ASSOCIATION OF SELECTED HAEMATOLOGICAL, BIOCHEMICAL AND GENETIC BIOMARKERS AMONG PATIENTS WITH ESSENTIAL HYPERTENSION AT CHUKA COUNTY REFERRAL HOSPITAL, KENYA

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Association of Selected Haematological, Biochemical and Genetic Biomarkers among Patients with Essential Hypertension at Chuka County Referral Hospital, Kenya

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other university.

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DEDICATION

This work is dedicated to my parents Mr. and Mrs. Mbaabu and my family for giving me an easy time during my studies.

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ACRONYMS AND ABBREVIATIONS

ADH	Aldosterone Hormone
AHA	American Heart Association
ACC	American College of Cardiology
AGTR1	Angiotensin II Type 1 Receptor Gene
AT1R	Angiotensin II type I receptor
AT2R	Angiotensin II type II receptor
BMI	Body Mass Index
BP	Blood Pressure
CBC	Complete Blood Count
CGH	Chuka General Hospital
CRP	C - reactive protein
CVD	Cardiovascular Disease
DBP	Diastolic Blood Pressure
DNA	Deoxyribonucleic Acid
EDTA	Ethylenediamine Tetraacetic Acid.
EH	Essential Hypertension.
GWAS	Genome Wide Association Studies.
HBP	High Blood Pressure.

HTN	Hypertension
JKUAT	Jomo Kenyatta University of Agriculture and Technology.
JNC	Joint National Committee
KHSS	Kenya Health Sector Strategic Plan
KNBS	Kenya National Bureau of Statistics
LMICs	Low and Middle Income Countries
LNK	Lymphocyte adaptor protein
MPV	Mean platelet volume
NCD	Non- Communicable Disease
NLR	Neutrophil to Lymphocyte Ratio
PAI-1	Plasminogen Activator Inhibitor 1
PCR	Polymerase Chain Reaction
PDW	Platelet Distribution Width
RAAS	Renin-angiotensin-aldosterone system
RFLP	Restriction Fragment Length Polymorphysim
RBC	Red blood cell
RDW	Red cell distribution Width
ROS	Reactive oxygen species
SBP	Systolic blood pressure

SNP Single Nucleotide Polymorphism

WHO World Health Organization

ABSTRACT

Hypertension is one of the leading causes of heart disease such as coronary artery disease and heart failure along with stroke and kidney damage among other conditions. Known as the “silent killer", hypertension is largely an asymptomatic disease that becomes evident only when it progresses to severity and begins to cause tissue damage in multiple organs. A significant proportion of the general population is not aware of their hypertension status and are therefore not under management to prevent development of complications that are associated with severe untreated essential hypertension. It is approximated that in almost 90% of cases, the underlying causes of hypertension are unknown (perhaps genetic or other environmental factors) and accounts for majority of cases. Primary or essential hypertension (EH) is the name given to this condition. This is in contrast to secondary hypertension that is the result of known medical conditions. Multiple risk factors for hypertension include: age, level of physical activity and genetics. Early detection of essential hypertension is crucial to ensure measures are taken to mitigate against the devastating consequences. The purpose of the current study was therefore to determine whether there is an association between AGTR1 (rs5186) SNP, C - reactive protein, selected hematological biomarkers and EH in a Kenyan population. These markers would contribute to monitoring progression and effective control of essential hypertension. This was a case control study conducted from March to July, 2022 at Chuka County Referral Hospital, Kenya. This being a case-control experiment, comprised of age and gender matched normotensive blood donors as the controls and cases being hypertensive patients. The study included 136 cases and 136 controls. Study participants were recruited by convenience sampling using selection criteria to attain the sample size required. Blood samples were collected and biomarkers analyzed. DNA was extracted and analyzed by PCR and Restriction Fragment Length Polymorphism. Data analysis was performed using Statistical analysis system (SAS) software. Independent t-test and Mann Whitney U test were used to analyse continuous data while for the categorical data, fishers’ exact test and Odds ratio was calculated to determine association between the groups. *P* values less than 0.05 were considered as statistically significant. The results showed that mean values of mean platelet volume (MPV), Neutrophil to Lymphocyte ratio (NLR) and the median values of C-reactive protein (CRP) and Red Cell Distribution Width (RDW) were significantly higher in hypertensive group compared to healthy control group. Platelet Distribution Width (PDW) (p=0.52) was found not to be statistically significant. The results also indicated that the 98.6% of the study population had the wild type AA genotype, and 1.4% was AC heterozygous carriers of the A1166C polymorphism. The results showed that there was no statistically significant association between AGTR1 (rs5186) SNP frequency and EH in Tharaka Nithi County, Kenya. ($p=0.6236$, ORs=0.4952(95%CI: 0.0442-5.5456). Therefore, it is important for clinicians to be aware that these biomarkers could be elevated due to essential hypertension in the absence of other inflammatory and chronic diseases. Derangements in CRP, RDW, MPV and NLR can help clinicians question a likelihood of essential hypertension in undiagnosed cases. This can help in prompt initiation of management and control of the disease. This study also recommends that further research to investigate other possible mutations in other genes which could be associated with essential hypertrnsion in Kenyan population.

CHAPTER ONE

INTRODUCTION

1.1 Background

Hypertension (blood pressure ≥ 130/80 mmHg) is a common non-communicable disease (NCD) where systemic arterial circulation experiences persistent elevated intravascular pressure. Severe and untreated hypertension is associated with heart disease, stroke and death (WHO, 2015). Essential hypertension (EH) also referred to as primary hypertension accounts for about 90% of cases of the disease and is hypertension for which the underlying causes remain unknown (Shere *et al*., 2017). The minority of hypertension cases 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 (Kuen and Williams, 2010*; Weber et al.,* 2014*)*

Hypertension (and other NCDs) has historically been regarded as lifestyle diseases associated with developed economies. However, in the past decade NCDs have increasingly become a critical concern in low and middle income countries (LMICs) (Engelgau *et al.,* 2018) In many developing countries settings, they are surpassing infectious diseases as the primary causes of morbidity and mortality. 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 percent) of the population than in high-income countries (28.5 percent) (WHO, 2019).

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% (WHO, 2021). This high prevalence of Hypertension in Africa could be attributed to increased urbanization and a concurrent rise in unhealthy, sedentary lifestyles in various African countries. In Kenya, according to a national survey conducted by Mohamed *et al*, estimated the overall age-standardized prevalence of hypertension was 24.5%. (Mohamed *et al*., 2018). The

survey utilized a sample size of 4,433 participants with national geographic coverage and thus gave a clear picture of hypertension status in Kenya.

In addition, a report by Kenya Health Sector Strategic Plan revealed that hypertension had affected about 22.7% of people in Kenya of which only 16.7 % had been diagnosed, and were on effective treatment coverage. 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 central region of Kenya at 37.2% followed by eastern region at 28.4%. Tharaka Nithi County is in the Eastern region and is considered to be one of the counties with high prevalence of Hypertension in Kenya. (KHSSP 2014-2018).

There are multiple hematological indicators for high blood pressure which may provide insight into the fundamental biological processes that lead to onset and progression of hypertension. These blood biomarkers give a better understanding on the pathophysiology, diagnosis, Essential Hypertension (EH) development and treatment efficacy (Androulakis *et al*., 2013). Some of these biomarkers include C-reactive protein (CRP) 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 (Yeldu *et al*., 2018). Other biochemical and hematological markers including serum uric acid, urinary albumin, red cell distribution width (RDW) and the neutrophil lymphocyte ratios (NLR) have been observed to be higher in hypertensive individuals. In addition, other biomarkers such as plasminogen activator inhibitor 1, fibrinogen, urine albumin creatine ratio, D-dimers and plasma renin have been reported to have an association with essential hypertension (Shere *et al*., 2017).

Genetics plays a critical role in the development of primary hypertension (Levy *et al*., 2010). 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 enhances the risk of EH. 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. 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*) (Mulerova *et al*,2020; Freeman, 2013). 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 (Fajar *et al*.,2019);(Liu *et al*.,2015). The association between the A1166C single nucleotide polymorphism (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) (Yako *et al*.,2015). It is unknown whether the results found in those populations hold true for hypertensive populations in East Africa.

Several studies have observed that in many African countries including Kenya, many individuals are unaware of their health status especially blood pressure(Africa, 2018) Given that early stages of hypertension mostly present with no symptoms this "silent killer" remains undiagnosed. There is therefore urgent need to increase awareness of the disease, the associated risk factors and for new improved and accurate diagnostics for early detection, progression and control of essential hypertension. This study therefore, aimed at addressing this knowledge gap through investigation of a combination of selected hematological markers {Mean Platelet Volume (MPV), Platelet Distribution Width (PDW), Neutrophil Lymphocyte Ratio (NLR) and Red Cell Distribution Width (RDW)}, CRP and the *AGTR1* A1166C SNP among patients with EH at Chuka County Referral Hospital in Tharaka Nithi county, Kenya. These

biomarkers were selected based on their strength of association with essential hypertension in other studies as well as the cost of analyzing the biomarkers. The information about these biomarkers would enable improved understanding of the aetiology of EH and support better case management.

1.2 Statement of the Problem

Hypertension is one of the leading causes of disease and mortality among non-communicable diseases, also known as the "silent killer". An estimated 1.13 billion individuals suffer from hypertension worldwide of which two-thirds of the population lives in low- and middle-income countries (Mills and Stefanescu, 2020.) This could be attributed to could be attributed to an increased urbanization and a concurrent rise in unhealthy, sedentary lifestyles and poor health systems in low and middle income countries while in developed countries there is a well-established healthcare system. Untreated severe hypertension has been associated with increased risk of heart disease, stroke, and death. Essential hypertension (EH) also referred to as primary hypertension, accounts for about 90-95% cases of the disease and is hypertension for which the underlying causes remain unknown and where it often presents with no symptoms.

Hypertension is largely an asymptomatic disease that becomes evident when it progresses to severe hypertension and begins to cause tissue damage in multiple organs. A large proportion of the general population is not aware of their hypertension status and are therefore not under management to prevent development of complications that are associated with severe untreated hypertension. Effective management would entail early diagnosis and early intervention to mitigate high costs and poor prognosis of late detection.

Mohamed *et al*., (2018) found Hypertension prevalence of 24.5 % in Kenya, a study that utilized 4443 participants across the forty-seven counties. A report by KHSSP, showed that central Kenya had the highest prevalence of hypertension at 37.2%, followed by Eastern region at 28.4%. (KHSSP report, 2014-2018). Tharaka Nithi county is in the Eastern region and is considered to be one of the counties with high prevalence of Hypertension.

1.3 Justification

Since majority of the population are unaware of their blood pressure status, there is a likelihood that they could have essential hypertension which can lead to hypertension related complications such as cardiovascular diseases, kidney damage among others. There is therefore need to identify hematological, biochemical and genetic biomarkers that are associated with essential hypertension. This may ultimately aid in early detection and initiating appropriate treatment and management to prevent complications of untreated essential hypertension. These biomarkers may also contribute to monitoring progression and effective control of essential hypertension. Identification of genetic markers would enable an understanding of the etiology of essential hypertension and this would help in screening and surveillance of the disease.

Hematological, biochemical and genetic markers may reveal the underlying processes associated with the onset, progression and complications of essential hypertension. Genetic changes may alter the normal physiological mechanisms and enhance the development of a disease (Levy et al., 2010). Genome wide association studies have found mutations in various genes to be linked to EH. These genes include: angiotensin I converting enzyme (ACE) gene, the angiotensinogen gene, 11 βhydroxysteroid dehydrogenases types 1 and 2 (11βHSD1 and 11βHSD2) genes and the angiotensin II type 1 receptor gene (*AGTR1*) among others (Mulerova, 2020; Freeman, 2013). Fajar *et al*., (2019) found an association between AT1R and increased risk of essential hypertension. A systematic review and meta-analysis (Yako *et al*., 2018) found that polymorphisms in AT1R gene have been associated with the risk of development of hypertension. Therefore, the association between the A1166C SNP in the AT1R gene and high blood pressure has not been consistently reported in various populations. Studies conducted in Africa have focused on North (Egypt, Tunisia) and West African (Nigeria, Burkina Faso) populations; none have been carried out in East African populations (Yako *et al*., 2018). It is unknown whether the results found in those populations hold true for hypertensive populations in East Africa.

Hematological and blood biochemical markers have also been associated with development and progression of essential hypertension in cross sectional and case

control study designs. Findings of a study by Yeldu *et al*., (2018), indicate inflammatory C-reactive protein (CRP) is positively correlated with hypertension and Cardiovascular diseases. Some haematological parameters in hypertensive have significantly been found to be elevated compared to normotensive individuals. In a study by Enawgaw *et al*., found that there was a significant increase in mean corpuscular hemoglobin concentration, red blood cell distribution width , mean platelet volume , and platelet distribution width in the hypertensive group compared to healthy individuals (Enawgaw *et al*., 2017)

In Kenyan population particularly Tharaka Nithi county, no similar studies that had been done on hematological, biochemical and genetic biomarkers for early detection, progression and control of essential hypertension. It was therefore unclear if these biomarkers could enable detection, progression and control of essential hypertension among patients at Chuka County referral hospital, Kenya

This study aimed at addressing this knowledge gap through investigation of a combination of selected hematological markers (such as MPV, PDW, NLR and RDW), biochemical marker (CRP) and genetic (*AGTR1* A1166C SNP) among patients with essential hypertension at Chuka County referral hospital in Kenya. This information would enable improved understanding of the aetiology of EH and support better case management.

1.4 Research Questions

i. What is the association between Red cell distribution width, Mean platelet volume platelet distribution width, Neutrophil to Lymphocyte ratio and essential hypertension among patients at Chuka County Referral Hospital?
ii. What is the association between serum levels of C-Reactive protein (CRP with essential hypertension among patients at Chuka County Referral Hospital?
iii. What is the frequency and association between angiotensin II type 1 receptor gene (AGTR1) SNP (A1166C) and EH among patients at Chuka County Referral Hospital?

1.5 Hypothesis

H_1. There is an association between MPV, NLR, PDW, RDW, CRP and AGTR1 mutations and the risk of developing essential hypertension.

1.6 Objectives

1.6.1 General Objective

To determine the association between selected hematological, C-reactive Protein, and genetic biomarkers among patients with Essential Hypertension at Chuka County Referral Hospital.

1.6.2 Specific Objectives

i. To determine the association between red cell distribution width, mean platelet volume, Neutrophil to lymphocyte ratio and platelet distribution width and Essential Hypertension among patients and controls at Chuka County Referral Hospital.
ii. To determine the association between C - reactive protein (CRP) and Essential Hypertension among patients and controls at Chuka County Referral Hospital.
iii. To determine the association and frequency between AGTR1 SNP (A1166C polymorphism) and Essential Hypertension among patients and controls at Chuka County Referral Hospital.

1.7 Limitations of the Study

This study was limited by difficulty in recruiting controls (blood donors) of age sixty (60) years and above. This is due to the fact that majority of the controls (blood donors) were of lower age bracket. Additionally, majority of patients with essential hypertension (cases) were above age 40 years with more of them being elderly as compared to younger age groups. This made it difficult to attain 100% age-gender matching among study participants.

CHAPTER TWO

LITERATURE REVIEW

2.1 Definition of Systemic Arterial Hypertension

Systemic Arterial Hypertension (SAH) has been severally redefined. According to America Heart association (AHA), it has recently been defined as elevated pressure with systolic pressure at or above 130 mm Hg and diastolic pressure is at or above 80mm Hg (American College of Cardiology, 2017). This was after findings from several randomized clinical trials and large scale prospective studies indicated a significant increase in Cardiovascular Disease (CVD) risk with rising blood pressure even when systolic blood pressure is as low as 115 mmHg. Hypertension is known to be the major cause disability and death worldwide. It is also a main risk for cardiovascular illnesses (Schmierder, 2010).

2.2 Classification of Systemic Arterial Hypertension

Based on etiology, systemic arteriole hypertension may be classified as essential or secondary hypertension. In essential hypertension, there is no identifiable specific medical condition as a cause of the raised blood pressure whereas in secondary type hypertension, the increased blood pressure can be attributed to an established medical condition (Camm *et al*., 2018). The Joint National Committee (JNC) classifies hypertension in adults (≥18 years) based on properly measured blood pressure readings. In this classification, Systemic Arterial Hypertension is categorized into either: prehypertension, stage one hypertension and stage two hypertension.

2.2.1 Essential Hypertension

Idiopathic, essential or primary hypertension is the elevated pressure not attributable to a medical condition such as pheochromocytoma, aldosteronism, renovascular disease, renal failure (monogenic) causes. It accounts for about 90-95% of all cases of hypertension (Camm *et al*., 2018). Although the causes of primary hypertension are not clearly known, genetic, behavioral and environmental variables have been linked to high blood pressure in essential hypertension. The genetic influence on essential

hypertension has been shown by familial studies revealing associations of blood pressure and first degree relatives who include siblings, parents and children. The genetic changes that cause inherited "essential" hypertension are still mostly unknown. Possible mutations related to inherited essential hypertension include those leading to increased lithium-sodium counter transport, low kallikrein excretion in the urine, fasting plasma insulin levels, high-density lipoproteins (HDL), Low density Lipoproteins (LDL) subtractions, fat pattern index, and BMI.

A variation in the angiotensinogen gene has been associated to high blood pressure in hypertensive siblings, according to Fang *et al*. The polymorphism involves nucleotide 704, where thymidine is substituted for cytosine, resulting in the replacement of methionine for threonine at position 235 (M235T). This is linked to higher plasma angiotensinogen levels (Fang *et al*., 2010). Essential hypertension has also been linked to polymorphisms and mutations in the angiotensin-converting enzyme, 2-adrenergic receptor, -adducin, angiotensinase C, renin-binding protein, G-protein 3-subunit, atrial natriuretic factor, and insulin receptor (Luft, 1998).

Environmental and behavioral factors shown to be associated with essential hypertension include: obesity, insulin resistance, high alcohol intake, high salt intake, physical inactivity, psychological stress, dyslipidemia, and low potassium or calcium intake in susceptible subjects (Oscar *et al*., 2000). Genetic factors also influence some of these behavioral patterns which may lead to elevation in blood pressure such as tendencies towards obesity and alcoholism. It is therefore postulated that essential hypertension results from an interaction between genetic, behavioral and environmental factors.

2.2.2 Secondary Hypertension

High blood pressure that has an identifiable and possibly a reversible cause is called secondary hypertension. It accounts for about 5-10% of high blood pressure and it's more prevalent among the young population. It is estimated that 30% of those aged 18 to 40 and hypertensive have the secondary hypertension type (Charles *et al*., 2017). Identifiable medical causes of secondary hypertension are endocrine disorders and non-endocrine disorders. Non-endocrine disorders include kidney disease, renal artery

stenosis, fibromuscular dysplasia and sleep apnea while some of the endocrine disorders include primary aldosteronism, cushings syndrome, haemochromocytomas, diabetes mellitus and thyroid diseases among others (Grossman *et al.*, 2015).

2.2.3 Joint National Committee (JNC) Classification of Systemic Arterial Hypertension

Joint National Committee (JNC) classifies hypertension in adults (≥18years) based on an average of two or more precisely recorded blood pressure measurements from at least two clinical visits. In this classification, systemic arterial hypertension is categorized into one of three groups: prehypertension, stage one HTN and stage two HTN as shown in table 1. Pre-hypertension is not a disease category but rather a cluster of individuals at high risk of developing hypertension. They are not candidates for medication therapy, but they should adopt a healthier lifestyle to lower their chances of getting hypertension in the future. Persons with hypertension (stages one and two) should be treated with drug therapy together with lifestyle modification.

Table 2.1: Classification of Systemic Arterial Hypertension by JNC

Stages	Systolic reading	Diastolic reading
Normal BP	<130 mmHg	<85 mmHg
Pre-HTN	130-139 mmHg	85-89 mmHg
HTN stage I	140-159 mmHg	90- 99 mmHg
HTN stage II	>160 mmHg	>100 mmHg

2.2.4 Other Classifications of Systemic Arterial Hypertension.

Other descriptive terms used in the diagnosis and management of hypertension are: Resistant Hypertension, Malignant Hypertension and Isolated Hypertension. When blood pressure stays above normal despite the administration of more than three drugs, this hypertension is referred to as resistant hypertension. Resistant high blood pressure is estimated to affect ten percent of patients with high blood pressure (Julian *et al*., 2015). Resistant hypertension patients may also have secondary hypertension where the etiology is yet to be recognized, prompting a clinician to further investigate the secondary causes. However, resistant hypertension can be treated successfully with multiple medications that are well known to eliminate the secondary cause.

The most severe type of hypertension is malignant hypertension. It is defined as HTN with the diastolic pressure >130 mmHg and evidence of end organ damage (Domek *et al*, 2019). The incidence of malignant HTN is low about one to two cases in one hundred thousand. The rates are likely to be high in black individuals (Shantsila & Lip, 2017). Malignant hypertension is usually an emergency medical state and always requires instant medical attention.

Isolated systolic hypertension refers to measured systolic pressure above 140 mmHg and diastolic pressure below 90 mmHg. This is the most common form of hypertension among the elderly (Tan & Thakur, 2021) but can also affect the young and middle-aged individuals (Franklin *et al*., 2015).

2.3 Epidemiology of Essential Hypertension

Hypertension affects about 1.28 billion persons globally and according to the estimates, 46% of persons with hypertension are completely unaware of their disease (WHO, 2021). Hypertension has been found to be more prevalent in low- and middle-income nations (31.5 percent of the population) compared to countries with a high level of income (28.5 percent of the population) (WHO, 2019). According to WHO, Africa has the greatest prevalence of hypertension in the world at 27%. This was in contrast to the Americas with the lowest prevalence of 18% (WHO, 2021 Factsheet). This could be attributed to increased urbanization and a concurrent rise in unhealthy, sedentary lifestyles in African countries. According to a systematic review and meta-analysis by (Kandala & Uthman, 2015), the prevalence of hypertension in low and middle-income countries was found to be 32.3%.

In Kenya, according to a national assessment conducted by Mohamed *et al.* (2018) the occurrence of hypertension in Kenya is 24.5%. The study utilized a sample size of 4433 participants with national geographic coverage and thus gave a clear picture of hypertension status in Kenya. In addition, a report by Kenya Health Sector Strategic Plan revealed that hypertension had affected a majority of people in Kenya and only 16.7 % had been diagnosed, and were on effective treatment coverage. In that report, the proportion of individuals who had raised blood pressure in the population, identified as hypertensive, put on treatment and whose blood pressure had been

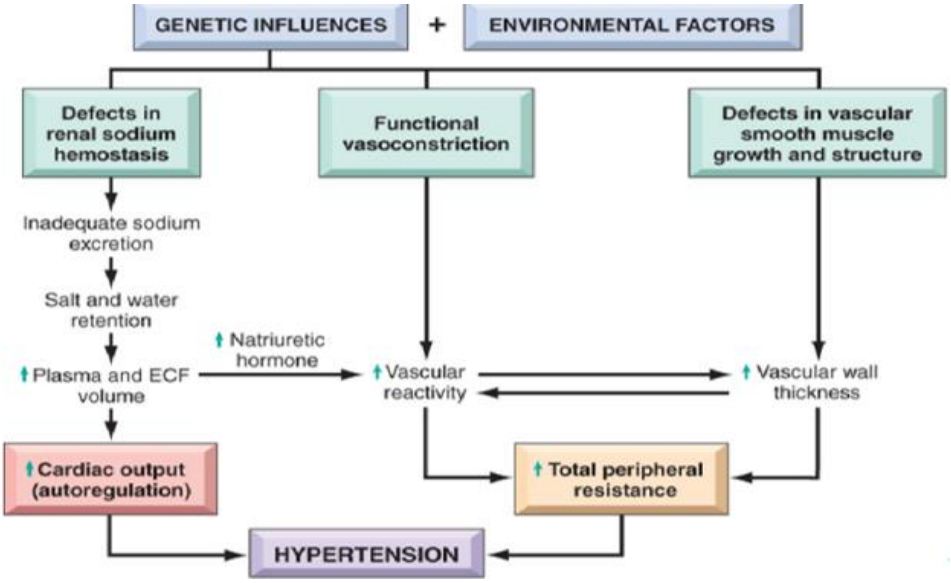
controlled was only 4%. Hypertension was more prevalent in central region of Kenya at 37.2% followed by eastern region at 28.4% (KHSSP 2014-2018). Tharaka Nithi county is in the Eastern region and is considered to be one of the counties with high prevalence of Hypertension in Kenya.

2.4 Pathogenesis of Essential Hypertension

Hypertension is a disease with a complicated pathophysiology and the maintenance of normal blood pressure involves several physiological systems and their disruption may take part in the progression of the primary hypertension. Some of the mechanisms include peripheral resistance, cardiac output, the autonomic nervous system or sympathetic hyperactivity, endothelium dysfunction, renin-angiotensin-aldosterone system, nitric oxide and vasoactive substances. The development of essential hypertension is also influenced by genetic and environmental variables. (Camm *et al*., 2018).

2.4.1 The Cardiac Output and Peripheral Resistance

The stability between peripheral vascular resistance and cardiac output is crucial for maintaining normal blood pressure. There must be a balance between cardiac output (stroke volume and pulse rate) and the peripheral resistance. The majority of essential high blood pressure patients have a normal cardiac output but a high peripheral resistance. Small arterioles, which have smooth muscle cells on their walls, determine peripheral resistance. The contraction of smooth muscle cells is thought to be linked to an increase in intracellular calcium concentration, which could explain why calcium channel blockers have a vasodilatory effect. Long-term smooth muscle narrowing is considered to cause structural modifications in the arteriolar artery walls, which may be initiated by Angiotensin, resulting to an increased peripheral resistance which is not reversible. It is hypothesized that in early stage high blood pressure, peripheral resistance is normal and the raise blood pressure is triggered by a rise in cardiac output, which is associated to sympathetic over activity (Beevers *et al*., 2001). The consequent increase in peripheral arterioles resistance may thus progress in a way to stop the elevated pressure that is passed to the capillaries where cell homeostasis would be significantly affected.



(*Adopted from* Kumar *et.al.* Robbins Basic Pathology 8th edition)

Figure 2.1: Factors Involved in Pathophysiology of Hypertension

The figures show how the body maintains normal blood pressure by balancing peripheral vascular resistance and cardiac output. Increased arterial blood pressure is due to various defects such as defects in renal sodium homeostasis, defects in vascular smooth muscle growth and structure. These defects lead to increased vascular wall thickness and vascular reactivity leading to increased total peripheral resistance. Increased plasma volume and ECF fluid leads to increased cardiac output. The imbalance between cardiac out and total peripheral resistance leads to development of hypertension.

2.4.2 Sympathetic Hyperactivity

The Sympathetic nervous system (SNS) plays a vital role in regulating arterial blood pressure since it stimulates the vessels. SNS stimulation can result in dilation and constriction of arterioles. Therefore, the autonomic nervous system has a significant role in normalizing blood pressure. The system is also significant since it mediates the short-lived alterations in pressure in case of anxiety and reduced exercise activities. Hypertension is often preceded by an increase in sympathetic nervous system activity. Insulin-glucose excess, dietary sodium chloride, nitric oxide deficit, and acute elevations in plasma osmolality are some of the conditions that causes sympathetic

nerve activity to rise (Wyss, 1993). Several neurotransmitters and neuromodulators are out of balance during the progression of hypertension, which contributes to raise noradrenaline release to the sympathetic neurons' postsynaptic targets, which regulate blood vessels, both directly and indirectly.

2.4.3 The Renal System

The kidneys are the target organs of the hypertension processes. The condition involves interactions of several organ systems and many mechanisms of independent and interdependent pathways (Hall *et al*., 2012). Because it controls extracellular volume, the nephron has a crucial role in the controlling arterial pressure. Pressure in the kidneys also controls arterial flow and blood pressure and controls the activity of many vasoactive systems, including the RAS system, and directly modulates salt excretion (Wadei & Textor, 2012). Therefore, kidneys play a role in progression of essential hypertension If kidneys are not able to control excess salt in the system, stimulation of the RAS and the SNS, it results to increased blood pressure (Cain *et al*., 2002). Essential hypertension is characterized by asymptomatic and symptomatic period which advances to a complicated hypertension resulting to organ damage.

There is increasing evidence that rise in blood pressure which is caused by endothelial dysfunctions and consequent remodeling is stimulated by a state of prolonged inflammation in blood vessels that results to production of cytokines and generation reactive oxidative stress (Androulakis *et al*., 2009). The functional and structure alterations in the resistance vessels usually lead to raised peripheral resistance leading to development of hypertension. The immune system also has a role in the development of hypertension. The cells of the immune system get stimulated and penetrate organs which include kidneys and the vasculature as the disease progresses (Guarner-Lans *et al*., 2020).

2.4.4 Endothelial Dysfunction

There is increasing evidence that rise in blood pressure which is caused by endothelial dysfunctions and consequent remodeling is stimulated by a state of prolonged inflammation in blood vessels, that results to production of cytokines, oxidative stress, and generation reactive oxidative stress (Androulakis *et al*., 2009). The functional and structural alterations in the resistance blood vessels usually lead to a raised peripheral resistance thus leading to development of hypertension. The immune system has a role to play in progression of EH. As the condition advances, immune cells are activated and they infiltrate the vasculature and kidneys. T cells have also a key role in the progression of salt-sensitive hypertension (Guarner-Lans *et al*., 2020).

Endothelial cells in the arteries produce a variety of powerful vasoactive agents, comprising the vasodilator and vasoconstrictor molecules such nitric oxide and endothelins, which play a significant role in cardiovascular regulation. Endothelial function modulation is an appealing treatment approach for reducing some of the most serious hypertension consequences.

2.4.5 The Renin-Angiotensin-Aldosterone-System

The renin-angiotensin aldosterone system (RAAS) is at the center of the regulation blood volume and systemic vascular resistance and thus this impact cardiac output and arterial pressure. The system is composed of renin, angiotensin and aldosterone. Renin is produced by the kidneys when the renal blood flow reduces, sympathetic nerve activation and decreased delivery of sodium to the kidneys. The liver secretes angiotensinogen which is an inactive protein. The renin secreted into blood by kidney in response to various physiological processes converts the angiotensinogen to angiotensin I (Ang I) which is then converted to angiotensin II (Ang II) by the angiotensin-converting enzyme (ACE) which is found in the lung's vascular endothelial cells. Angiotensin II has several functions such as constriction of resistance blood vessels thus increasing systematic vascular resistance and arterial pressure. In addition, Ang II triggers sodium transport at renal tubular sites thus elevating sodium and water retention. It also acts on adrenal cortex to release aldosterone and kidneys to raise retention of sodium and fluids. Increased renin-angiotensin-system activation

leads to an increased blood pressure due to uncontrolled vasoconstrictions (Guarner-Lans *et al*., 2020).

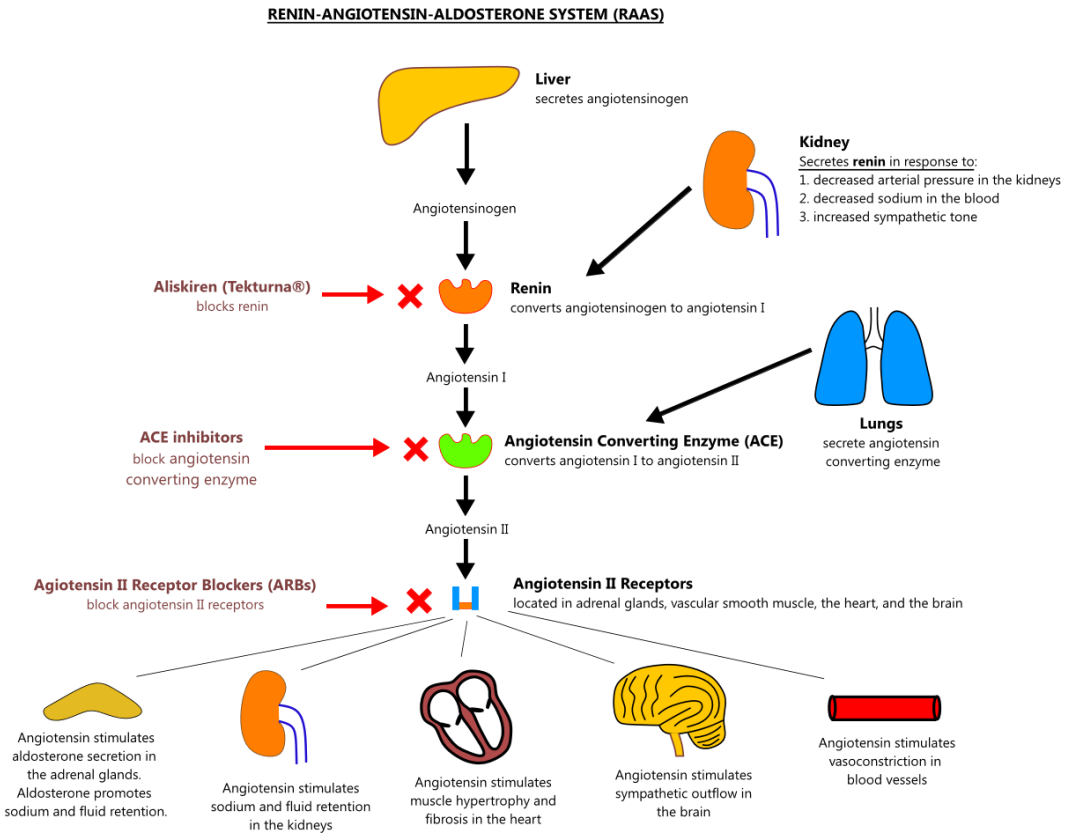


Figure 2.2: The Renin-Angiotensin-Aldosterone System

Adopted from: https://www.straighthealthcare.com/renin-angiotensin-aldosterone-system-figure.html

Angiotensin II is known to exert its action through binding to receptors in the body. It binds to one of two G-protein receptors which are Ang II type 1 receptors and Angiotensin II type 2 receptors. Ang II mostly occurs via AT1R receptors which is found in the endothelium of arterioles throughout the circulation to attain vasoconstriction. The signaling occurs via G protein and activates phospholipase C and consequently raise intracellular calcium (Dhanachandra Singh & Karnik, 2017). This in turn leads to a rise in total peripheral resistance and eventually blood pressure. Dysfunctions and alterations of the angiotensin receptors will affect the action of angiotensin II thus leading development of hypertension

2.4.6 The Renin Angiotensin System in Physiological and Pathological or Disease State

Some of the physiological roles of RAS system include: the maintenance of blood pressure stability and extracellular fluid homeostasis. Most of the RAS actions are done by Ang II with its receptors in a multiple tissue as well as organs. In physiological state, the Angiotensinogen produced in the liver is converted into Ang I by renin. Ang I is converted to Ang II by Angiotensin converting enzyme (ACE). The ACE is mostly produced by endothelial cells in the lungs. The ACE2 is a peptidase that plays the role of converting the Ang II to Ang 1e7) which results to a protective signal.

In presence of mutations, there is overexpression of AT1R. These mutations occur at a region where a lot of regulation factors are found and thus. Over-expression of the AT1R in individuals leads to activation of pathway leading to development of essential hypertension

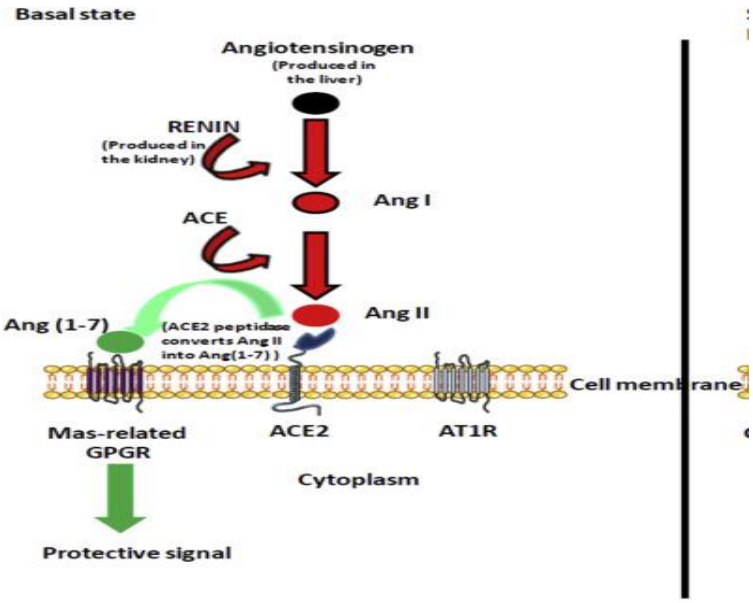


Figure 2.3: The Renin-Angiotensin System in Normal State

Source: (*Devaux et al.*, 2020)

The Figure shows the RAAS in basal state where Angiotensinogen is converted into Ang 1 by renin and to Ang II by angiotensin converting enzyme. The ACE2 peptidase converts further the angiotensin II into Ang (1-7) resulting into protective signal.

2.4.6.1 The role of AT1R and AT2R Receptors in Regulation of blood Pressure

The Angiotensin II is a protein produced by the liver and exerts most of it actions through binding to receptors. There are two types of specific surface receptors to which Ang II exerts it effects. These include Angiotensin 1 Receptor (AT1R) and Angiotensin 2 Receptor (AT2R). The AT1R is expressed more in the body compared to the AT2R and therefore its plays a great role in regulation of blood pressure (Dhanachandra Singh & Karnik, 2017).

The AT2R is known for inhibition of cell growth and proliferation and promotion of differentiation counteracting the effects of Ang II at the AT1R. The AT1R is the most active receptor where the angiotensin binds and exerts it functions and therefore plays a major role in the Renin-Angiotensin system pathway that ensures regulation of blood pressure, electrolyte balance and body fluid. The AT1R among other functions causes vasoconstriction of blood vessels which is key in regulating pressure. Any dysfunction of the AT1R would lead to the development of essential hypertension.

However, the two specific surface receptors have opposing actions. The angiotensin functions at the AT1R are opposed at the AT2R. While the AT1R causes vasoconstriction to increase blood pressure, the AT2R causes vasodilation to lower the blood pressure. (Carey *et al.*, 2000).

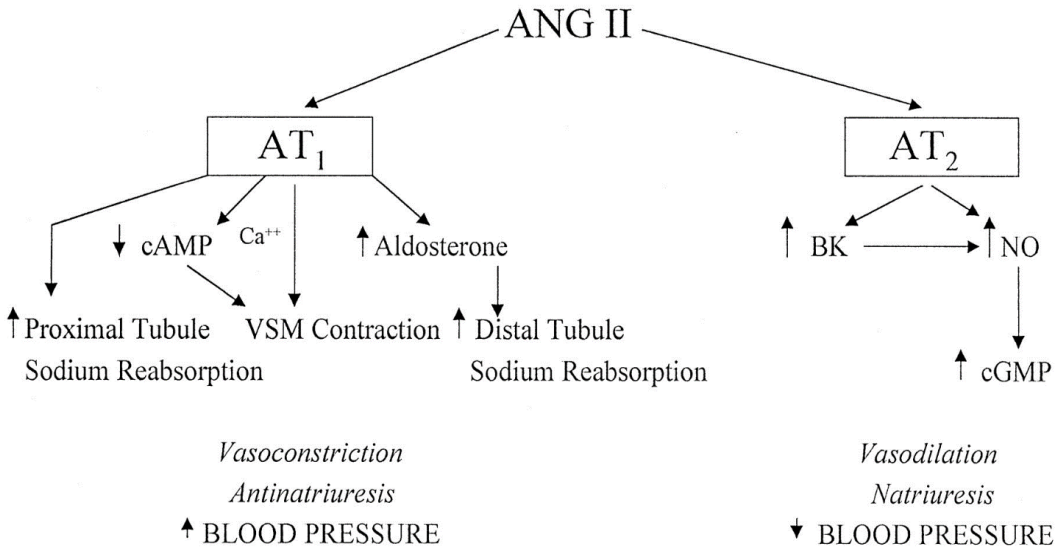


Figure 2.4: The Role of AT1R and AT2R Receptors in Blood Pressure Regulation

Source: (Carey et al., 2000)

The figure shows how the angiotensin II functions by binding to the receptors which has opposing actions. The angiotensin functions at the AT1R are opposed at the AT2R. While the AT1R causes vasoconstriction to increase blood pressure, the AT2R causes vasodilation to lower the blood pressure.

2.4.6.2 The Angiotensin II Type One Receptor and Hypertension Treatment

Angiotensin II which is one of the components of RAS plays a crucial role in the systemic arterial pressure regulation and also thus influences vascular structure and function and disease progression. An alteration to its high-affinity AT1 receptor binding site is the main mechanism by which this is mediated. Angiotensin II is a great candidate for pharmacologic blocking since blood pressure reduction will take place not only through vasodilation and increased natriuresis but also through prevention of structural alterations that can modify vascular compliance. Not only through vasodilation and increased natriuresis but also through prevention of structural alterations that can modify vascular compliance. (Weir and Dzau, 1999). Angiotensin

(Ang) II AT1 receptor antagonists are now known to be an effective method of decreasing blood pressure in hypertensive individuals by blocking the renin-angiotensin system. Burnier, M. (2001). Angiotensin II receptor type AT1 blockers were created as a result of ongoing research into new pharmacological medicines for the treatment of hypertension. Vasoconstriction, aldosterone production and release, sodium reabsorption by the kidneys, cardiac cell growth, vascular smooth muscle proliferation, an increase in peripheral noradrenergic action and central sympathetic nervous system activity, stimulation of vasopressin release, and inhibition of renin release from the kidney are some of the most significant functions mediated by AT1 receptors. Angiotensin II's interaction with its AT1 receptor is inhibited by angiotensin II receptor type AT1 blockers. Contrary to angiotensin-converting enzyme inhibitors, these medications do not affect bradykinin or substance P levels, therefore they lower blood pressure without having the side effect of coughing. These medications can therefore be used to treat hypertension individuals who need therapy with a drug blocking the impact of angiotensin-converting enzyme but are unable to utilize angiotensin-converting enzyme inhibitors due to the side effect of cough. (Contreras et al., 2003)

2.4.7 Genetic, Environmental and Behavioral Factors Associated with Hypertension

Several genetic factors are commonly involved in the progression of essential hypertension in a given person. As a result, pinpointing the relative aids of each of these genes is extremely challenging. Nonetheless, persons with one or two hypertensive parents are almost twice as likely to develop hypertension, and several epidemiological studies estimate that hereditary variables account for about 30% of blood pressure variation between populations. Smoking is also risk factor for other heart ailment and therefore may be a risk factor for the progression of high blood pressure (Halperin *et al.*, 2008).

Income, education, and housing are all social determinants of health that have a negative effect on behavioral risk factors and, as a result, influence the advancement of hypertension. Unemployment or the fear of unemployment, for example, can elevate

stress levels, which can cause high blood pressure. Due to a lack of access to diagnostics and treatment, living and working conditions can also delay prompt identification and treatment, as well as obstruct the prevention of consequences. Hypertension is also more likely to develop due to unhealthy circumstances that encourage fast food intake, sedentary behavior, cigarette use, and the hazardous use of alcohol. (WHO, 2013).

2.5 Clinical Manifestations and Complications of Essential Hypertension

High blood pressure is also called a "silent killer". A majority of people with hypertension are unaware of the condition because it mostly presents with no signs and symptoms. It is therefore essential to have blood pressure checked often even among healthy individuals. When it presents with symptoms, the notable ones include; buzzing in the ears, early severe headaches, nose bleeding, irregular heartbeats, and blurred visions. Severe form of high blood pressure can present with symptoms such as muscle tremors, fatigue, confusion, anxiety, nausea, vomiting, confusion, and chest pains. Uncontrolled and advanced systemic arteriole hypertension can result in complications arising from end organ damage. These complications include coronary artery disease (CAD), myocardial infarction, ischemic or hemorrhagic stroke, kidney disease, hypertensive retinopathy and pulmonary disease among others.

2.6 Diagnosis of Hypertension

The clinical diagnosis of hypertension is based on the latest classifications of a reliable systolic pressure above 130 mm/Hg and greater than 80mm/Hg diastolic pressure (Welton *et al*., 2017). This is done by taking accurate blood pressure (BP) measurement. According to world health organization (WHO), affordable and reliable electronic devices should be used in taking blood pressure measurements (WHO, 2003). Due to mercury toxicity, it is recommended that mercury devices be replaced by electronic devices (Parat *et al*., 2010). Aneroid devices such as sphygmomanometers must be calibrated periodically and all users must be trained and assessed in taking bp measurements using such devices. BP measurements must be taken more than once before diagnosing hypertension. The measurements are taken simultaneously, at least two minutes apart and with the individual being seated and

relaxed. Systematic and random mistakes are common in BP estimation, although simple guidelines-recommended procedures can help to minimize these inaccuracies. (Whelton *et al*., 2017).

2.7 Treatment of Hypertension

After a diagnosis of hypertension is confirmed, immediate treatment is necessary in order to control BP and thus reduce the risk for cardiovascular and other related complications. The 2017 ACC and AHA BP guidelines recommends use of non-pharmacological for all adults diagnosed with high blood pressure (Whelton *et al*., 2017). Non-pharmacological methods of treatment of hypertension include lifestyle modifications such as healthy diet, salt reduction, moderation of alcohol consumption, regular physical activity, weight reduction, smoking cessation and minimal stress among others.

Pharmacotherapy is advised for all pre-hypertensive adults with cardiovascular risk factors and those with Stage 1 and 2 Hypertension (Whelton *et al*., 2017). Some of the drugs used in the management of are the beta-blockers which function by making the heart beat more slowly and with less force hence decreasing blood pressure. Calcium channel blockers prevent calcium from inflowing the muscle cells of the heart and blood vessels and this reduces blood pressure. Others include thiazide diuretics, ACE inhibitors and angiotensin II receptor antagonists (ARBs).

Antihypertensive therapy use varies by regions, similar to hypertension awareness. Generally, 36.9% of hypertensive individuals globally were on treatment in 2010. Antihypertensive drug use was shown to be higher in high-income areas than it was in low-income areas (55.6 percent vs. 29.0 percent) (Mills *et al.*, 2016).

2.8 Prevention of Hypertension

Hypertension can be prevented by use of strategies that usually target the population especially the individuals who are at risk of hypertension. Health promotion and mass screening to detect those individuals at risk and effective management of the condition is imperative. Lifestyle intervention strategies to those at risk is one of the ways which

can be applicable to those who are at higher risk of hypertension compared to the young populations which is at lower risk. Furthermore, when implemented early enough, a prevention strategy has the best and longest-term potential for preventing the precursors that lead to hypertension and the difficulties that accompany it.

It is recommended that the general population and those at risk to take less salty meals at all times. The recommended healthy salt intake is 10 grams of Sodium Chloride (NaCl) per day. This is due to the fact that sodium is known hold excess fluid and increase blood volume thus burdening the heart. Eating a balanced diet is also encouraged which include more fruits and vegetables. Smoking should be avoided by all means because it has been proven to increase the risk of heart-related diseases. The chemicals in smoke accumulate and build up in arteries limiting smooth flow of blood. Moderate alcohol consumption is highly recommended in prevention of hypertension. Increased alcohol consumption leads to increased blood pressure. It is recommended that a man should take two units of alcohol and women one unit per day to prevent hypertension. Weight loss and physical exercises ensures a strong heart thus reducing stress on the arteries and this lowers blood pressure (WHO, 2019).

2.9 Essential Hypertension Biomarkers

Hypertension (HTN) is the single most important cause of cardiovascular and morbidity worldwide. Nearly 45% of all cardiovascular and 51% of stroke deaths are due to HTN. Essential hypertension is a silent disease which the cause is unknown. Identification of EH biomarkers would aid in early detection and accurate causes of EH among patients. Therefore, there is a need for biomarkers that could predict the risk of development of HTN and improve case management for the patient. This can be achieved through better understanding of aetiology of EH. The blood biomarkers for EH may include hematological, biochemical and genetical.

2.9.1 Hematological Biomarkers of Essential Hypertension

The growing and existing body of indication shows that the progression and advancement of hypertension is due to chronic inflammation of blood vessels (Savoia

& Schiffrin., 2006). Some hematological parameters associated with oxidative stress and inflammation has been investigated.

2.9.1.1 Red Cell Distribution Width

The red cell distribution width (RDW) determines the size and volume range of RBCS. Red Cells (RBCs) transport oxygen from the lungs to all body's cells. Any abnormality in the functional and physicochemical properties of red blood cells (RBCs) may trigger the defects that are strongly related to hypertension, stroke, and other heart diseases (Odashiro *et al*., 2015). The RBC membrane is elastic and, in some conditions such as hereditary spherocytosis or sickle cell ailment it changes shape. Variation in cell volume among the erythrocytes or anisocytosis, is reflected in the complete blood count by the RDW. RDW is one of the basic hematological parameters and measures the variations in the size of circulating red cells. RDW is therefore a direct measure of the width of the distribution. (Salvagno *et al*., 2015).

RDW is an inflammation marker and prognostic influence a variety of disorders including Essential Hypertension. In several disease conditions such as: stroke, heart failure, peripheral artery disease among others, it has been shown in research to be able to predict prognosis. Bilal *et al*. found that in hypertensive patients, mean RDW levels are greater; supporting the theory that hypertension is influenced by inflammation. This backs up the theory that inflammation and RDW are closely interrelated, and that persistent inflammation causes higher RDW levels (Bilal *et al*., 2016).

In another cross-sectional study by Seo *et al*., high RDW was significantly and independently related with the progression of hypertension (Seo *et al*., 2019). Enawgaw *et al* also found that RDW is greatly elevated in hypertensive patients compared to normotensive people (Enawgaw *et al*., 2017). Several studies suggest that higher RDW, which is a measure of the variability in the circulating RBC size, may result from ineffective erythropoiesis due to prolonged inflammation during hypertension (Fornal *et al*., 2014).

2.9.1.2 Mean Platelet Volume and Platelet Distribution Width

Studies have shown that there is a substantial difference in platelet indices in hypertensive and normotensive individuals (Tesfay *et al*., 2019) This is an indication that alteration of hematological markers has a strong relationship with the prognosis of hypertension. Platelet indices such as platelet distribution width (PDW) and mean platelet volume (MPV) are the indices which are increased during platelet stimulation. MPV is a measure of the typical size of platelets in the blood and is part of the full haemogram test.

PDW is a unique sign of clotting activation that describes platelet size heterogeneity. Platelet stimulation has been linked to cardiovascular morbidity and mortality in studies. Research has demonstrated that platelets play role in mediating immune response and retaining the atherosclerosis, inflammation and vascular homeostasis (Rodriques *et al*., 2014). Platelet activation occurs in people with high blood pressure due to systemic inflammation and immunological dysfunction. (Zheng *et al*., 2015). Activated platelets are also thought to conglutinate the walls of wounded pulmonary vessels and contribute in the formation of in situ thrombosis, as well as produce growth factors and cytokines that impact pulmonary vascular remodeling. In addition, it is postulated that elevated platelet indices during hypertension could be due malfunction of the vascular endothelium which is associated with the pathophysiological mechanisms of HBP and might result to the platelet activation and local thrombosis.

Recent research evidence suggests that there is link between MPV and hypertension. Recently research has shown that MPV is considerably increased in individuals with heart disease (Zheng *et al*., 2015). The findings of a study by Nadar *et al*. reported that MPV was considerably elevated in patients who had high blood pressure than in control persons and those had organ injury had considerably enlarged platelets than those who had no organ impairment. A study by (Coban *et al*., 2004) findings revealed that MPV was greatly increased in essential hypertensive and white-coat hypertensive compared to normal persons, and that MPV was relatively higher in primary than in white-coat hypertensive patients. In another study by Cao *et al* the findings also showed an association between hypertension and MPV (Cao *et al*., 2012). These

findings agreed with a study done by Enawgaw *et al* that PDW and MPV were considerably elevated in hypertensive patients compared to healthy individuals (Enawgaw *et al*., 2017).

2.9.1.3 Neutrophil to Lymphocyte Ratio

Neutrophil to Lymphocyte (NLR) is a hematological marker that has recently drawn attention because of its link to cardiovascular and non-cardiac disorders. It is calculated as a simple ratio between absolute neutrophil and lymphocyte counts estimated in peripheral blood. NLR imitates the balance between two features of the immune system: acute and prolonged inflammation. According to Liu *et al* there is greater association between NLR and the risk of progression of primary hypertension (Liu *et al*., 2015). These results in this study were also consistent with a study done Karagoz *et al* that showed that NRL was elevated in hypertensive patients (Karagoz *et al.,* 2015). NLR has also been shown to be a useful biomarker for predicting the prognosis of hypertension. It has been established that hypertensive individuals with high NLR quartiles have a higher all-cause mortality rate. (Sun *et al*., 2017).

2.9.2 Biochemical Markers for Essential Hypertension

The vascular dysfunction is a very established system that has an important function to play in the development of essential hypertension (Currie *et al*., 2016). Any change in normal vascular endothelial function reduces vasodilator responses. As a result of this, the vasculature suffers structural damage, which eventually leads to remodeling and stiffness. Indicators of vascular dysfunctions aid in the study of the pathophysiology of the disease as well as the assessment of disease progression, treatment effectiveness, and the development of hypertension-related comorbidities. Several circulating blood biomarkers have been investigated for measuring endothelium dysfunctions, progression, antihypertensive drug effectiveness, and hypertension-related complications. Several circulating blood indicators have been investigated for this purpose. (Shere *et al*., 2017).

2.9.2.1 C-reactive Protein (CRP)

CRP is produced by liver hepatocytes in response to inflammation during the acute phase. Inflammation influences the pathogenesis and development of hypertension. Essential hypertension is as a result of resistance of smooth blood flow in arteries due to increased peripheral resistance which undergoes vascular remodeling leading to changes in structure and function in the endothelium (Savoia & Schiffrin, 2006). These alterations are frequently seen in the early onset of hypertension as a result of widespread inflammation. Essential hypertension causes endothelial dysfunction through hemodynamic changes resulting to elevated levels of inflammatory markers such CRP. However, inflammation can also be as a result of other chronic medical conditions and medications. These include: autoimmune diseases, secondary hypertension, kidney disease, diabetes mellitus, arthritis and other joint diseases, cancers, sickle cell disease, allergies and rheumatoid arthritis among others.

The association between high C - reactive protein and essential hypertension (EH) has been recognized since CRP is both an atherothrombotic process and a mediator (Cortez & Muxfeldt, 2016). A large cohort of approximately 15,000 females in good health was studied for eight years and the findings revealed that raised CRP levels (≥3mg/L) along with increasing BP were found to be good independent future predictors of CVD problems. Thus, CRP had increased prognosis value at all BP levels (Blake *et al*., 2003). In another study conducted by Sesso *et al*., evaluated about 20,000 participants for eight years and it was found out that about 5,000 persons developed hypertension. According to findings in this study, it was concluded that increased CRP levels increased chances of developing hypertension compared to those with decreased levels. (Sesso *et al*., 2003).

In another study Wang *et al*. (2016) investigated about three thousand five hundred individuals to determine which blood markers had the strongest correlation with hypertension risk and features of a multiple marker perspective for predicting the occurrence of hypertension. Some of the inflammatory biomarkers which were studied were urinary albumin/creatine ratio, plasma renin, hemocysteine, Plasminogen activator Inhibitor-I (PA1-1) and CRP among others. The obtained outcome was

notable for a combination of three analyzed markers and these were CRP, urinary albumin creatine ratio and PAI-I which were linked to a higher risk of hypertension. A study in done in Nigeria also noted that CRP, fibrinogen, BMI, SDP and DBP were considerably higher in hypertensive subjects than normotensive participants (Yeldu *et al*., 2018).

2.9.3 Genetic Biomarkers of Essential Hypertension

Genetics also plays a critical role in the development of primary/essential hypertension (Levy et al., 2010). The human genome has mechanisms including genetic redundancies to accommodate genetic changes that may occur during DNA replication, transcription and translation. However, despite mechanisms to ensure relative stability in the genetic information mutation do occur that alter normal physiological mechanisms and enhance risk development of the disease. The recognition of the genomic biomarkers may provide an approach for the early detection of those individuals at risk of disease before progression to advanced hypertension. There is notable evidence that essential hypertension occurs as a polygenic trait with multiple genes exerting diverse effects on the blood pressure control and dysregulation following a complicated non-Mendelian mode of inheritance (Padmanabhanet, 2015). There are approximately twenty-five rare mutations and 53 SNPs which are believed to cause hypertension. (Ahn & Gupta, 2018)

2.9.3.1 The Angiotensin II Type 1 Receptor (AT1R) Gene

The angiotensin II protein is a vasoconstrictor whose function is mediated through binding to angiotensin II type 1 receptor (AT1R), a membrane associated G protein receptor. The AT1R human gene is 55 kb long, with five exons and four introns and is highly polymorphic. The A1166C polymorphism occurs when an adenine residue is substituted for cytosine at location 1166 in the gene's 3' untranslated regions on chromosome 13. The A allele results in the loss of the Dde1 enzyme restriction site which on the other hand is present in the presence of cytosine (C) allele.

Various studies have identified polymorphisms in various genes that could be associated with hypertension such as C573T and A1166C which are AT1R single-

nucleotide polymorphisms (SNPs). (Chaves *et al*., 2001). Inconsistent relationship between the A1166C polymorphism of the AT1R gene and hypertension has been described among various populations. In a study done in Nigeria, it was found that Hypertension was not associated with the A1166C polymorphism (Kooffreh *et al*., 2013). Another study revealed that AT1R A1166C remained a very valuable single nucleotide polymorphism since it was linked to the development of essential hypertension (Fajar *et al*., 2019). These inconsistent findings could be due to different study designs or populations involved. The findings in various studies have been conflicting but, in most studies, AT1R has been shown to have an association with the progression of hypertension.

2.9.3.2 The Angiotensin II Type 1 Receptor Gene (AGTR1) and Treatment of Hypertension

The AGTR1 gene provides instructions the synthesis of Angiotensin II receptor type 1 which is among the components of RAAS that plays a key role in regulation of blood pressure and balance of fluids and salts in the body. Through a series of steps, the RAS produces a molecule called Angiotensin II which attaches to AT1R stimulating chemical signaling. The signaling causes blood vessels to constrict which results in increased blood pressure, aldosterone production and absorption of water and salts by kidneys.

SNPs within RAS can give insight into how hypertension develops. SNPs around the promoter region of the AGTR1 gene can lead to an increased expression of AT1R receptors resulting to the development of EH. SNPs can also aid in identifying novel molecule target within the system which hypertension drugs can be tailored. Blockade of the renin-angiotensin system with angiotensin (Ang) II AT_1 receptor antagonists is now recognized as an effective means of lowering blood pressure in hypertensive patients. In addition, several large clinical trials performed with these agents have demonstrated that blocking Angiotensin type 1 receptors can confer a benefit in terms of morbidity and/or mortality in patients with essential hypertension and left ventricular hypertrophy as well as in patients with type 2 diabetic nephropathy (Burnier M 2001).

Hypertension is an important risk factor for the occurrence of cardiovascular events. The hyperactivity of the renin-angiotensin-aldosterone system is considered a cardiovascular risk factor in subjects with essential hypertension. One of the factors that lead to the development of EH is the intrinsic vascular abnormality, in which the renin-angiotensin-aldosterone system is obviously the milieu for the occurrence of the pathologic changes in blood vessel walls. Many drugs with different mechanisms of action have been used for the treatment of hypertension and its vascular complications (Contreras et al., 2003). Nevertheless, the utilities of many drugs are limited by their adverse effects. Continuous research in the search for new pharmacological agents for the treatment of hypertension has led to the development of angiotensin II receptor type AT1 blockers. The most important functions mediated by AT1 receptors include: vasoconstriction, induction of the production and release of aldosterone, renal reabsorption of sodium, cardiac cellular growth, proliferation of vascular smooth muscle, increase of peripheral noradrenergic action and the central activity of the sympathetic nervous system, stimulation of vasopressin release, and inhibition of renin release from the kidney. The angiotensin II receptor type AT1 blockers inhibit the interaction of angiotensin II with its AT1 receptor. These agents lower blood pressure without producing cough as a side effect since, unlike the angiotensin-converting enzyme inhibitors they do not influence the levels of bradykinin or substance P. Hence, these drugs are suitable for the treatment of hypertensive patients who require therapy with a drug blocking the effect of angiotensin-converting enzyme but cannot use angiotensin-converting enzyme inhibitors due to cough as a side effect. (Weir and Dzau, 1999).

2.10 Conceptual Framework of the Study

Given that this was analytical study the aim was to clarify the relationship between different biomarkers including genetic (AGTR1), biochemical (CRP) and selected hematological markers and essential hypertension. The study sought to examine differences in these markers in normotensive and hypertensive individuals (Figure 2.6). The study focused on independent variables which included hematological, biochemical and genetic markers. They were plasma C-reactive protein, mean platelet volume, platelet distribution width, neutrophil to lymphocyte ratio, red cell distribution

width and angiotensin II receptor gene (1166 A>C). The dependent variable was essential hypertension. By establishing the relationship between these biomarkers and essential hypertension, the contribution of each biomarker to detection, progress and control of essential hypertension was determined.

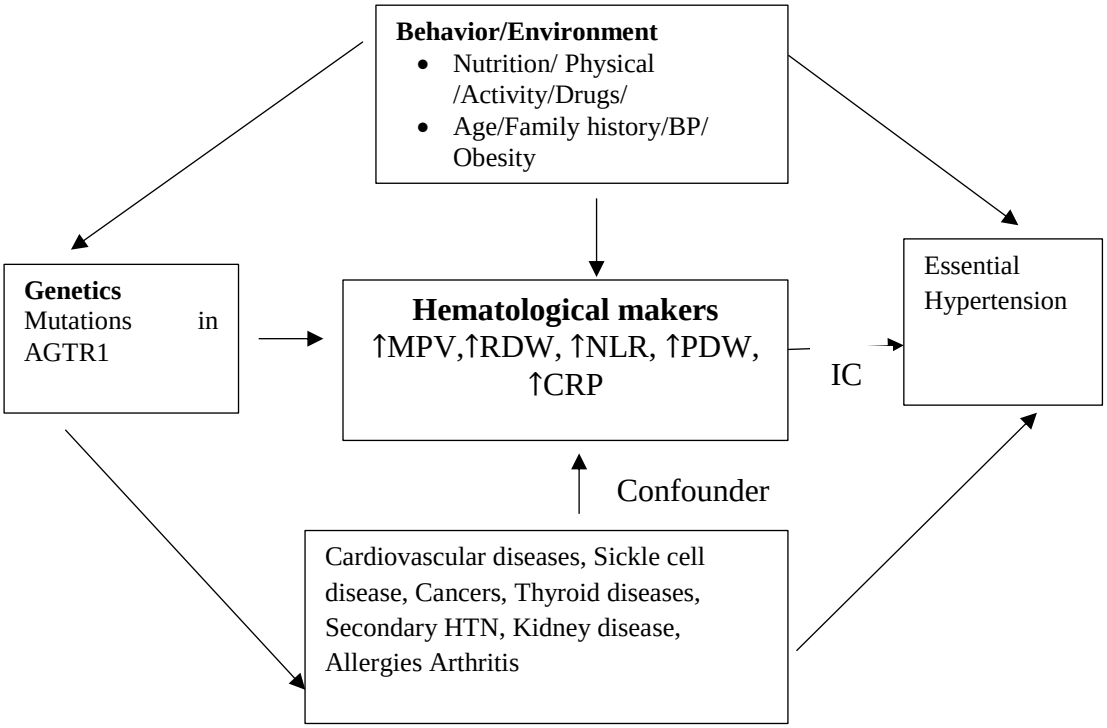


Figure 2.5: Conceptual Framework of the Study

IC = Independent of Confounders

CHAPTER THREE

METHODOLOGY

3.1 Study Setting

The study site was Chuka County Referral Hospital. It is located in Chuka town, which is the largest town in Tharaka Nithi County in Mount Kenya region. The county referral hospital has a bed capacity of 100 patients. According to 2019 census, the County has a population of 393,177 (KNBS, 2019). It covers a total area of 2609 square kilometers and borders Embu to the South West, Meru to North and North East, Kitui to the East and South East. The County is divided into four sub-counties which are; Tharaka North, Tharaka South, Meru South and Mara. This county is among the regions of Kenya having a high prevalence of hypertension of 28%.

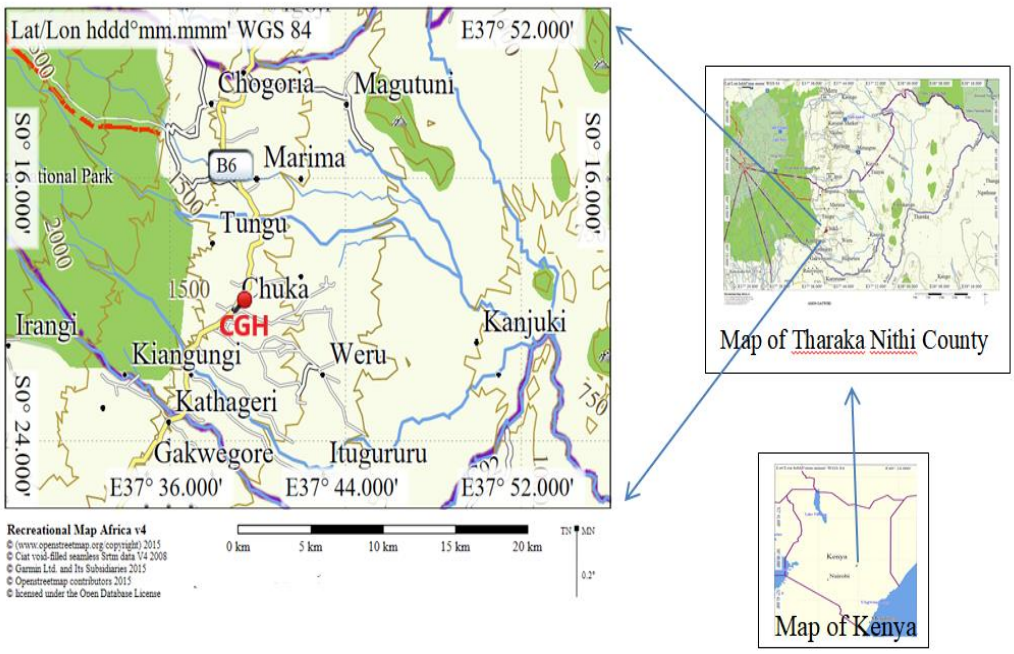


Figure 3.1: Map Showing the Location of Chuka County Referral Hospital (CCRH)

3.1.1 Inclusion and Exclusion Criteria

3.1.1.1 Inclusion Criteria

i. Cases were adult patients aged 18 years and above both male and female, who had essential hypertension. The study included those who were newly diagnosed and those who had been diagnosed with EH and enrolled for hypertension clinics at Chuka County Referral Hospital.
ii. Controls were age and gender matched normotensive adults, preferably blood donor. Frequency matching was used where controls were selected with same distribution as cases in terms of age and gender.

3.1.1.2 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.

Pregnant women and non-consenting patients were also excluded from participation in the study.

3.2 Study Design

The study was a case-control study with controls comprising normotensive blood donors and cases being hypertensive patients attending Chuka County Referral Hospital medical outpatient clinics between March and July 2022

3.3 Sample Size Determination

The sample size was calculated using sample size calculators for designing clinical research at https://sample-size.net/ as shown below. This was a case-control study to compare the proportions individuals having SNPs associated with hypertension among hypertensive patients (cases) and normotensive individuals (controls). The hypothesis (two-sided α=0.05; β=0.2) to be tested using the Chi-squared statistic is that the SNP

frequencies (dichotomous outcome) are the same between the two groups. The sample size was calculated to test the hypothesis that there was at 5% difference in the prevalence of SNPs between cases and controls (ratio 1:1). There was the same number of cases (hypertensive patients, q_1=0.5) same number of controls (normotensive individuals, q_o=1-q_1 = 0.5). The proportion of hypertension in the general population, Q1=0.28(KHSSP 2014-2018) and a risk ratio, RR= 5.00 was used. This required 136 cases and 136 controls

Note: The prevalence of hypertension (P) in the general population was specified and used in the sample size calculator from https://sample-size.net/sample-size-proportions-alternative/

Comparison of the proportions will be done using the Chi-squared statistic. The study included Group 1 (exposed) and Group 0 (unexposed). P was known (the prevalence of the outcome in the population) and Q_1 (the proportion of exposed individuals in the population), and you can estimate the risk ratio (RR). The calculator uses these to derive P_1 (outcome proportion in exposed) and P_0 (outcome proportion in unexposed) and, from there, the sample sizes. Note the distinction between Q_1 (proportion of exposed individuals in the population) and q_1 (proportion of exposed subjects in the study).

Instructions: Enter parameters in the green cells. Answers appeared in the blue box below.

α (two-tailed) =	0.05	Error type 1
β =	0.20	Error type 2
q_1 =	0.50	Subjects that are in exposed group.
q_0 =	0.50	Subjects that are in unexposed

Q_1 = 0.28 Proportion of individuals in the population who are exposed.

P = 0.05 Prevalence of the outcome in the population.

RR = 5.00 Estimated risk ratio, (P_1/P_0). For the parameters currently entered, this value must be greater than 0.00.

Calculate

Q_0 = Proportion of individuals in the population who are unexposed = 1 - Q_1 = 0.7200.

Limits on the risk ratio RR:

Lower limit: Use ($P-Q_0$)/($100-Q_0$) = -2.3929 if it is positive, otherwise use 0. **Lower limit: 0.00**

Upper limit: If $P > Q_1$, use ($100-Q_1$)/($P-Q_1$) = -3.1304, otherwise no upper limit. **Upper limit: None**

The standard normal deviate for $\alpha = Z_\alpha$ = 1.9600

The standard normal deviate for $\beta = Z_\beta$ = 0.8416

P_1 = The probability of the outcome in exposed people = $P * RR / (1 + Q_1 * (RR - 1))$ = 0.1179

P_0 = The probability of the outcome in unexposed people = $P / (RR - Q_0 * (RR - 1))$ = 0.0236

Pooled proportion = PP = $(q_1*P_1) + (q_0*P_0)$ = 0.0868

$A = Z_\alpha\sqrt{PP(1-PP)(1/q_1 + 1/q_0)}$ = 1.1735

$B = Z_\beta\sqrt{P_1(1-P_1)(1/q_1) + P_0(1-P_0)(1/q_0)}$ = 0.4242

$C = P_1 - P_0 = 0.0943$

Total group size = $N = (A+B)^2/C^2 = 230$

Continuity correction (added to N for Group 0) = $CC = 1/(q_1 * |P_1 - P_0|) = 21$

Table 3.1: Sample size (with continuity correction)

	N	Outcome+	Outcome-
Group 1:	136	16	120
Group 0:	136	3	133
Total:	272	19	253

Sample size (without continuity correction)

3.4 Sampling Method

Study participants were recruited by convenience sampling using the selection criteria to attain the sample size required. A blood consent form was given to each participant to read and understand the study (details in Appendix III.) The consent form was also translated into Kiswahili for those who couldn't comprehend English Details in Appendix IV Those who couldn't read and write had the consent form translated to them and they thumb printed after consenting.

3.5 Laboratory Procedures

3.5.1 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 which was obtained by measuring weight and height. To detect obesity, the participants' weight and height was measured and used to estimate body mass index. Details in Appendix I and II

3.5.2 Medical Health Records

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 participant, medical records of the participant were retrieved and reviewed to ensure that the right participants were recruited for the study. 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.

3.5.3 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 were chosen.

3.5.4 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 5000g for three minutes and serum was transferred to a fresh 1.8ml tube for C-reactive protein determination.

3.5.5 Complete Blood Count Analysis

Complete blood counts were performed at Chuka County referral hospital hematology laboratory using a five-part automated Dymind haematological analyzer (Shenzhen Dymind Biotechnology Co LTD, China). The analyzer generated a measured change in resistance when the diluent is mixed with blood. An electric current flowed through an opening where the cells under analysis were also introduced. The diluents were displaced when the cells pass through the aperture, and because cells are poor conductors of electricity, resistance rises, which is measured as a voltage pulse and recorded digitally. A vacuum pump drew the cell suspension into a tubing system through the opening. The selected hematological biomarkers (RDW, MPV, NLR and PDW) were analyzed and recorded for each study participant.

3.5.6 Measurement of Plasma Levels of C-Reactive Protein (CRP)

C - reactive protein testing was done at Guru Nanak Hospital clinical chemistry laboratory on the Mindray BS 230 (Meron Scientific Private Ltd, India) which 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 stored serum sample was brought to room temperature for it to thaw. A two hundred microlitre aliquot of blood was pipetted and transferred to a sample cup. Information about the sample was keyed into the machine and the sample was placed into the machine following standard procedures provided in the operator's manual. This was run along with control material. The serum CRP was quantitatively determined and the results were displayed on the machine. This was done to all samples and results were recorded accordingly.

3.5.7 Quality Assurance

To ensure accurate and reliable study results, all quality assurances processes such as pre-analytical, analytical and post analytical processes 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 these ensured reliable and accurate results were obtained.

3.5.8 Transportation and Storage of Blood for DNA extraction

The EDTA blood for DNA extraction was continuously stored at -20◦C at Chuka County Refferal Hospital until shipped for DNA extraction procedures. The DNA extraction was done at Chuka University Research Laboratory. The samples were transported from Chuka Hospital to Chuka University at 2-8 degrees Celsius and all local and national transport regulations were strictly be adhered to. The samples were packaged using the triple packaging system. Details in appendix V.

3.5.9 DNA Extraction

DNA was extracted from the 272 samples using the Isolate II Genomic DNA kit (Bioline Meridian) following the standard operating procedures as described by the manufacturer. Two hundred microliters of whole blood sample was added into a microcentrifuge tube, mixed with 200 µl lysis Buffer G3 and 25 µl Proteinase K by vortexing for 20 seconds followed by incubation at 70ºC for 15 minutes. After incubation, 200µl of ethanol (96-100%) was added. To enhance DNA binding, for each of the isolate II Genomic DNA spin column was placed in a collection tube and the sample was loaded into the column. All the lysate was loaded and then centrifuged for 1 minute at 11000 x g. This was repeated at a higher force (18000 xg) if the sample was not completely filtered through the matrix. The column was placed in a new collection tube (2ml). The washing of the silica membrane was done by addition of 500ul of buffer GW1 and centrifuged for 1minute at 11000xg. The flow was discarded and the collection tube reused. Six hundred microliters of buffer GW2 was added to the column and centrifuged for 1min at 11000 x g. The flow was discarded and the collection tube was reused. To remove all wash buffers from the column further centrifugation was done at 11000x g to remove residue ethanol. The isolate II Genomic DNA spin column was placed in a 1.5ml microcentrifuge tube. The DNA was eluted by adding 100ul of the pre-heated elution buffer G directly into the silica membrane.

This was incubated for 1 minute at room temperature and centrifuged for 1minute at 11000xg. The presence and the extracted genomic DNA was checked by running it on 1% agarose gel electrophoresis. The eluted and confirmed genomic DNA was stored at -20°C for PCR and restriction fragment length polymorphism (RFLP) analysis.

3.5.9.1 PCR Amplification of the AGTR1 Gene

A 359bp region of *AGTR1* was amplified by polymerase chain reaction (PCR) using the following primer pair: 59-ATAATGTAAGCTCATCCACC- 39 (forward primer) and 59-GAGATTGCATTTCTGTCGGT- 39 (reverse primer). The primers were obtained from INQABA Biotec Limited. Amplification of the 212 samples 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, 2.5ul 10x Buffer, Forward Primer 0.5 (10µM), Reverse Primer 0.5 (10µM), 0.5ul of 10mMdNTPs, 0.125µl of the *Taq* DNA polymerase and 10µl template genomic DNA (Table 3.1) The PCR 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 against a 1kb molecular ladder.

Table 3.2: DNA Amplification Components.

Reagent	Amount
nuclease-free water	10.875 µl
10X Buffer	2.5 µl
Forward primer.	0.5 µl (10 µM)
Reverse Primer	0.5 µl (10 µM)
dNTPS	0.5 µl
Tag DNA polymerase	0.125 µl
Template DNA.	10 µl

3.5.9.2 DdeI Restriction Digest 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 of 212

samples were digested using *Desulfovibrio desulfuricans 1* (*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^{TM} Buffer (New England Biolabs). This was topped up with nuclease free water to make a 25ul reaction (Table 3.2) and gently mixed by pipetting up and down followed by centrifuging briefly to bring the contents down the tube. The tubes were then 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) as the standard. The 10 µl of the digests products was subjected to 2% agarose gel electrophoresis where the electrophoresis tank power pack was set at 80V and ran for forty-five minutes. After that the imaging was done on Bio-Rad Imaging system (Bio-Rad Laboratories, Inc. USA).

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.

Table 3.3: *DdeI* Restriction Digest Setup for a Single Reaction

Components	**Volume**
PCR products	10ul
10X NE Buffer	2.5ul
DdeI (Restriction Enzyme)	0.25ul
Nuclease Free water	12.25ul
Total	**25ul**

3.6 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.

3.7 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) (Appendices V and VI respectively). Authorization was obtained from Chuka County Referral Hospital administration. (Appendix VÌI) 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.

Confidentiality was highly maintained during the study. During sample collection, labelling was done by use of unique codes and during data analysis, it was be encrypted with security passwords to ensure no unauthorized access to the data. The study findings were published in a peer reviewed journal and it didn't reveal the identity of the participants in any way. The participant's risks and rewards in the study were clearly explained to them in a language they could understand as part of the informed consent process. The risk of participating was minimal since blood was collected once from a participant by a highly qualified laboratory staff. No incentives were offered to participants in the study. Participants were allowed to withdraw from the study any

time during the study and they were not to suffer any consequences for withdrawal. The participant's blood samples were disposed after use by incineration.

CHAPTER FOUR

RESULTS

4.1 Socio-Demographic and Clinical Characteristics of the Study Participants

The study included a total of 272 study participants that comprised of 136 hypertensive patients with mean age of 50.96 ± 14.81 years and 136 controls with mean age of 38.78± 2.96 years. A total of 75 (28%) of participants were males and a total of 197(72%) were females in both hypertensive and healthy control groups. The mean value of the systolic and diastolic blood pressure of hypertensive patients were 145.74 ±18.53 and 84.64 ±13.21 mmHg respectively while for the control group were 123.6±9.59 and 73.24± 8.05 mmHg respectively. The mean body mass index of the patients was 27.42 ± 4.92 kg/M^2 while and 25.22 ± 4.13 kg/M^2 for the control group. From both hypertensive and control group, there was a total of 162(60%) who had first degree relative having a history of hypertension while 101(37%) had no family history of hypertension and 9(3%) had no information about family history of hypertension.

Hypertension was more prevalent in old age while majority of blood donors are 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, thirty cases and thirty 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 for statistical analysis.

Table 4.1: Socio-Demographic and Clinical Characteristics of Study Participants (n = 212)

Variable		Controls (%)	Cases (%)
Gender	Male	41(30)	34(25)
	Female	95(70)	102(75)
Age	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(51.0)	31(23)
	Unknown	0(0.0)	9(7)
Mean BMI (kg/m^2) ± SD	-	25.22±4.20	27.42±4.89
Mean BP (mmHg) ± SD	SBP	123.6±9.59	145.74±17.39
	DBP	73.24±7.76	84.64±11.99

4.2 Selected Hematological Biomarkers among Patients with Essential Hypertension and Controls at Chuka County Referral Hospital

A complete blood count analysis was done on samples from all the study participants. The normality of the distribution of the biomarker measurements was tested and only MPV and NLR were found to be normally distributed while RDW and PDW had skewed distributions. As result, 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 RDW and PDW were 41.95±1.08, 11.5±2.3 and 44.95±1.21, 11.0±2.2 in controls and cases respectively. Mean values for NLR and MPV were 2.09±1.3, 9.36±1.14 and 2.74±1.4, 10.71±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. ($p = .519$) (Table 4.2, Figure 4.1).

Table 4.2: Comparison of Selected Hematological Biomarkers between cases and control groups among patients attending Chuka County referral hospital

Variables	Cases n=106	Controls n=106	*p* value
	Median ±*IQR*	**Median ±*IQR***	
RDW	44.998±2.25	41.399±1.09	<0.0001
PDW	11.2±2.3	11.6±2.4	0.5189
	Mean ±*SD*	Mean ±*SD*	
MPV	10.61±1.13	9.45±1.14	<0.0001
NLR	2.78±1.4	2.09±1.0	<0.0001

Key: RDW=Red Cell Distribution Width, MPV= Mean Platelet Width, PDW=Platelet Distribution Width, NLR= Neutrophil to Lymphocyte Ratio,

4.3 Determination of Serum Levels of C-Reactive Protein (CRP) in Patients with Essential Hypertension and controls at Chuka County Referral Hospital

Serum CRP levels were analyzed for all 106 cases and 106 controls. The normality test was done and CRP was found to have skewed distribution and as a result was express as median values (plus the interquartile range). Mann Whitney U test was used to determine statistical significance by comparing the medians between the two groups. The difference between cases and the control group for CRP was statistically significant (P < .001) (Table 4.3, Figure 4.1)

Table 4.3: Comparison of C - Reactive Protein Medians between Cases and Control Groups among Patients Attending Chuka County Referral Hospital

Variables	Cases n=136	Controls	*p* value
	Median ±IQR	**Median ±IQR**	
CRP	2.9±4.85	0.5±1.8	<0.0001

Key: CRP= C-reactive Protein

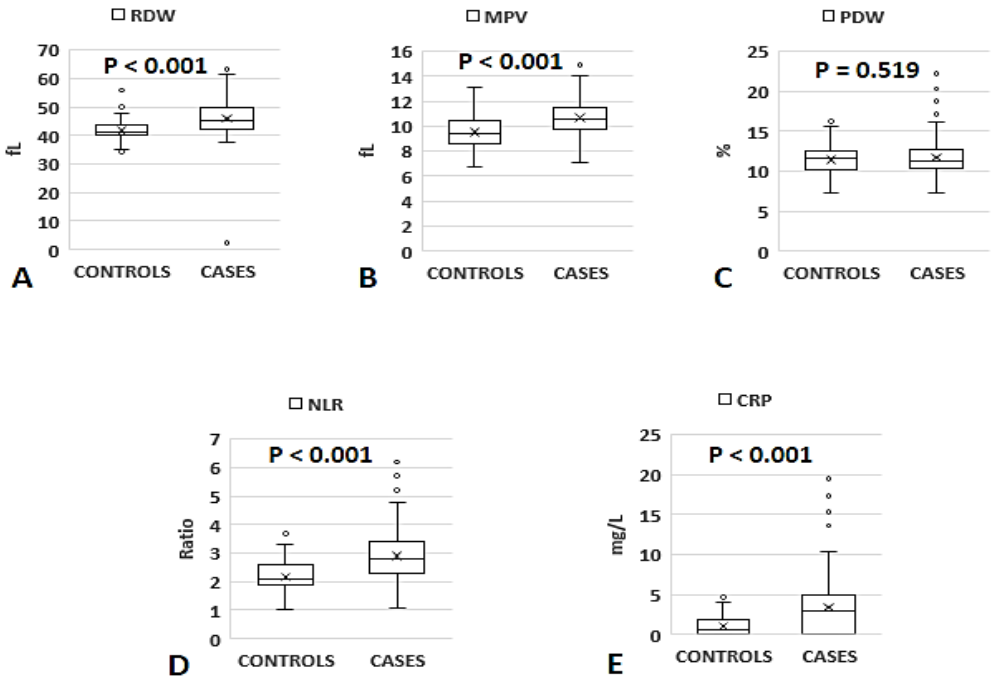


Figure 4.1: Comparative levels of Selected Hematological and CRP Biomarkers between Controls and Cases

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.

4.4 Frequency and Association of Angiotensin II Type 1 Receptor Gene SNPs (A1166C Gene Polymorphism) with Essential Hypertension among Patients Attending Chuka County Referral Hospital

In a total of one hundred and six (106) cases and one hundred and six (106) controls, DNA amplification of the *AGTR1* gene using the specific primers was done successfully and resulted in a 359bp DNA product (Figure 4.2) and on subsequent digestion of the amplified fragment (amplicon) with *DdeI* restriction endonuclease, DNA fragments of 359bp (AA), 359bp, 220bp and 139bp (AC) length were observed.

Thus, each of samples subjected to restriction digestion produced one of the two different electrophoretic patterns as shown in (Figure 4.3).

The frequencies of the AA, AC and CC genotypes were 98.1%, 1.9% 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% respectively. Out of the two hundred and twelve (212) participants whose amplicons (359bp) were digested, three (3) were found to have the heterozygous genotype (AC) and the other two hundred and nine (209) had the homozygous normal (AA) (Table 4.4). This represented a frequency of 1.42% of the mutant alleles and 98.58% had the wild type alleles in the studied population. Among the three individuals with the mutant alleles, two were females and one was a male. Additionally, two of the three individuals were from cases and the other was a control. Odds ratio was used to determine the association between the AGTR1 gene mutations and essential hypertension. There was no significant association between AGTR1 mutations and EH in the studied population in genotype and allele frequencies. (*ORs*= 0.4952, *P*= 0.6076).

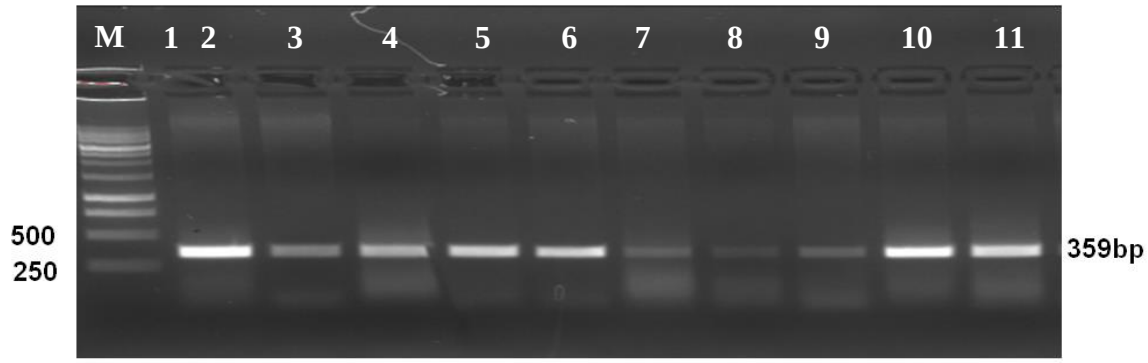


Figure 4.2: Agarose Gel Electrophoresis of *AGTR1* PCR Amplicons

The selected images of PCR amplification of the 359bp fragment of human *AGTR1* gene as seen on the agarose gel electrophoresis. The first lane from left (M) is the molecular DNA ladder marker that was used as the standard and all the other lanes (1-11) represents the 359bp PCR products (amplicons). (M = 1kb molecular DNA ladder marker)

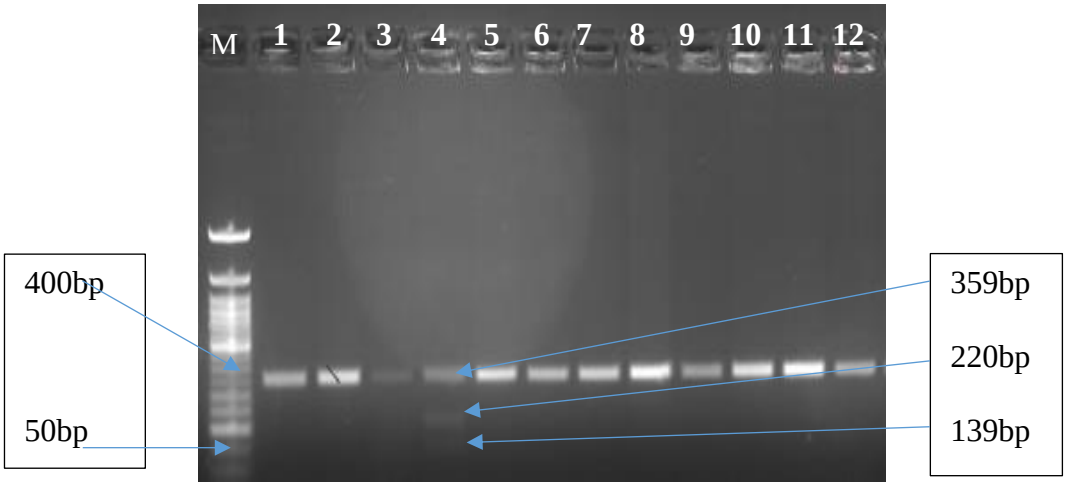


Figure 4.3: RFLP Analysis of *AGTR1* PCR Amplicons

The 359bp PCR amplicons were digested using the Dde1 restriction endonuclease. The homozygous AA normal genotype (single 359bp band; Lanes1-3, 5-12) and the heterozygous AC mutant genotype (threes bands of 359bp, 220bp and 139bp; Lane 4) were detected. (M = 50bp molecular DNA ladder marker).

Table 4.4: Genotype frequencies of A1166C Variants of the Angiotensin II Type 1 Receptor Gene in Cases and Controls

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

[a]P = p value, [b]OR = Odds ratio, [cC] I= Confidence Interval

CHAPTER FIVE

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 hypertension 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.

A key knowledge gap especially for essential hypertension (EH), 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 in the 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) but not for Platelet Distribution Width (PDW) and *AGTR1* mutations (p =0.519, ORs =0.4952 respectively).

Based on the results of our study, the cases had a statistically significant higher median values of Red cell distribution width (p =<0.0001) when compared to the healthy controls group. These findings agree with other similar studies by done in Eastern and Northwest Ethiopia respectively (Sileshi *et al*. 2021 and Enawgaw *et al*., 2017) which reported that RDW was significantly increases in hypertensives compared to normotensives. In another large retrospective cohort study conducted by Seo and other authors clearly demonstrated that increased RDW was associated with an increased risk of hypertension incidence (Seo *et al*., 2019). The association was independent of established risk factors and was progressive with increased RDW. However, a study in Iran population reported conflicting findings (Emamiam *et al*., 2017). Evidence has shown that increased RDW is as a result of ineffective erythropoiesis that is caused by chronic inflammation (Zhou *et al.,* 2017). It has been discovered that inflammatory cytokines prevent erythrocytes from maturing, allowing immature red cells to enter the circulation and increasing the variability in size (Turchetti et al., 1999). 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 (Fornal *et al*., 2014).

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 (Sileshi *et al*., 2021). These findings are also consistent with another study conducted by Enawgaw and other researchers (Enawgaw *et al*., 2017). 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 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 than mature ones, their existence represents a risk factor for the occurrence of coronary thrombosis and myocardial infarction (Inanc *et al*., 2010).

Our study also showed that the mean values of NLR were significantly elevated in cases compared to control group (p <.001). These findings are consisted with results of previous cohort studies conducted in Chinese populations. (Fornal *et al*., 2014, Inanc *et al*., 2010, Liu *et al*., 2015). It was also noted that NLR can be a good predictive value in preeclampsia (Mensah *et al*., 2022). This difference 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 (Jhuang *et al.*,2019).

This study also found that median values of CRP were significantly elevated in cases compared to controls (p =<0.0001). These results concur with results from previous studies done by Wang et al., (2016), Yeldu *et al*., (2018), and a prospective study by Alam *et al*., (2018). However, a previous study by Sesso *et al*., (2015) reported that CRP was not associated with higher risk of developing hypertension in middle-aged and older men. 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. HTN may in turn induce inflammation and raised CRP levels. (Shafi *et al*., 2010).

This study also found out that there was no statistically significant difference in median values of PDW in cases and control group (p=0.52). These findings are inconsistent with previous studies done by Yang *et al.,* (2016);Enawgaw *et al.,* (2017) and Sileshi et al., (2021).

The study also investigated the angiotensin II type one receptor gene mutations and the A1166C genotype frequency was determined. The RAAS is known to be the main system that plays a great role in the development of essential hypertension (Yim and Yoo, 2008). Angiotensin II is one of the RAAS precursors which is essential in triggering negative effects in hypertension through AT1R (Singh and Karnik ,2016). A1166C is one of the AT1R genes that has received a lot of attention up to date.

The distribution of genotyped (AA and AC) among the studied population was familiar to what was reported in Nigeria(Koffrey *et al.*,2013) that involved one thousand two hundred and twenty-four participants noted that polymorphisms in AT1R gene are not

associated with essential hypertension. The findings of the current study are also similar to an unpublished thesis research by Freeman Julia Carol 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 done in Kenya in 2013, the population in Kasiagu region may differ from that of Tharaka Nithi County which is predominantly of Bantu origin. (Freeman, 2013). These results are in contrast to other studies in non-African populations which have reported that *AGTR1* polymorphisms are associated with essential hypertension. (Chandra *et al*., 2014; Parchwani *et al*., 2018). However other studies in non-African populations have reported contrary results from the current study. In study done by Chandra *et al* in India reported that AT1R polymorphisms are associated with essential hypertension. Yako *et al* in a systematic review and meta- analysis noted that AGT (rs699) and AGTR1 (rs5186) were the single nucleotide polymorphisms which were likely to predispose African populations to hypertension (Yako *et al.,* 2018). Similar meta-analysis studies done by Fajar *et al*., (2019) and Liu et al., (2015) reported that polymorphisms in AT1R was associated with hypertension but no association was found in African population. The results of our study also differed with those reported by Parchwani *et al.,* 2018 which suggested that the AT1R gene A1166C polymorphisms seemed to be useful genetic determinants of hypertension etiopathogenesis and the AT1R genetic variants might be predictors of susceptibility in the families that are affected.

These findings point to distinct differences in the *AGTR1* polymorphism profiles in African populations compared to non-African populations suggesting that alternative pathophysiological pathways could be involved in the onset of essential hypertension. Notably, the CC genotype was completely absent in the Kenyan population similar to observations in Cameroon, Ghana, Nigeria and Burkina Faso (Yako *et al.,* 2018;Liu *et al.,* 2015;Ghogomu *et al.,* 2016;Tchelougou *et al.,* 2015). 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 healthy controls (Mehri *et al*., 2012

CHAPTER SIX

SUMMARY OF KEY FINDINGS, CONCLUSION AND RECOMMENDATIONS

6.1 Summary of Key Findings

Hypertension is one of the leading causes of heart disease such as coronary artery disease and heart failure along with stroke and kidney damage among other conditions. Known as the “silent killer", hypertension is largely an asymptomatic disease that becomes evident only when it progresses in severity and begins to cause tissue damage in multiple organs. A significant proportion of the general population is not aware of their hypertension status and are therefore not under management to prevent development of complications. This study was conducted at Chuka County Referral hospital in Tharaka Nithi County to determine whether there is an association between selected hematological markers such as MPV, RDW, PDW and NLR, C-reactive protein and Angiotensin II type 1 receptor gene mutations(AGTR1) and essential hypertension. The study was a case control experiment involving hypertensive patients as the cases and normotensive blood donors as controls.

The results of this study showed that mean values of mean platelet volume (MPV), Neutrophil to Lymphocyte ratio (NLR) and the median values of C-reactive protein (CRP) and Red Cell Distribution Width (RDW) were significantly higher in hypertensive group compared to healthy control group. Platelet Distribution Width (PDW) (P=0.53) was found not to be statistically significant.

The results also indicated that the 98% of the study population had the wild type AA genotype, and 1.2% was AC heterozygous carriers of the A1166C polymorphism. The results showed that AGTR1 gene mutations was not associated with EH (ORs=0.4952).

6.2 Conclusion

1. This study notes that the median values RDW and the mean values of MPV and NLR were significantly higher in the cases compared to the control group. PDW showed no statistically significant difference between the two groups.
2. The median values of C - reactive protein were significantly higher in the cases compared to the control group.
3. The frequency of Angiotensin II type one receptor gene (rs5186) mutation was 1.42% in the studied population.AGTR1 SNP was not associated with essential hypertension incidence in the studied population..

6.3 Recommendations.

The study recommends that:

1. Clinicians should be weary that CRP, RDW, MPV and NLR can be elevated due to essential hypertension in the absence of chronic and inflammatory diseases and derangement of the biomarkers should help them question a likelihood of essential hypertension in undiagnosed cases and initiate prompt management of the disease.
2. The current study recommends a further study on more possible biochemical biomarkers of essential Hypertension.
3. Possible future studies to investigate gene expression profiles of *AGTR1* among individuals and mutations in other genes which could be associated with essential hypertension in Kenyan populations.

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APPENDICES

Appendix I: Study Questionnaire.(*ENGLISH)*

Title: SHematological and Genetic Biomarkers among Patients with Essential Hypertension and their age/gender matched controls at Chuka level 5 hospital**.**

BASELINE DATA RELATED TO ESSENTIAL HYPERTENSION

INTRODUCTION

I am Amos Mutwiri Mbaabu, a Masters student from JKUAT, College of health sciences and currently doing a clinical research on Hematological and Genetic biomarkers among patients with Essential Hypertension at Chuka Level 5 hospital in Tharaka Nithi County. The purpose of this study is to identify a combination of hematological and genetic biomarkers that have a role in early detection, progress and control of essential hypertension.

This questionnaire is designed to collect baseline data related to hypertension. Therefore, I kindly request you to feel free and fill this questionnaire and the information and data obtained will be treated with at most confidentiality. Your honesty will be very useful in generating accurate data in this study.

SPECIFIC INSTRUCTIONS

You are expected to read and understand each question and then respond appropriately by ticking or writing down your answer.

BASELINE CHARACTERISTICS

1. Gender (Choose one)

   Male ☐ Female ☐

2. How old are you? (Choose one)

   18-29 ☐ 30-44 ☐ 45-59 ☐ 60-65 ☐

3. Do you have any history of Hypertension? (Choose one)

YES ☐ NO ☐

4. Do you have any first degree relative suffering from Hypertension? (parents, brother, sister, child) (Choose one)

YES ☐ NO ☐

5. To determine your Body Mass Index, kindly Measure your weight (KG) and Height (M)

Weight…… (kg) Height……(m)

6. Do you have any health condition? If yes, please indicate the condition.

……

7. Blood pressure……mm Hg

Thank you.

Appendix II: Somo Hojaji. (Kiswahili)

Kichwa: Alama za Kibiolojia za Hematolojia na Jenetiki miongoni mwa Wagonjwa walio na Shinikizo la damu Muhimu: Utafiti wa udhibiti wa kesi katika hospitali ya Chuka level 5.

DATA YA MSINGI INAYOHUSIANA NA SHINIKIZO LA DAMU MUHIMU

UTANGULIZI

Mimi ni Amos Mutwiri Mbaabu, mwanafunzi wa Shahada ya Uzamili kutoka JKUAT, Chuo cha Sayansi ya Afya na kwa sasa anafanya utafiti wa kimatibabu kuhusu viashirio vya Hematological, Biochemical na Genetic kati ya wagonjwa walio na Shinikizo la damu Muhimu katika hospitali ya Chuka Level 5 katika Kaunti ya Tharaka Nithi. Madhumuni ya utafiti huu ni kutambua mchanganyiko wa alama za damu, biokemikali na maumbile ambazo zina jukumu katika kugundua mapema, maendeleo na udhibiti wa shinikizo la damu muhimu.

Hojaji hii imeundwa kukusanya data ya msingi inayohusiana na shinikizo la damu. Kwa hivyo, ninakuomba ujisikie huru na ujaze dodoso hili na habari na data iliyopatikana itashughulikiwa kwa usiri zaidi. Uaminifu wako utakuwa muhimu sana katika kutoa data sahihi katika utafiti huu.

MAAGIZO MAALUM

Unatarajiwa kusoma na kuelewa kila swali na kisha kujibu ipasavyo kwa kuweka alama au kuandika jibu lako.

SIFA MSINGI

1.Jinsia (Chagua moja)

Mwanaume ☐ Mwanamke ☐

2.Una umri gani? (Chagua moja)

18-29 ☐ 30-44 ☐ 45-59 ☐ 60-65 ☐

3.Je, una historia yoyote ya Shinikizo la damu? (Chagua moja)

NDIYO ☐ Hapana ☐

4.Je, una jamaa yeyote wa shahada ya kwanza anayesumbuliwa na Shinikizo la damu? (wazazi, kaka, dada, mtoto) (Chagua moja)

NDIYO ☐ Hapana ☐

5 Je! unajua Fahirisi ya Misa ya Mwili wako..... NDIYO….HAPANA…..Ikiwa hakuna Kipimo kwa upole uzito wako Urefu (M) Uzito(kilo)

6.Je, una hali yoyote ya afya? Ikiwa ndio, tafadhali taja hali hiyo.

……

7. Shinikizo la damu

Asante.

Appendix III: Participant Blood Sample Informed Consent Form.

Title of Study: Hematological and genetic biomarkers among patients with essential hypertension and their age/gender matched controls at Chuka Level 5 Hospital.

Hematological markers are blood count parameters such red cell distribution width, mean platelet volume platelet distribution width and plasma c-reactive protein which could be associated with essential hypertension. Genetic markers such single nucleotide polymorphisms associated with essential hypertension will be investigated.

Researcher name: Amos Mutwiri Mbaabu (Jomo Kenyatta University of Science and Technology)

It is entirely up to you whether or not you choose to take part in this research. Before deciding whether or not to participate, please carefully read this participant blood sample informed consent form. You are welcome to ask as many questions as you like.

Why have you given me this form?

This informed consent form is about a clinical research that is being done in people who are hypertensive and blood donors who are normotensive and healthy.

You are being requested to sign this consent form once you agree to participate and give your blood sample

Why is this blood sample being collected?

The sample will be used to identify hematological and genetic biomarkers that may have a role in detection, progression and control of arterial hypertension among patients at chuka level 5 hospital

What exactly am I supposed to do?

5mL of your blood will be taken from your arm if you accept to participate in this study.

How long will I be a research participant?

The research will take about three months to complete.

What risks am I likely to encounter?

When the blood sample is obtained, you may feel some discomfort for a short while. The place where the needle is inserted has a slight risk of bruising, infection, or edema.

What is the benefit of participating in this research?

There will be no direct benefit/compensation from participating in this study. The results will give information on biomarkers that enable early detection, progression and control of hypertension.

If I consent, can I withdraw from the study later?

It is voluntary to take part. You can withdraw from the study at any point.

Will I be paid for my participation?

The will be no payment for taking part in this study and there are no costs to you.

How will you protect my information?

- By agreeing to participate in this study, your medical records or file will be reviewed and any information that identifies you will be highly encrypted.
- All data collected in the study will be identifiable by a unique identifier and will exclude any information that can be used to identify you, such as your name. Your data will be coded, and your study records will be kept safely.

If I have any further questions, whom should I contact?

Please contact the following people if you have any queries about the study:

Mr. Amos Mutwiri Mbaabu: +254715134472: amohmbaabu@gmail.com

Dr. Amos Mbugua +254702961963: ambugua@jkuat.ac.ke

Acceptance of Research Participation

- I'm aware that I'm being asked to provide consent to collect a blood sample that will be used for hematological and genetic biomarker testing.
- This research was explained to me by _____.
- Each page of this Participant Blood Sample Informed Consent Form has been read to me, or I have had it read to me.
- All of my inquiries were satisfactorily addressed.
- I have the right to withdraw my participation and/or consent from the study at any time if I change my mind.
- I willingly accept to take part in this research.

Name Signature Thumb print Date

Statement from the Investigator

I gave the participant a thorough explanation of the study. The participant is aware of the nature, requirements, risks, and benefits of participation in this research, to the best of my knowledge.

Name Sign Date

Assistance Declaration

Was there any assistance provided to the participant during the consent process?
□ Yes □ No

- The participant/substitute decision-maker was read the permission form, and the person signing below certifies that the study was accurately explained to him/her and that he/she appeared to comprehend it, and that the participant freely gave consent.
- The whole content of this consent form was translated to the participant by the person appending the signature.

Name of Assistant Sign Thumb print Date

Appendix IV: Kiswahili Blood Sample Informed Consent Form.

HOSPITALI YA CHUKA NGAZI YA 5

FOMU YA RIDHAA YA MSHIRIKI

Kichwa cha Utafiti: Alama Za Hematolojia, Biochemical Na Jenetisi Miongoni Mwa Wagonjwa Mwenye Shinikizo La Damu Katika Hospitali Ya Chuka Ngazi Ya 5.

Jina la mtafiti: Amos Mutwiri Mbaabu (Chuo Kikuu Cha Jomo Kenyatta Cha Kilimo Na Teknolojia)

Kushiriki katika utafiti huu ni kwa hiari. Tafadhali soma sampuli hii ya damu ya mshiriki fomu ya kibali kwa uangalifu kabla ya kuamua kama ungependa kushiriki. Jisikie huru kuuliza maswali mengi upendavyo.

Kwa nini napewa hii fomu?

Fomu hii ya idhini iliyo na ufahamu inahusu utafiti wa kimatibabu ambao unafanywa kwa watu walio na shinikizo la damu na watoa damu ambao wana viwango vya kawaida na wenye afya.

Unaombwa kutia sahihi kwenye fomu hii ya idhini mara tu unapokubali kushiriki na kutoa sampuli yako ya damu

Kwa nini sampuli hii ya damu inakusanywa?

Sampuli hiyo itatumika kubaini viashirio vya kihematolojia na kijenetiki ambavyo vinaweza kuwa na jukumu la kugundua, kuendelea na kudhibiti shinikizo la damu miongoni mwa wagonjwa katika hospitali ya chuka level 5.

Ni nini kinachotarajiwa kwangu?

Ukikubali kushiriki katika utafiti huu, 5mL ya damu yako itatolewa kutoka kwa mkono wako.

Je, nitahusika kwa muda gani katika utafiti?

Utafiti utachukua takriban miezi mitatu.

Je, ni hatari gani ninazoweza kupata?

Unaweza kupata usumbufu wa muda wakati sampuli ya damu inachukuliwa. Kuna hatari ndogo ya michubuko, maambukizi au uvimbe kwenye tovuti ambayo sindano imeingizwa

Je, ninaweza kutarajia kufaidika kwa kushiriki katika utafiti huu?

Hutapokea manufaa yoyote ya moja kwa moja kutokana na kushiriki katika utafiti huu. Matokeo yanayofanywa yatatoa habari juu ya alama za kibayolojia zinazowezesha utambuzi wa mapema, maendeleo na udhibiti wa shinikizo la damu.

Ikiwa ninakubali sasa, ninaweza kubadilisha mawazo yangu na kujiondoa baadaye?

Kushiriki kwako katika utafiti huu ni kwa hiari. Njia mbadala ya utafiti huu si kushiriki

Je, nitalipwa kwa ushiriki wangu au kutakuwa na gharama zozote za ziada kwangu?

Hutapokea malipo kwa kushiriki katika utafiti huu na hakuna gharama kwako.

Je, taarifa zangu za kibinafsi zinalindwa vipi?

- Ukiamua kushiriki katika utafiti huu, mpelelezi na wafanyakazi wa utafiti watakagua rekodi zako za matibabu au faili na maelezo ambayo yanakutambulisha yatatolewa iwapo tu itahitajika kisheria.
- Taarifa zote zilizokusanywa wakati wa ushiriki wako katika utafiti huu zitatambuliwa kwa nambari ya kipekee ya utafiti na hazitakuwa na taarifa zinazokutambulisha. rekodi za masomo zenye msimbo zitahifadhiwa kwa usalama.

Nitawasiliana na nani ikiwa nina maswali zaidi?

Ikiwa una maswali yoyote kuhusu utafiti huu, tafadhali wasiliana na:

Amos Mutwiri Mbaabu: +254715134472: amohmbaabu@gmail.com

Dkt Amos Mbugua: +254702961963:ambugua@jkuat.ac.ke

Idhini ya Kushiriki katika Utafiti

- Ninaelewa kuwa ninaombwa kutoa idhini ya kukusanya sampuli ya damu ambayo itatumika kwa uchunguzi wa alama za kihematolojia na kijenetisi.
- Utafiti huu ulinifafanuliwa na _____.
- Nimesoma, au nimesomewa, kila ukurasa wa Fomu hii ya Idhini ya Sampuli ya Damu ya Mshiriki.
- Maswali yangu yote yamejibiwa kwa kuridhika kwangu.
- Nikiamua baadaye kwamba ningependa kuondoa ushiriki wangu na/au kibali kutoka kwa utafiti, ninaweza kufanya hivyo wakati wowote.
- Ninakubali kwa hiari yangu kushiriki katika utafiti huu.

Jina la mshiriki Sahihi kidole gumba

Tarehe

Taarifa ya Mpelelezi

Nimeeleza kwa makini utafiti kwa mshiriki. Kwa ufahamu wangu, mshiriki anaelewa asili, mahitaji, hatari na faida zinazohusika katika kushiriki katika utafiti huu.

Jina la Mpelelezi sahihi Tarehe

Azimio la Msaada

- Je, mshiriki alisaidiwa wakati wa mchakato wa idhini? Ndiyo : Hapana

- Fomu ya idhini ilisomwa kwa mshiriki/mtoa maamuzi mbadala, na mtu anayetia saini hapa chini anathibitisha kwamba utafiti ulielezwa kwa usahihi, na inaonekana kueleweka, na idhini ilitolewa na mshiriki/mtoa maamuzi mbadala.

- Mtu anayetia sahihi hapa chini alitenda kama mfasiri wa mshiriki/mtoa maamuzi mbadala wakati wa mchakato wa kutoa idhini. Anathibitisha kuwa wametafsiri kwa usahihi taarifa kwa mshiriki/mtoa maamuzi mbadala, na anaamini kuwa mshiriki/mtoamaamuzi mbadala ameelewa taarifa iliyotafsiriwa.

Jina la Mtu Anayesaidia **Sahihi** **Tarehe**

Appendix V: Packaging and Transportation of Blood Samples from Chuka Hospital to JKUAT.

Packaging of the Blood Specimens.

Triple Packaging system was used and consisted of: primary receptacles (blood in EDTA tubes) secondary packaging and outer packaging.

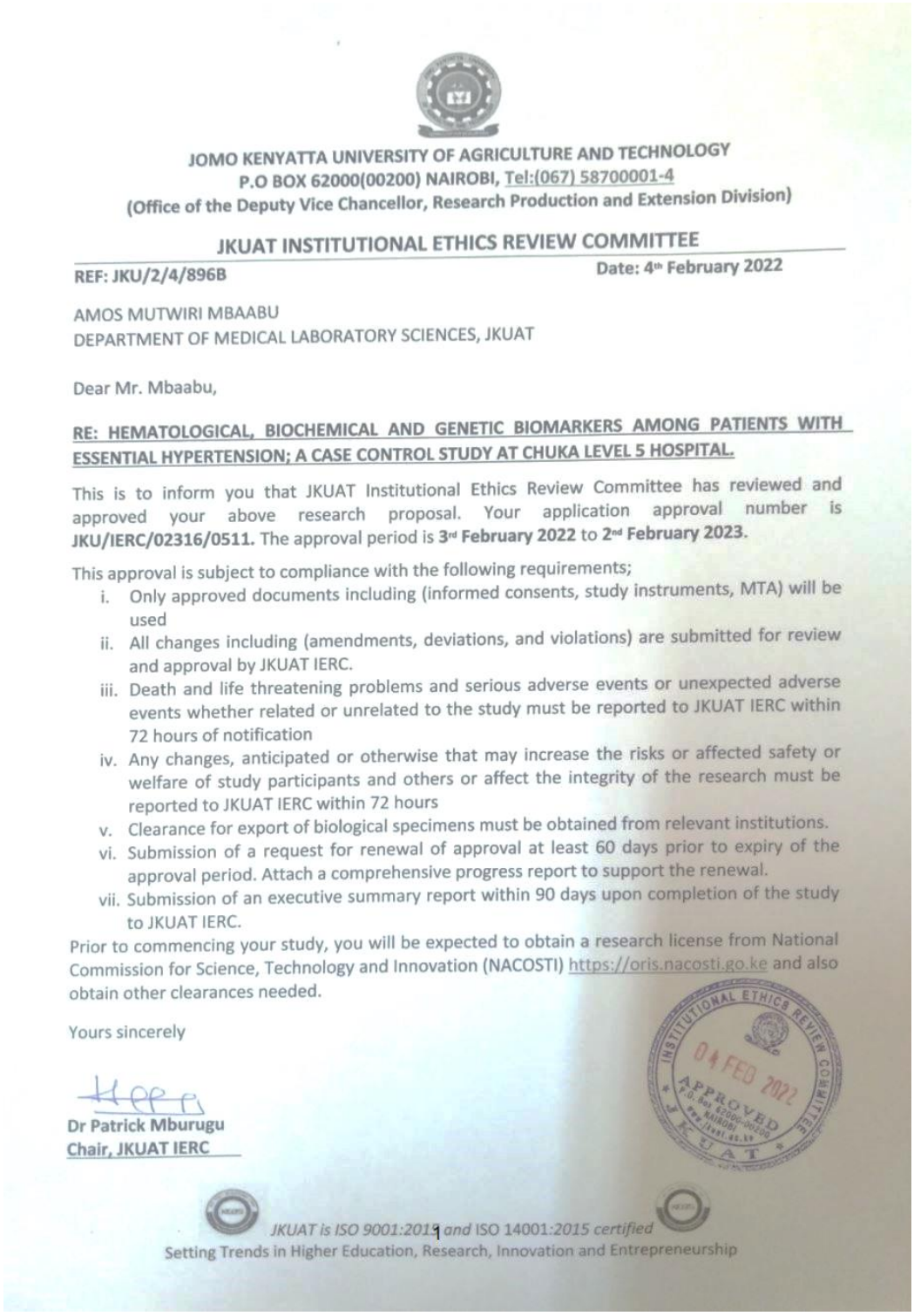
Secondary Packaging for Blood Tubes

All samples (blood tubes) were put in a transporting rack together, with absorbent material inserted between the blood tubes and the first layer of secondary packaging. To prevent tube-to-tube contact, each tube will be segregated from others. The absorbent material will be wrapped around the first layer of secondary packaging and sealed.

Outer Packaging for Blood Tubes

Additional absorbent material was inserted in the shipper's bottom for cushioning. On top of the absorbent material, a single layer of refrigerator packs will be applied. The refrigerator packs will be stacked on top of the boxed specimens. An extra cushioning material will be utilized to keep the shipper from shifting while in transportation. A second refrigerator will be placed on top of the secondary packaging to keep the delivery temperature between 2 and 10 degrees Celsius. The blood shipment manifest will be placed in a sealable plastic bag on top of the shipper's packets. Lid will be placed on the shipper. Labels and Markings will be fixed next to shipper’s address. A label (UN 3373) and the words “Biological Substance, Category B” will be placed next to the label on the front of the shipper. The samples will be transported by road from Chuka level 5 hospital to Pausti research laboratories-JKUAT.

Appendix VI: JKUAT Institutional Ethics Review Committee Approval



JOMO KENYATTA UNIVERSITY OF AGRICULTURE AND TECHNOLOGY
P.O BOX 62000(00200) NAIROBI, Tel:(067) 58700001-4
(Office of the Deputy Vice Chancellor, Research Production and Extension Division)

JKUAT INSTITUTIONAL ETHICS REVIEW COMMITTEE

REF: JKU/2/4/896B **Date: 4th February 2022**

AMOS MUTWIRI MBAABU
DEPARTMENT OF MEDICAL LABORATORY SCIENCES, JKUAT

Dear Mr. Mbaabu,

RE: HEMATOLOGICAL, BIOCHEMICAL AND GENETIC BIOMARKERS AMONG PATIENTS WITH ESSENTIAL HYPERTENSION; A CASE CONTROL STUDY AT CHUKA LEVEL 5 HOSPITAL.

This is to inform you that JKUAT Institutional Ethics Review Committee has reviewed and approved your above research proposal. Your application approval number is **JKU/IERC/02316/0511.** The approval period is **3rd February 2022** to **2nd February 2023.**

This approval is subject to compliance with the following requirements;

i. Only approved documents including (informed consents, study instruments, MTA) will be used
ii. All changes including (amendments, deviations, and violations) are submitted for review and approval by JKUAT IERC.
iii. Death and life threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to JKUAT IERC within 72 hours of notification
iv. Any changes, anticipated or otherwise that may increase the risks or affected safety or welfare of study participants and others or affect the integrity of the research must be reported to JKUAT IERC within 72 hours
v. Clearance for export of biological specimens must be obtained from relevant institutions.
vi. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal.
vii. Submission of an executive summary report within 90 days upon completion of the study to JKUAT IERC.

Prior to commencing your study, you will be expected to obtain a research license from National Commission for Science, Technology and Innovation (NACOSTI) https://oris.nacosti.go.ke and also obtain other clearances needed.

Yours sincerely

Dr Patrick Mburugu
Chair, JKUAT IERC

INSTITUTIONAL ETHICS REVIEW COMMITTEE
04 FEB 2022
APPROVED
P.O. Box 62000-00200 NAIROBI
www.jkuat.ac.ke
JKUAT

JKUAT is ISO 9001:2015 and ISO 14001:2015 certified
Setting Trends in Higher Education, Research, Innovation and Entrepreneurship

Appendix VII: Research License from NACOSTI

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: **840124**

Date of Issue: **17/February/2022**

RESEARCH LICENSE

This is to Certify that Mr.. AMOS MUTWIRI MBAABU of Jomo Kenyatta University of Agriculture and Technology, has been licensed to conduct research in Tharaka-Nithi on the topic: HEMATOLOGICAL, BIOCHEMICAL AND GENETIC BIOMARKERS AMONG PATIENTS WITH ESSENTIAL HYPERTENSION: A CASE CONTROL STUDY AT CHUKA LEVEL 5 HOSPITAL for the period ending : 17/February/2023.

License No: **NACOSTI/P/22/15848**

840124

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Verification QR Code

NOTE: This is a computer generated License. To verify the authenticity of this document, Scan the QR Code using QR scanner application.

Appendix VIII: Chuka County Referral Hospital Research Clearance

THARAKA NITHI COUNTY GOVERNMENT
DEPARTMENT OF HEALTH SERVICES AND SANITATION
CHUKA REFERRAL HOSPITAL
OFFICE OF THE MEDICAL SUPERINTENDENT
P.O. BOX 8 – 60400
CHUKA
CELL: 0728 226 333 **EMAIL**: medsuptchuka@gmail.com

When replying please quote:
REF: CKA/MED/T/16/VOL.VI/269

Date: 21st February 2022

MR. AMOS MUTWIRI MBAABU
P.O BOX 109 – 60400
CHUKA

Dear Sir,

RE: NO OBJECTION TO REQUEST FOR AUTHORIZATION TO CONDUCT RESEARCH AT CHUKA REFERRAL HOSPITAL

The above subject refers.

Following your request dated 17th February 2022, for an opportunity to carry out research at Chuka Referral Hospital on "**Hematological, Biochemical and Genetic Biomarkers Among Patients with Essential Hypertension,**" for a time period of three (3) months from 28th February 2022, your request has been granted. This is subject to the following;

- That you will adhere to all the Ethical Guidelines for your research as stipulated in the Jomo Kenyatta University of Agriculture and Technology University Institutional Ethics Review Committee recommendations.
- That you will commit to share your research findings with the Hospital for purposes of Quality Improvement in Service Delivery.
- That the data collection exercise will not interfere with service delivery at the hospital.
- That you are required to bring consumables for the research as indicated in your request
- Payment of the research fee.

If the conditions stipulated above are acceptable to you, kindly sign below:

NAME AMOS MUTWIRI MBAABU SIGN _____ DATE 21/02/2022

JANICE GATAKAA GITARI
HRMDO – HEALTH
FOR: MEDICAL SUPERINTENDENT
CHUKA REFERRAL HOSPITAL

Copy to:
- HAO – Chuka Referral Hospital
- Nursing Services Manager-Chuka Referral Hospital
- Stephen Miano – MLT Services – In charge