. Med.*, 2019; 57(6): 838–844, , doi: 10.1515/cclm-2018-0670.

16 Birhan T. A., "Association of angiotensin-converting enzyme gene insertion / deletion polymorphisms with risk of hypertension among the Ethiopian population," 2022.

17 Al-Hassani O. M. H., "Detection of AGT Gene Polymorphism in Patient with Hypertension in Mosul City," *Iraqi J. Biotechnol.*, 2019; 18 (2): 1-6 .

18 Mocan O., Radulescu D. A. N., Buzdugan E., Cozma A., Leucuta D. C., and Procopciuc L. M., "Association Between M235T -AGT and I / D -ACE Polymorphisms and Carotid Atheromatosis in Hypertensive Patients : A Cross-Sectional Study," 2020; 2819 v2811–2819, , doi: 10.21873/invivo.12107.

19 Karthikeyan *et al.* M., "Angiotensin gene polymorphisms (T174M and M235T) are significantly associated with the hypertensive patients of Tamil Nadu, South India," *Int. J. Hum. Genet.*, 2013; 13 (4): 201–207, , doi: 10.1080/09723757.2013.11886218.

20 Fajar J. K.*et al.*, "The genes polymorphism of angiotensinogen (AGT) M235T and AGT T174M in patients with essential hypertension: A meta-analysis," *Gene Reports*, 2019; 16 (April): 100421, , doi: 10.1016/j.genrep.2019.100421.

21 Shamaa M. M., Fouad H., Haroun M., Hassanein M., and Hay M. A. A., "Association between the Angiotensinogen (AGT) gene (M235T) polymorphism and Essential Hypertension in Egyptian patients," *Egypt. Hear. J.*, 2015; 67 (1): 1–5, , doi: 10.1016/j.ehj.2013.10.001.

22 Kolovou V.*et al.*, "Angiotensinogen (AGT) M235T, AGT T174M and Angiotensin-1- Converting Enzyme (ACE) I/D Gene Polymorphisms in Essential Hypertension: Effects on Ramipril Efficacy," *Open Cardiovasc. Med. J.*, 2015; 9(1): 118–126, , doi: 10.2174/1874192401509010118.

23 Say Y. H., Ling K. H., Duraisamy G., Isaac S., and Rosli R., "Angiotensinogen M235T gene variants and its association with essential hypertension and plasma renin activity in Malaysian subjects: A case control study," *BMC Cardiovasc. Disord.*, 2005: 5 1–10, , doi: 10.1186/1471-2261-5-7.

24 Cheng J. L., Wang A. L., and Wan J., "Association between the M235T polymorphism of the AGT gene and cytokines in patients with hypertension," *Exp. Ther. Med.*, 2012; 3(3): 509–512, , doi: 10.3892/etm.2011.433.

25 Kooffreh M. E., Anumudu C. I., Akpan E. E., Ikpeme E. V., and Lava Kumar P., "A study of the M235T variant of the angiotensinogen gene and hypertension in a sample population of Calabar and Uyo, Nigeria," *Egypt. J. Med. Hum. Genet.*, 2013; 14 (1): 13–19, , doi: 10.1016/j.ejmhg.2012.06.007.

26 Tran T. T.*et al.*, "Association between AGT M235T and left ventricular mass in vietnamese patients diagnosed with essential hypertension," *Front. Cardiovasc. Med.*, 2021; 8 (February): 1–7, , doi: 10.3389/fcvm.2021.608948.

27 Yosif et al., "Angiotensinogen Gene Polymorphism (M235T) in Sudanese Hypertensive Patients.," *Int. J. Adv. Res.*, 2016; 4 (11): 446–450, , doi: 10.21474/ijar01/2103.

28 Ying C. Q.*et al.*, "Association of the renin gene polymorphism, three angiotensinogen gene polymorphisms and the haplotypes with essential hypertension in the mongolian population," *Clin. Exp. Hypertens.*, 2010; 32 (5): 293–300, , doi: 10.3109/10641960903443517.

29 Lerthiranwong T.and Piyamongkol W., "The Association of Angiotensinogen (AGT M235T) Gene Polymorphism and Essential Hypertension in Thai Post- Menopausal Women," 2017; 25 (1): 52–61,.

30 Mohana V. U., Swapna N., Surender R. S., Vishnupriya S., and Padma T., “Gender-related association of AGT gene variants (M235T and T174M) with essential hypertension-A case-control study,” *Clin. Exp. Hypertens.*, 2012; 34 (1): 38–44, , doi: 10.3109/10641963.2011.618207