

1 **Selected Hematological markers and C-reactive**
2 **Protein, not *AGTR1* SNP, are associated**
3 **with Essential hypertension in Tharaka Nithi**
4 **County, Kenya**
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10 **ABSTRACT**

Background: Essential Hypertension (EH) accounts for majority of hypertension cases globally. Genetic factors along with hematological and biochemical changes may underlie EH and these have not been well studied in Kenya. A meta-analysis in African populations (excluding East Africa) identified the 1166A>C (rs5186) single nucleotide polymorphism (SNP) in the angiotensin II type 1 receptor gene (*AGTR1*) that encodes the angiotensin II type 1 receptor as likely to predispose some Africans to hypertension.

Aim: The purpose of the study was to determine whether the *AGTR1* (rs5186) mutation, C-reactive protein (CRP) and selected hematological biomarkers may be associated with the onset of EH in Tharaka Nithi County, Kenya.)

Study design: A case control study design was adopted.

Place and Duration of Study: The research was conducted from March to July 2022 at Chuka County Referral Hospital in Tharaka Nithi County.

Methodology: A total of 272 participants, both hypertensive and normotensive, were recruited and blood samples obtained. DNA was extracted and analyzed by PCR RFLP. Independent T-test, Mann Whitney U test and Odds ratios were used to compare the two groups. P values less than 0.05 were considered statistically significant.

Results: Median values for Red Cell Distribution Width (RDW), C-reactive Protein (CRP) and mean values for Mean Platelet Volume (MPV) and Neutrophil to Lymphocyte ratio (NLR) were significantly higher ($P < .001$) in hypertensive group compared to normotensive individuals. Mean Platelet Distribution Width (PDW) was not significantly different between cases and controls. ($P=.519$) There was no significant association between the *AGTR1* 1166A>C (rs5186) SNP frequency and EH in both groups ($P=0.6236$, $OR=0.4952(95\%CI:0.0442-5.5456)$)

Conclusion: The *AGTR1* (rs5186) SNP is not associated with EH in Tharaka Nithi County, Kenya. EH is associated with elevated levels of CRP, RDW, MPV and NLR in the absence of other inflammatory and chronic diseases. Further studies of the genetics of hypertension in Kenya need to be conducted.

11
12 Keywords: [Essential Hypertension, Biomarkers, C-reactive protein, Angiotensin II type one receptor, Kenya]
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1. INTRODUCTION

Hypertension is a common non-communicable disease (NCD) where systemic arterial circulation experiences persistent elevated pressure. According to America Heart association, it has recently been defined as elevated pressure when systolic pressure is at/or 130mm Hg and diastolic pressure is at/or above 80mm Hg. [1]. This was after findings from several randomized clinical trials and large scale prospective studies indicated a significant increase in cardiovascular disease risk with rising blood pressure even when systolic blood pressure is as low as 115 mmHg [1]. Severe and untreated hypertension is linked to a higher risk of developing heart disease, stroke and death [2]. Essential hypertension (EH), also referred to as primary hypertension accounts for over 90% of cases of the disease and the underlying causes remain unknown [3]. The minority of hypertension cases (<10%) are due to secondary hypertension which is elevated blood pressure as a result of a defined specific disease or condition. These may include kidney disease, diabetes, thyroid disorders or obstructive sleep apnea [4], [5]. Hypertension and other NCDs are lifestyle associated diseases that are increasingly becoming a critical concern in low and middle income countries (LMICs). The World Health Organization found that in 2010, elevated blood pressure was predicted to affect thirty-one percent of the population globally. It was noted that hypertension was more common in less developed countries (31.5%) of the population than in high-income countries (28.5%) [6].

The World Health Organization reported in 2021 that Africa had the highest prevalence of hypertension in the world at 27%. This was in contrast to the Americas with the lowest prevalence of 18% [7]. This high prevalence of HTN in Africa could be attributed to a number of factors including increased urbanization with a concurrent rise in unhealthy, sedentary lifestyles in various African countries. In a national survey published in 2018 by Mohamed S.F and others, the estimated the overall age-standardized prevalence of hypertension was 24.5%. [8]. This was in line with an earlier report in 2016 by Kenya Health Sector Strategic Plan [9] which revealed that hypertension had affected approximately 23% of the Kenyan population but that only 16.7% of these individuals had been diagnosed [9]. According to the report, the proportion of hypertensives who were on effective treatment and whose blood pressure had been controlled was only 4%. Hypertension was more prevalent in the central region of Kenya at 37.2% followed by the eastern region at 28.4%. [9]. Tharaka Nithi county is located in the eastern region and is considered to be one of the counties with a high prevalence of hypertension in Kenya.

There are multiple haematological indicators for high blood pressure which may provide insight into the fundamental biological processes that lead to hypertension onset and progression [10]. Blood biomarkers have been reported to give a better understanding of the pathophysiology, diagnosis, progression and treatment efficacy EH [10]. Some of these biomarkers include C-reactive protein which is a systemic inflammatory marker and evidence in some cross-sectional studies in Nigeria has shown that it is associated with increased risk of the development of hypertension. [11]. Other biomarkers including serum uric acid, urinary albumin, red cell distribution width and the neutrophil lymphocyte ratio have been observed to be higher in hypertensive individuals. In addition, other biomarkers such as plasminogen activator inhibitor 1, fibrinogen, urine albumin creatinine ratio, D-dimers and plasma renin have been reported to have an association with EH [3].

Genetics also plays a critical role in the development of primary hypertension. [12]. Genetic changes may alter normal physiological mechanisms by altering gene or protein expression for critical components of biological pathways involved in blood pressure regulation and thus enhance the risk of EH [13]. Single nucleotide polymorphisms in various genes that encode components of the Renin, aldosterone, angiotensin system (RAAS) have been found to modulate blood pressure regulation and impact the development of EH [14]. Consequently, genetic biomarkers may reveal the underlying processes associated with the early onset, progression and complications of EH. Genes that have been linked to EH include the angiotensin I converting enzyme (ACE) gene, the angiotensinogen gene, 11 β hydroxysteroid dehydrogenases types 1 and 2 (11 β HSD1 and 11 β HSD2) genes and the angiotensin II type 1 receptor gene (*AGTR1*) among others [13] [14]. Angiotensin II is a vasoconstrictor that works primarily by binding to angiotensin type one receptor (AT1R). AT1R is a key component in the RAAS through promotion of intracellular signaling pathways that contribute to the onset of hypertension, endothelial dysfunction and cardiovascular diseases. The single nucleotide polymorphism where the cytosine (C) at position 1166 is replaced with an adenine (A) in the angiotensin II type 1 receptor gene (*AGTR1*) which codes for the angiotensin II type 1 receptor (AT1R) have been associated with EH in Asian populations [15-16]. The association between the A1166C SNP in *AGTR1* and high blood pressure has not been consistently reported in various populations. Most of the studies conducted in Africa have focused on populations in North Africa (Egypt, Tunisia) and West Africa (Nigeria, Burkina Faso) [15, 17-19]. It is unknown whether the results found in those populations hold true for hypertensive populations in East Africa. This study aimed at addressing this knowledge gap through investigation of a combination of selected hematological markers (such as MPV, PDW, NLR and RDW), CRP and the *AGTR1* A1166C SNP among Kenyan individuals with essential hypertension. This information would enable improved understanding of the aetiology of EH and support better case management.

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80 **2. MATERIALS AND METHODS**
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83 **2.1. Study setting**
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85 The study site was Chuka County Referral Hospital, located in Chuka, the largest town in Tharaka Nithi County, Kenya.
86 This county is among the regions of Kenya having a high prevalence of hypertension of 28%.
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89 **2.2. Study Population**
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91 The study enrolled hypertensive patients and normotensive healthy individuals (blood donors) attending Chuka County
92 Referral Hospital, Kenya between March 2022 and July 2022.
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94 **2.2.1. Inclusion Criteria**
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96 Cases were both male and female adult patients aged 18 years and above, who presented with essential hypertension.
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98 Controls were healthy blood donors who were age and gender matched with the cases. Frequency matching was used
99 where controls were selected with same distribution as cases in terms of age and gender.
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101 **2.2.2. Exclusion Criteria**
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103 Patients who had secondary hypertension, kidney disease, diabetes mellitus, sleep apnea, arthritis and other joint
104 diseases, cancers, sickle cell disease, allergies, chronic obstructive pulmonary disease (COPD) and rheumatoid arthritis
105 were excluded from the study.
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107 In addition, pregnant women and non-consenting patients were also not considered for enrollment in the study.
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110 **2.3. Study Design**
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112 The study was a case-control study with controls comprising normotensive healthy blood donors and cases being
113 hypertensive patients attending medical outpatient clinics at Chuka County Referral Hospital. This design was suited to
114 identify hematological and genetic biomarkers that may be associated with EH.
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116 **2.4. Sampling Method**
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118 Study participants were recruited by convenience sampling using the selection criteria to attain the required sample size.
119 A written informed consent form was given to each participant to read and understand the study. The consent form was
120 also translated into Kiswahili for those who could not comprehend English. Those who could not read and write had the
121 consent form verbally translated for them and they thumb printed after consenting.
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123 **2.5. Sample Size Determination**
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125 The sample size was calculated using the online sample size calculators at <https://sample-size.net/>. This was a case-
126 control study to compare the frequency of the rs5186 SNP among hypertensive patients (cases) and normotensive
127 individuals (controls). The sample size was calculated to test the hypothesis, (two-sided $\alpha=0.05$; $\beta=0.2$) that there was at
128 least a 5% difference in the prevalence of the *AGTR1* 1166A>C (rs5186) SNP frequency between cases and controls
129 (ratio 1:1). An equal number of cases (hypertensive patients, $q_1=0.5$) and controls (normotensive individuals, $q_0=1-q_1 =$
130 0.5) were enrolled. The estimated prevalence of hypertension in the general population was 28% [8]. This resulted in a
131 sample size of 272 study participants with 136 cases and 136 controls.
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135 **2.6. Sample collection and measurement of biomarkers**
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137 **2.6.1. Baseline data related to Hypertension**
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139 A well-structured questionnaire was used to obtain the demographic information of study participants which included: age,
140 gender, family history of hypertension, and body mass index (BMI).
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142 **2.6.2. Medical health records review**
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144 All study participants, including those who were recently diagnosed with hypertension, were recruited during their
145 scheduled appointments in the medical outpatient clinic days. After seeking informed consent from the study participants,
146 medical records of the participants were retrieved and reviewed. Health record information was used to scrutinize
147 recruited patients and exclude those who had other conditions that would make them ineligible for Inclusion in the study.
148 Clinical data for blood donors was obtained from the blood donation questionnaire which is routinely used to determine
149 eligibility as a blood donor.
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151 **2.6.3. Blood Pressure Measurement**
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153 The participant's blood pressure was measured using an automated oscillometric cuff on either their right or left arm, and
154 the results were displayed on the device's external display. After 2 minutes, a second BP measurement was taken, and if
155 the values changed by more than 5 mmHg, the readings were taken again until two consecutive stable readings were
156 obtained. After 2 minutes, the participants' BP was taken on the opposite arm, and if there was a measurement disparity
157 between the two arms, the readings from arm with the higher value was chosen.
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159 **2.6.4. Blood Specimen Collection**
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161 Five milliliters of venous blood sample was collected from each selected and consented participant using a sterile
162 disposable syringe and 21-gauge needle. Three milliliters of collected whole blood was transferred into an EDTA tube for
163 full blood count analysis and extraction of genomic DNA. The remaining two milliliters of blood was transferred into the red
164 top plain tube for serum C-reactive protein determination. The plain tube blood sample was allowed to clot, centrifuged at
165 5000g for three minutes and serum was transferred to a fresh 1.8ml tube for C-reactive protein determination.
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167 **2.6.5. Complete blood count analysis**
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169 Complete blood counts were performed using a five-part automated haematology Dymind analyzer (Shenzhen Dymind
170 Biotechnology Co LTD, China). To ensure accurate and reliable study results, all quality assurance processes such as
171 pre-analytical, analytical and post analytical processes were strictly adhered to. All levels (normal, low and high) of daily
172 quality controls for the equipment were run before analyzing participant samples. The samples were only analyzed after
173 control values were within acceptable limits. The samples were also analyzed in a laboratory that takes part in external
174 quality assessment. Both serum CRP and complete blood count were subjected to these quality procedures and this
175 ensured reliable and accurate results were obtained.
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177 **2.6.6. Measurement of plasma levels of C-reactive protein (CRP)**
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179 C-reactive protein testing was done on the Mindray BS 230 (Meron Scientific Private Ltd, India). The Mindray employs an
180 immunoturbidimetric *in vitro* test for determination of CRP levels in human serum. Human CRP binds to monoclonal anti-
181 CRP antibodies coated latex particles. Turbidimetric analysis is used to identify the aggregates. A 200µl aliquot of serum
182 was pipetted and transferred to a sample cup. Information about the sample was keyed into the machine and the sample
183 was placed into the machine following standard procedures provided in the operator's manual. The serum CRP was
184 quantitatively determined and the results were displayed on the machine. This was done to all samples and results were
185 recorded accordingly.
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187 **2.7. DNA extraction and PCR amplification**
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189 DNA was extracted using the Isolate II Genomic DNA kit (Bioline Meridian) following the standard operating procedures
190 as described by the manufacturer. The eluted genomic DNA was stored at -20°C. A 359bp region of *AGTR1* was
191 amplified by polymerase chain reaction (PCR) using the following primer pair: 59-ATAATGTAAGCTCATCCACC- 39
192 (forward primer) and 59-GAGATTGCATTTCTGTCCGGT- 39 (reverse primer). Amplification was carried out using a BIO-
193 RAD Thermal Cycler C 1000 Touch (Bio-Rad Laboratories, Inc. USA) in a final volume of 25µl containing 10.875µl
194 nuclease-free water, 2.5ul 10x Buffer, Forward Primer 0.5 (10µM), Reverse Primer 0.5 (10µM), 0.5ul of 10mM dNTPs,

0.125µl of the *Taq* DNA polymerase and 10µl template genomic DNA. The cycling conditions were a pre-denaturation cycle at 94°C for 4 minutes, 35 cycles of subsequent denaturation at 94°C for 45 seconds, annealing at 57°C for 45 seconds and extension at 68°C for 1 minute followed by 5 minutes' final extension at 68°C. The PCR products were analyzed on 2% agarose gel electrophoresis.

2.8. RFLP Analysis of *AGTR1* amplicons

Genotyping of variants at the rs5186 locus was carried out through restriction fragment length polymorphism of the *AGTR1* PCR amplicons. The 359-bp amplicons were digested using *DdeI* whose recognition site is 5'CTNAG3'. A restriction endonuclease digest of a 10µl aliquot of PCR amplicon was carried out by addition of 0.25µl of *DdeI* endonucleases, 2.5µl of 10X rCutSmart™ Buffer (New England Biolabs) and nuclease free water to give a final reaction volume of 25µl. This reaction mixture was incubated at 37°C on water bath for 15 minutes. Following digestion, the restriction fragments were then separated and distinguished on a 2% agarose gel stained in ethidium bromide alongside a 50bp DNA ladder (New England BioLabs). Images of the gels were captured after placing the gel on a UV transilluminator. *AGTR1* (A1166C) genotype variants were identified on the basis of band patterns on the agarose gel after *DdeI* digestion as follows: the homozygous normal (AA) genotype lacks a *DdeI* restriction site on both chromosomes and produces a single 359bp band; the homozygous mutant (CC) genotype possesses a *DdeI* restriction site on both chromosomes and results in two bands of 220bp and 139bp respectively; the heterozygous genotype (AC) possesses a *DdeI* restriction site on only one of the two chromosomes and results in three bands of 359bp, 220bp and 139bp.

2.9. Data Analysis

Data was entered into MS Excel and analyzed using version 9.4 of the SAS statistical software. The results were presented as tables. The normality of the distribution of C-reactive protein (CRP), Red cell distribution width (RDW), Mean Platelet volume (MPV), Platelet distribution Width (PDW) and Neutrophil to lymphocyte ratio (NLR) values were tested. MPV and NLR values were normally distributed while the other variable had a skewed distribution. As a result, CRP, RDW and PDW values were expressed as median plus interquartile range and compared using the Mann Whitney U test. MPV and NLR values were expressed by mean ± SD and tested using the independent t test. For categorical data (*AGTR1* gene), Odds ratios with 95% confidence intervals were calculated to determine the association between EH and the *AGTR1* gene A1166C polymorphism.

3. RESULTS

3.1. Sociodemographic characteristics of the study participants

272 participants were enrolled for the study, 136 cases and 136 healthy controls. Hypertension is more prevalent in old age while majority of blood donors tend be in the younger age bracket. Consequently, it was difficult to obtain suitable age-matched controls for all the cases. For that reason, sixty (60) participants both cases and controls were dropped due to matching challenges. However, the dropping off the participants only affected genetic biomarker due to low frequencies in the population. The other biomarkers (hematological & biochemical) were within sample size estimation rules. Therefore, 212 participants were age and gender matched and used was used for statistical analysis.

Table: 1 Sociodemographic characteristics of the study participants

VARIABLE		CONTROLS (%)	CASES (%)
Gender	Male	41 (30)	34 (25)
	Female	95 (70)	102 (75)
Age groups	18-35	34 (32)	31 (29)
	36-55	49 (46)	51 (48)
	>55	23 (22)	24 (23)

Mean age \pm SD		42.8 \pm 12.31	44.8 \pm 11.34
HTN History	Yes	66 (49)	96 (70)
	No	70 (51)	31 (23)
	Unknown	0 (0)	9 (7)
Mean BMI (kg/m ²) \pm SD		25.73 \pm 4.20	27.53 \pm 4.89
Mean BP (mmHg) \pm SD	SBP	124.1 \pm 9.42	145.5 \pm 17.39
	DBP	74.1 \pm 7.76	85.4 \pm 11.99

3.2. Hematological and biochemical biomarkers and essential hypertension

A complete blood count and C-reactive protein analysis was done on samples from the study participants. The normality of the distribution of the biomarker measurements was tested and only MPV and NLR were found to be while RDW, PDW and CRP had skewed distributions. As result, CRP, RDW and PDW were expressed as median values (plus the interquartile range) and compared by Mann-Whitney U test. MPV and NLR were expressed by means \pm SD and the data analyzed using the independent t test. The median values (\pm IQR) for CRP, RDW and PDW were 0.6 \pm 1.8, 41.95 \pm 1.08, 11.5 \pm 2.3 and 2.9 \pm 4.8, 44.95 \pm 1.21, 11.0 \pm 2.2 in controls and cases respectively. Mean values for NLR and MPV were 2.09 \pm 1.3, 9.36 \pm 1.14 and 2.74 \pm 1.4, 10.71 \pm 1.14 in controls and cases respectively. The difference between cases and the control group for all these biomarkers was statistically significant ($P < .001$) except for. PDW which showed no statistical significance. ($P = .519$) (Figure 1).

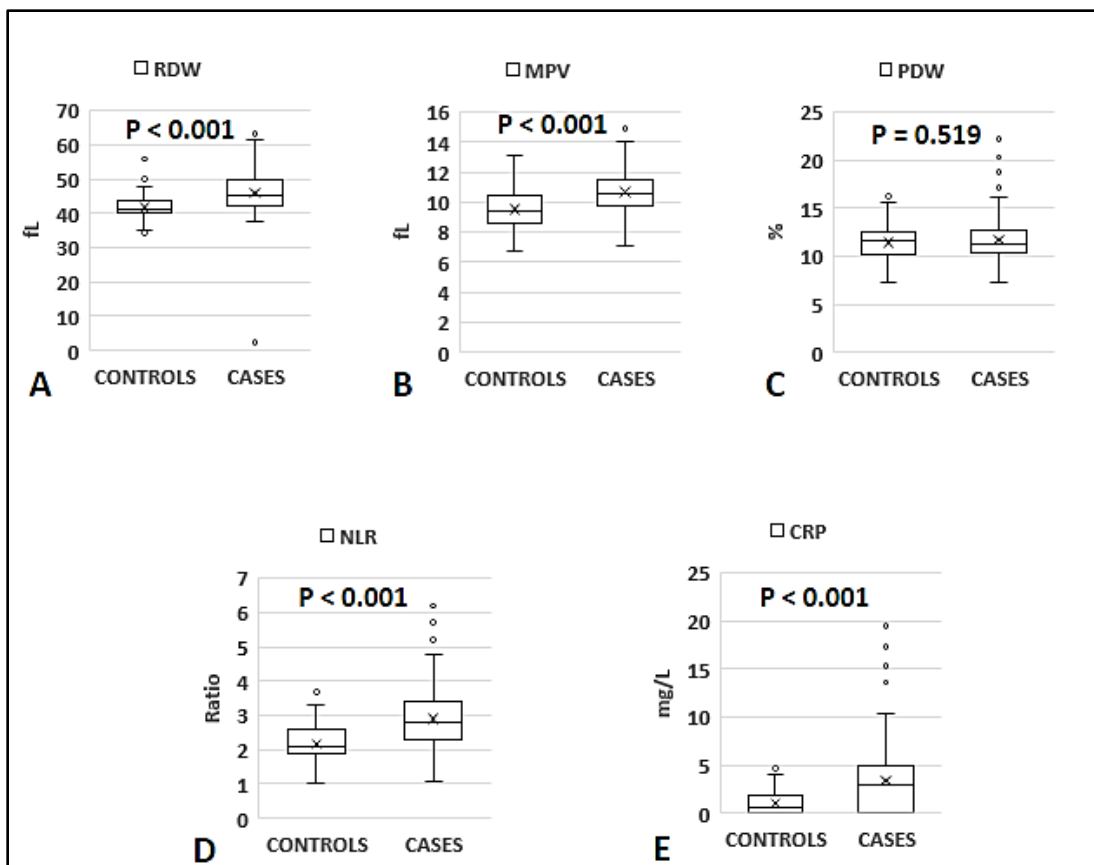


Figure 1. Comparative levels of haematological biomarkers between normotensive (controls) and hypertensive (cases) individuals. Biomarkers included Red Cell Distribution Width (RDW, Panel A); Mean Platelet Volume (MPV, Panel B); Platelet Distribution Width (PDW, Panel C); Neutrophil to Lymphocyte Ratio (NLR, Panel D) and C-reactive

266 Protein (CRP, Panel E). Significantly ($P < 0.05$) higher levels of all the biomarkers except for PDW, were present in the
267 cases compared to the controls. "x" represents the mean value.
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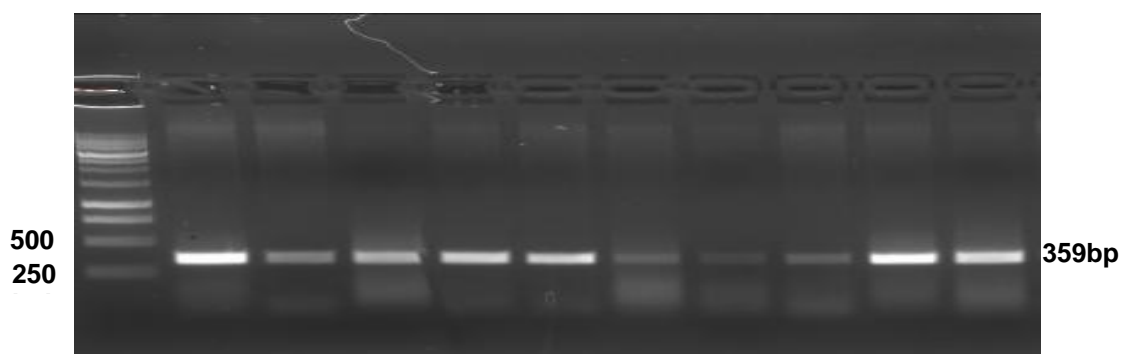
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271 3.3 Frequency and association of Angiotensin II type 1 receptor gene SNPs (A1166C gene polymorphism) with 272 Essential Hypertension among patients attending Chuka County Referral Hospital.

273 In a total of one hundred and six (106) cases and one hundred and six (106) controls, DNA amplification of the *AGTR1*
274 gene using the specific primers done successfully and resulted in a 359bp DNA product (figure 2) and on subsequent
275 digestion of the amplified fragment (amplicon) with *DdeI* restriction endonuclease, DNA fragments of 359 (AA), 359, 220
276 and 139bp (AC) length were observed (figure 3). The frequencies of the AA, AC and CC genotypes were 98.1%, 1.9%
277 and 0.0% in cases respectively. The frequencies of the AA, AC and CC genotypes in controls were 99.1%, 0.9 and 0.0%
278 respectively. Out of the two hundred and twelve (212) participants whose amplicons (359bp) were digested, three (3)
279 were found to have the heterozygous genotype (AC) and the other two hundred and nine (209) had the homozygous
280 normal (AA). This represented a frequency of 1.42% of the mutant alleles and 98.58% had the wild type alleles in the
281 studied population. There was no significant association between *AGTR1* mutations and EH in the studied population
282 ($ORs = 0.4952$) in genotype and allele frequencies. (Table 2).
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288 **Figure 2: Agarose gel electrophoresis of *AGTR1* PCR amplicons.** The selected images of PCR amplification of the
289 359bp fragment of human *AGTR1* gene as seen on the agarose gel electrophoresis. The first lane from left is the
290 molecular DNA ladder marker that was used as the standard and all the other lanes represents the 359bp PCR products
291 (amplicons). (M = 1kb molecular DNA ladder marker)

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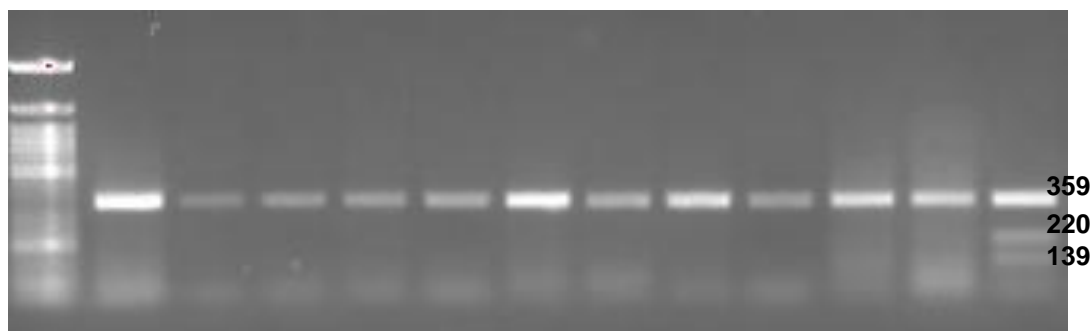
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301 **Figure 3: Agarose gel electrophoresis of Restriction Digests.** The figure shows digestion of the 359bp PCR product
302 by the *DdeI* restriction endonuclease. The images are showing the two genotypes obtained from study cases and

303 controls (AA, AC) seen on the ethidium bromide-stained gel. The homozygous (AA) has a band of 359bp and the
304 heterozygous (AC) has three bands of 359bp, 220bp and 139bp. (M = 50bp molecular DNA ladder marker)
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Table 2: Genotype frequencies of A1166C variants of the angiotensin II type 1 receptor gene in hypertensive and normotensive study participants

Group	Genotypes		Totals	P	OR	95% Confidence limits	
	Normal: AA	Mutant: AC				Lower Bound	Upper bound
Hypertensives	104(98.1%)	2(1.9%)	106	0.6236	0.4952	0.0442	5.5456
Normotensives	105(99.1%)	1(0.9%)	106				
Total (%)	209 (98.6)	3 (1.4)	212 (100)				

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4. DISCUSSION

Recent reports by the World Health Organization on the global prevalence of hypertension have pointed to the highest prevalence being found in the African continent. This seems to support the hypothesis of an “epidemiological transition” in Africa. This is defined as a shift from acute infectious and deficiency diseases often associated with underdevelopment to an increase in chronic non-communicable diseases (such as HTN) due to increased affluence in segments of the population. Nevertheless, despite the underlying reasons, there is need for detection and effective management of hypertension in African countries including Kenya. A 2018 hypertension study reiterated the gaps in awareness, treatment and control suggesting that substantial research needs to be conducted to fill the data gap so as to empower the general population, health practitioners and policy makers to better control hypertension in Kenya.

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A key knowledge gap especially for essential hypertension (EH) - hypertension for which the underlying causes remain unknown - is the identification of the underlying aetiological factors. This information would enhance early detection and improved case management. Multiple genetic, hematological and biochemical changes may underlie EH. The association between the A1166C SNP in *AGTR1* and high blood pressure has not been substantially interrogated in Kenya (and East Africa in general) with most investigations having been conducted North (Egypt, Tunisia) and West African (Nigeria, Burkina Faso) populations. The purpose of the study was therefore to determine whether the *AGTR1* (rs5186) mutation, C-reactive protein (CRP) and selected hematological biomarkers may be associated with the onset of EH in Tharaka Nithi County, Kenya. From the results obtained from our study, statistically significant associations with EH were found in Red Cell Distribution Width (RDW), Mean Platelet Volume (MPV), Neutrophil to Lymphocyte ratio (NLR) and C-reactive Protein ($P < .001$) and not for Platelet Distribution Width (PDW) and *AGTR1* mutations ($P = 0.519$, ORs = 0.4952 respectively).

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Based on the results of the current study, the cases had a statistically significant higher median values of Red cell distribution width ($P < .001$) when compared to the healthy control group. These findings are in agreement with other similar studies by [20-21] in Eastern and Northwest Ethiopia respectively. Another large retrospective cohort study conducted by Seo and other authors [22] demonstrated that increased RDW was associated with an increased risk of hypertension incidence. The association was independent of established risk factors and was progressive with increased RDW. However, a study in Iran population reported conflicting findings [23]. Evidence has shown that increased RDW is as a result of ineffective erythropoiesis that is caused by chronic inflammation. [24]. It has been shown that inflammatory cytokines prevent erythrocytes from maturing, allowing immature red cells to enter the circulation and increasing the variability in size [25]. Additionally, increased RDW might signify improved erythropoiesis brought on by circulating amounts of neurohormonal mediators, which result in a rise in the heterogeneity of circulating red cells [26].

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The present study revealed that the mean values of MPV were significantly different in hypertensive patients ($P < .001$) compared to the control group. The findings of a study carried out in Harar, Eastern Ethiopia in the year 2021 involving adult hypertensive patients and healthy blood donors, are consistent with the current study. [20]. These findings are also consistent with another study conducted by Enawgaw and other researchers [21]. Vascular damage in people with hypertension may be one of the potential causes of the elevated MPV since endothelial damage brought on by high blood pressure triggers platelet activation and platelet production to increase. There is evidence that indicate that the site of an injured blood vessels, platelet consumption is increased and this leads to escape of large platelets from the bone marrow resulting to an increase in platelets and MPV values. Due to the fact that larger platelets are haemostatically more active

354 than mature ones, their existence represents a risk factor for the occurrence of coronary thrombosis and myocardial
355 infarction [27].

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357 Our study also showed that the mean values of NLR were significantly elevated in cases compared to control group (P
358 <.001). These findings are consisted with results of previous cohort studies conducted in Chinese populations [26-29]. It
359 was also noted that NLR can be a good predictive value in preeclampsia. [30]. This difference might occur because NLR
360 is a biomarker of systemic inflammation. NLR is an indicator of persistent low-grade inflammation, and in some situations,
361 an elevated NLR could be linked to hypertension since it also promotes persistent inflammation. This current study also
362 found that median values of CRP were significantly elevated in cases compared to controls (P <.001). These results
363 concur with results from previous studies [31-32]. However, a previous study reported that CRP was not associated with
364 higher risk of developing hypertension in middle-aged and older men [33]. The increased CRP in cases may be explained
365 by the endothelium's continued inability to produce prostacyclin and nitric oxide, which causes the endothelium's
366 vasodilator and antithrombotic properties to decline. Hypertension may in turn induce inflammation and raised CRP levels.
367 [34]. The current study also found out that there was no statistically significant difference in median values of PDW in
368 cases and the control group (P= 0.519). These findings are inconsistent with previous comparative cross section studies
369 done hypertensive and normotensive healthy adults [20-21].

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371 Our study also investigated mutations in *AGTR1* gene and the A1166C genotype frequency was determined. 99.1% of
372 the controls had the homozygous normal (AA) genotype while only 0.9% were heterozygous (AC). There was no
373 statistically significant difference in genotype frequencies among cases (98.1%, AA and 1.9%, AC) in cases (P>0.05).
374 These findings are similar to a study done in Nigeria noted that polymorphisms in *AGTR1* gene are not associated with
375 essential hypertension. [35]. The findings of the current study are also similar to an unpublished thesis research by
376 Freeman, Julia Carol conducted in Kasiagu, a region in south-eastern Kenya, Taita Taveta County in 2013 that reported
377 no association between *AGTR1* mutations and EH. Although the study was done in Kenya in 2013, the population in
378 Kasiagu region may differ from that of Tharaka Nithi County which is predominantly of Bantu origin [14]. These results are
379 in contrast to other studies in non-African populations which have reported that *AGTR1* polymorphisms are associated
380 with essential hypertension [36-37].

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382 These findings point to distinct differences in the *AGTR1* polymorphism profiles in African populations vis a vis non-
383 African populations suggesting that alternative pathophysiological pathways could be involved in the onset of essential
384 hypertension. Notably, the CC genotype was almost completely absent in the Kenyan population similar to observations in
385 Cameroon, Ghana, Nigeria and Burkina Faso [16-19]. This is in contrast to findings in Tunisia where the CC genotype
386 (which has been associated with higher risk of essential hypertension) was present at a prevalence of 43.7% in
387 hypertensive participants and 18.3% in healthy controls. [38]. Possible future studies could investigate gene expression
388 profiles of *AGTR1* among individuals with essential hypertension in Africa. Indeed, a recent study on angiotensin
389 receptors (ATR1, ATR2, and ATR4) noted differential AT1R expression in HIV-infected pre-eclamptic women of African
390 descent [39]. Additional studies on the role of other genetic polymorphisms that may be associated with EH in Africa will
391 also need to be conducted to further contribute to a better understanding of the impact of genetics on hypertension in
392 African populations.

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394 However, our study had some limitations because it was difficulty to get controls of age 60 and above. This is due to the
395 fact that majority of blood donors are of lower age bracket. Additionally, majority of hypertension patients are above age
396 40 years and therefore there were more cases in elderly compared to young age group hence we could not attain 100%
397 age/gender matching for all enrolled study participants.

401 5. CONCLUSION AND RECOMMENDATIONS

402
403 This study notes that the median values of CRP and RDW and the mean values of MPV and NLR were significantly
404 higher in the cases compared to the control group. PDW showed no statistically significant difference between the two
405 groups. Angiotensin II type one receptor gene (rs5186) SNP was not associated with essential hypertension incidence in
406 the studied population. Therefore, it is important for clinicians to be aware that these biomarkers could be elevated due to
407 essential hypertension in the absence of other inflammatory and chronic diseases. Derangements in CRP, RDW, MPV
408 and NLR can help clinicians question a likelihood of Essential Hypertension in undiagnosed cases. This can help in
409 prompt initiation of management and control of the disease. This study also recommends that further research to
410 investigate other possible mutations in other genes which could be associated with essential hypertension in Kenyan
411 population.

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418 **COMPETING INTERESTS**

419 Authors declared that there were no competing interests.
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422 **AUTHORS' CONTRIBUTIONS**

423 This work was carried out in collaboration among all authors. Amos Mbaabu conducted the data collection, data analysis
424 and compilation of the draft manuscripts and the final manuscript. Caroline Mangare and Collins Wangulu contributed to
425 supervision, data analysis, editing of draft manuscript and approved the final manuscript. Amos Mbugua contributed to
426 conceptualization, study design, supervision, data analysis, editing of draft manuscript and approved the final manuscript.
427

428 **CONSENT AND ETHICAL APPROVAL**

429 Ethical approval was obtained from Jomo Kenyatta University of Agriculture and Technology Institutional Ethics Review
430 Committee (JKU/IERC/02316/0511) and National Commission for Science, Technology and Innovation
431 (NACOSTI/P/22/15848). Authorization was obtained from Chuka County Referral Hospital administration. An informed,
432 written and voluntary consent was sought from the participants and parents/guardians of participants aged 18 years and
433 above before involvement into the study.
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