Selected haematological markers and C-reactive protein, not *AGTR1* SNP, are associated with essential hypertension in Tharaka Nithi County, Kenya

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Authors' contributions

This work was carried out in collaboration among all authors. Author A. Mbaabu conducted the data collection, data analysis and compilation of the draft manuscripts and the final manuscript. Authors CM and CW contributed to supervision, data analysis and editing of draft manuscript. Author A. Mbugua contributed to conceptualization, study design, supervision, data analysis and editing of draft manuscript and approved the final manuscript. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Background: Essential Hypertension (EH) accounts for majority of hypertension cases globally. Genetic factors along with haematological 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 haematological 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.

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 130 mm Hg and diastolic pressure is at/or above 80 mm 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, 11βhydroxysteroid dehydrogenases types 1 and 2 (11βHSD1 and 11βHSD2) 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 haematological 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.

2. MATERIALS AND METHODS

2.1 Study Setting

The study site was Chuka County Referral Hospital, located in Chuka, the largest town in Tharaka Nithi County, Kenya. This county is among the regions of Kenya having a high prevalence of hypertension of 28%.

2.2 Study Population

The study enrolled hypertensive patients and normotensive healthy individuals (blood donors) attending Chuka County Referral Hospital, Kenya between March 2022 and July 2022.

2.2.1 Inclusion criteria

Cases were both male and female adult patients aged 18 years and above, who presented with essential hypertension.

Controls were healthy blood donors who were age and gender matched with the cases. Frequency matching was used where controls were selected with same distribution as cases in terms of age and gender.

2.2.2 Exclusion criteria

Patients who had secondary hypertension, kidney disease, diabetes mellitus, sleep apnea, arthritis and other joint diseases, cancers, sickle cell disease, allergies, chronic obstructive pulmonary disease (COPD) and rheumatoid arthritis were excluded from the study.

In addition, pregnant women and non-consenting patients were also not considered for enrollment in the study.

2.3 Study Design

The study used a case-control design with controls comprising normotensive healthy blood donors and cases being hypertensive patients attending medical outpatient clinics at Chuka County Referral Hospital. This design was suited to identify haematological and genetic biomarkers that may be associated with EH.

2.4 Sampling Method

Study participants were recruited by convenience sampling using the selection criteria to attain the required sample size. A written informed consent form was given to each participant to read and understand the study. The consent form was also translated into Kiswahili for those who could not comprehend English. Those who could not read and write had the consent form verbally translated for them and they thumb printed after consenting.

2.5 Sample Size Determination

The sample size was calculated using the online sample size calculators at https://sample-size.net/. This was a case-control study to compare the frequency of the rs5186 SNP among hypertensive patients (cases) and normotensive individuals (controls). The sample size was calculated to test the hypothesis, (two-sided $\alpha=0.05$; $\beta=0.2$) that there was at least a 5% difference in the prevalence of the *AGTR1* 1166A>C (rs5186) SNP frequency between cases and controls (ratio 1:1). An equal number of cases (hypertensive patients, $q_1=0.5$) and controls (normotensive individuals, $q_0=1-q_1 = 0.5$) were enrolled. The estimated prevalence of hypertension in the general population was 28% [8]. This resulted in a sample size of 272 study participants with 136 cases and 136 controls.

2.6 Data Collection

2.6.1 Baseline data related to hypertension

A well-structured questionnaire was used to obtain the demographic information of study participants which included: age, gender, family history of hypertension, and body mass index (BMI).

2.6.2 Medical health records review

All study participants, including those who were recently diagnosed with hypertension, were recruited during their scheduled appointments in the medical outpatient clinic days. After seeking informed consent from the study participants, medical records of the participants were retrieved and reviewed. Health record information was used to scrutinize recruited patients and exclude those who had other conditions that would make them ineligible for Inclusion in the study. Clinical data for blood donors was obtained from the blood donation questionnaire which is routinely used to determine eligibility as a blood donor.

2.6.3 Blood pressure measurement

The participant's blood pressure was measured using an automated oscillometric cuff on either their right or left arm and the results were displayed on the device's external display. After 2 minutes, a second BP measurement was taken, and if the values changed by more than 5 mmHg, the readings were taken again until two consecutive stable readings were obtained. After 2 minutes, the participants' BP was taken on the opposite arm, and if there was a measurement disparity between the two arms, the readings from arm with the higher value was chosen.

2.6.4 Blood specimen collection

Five milliliters of venous blood sample were collected from each selected and consented participant using a sterile disposable syringe and 21-gauge needle. Three milliliters of collected whole blood was transferred into an EDTA tube for full blood count analysis and extraction of genomic DNA. The remaining two milliliters of blood was transferred into the red top plain tube for serum C-reactive protein determination. The plain tube blood sample was allowed to clot, centrifuged at 5000xg for three minutes and serum was transferred to a fresh 1.8ml tube for C-reactive protein measurement.

2.6.5 Complete blood count analysis

Complete blood counts were performed using a five-part automated haematology Dymind analyzer (Shenzhen Dymind Biotechnology Co LTD, China). To ensure accurate and reliable study results, all quality assurance processes of the pre-analytical, analytical and post analytical phases were strictly adhered to. All levels (normal, low and high) of daily quality controls for

the equipment were run before analyzing participant samples. The samples were only analyzed after control values were within acceptable limits. The samples were also analyzed in a laboratory that takes part in external quality assessment. Both serum CRP and complete blood count were subjected to these quality procedures and this ensured the reliability and accuracy of results.

2.6.6 Measurement of CRP levels

C-reactive protein testing was carried out using the Mindray BS 230 clinical chemistry analyzer (Meron Scientific Private Ltd, India). The Mindray employs an immunoturbidimetric in vitro test for determination of CRP levels in human serum. Human CRP binds to monoclonal anti-CRP antibodies coated latex particles. Turbidimetric analysis is used to identify the aggregates. A 200µl aliquot of serum was pipetted and transferred to a sample cup. Information about the sample to be analyzed was keyed in and the serum was loaded into the machine following standard procedures provided in the operator's manual. The serum CRP was quantitatively determined and the results were displayed on the machine. This was conducted for all samples and results were recorded accordingly.

2.7 DNA Extraction and PCR

Genomic DNA was extracted using the Isolate II Genomic DNA kit (Bioline Meridian) following the standard operating procedures as described by the manufacturer. The eluted genomic DNA was stored at -20°C. A 359bp region of the *AGTR1* gene was amplified by polymerase chain reaction using the following pair of primers: 5'-ATAATGTAAGCTCATCCACC-3'(forward primer) and 5'-GAGATTGCATTTCTGTCGGT-3' (reverse primer). Amplification was carried out using a BIO-RAD Thermal Cycler C 1000 Touch (Bio-Rad Laboratories, Inc. USA) in a final volume of 25µl containing 10.875µl nuclease-free water, 2.5µl 10xBuffer, 0.5µl Forward Primer (10µM), 0.5µl Reverse Primer (10µM), 0.5µl 10mMdNTPs, 0.125µl *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 amplicons were analyzed by electrophoresis on a 2% agarose gel.

2.8 RFLP Analysis of *AGTR1* Amplicons

Genotyping of variants at the rs5186 locus was carried out through restriction fragment length polymorphism (RFLP) of the *AGTR1* PCR amplicons. The 359-bp amplicons were digested using *DdeI*, a restriction endonuclease whose recognition site is 5'CTNAG3'. A restriction endonuclease digest of a 10µl aliquot of the PCR amplicon was carried out by addition of 0.25µl of *DdeI*, 2.5µl of 10X rCutSmartTM 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 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. 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. The categorical data (*AGTR1* genotypes) was analyzed using Fisher's exact test. P value < .05 was considered as statistically significant. 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

A total of 272 participants were enrolled for the study with 136 EH 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 participants which included both cases (30) and controls (30) were dropped due to matching challenges. However, exclusion off these participants may have only affected the assessment of the genetic biomarker due to its low frequency in the population. The adjusted sample size did not affect investigation of other biomarkers (haematological & biochemical). Therefore, 212 participants were age and gender matched and used was used for statistical analysis.

3.2 Biomarkers and Hypertension

Analysis of the complete blood count and C-reactive protein levels was carried out 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 ± SD and the data analyzed using the independent t test. The median values (±IQR) for CRP, RDW and PDW were 0.6±1.8 and 41.95±1.08; 11.5±2.3 in controls and 2.9±4.8; 44.95±1.21 and 11.0±2.2 in cases respectively. Mean values for NLR and MPV were 2.09±1.3 and 9.36±1.14 in controls 2.74±1.4 and 10.71±1.14 and cases respectively. The difference between cases and the control group for all these biomarkers was statistically significant ($P < .001$) except for PDW for which the difference was not statistically significant ($P = .519$) (Fig. 1).

3.3 *AGTR1* genotype frequencies

PCR amplification of the *AGTR1* gene was successfully conducted on a total on the 212 samples which included 106 cases and 106 controls. This resulted in the generation of DNA amplicons of the expected 359bp size (Figure 2). RFLP analysis involved *DdeI* digestion of the amplicons followed by agarose gel electrophoresis for *AGTR1* genotyping based on DNA fragment band patterns. The AA (single 359bp band) and AC genotypes (3 bands of 359pb, 220bp and 139bp) were observed (Figure 3). The frequencies of the AA, AC and CC genotypes were 98.1%, 1.9% and 0.0% in cases and 99.1%, 0.9 and 0.0% in controls respectively. Therefore, out of the 212 participants only three were found to have the heterozygous genotype (AC) while the other two hundred and nine (209) had the homozygous normal genotype (AA). This represented a frequency of 1.42% of the mutant alleles and 98.58% had the wild type alleles in the studied population. There was no statistically significant difference between cases and control group ($P=0.6214$; $OR=0.4952$) in genotype frequencies (Table 2).

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 gaps so as to empower the general population, health practitioners and policy makers to better control hypertension in Kenya.

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, haematological 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 in 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 haematological 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; P=0.519) and the *AGTR1* rs5186 mutation, (OR=0.4952).

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 an Iranian 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].

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 results are also similar to those obtained in 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 indicates that at the site of an injured blood vessels, platelet consumption is increased and this leading to the 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 than mature ones, their existence represents a risk factor for the occurrence of coronary thrombosis and myocardial infarction [27].

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

Table 1. Sociodemographic characteristics of the study participants

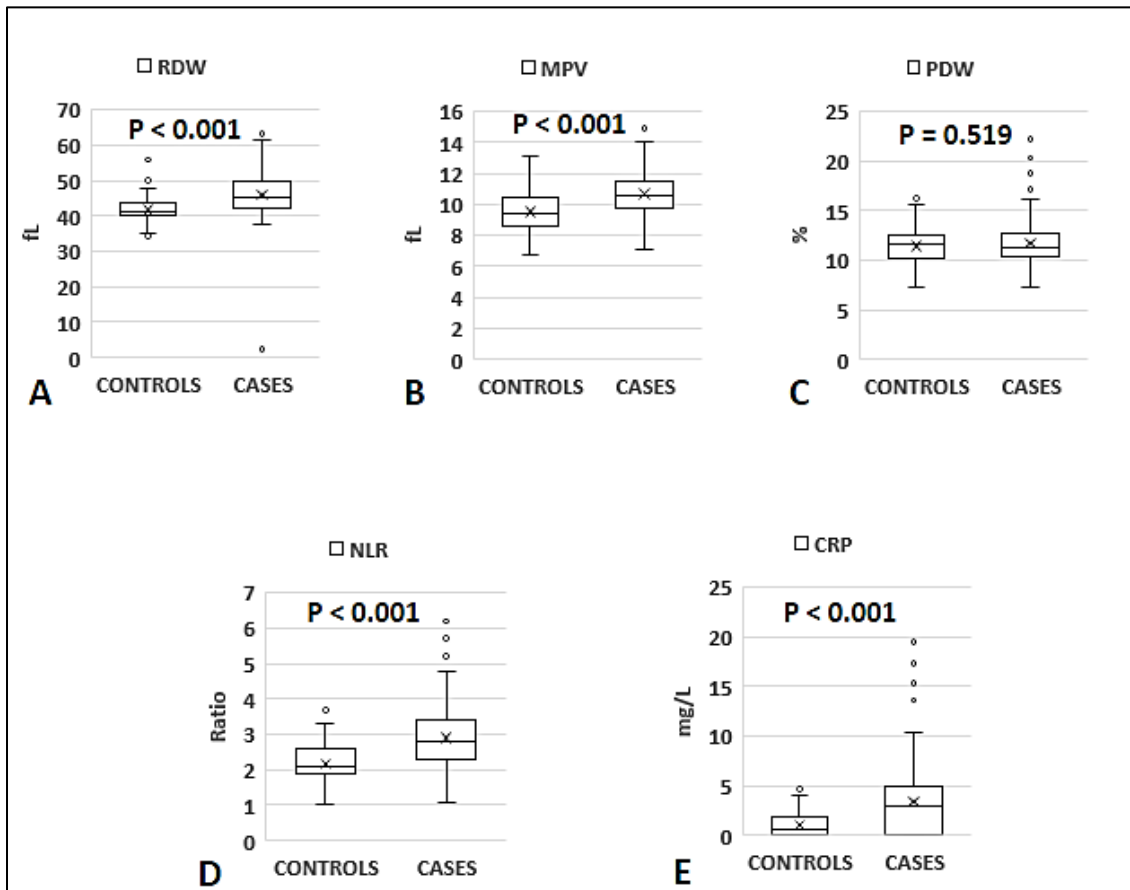| Variable		Controls (%)	Cases (%)
Gender	Male	41 (30)	34 (25)
	Female	95 (70)	102 (75)
Age groups	18-35	34 (32)	31 (29)
	36-55	49 (46)	51 (48)
	>55	23 (22)	24 (23)
Mean age ± SD		42.8 ±12.31	44.8 ±11.34
HTN History	Yes	66 (49)	96 (70)
	No	70 (51)	31 (23)
	Unknown	0 (0)	9 (7)
Mean BMI (kg/m^2) ± SD		25.73 ±4.20	27.53±4.89
Mean BP (mmHg) ± SD	SBP	124.1 ±9.42	145.5 ±17.39
	DBP	74.1 ±7.76	85.4 ±11.99

Fig. 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 protein (CRP, Panel E). Significantly (P<0.05) higher levels of all the biomarkers except for PDW, were present in the cases compared to the controls. "x" represents the mean value

Table 2. *AGTR1* genotype frequencies in hypertensive and normotensive study participants

Group	Genotypes		Totals	P[a]	OR[b]	95% CI[c]	
	AA (%)	AC (%)					
Hypertensives	104 (98.1)	2 (1.9)	106	0.6236	0.4952	0.0442	5.5456
Normotensives	105 (99.1)	1 0.9)	106				
Totals	209 (98.6)	3 (1.4)	212				

[a]P = p value, [b]OR = Odds ratio, [c]C I= Confidence Interval
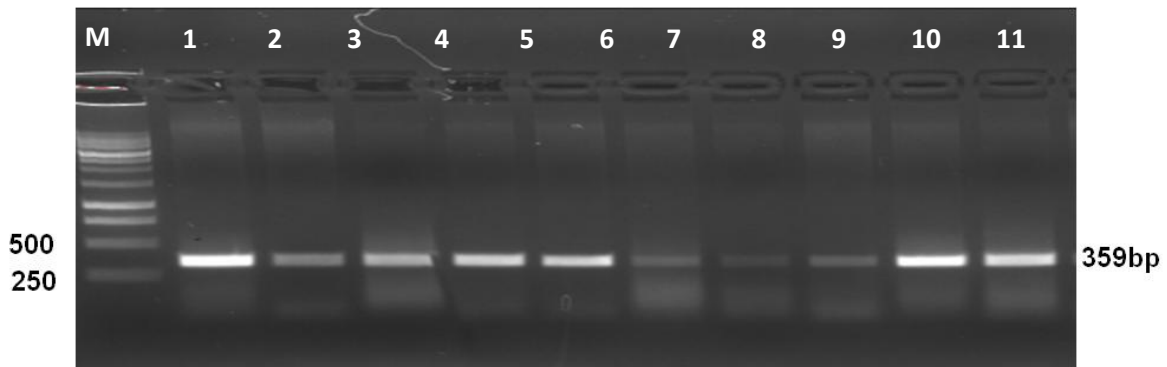
Fig. 2. Agarose gel electrophoresis of *AGTR1* PCR amplicons
The selected images of PCR amplification of the 359bp fragment of human AGTR1 gene as seen on the agarose gel electrophoresis. Lanes 1-11 represent 359bp PCR amplicons from 11 out of the 272 samples. (M = 1kb molecular DNA ladder marker).
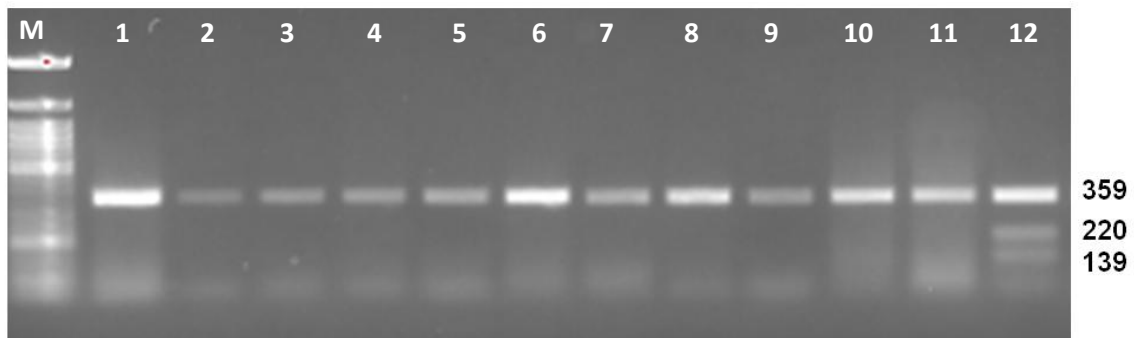

Fig. 3. RFLP Analysis of *AGTR1* PCR amplicons
The 359bp PCR amplicons were digested using the Dde1 restriction endonuclease. The homozygous AA normal genotype (single 359bp band; Lanes1-11) and the heterozygous AC mutant genotype (threes bands of 359bp, 220bp and 139bp; Lane 12) were detected. (M = 50bp molecular DNA ladder marker).

The rs5186 mutation in AGTR1 gene and the genotype frequencies were also determined in the current study. We found that 99.1% of the controls had the homozygous normal (AA) genotype while only 0.9% were heterozygous (AC). There was no statistically significant difference when compared to the genotype frequencies among cases where 98.1% had the AA genotype and only 1.9% had the AC genotype (P>0.05). These findings are similar to a study done in Nigeria involving 1224 participants which noted that polymorphisms in

AGTR1 gene were not associated with essential hypertension. [35]. The findings of the current study are also similar to those obtained in an unpublished thesis research investigation by Freeman, J.C. conducted in Kasiagu, a region in south-eastern Kenya, Taita Taveta County in 2013 that reported no association between *AGTR1* mutations and EH. Although the study was notably also conducted in Kenya, the population in Kasiagu region may differ from that of Tharaka Nithi County which is predominantly of Bantu origin [14]. These results are in contrast

to other studies in non-African populations which have reported that *AGTR1* polymorphisms are associated with essential hypertension [36-37].

Notably, the CC genotype was completely absent in the Kenyan population similar to observations in Cameroon, Ghana, Nigeria and Burkina Faso [16-19]. This is in contrast to findings in Tunisia where the CC genotype (which has been associated with higher risk of essential hypertension) was present at a prevalence of 43.7% in hypertensive participants and 18.3% in shealthy controls [38]. These findings point to distinct differences in the AGTR1 polymorphism profiles in African populations in comparison non-African populations (or African populations that may which may include individuals of Middle Eastern or Asian descent) suggesting that alternative pathophysiological pathways could be involved in the onset of essential hypertension in the African setting. Possible future studies could investigate gene expression profiles of AGTR1 among individuals with essential hypertension in Africa which would provide additional data to explore the hypothesis of differing genetic profiles in African populations. Indeed, a recent study on angiotensin receptors (ATR1, ATR2, and ATR4) noted differential AT1R expression in HIV-infected pre-eclamptic women of African descent [39]. Additional studies on the role of other genetic polymorphisms that may be associated with EH in Africa will also need to be conducted to further contribute to a better understanding of the impact of genetics on hypertension in African populations.

One limitation faced in our study was difficulty in obtaining age and gender matched controls for cases aged 60 years and above. This is due to the fact that majority of blood donors were young individuals. Conversely, majority of hypertension patients tended to comprise adults aged above 40 years. This age discrepancy necessitated the exclusion of the unmatched individuals from statistical analyses.

5. CONCLUSION AND RECOMMENDATIONS

This study noted that the median values of CRP and RDW and the mean values of MPV and NLR were significantly higher individuals with essential hypertension compared to normotensives. However PDW and the *AGTR1* gene (rs5186) SNP were not associated with essential hypertension incidence in the studied population.

Therefore, it is important for clinicians to be aware of biomarkers that could be elevated due to essential hypertension in the absence of other inflammatory and chronic diseases. Detection of derangements in CRP, RDW, MPV and NLR levels may aid clinicians in early detection of EH in undiagnosed cases. This would allow for prompt initiation of case management and control of the disease. This study recommends further research needs on other genes which could be associated with onset or progression of essential hypertension in the Kenyan population.

CONSENT AND ETHICAL APPROVAL

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

COMPETING INTERESTS

Authors declared that there were no competing interests.

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