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Determining the Causal Role of Malaria in Elevating Blood Pressure and Pulse Wave Velocity in Kenyan Adolescents and Adults

Anthony Oliwa Etyang

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LONDON SCHOOL OF HYGIENE & TROPICAL MEDICINE

Funded by Wellcome

Dedicated to all of my family

Abstract

Introduction: High blood pressure is recognized as a leading risk factor for stroke and death in sub-Saharan Africa (sSA). While many studies have examined the role of established risk factors such as obesity and salt consumption, less is known about other factors, such as infection, that could be of particular importance in sSA. Ambulatory blood pressure measurement has emerged as the optimal method in recent years in Western settings, but there has been limited use to date in sSA. This work presents the results of a study investigating whether malaria, which is widespread in sSA could contribute to the development of high blood pressure using ambulatory measurements.

Methods: Preliminary work involved determining the prevalence of hypertension in Kilifi, Kenya and examining the population-level effects of using ambulatory blood pressure monitoring (ABPM) for diagnosing hypertension. A literature review outlining the basis of the malaria-high blood pressure hypothesis and the Mendelian randomization method for testing the hypothesis was conducted. Sickle cell trait and alpha (+) thalassemia were chosen as instrumental variables to represent malaria exposure because they protect against malaria. Two studies were performed in Nairobi, Kenya among the same cohort to confirm that sickle-cell trait and alpha-thalassemia do not influence blood pressure in the absence of malaria and were therefore valid instrumental variables to test the malaria-high blood pressure hypothesis in Kilifi where there is malaria transmission. A Mendelian randomization study was then conducted in Kilifi, Kenya where 24-hour blood pressure and arterial

stiffness indices were compared in individuals with and without sickle cell trait and alpha thalassemia.

Results: The prevalence of hypertension in Kilifi, a rural area, was found to be as high as in urban areas of Kenya despite the low frequency of classical risk factors such as obesity and excessive salt consumption. Use of ambulatory blood pressure monitoring for diagnosing hypertension was found to improve the accuracy of detection of high blood pressure.

Neither Sickle-cell trait (SCT) nor alpha+ thalassemia influenced blood pressure or arterial stiffness indices among adolescents that had been lifelong residents of Nairobi, where there is no malaria transmission. Among individuals that had been lifelong residents of Kilifi, Kenya where there has been on-going malaria transmission, blood pressure was found to be lower among individuals with SCT, which protects against malaria episodes compared to those without SCT. The difference in BP by SCT status was larger in women than in men. There were no significant differences in arterial stiffness based on SCT status.

Conclusion: This work suggests that malaria contributes to the burden of hypertension in sSA, and the control of malaria may lead to a reduction in blood pressure in this group. Future work should focus on confirming the findings using alternative study designs such as examining blood pressure in cohorts born before and after complete malaria elimination in parts of the world where this has been achieved. Subsequent work would involve delineating the pathophysiological mechanisms involved in malaria induced BP elevation with a view to generating new drugs to control hypertension.

Declaration

Statement of Own Work

I, Anthony O. Etyang, confirm that the work presented in this thesis is my own. I conceived the ideas and developed them further with my supervisors. I supervised all of the data collection, which was performed by field workers that I trained. Where information has been derived from other sources, this has been indicated in the thesis. I have read and understood the School's definition of plagiarism and cheating given in the Research Degrees Handbook.

Anthony Oliwa Etyang

July 2017

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Preface

This thesis comprises four published and one as yet unpublished research papers focusing on the malaria-high blood pressure hypothesis. Although the papers are from and form one body of work, they also stand alone as independent research contributions. As such, there will be several instances where the setting, definitions and other descriptions are repeated. For the sole purpose of making the thesis easier to read and annotate, for the four published papers I have included the final word-processed document in this thesis rather than the published proofs. Internet links to the published versions are provided in the preface to each paper.

The introductory chapter looks at the importance of hypertension in Africa, followed by a description of how I came up with the malaria-high blood pressure hypothesis and considerations that I made in designing the studies to test it. Chapters 2-6 are a series of research papers beginning with a descriptive study of hypertension in Kilifi, Kenya (Chapter 2), a review of the literature in support of the hypothesis tested (Chapter 3) and finally 3 papers (Chapters 4-6) presenting data from studies done in order to test the hypothesis. Chapter 7 is a discussion of the results of the studies and their implications and it outlines what direction future research should take.

Contents

Chapter 1. Introduction	12
A. <i>The Changing Epidemiology of High Blood Pressure in Africa</i>	12
B. <i>Defining hypertension: use of cut-offs and examining BP as a continuous variable</i>	14
C. <i>Aetiology of Hypertension in Africa</i>	16
D. <i>Observations Suggesting the Role of Malaria in the Aetiology of Raised Blood Pressure</i>	19
Sub-Saharan African Populations have higher Age-Standardized Blood Pressures than other Populations	19
Obesity and excessive salt intake are not as common in Kilifi despite high levels of cardiovascular disease	20
Malaria is common in Kilifi and is associated with factors known to cause hypertension	21
E. <i>Blood pressure measurement</i>	23
Accuracy of blood pressure measurement	27
Ambulatory Blood Pressure Monitoring	28
F. <i>Determining past exposure to malaria</i>	31
G. <i>Background Information on the Study Populations used in this Thesis</i>	35
Summary	37
Chapter 2. Research paper I: Clinical and Epidemiological Implications of 24-hour Ambulatory Blood Pressure Monitoring for the Diagnosis of Hypertension in Kenyan Adults: A Population Based Study	46
Chapter 3. Research paper II: The Malaria-High Blood Pressure Hypothesis	76
Chapter 4. Research paper III: Blood Pressure and Arterial Stiffness in Kenyan Adolescents With α(+)Thalassemia	93
Chapter 5. Research paper IV: Blood Pressure and Arterial Stiffness in Kenyan Adolescents with the Sick Cell Trait	116
Chapter 6. Research paper V: Relationship between childhood malaria and adult blood pressure	142
Chapter 7. Discussion and Conclusion	188
A. <i>Increased risk of malaria is associated with increased blood pressure and increased odds of hypertension- replication and mechanistic studies needed</i>	188
B. <i>Ambulatory blood pressure monitoring can be performed in an African setting- but challenges exist</i>	194
C. <i>Sickle cell trait and alpha thalassemia do not directly influence blood pressure- unlikely to contribute to ethnic differences in cardiovascular disease</i>	198
D. <i>How do we interpret these findings in view of previous studies demonstrating that factors related to urbanization are responsible for increased blood pressure?</i>	200
E. <i>Public Health Relevance of the Findings in this Thesis and Potential Interventions</i>	201
F. <i>Proposed Future Work</i>	202
Appendix	205

List of Tables[§]

Table 1-1: Published studies of ABPM in Africa.....	30
Table 2-1: Characteristics of study subjects	67
Table 2-2: Mean BP, pulse pressure and AASI, by age, derived from screening BP and 24 hour ABPM	68
Table 2-3: Age specific prevalence of hypertension using screening measurement and ABPM	69
Table 2-4: Validity of measures of casual BP method compared to ABPM	70
Table 2-5: Interval likelihood ratios for screening systolic and diastolic blood pressures	71
Table 4-1: Characteristics of Study Participants	112
Table 4-2: Regression Analyses Investigating Possible Effect of Thalassemia Status on 24-Hour Systolic and Diastolic BP	113
Table 5-1: Characteristics of study subjects according to sickle trait status, Nairobi, Kenya 2015-2016	137
Table 5-2: Predictors of mean 24-hour systolic and diastolic BPs among adolescents in Nairobi, Kenya, 2015-2016	138
Table 6-1: Characteristics of Study Participants by SCT Status	161
Table 6-2: Effect of SCT on Ambulatory blood pressure indices in Kilifi and Nairobi	162
Table 6-3: Age specific effects of SCT on BP in Kilifi participants.....	163

[§] Tables and figures are numbered sequentially within each chapter e.g. Table 3-7 refers to the 7th table in Chapter 3

List of Figures[§]

Figure 1-1: Aetiology of hypertension and its consequences	18
Figure 2-1: Study recruitment profile	73
Figure 2-2: Comparison of screening and ABPM blood pressure distributions.....	74
Figure 2-3: Population prevalences of different BP indices.....	75
Figure 3-1: The malaria-high blood pressure hypothesis	92
Figure 4-1: Study Flow Chart.....	114
Figure 4-2: Twenty-four hour ABPM measures by alpha thalassemia status	115
Figure 5-1: Study Flow Chart.....	140
Figure 5-2: Twenty-four hour ABPM measures by sickle cell trait (SCT) carrier status in Nairobi, Kenya 2015-2016	141
Figure 6-1: Study Flow Chart.....	185
Figure 6-2: Age Standardized [‡] Effect of SCT on ABPM Indices in Kilifi and Nairobi	186

[§] Tables and figures are numbered sequentially within each chapter e.g. Table 3-7 refers to the 7th table in Chapter 3

List of acronyms and abbreviations used in this thesis

ABPM:	Ambulatory blood pressure monitoring
ACE:	Angiotensin converting enzyme
Ang-2:	Angiopoietin 2
BMI:	Body mass index
BP:	Blood pressure
DM:	Diabetes mellitus
DBP:	Diastolic blood pressure
HIV:	Human Immunodeficiency Virus
HTN:	Hypertension
KEMRI:	Kenya Medical Research Institute
KHDSS:	Kilifi Health and Demographic Surveillance System
KWTRP:	KEMRI-Wellcome Trust Research Programme
LBW:	Low birth weight
MR:	Mendelian randomization
MUAC:	Mid-upper arm circumference
NCD:	Non-communicable disease(s)
NUHDSS:	Nairobi Urban Health and Demographic Surveillance System
PWV:	Pulse wave velocity
SBP:	Systolic blood pressure
SCT:	Sickle cell trait
sSA:	sub Saharan Africa
UACR:	Urine albumin to creatinine ratio
WHO:	World Health Organization

Chapter 1. Introduction

A. The Changing Epidemiology of High Blood Pressure in Africa

Early studies of hypertension in sub-Saharan Africa suggested that population mean blood pressure levels in the region were low. In one of the earliest papers on high blood pressure in sub-Saharan Africa, published in the *Lancet* in 1929, C.P. Donnison reported a mean systolic blood pressure of 106 mm Hg and mean diastolic blood pressure of 67 mm Hg among 1,800 apparently healthy adults aged 15 years and above at Kisii District Hospital located in the highlands of Western Kenya.¹ This was in comparison to average values of 140/90 mmHg among Europeans and Americans at the time. He reported that only 2 subjects (who had not shown any signs of nervousness) had systolic blood pressures in the hypertensive range; one had a recording of 172 mm Hg and the other had a reading of 150 mm Hg. Unfortunately he was not able to follow up these 2 individuals and study them further. In a 1961 study by Shaper *et al* among the Samburu, Turkana and Rendile in semi-arid northern Kenya, no participant had a systolic blood pressure above 160 mm Hg.² Similar findings were reported by Beiser *et al* in Senegal in 1976³ and Pobee *et al* in rural Ghana in 1977.⁴

In recent years there has been a proliferation of studies suggesting an increasing burden of high blood pressure in Africa.⁵⁻¹⁰ These have been summarized in systematic reviews and meta-analyses, each addressing slightly different questions, that were conducted by Addo *et al*,¹¹ Twagirumukiza *et al*,⁶ and Ataklte *et al*.¹⁰ Although these studies all had some significant shortcomings, they revealed the following:

- limited population-level data on hypertension epidemiology in many African countries
- extensive variation in the prevalence of hypertension both within and between studies included in the meta-analyses
- an age-related increase in the prevalence of hypertension
- a higher prevalence of hypertension in urban areas compared to rural areas
- low levels of awareness of hypertension in those with blood pressure above the normal range
- low levels of treatment among those previously diagnosed as hypertensive
- low levels of blood pressure control among those on medications for hypertension

Partly in response to deficiencies identified in the individual studies that contributed to the meta-analyses cited above, a number of World Health Organization (WHO) supported STEPwise surveys have been conducted to determine the prevalence of hypertension and other risk factors for non-communicable diseases in several countries in Africa.¹² The surveys are referred to as STEPwise (STEPS) because the data are collected in 3 steps; step 1 uses a questionnaire to collect demographic and lifestyle data; step 2 involves measurements of height, weight, blood pressure, waist and hip circumference; and step 3 uses laboratory (biochemistry) investigations. Although no formal meta-analysis of the STEPs surveys has been conducted to date, these surveys have come up with essentially similar findings to previously conducted studies, as exemplified in one that was conducted in

Malawi.¹³ A point to note though is that a recently conducted STEPs survey in Kenya found a marginally higher prevalence of hypertension in rural areas compared to urban regions.¹⁴ The findings of the Kenyan STEPs survey are discussed in more detail in the first paper in this thesis.

The consequences of uncontrolled hypertension are significant. The Interstroke study established that hypertension accounts for the largest share of the population attributable risk for stroke¹⁵ in low and middle income countries. Stroke is a leading cause acquired disability in these countries, many of which are in Africa.¹⁶ The Interheart study similarly established that hypertension plays a major role in the development of myocardial infarction in black Africans.¹⁷ In a study looking at the causes of heart failure in 9 African countries, hypertension was identified as the leading risk factor for the condition.¹⁸ Hypertension is also associated with the increasing burden of renal disease in sub-Saharan Africa.^{5,19}

B. Defining hypertension: use of cut-offs and examining BP as a continuous variable

The aim of this section is to define the cut-offs used in defining hypertension in the studies in this thesis, as well as briefly discuss the pros and cons of using cut-offs versus examining blood pressure as a continuous variable. Among adult (age >16 years) participants in the studies in this thesis I have used clinic and ambulatory blood pressure thresholds that have been defined by the European Society of Hypertension.²⁰ These are as follows:

- Clinic systolic BP of 140 mm Hg or above and/or diastolic BP of 90 mm Hg or above. The technique used in clinic BP measurement is a casual one (as opposed to ambulatory blood pressure monitoring) and

therefore the term clinic BP is sometimes referred to as casual BP or screening BP.

- When using ambulatory blood pressure monitoring, a participant meeting any one of the following thresholds is defined as having hypertension:
 - i. 24-hours systolic BP ≥ 130 mm Hg and/or 24-hour diastolic BP ≥ 90 mm Hg
 - ii. Daytime systolic BP ≥ 135 mm Hg and/or daytime diastolic BP ≥ 85 mm Hg
 - iii. Nighttime systolic BP ≥ 120 mm Hg and/or nighttime diastolic BP ≥ 70 mm Hg

Among adolescents (age 11-16 years), the international consensus is to use percentile distributions to define hypertension as no outcome studies have been conducted to determine thresholds above which cardiovascular risk is increased. Using guidelines from the Paediatric working group of the European Society of Hypertension²¹, an adolescent with a clinic or ambulatory blood pressure value above the 95th percentile for their age and sex is considered to be hypertensive.

The advantage of using thresholds for defining hypertension is that it makes it possible to easily compare prevalences of hypertension in different study settings and also enables the application of simple treatment protocols based on a simple dichotomization of individuals into those with and without hypertension. This simplification however comes with several disadvantages:

- i) The thresholds used were defined using populations that are different from those used in this thesis. The implications of this are discussed in Chapter 7.

ii) It is now recognized that the risk of cardiovascular events increases linearly with blood pressure elevation even at levels below current cut-offs²², therefore the use of thresholds carries the risk of failing to identify and advise individuals with BP below current cut-offs that they need to put in place strategies to reduce their blood pressure.²³

C. Aetiology of Hypertension in Africa

Few studies have examined risk factors that are unique to sub Saharan Africa in the aetiology of hypertension. Risk factors identified to date are mostly similar to those implicated in other parts of the world. Dietary factors (excess salt and energy intake, insufficient potassium intake) and adiposity have been shown in numerous studies to contribute to the development of essential hypertension and likely account for most of the increase in the burden of hypertension that has been observed with increasing urbanization in Africa.^{24,25} Other factors that may be of particular relevance among African populations include:

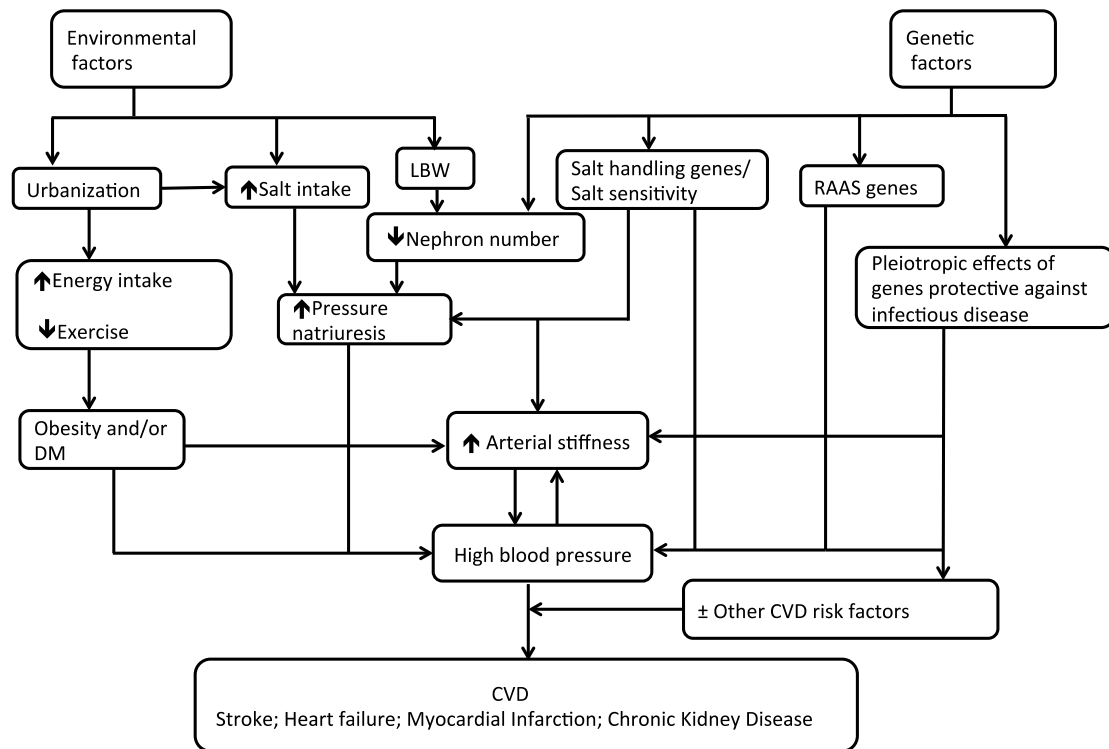
- iv. Low plasma renin: This is thought to be due to abnormalities in sodium handling such as excessive renal reabsorption of sodium (see below), and may explain the poor response to angiotensin converting enzyme inhibitor therapy that has been observed in African populations.^{5,26}
- v. Sodium sensitivity: This refers to an abnormal blood pressure response to increased sodium intake that may be due to hereditary and/or environmental factors, and appears to be more common in African populations.^{5,24,27}

- vi. Abnormalities in the renin angiotensin-aldosterone system:
Polymorphisms of the promoter region of the angiotensinogen and the aldosterone synthase gene have been identified in previously untreated black South Africans and may play a role in blood pressure elevation.²⁸ In addition a polymorphism in the ACE gene has been associated with protection against cerebral malaria while increasing the risk of hypertension^{29,30} (see section on the High blood pressure-malaria protection hypothesis below).
- vii. HIV infection: Africa has the highest incidence and prevalence of HIV worldwide and a number of studies demonstrate that HIV infection as well as drugs used in its treatment may result in elevation of blood pressure.³¹⁻³⁴
- viii. The prevalence of diabetes mellitus (DM) is on an upward trend in Africa.³⁵ DM is an established risk factor for the development of hypertension.^{36,37}

The emerging consensus is that the development of hypertension is multi-factorial featuring interplay between environmental and genetic factors, although the individual contributions of each are not well ascertained.^{5,24}

Figure 1-1 below is a schematic diagram that summarizes current opinion.

Figure 1-1: Aetiology of hypertension and its consequences



CVD: Cardiovascular disease

DM: Diabetes Mellitus

LBW: Low birth weight

RAAS: Renin-angiotensin-aldosterone system

The studies in this thesis tested a unique risk factor, malaria whose potential contribution to the development of hypertension has not previously been considered. In the next sections I describe how I came up with the hypothesis and considerations I made in deciding how to go about testing it. The final chapter of this thesis (Chapter 7) contains a discussion of the apparent contradiction between this hypothesis and previous studies.

D. Observations Suggesting the Role of Malaria in the Aetiology of Raised Blood Pressure

The idea that malaria could be linked to hypertension may appear speculative at first, but it was informed by a series of observations, which were however not conclusive, therefore necessitating a formal study of the subject. I highlight these observations here. The hypothesis is discussed in more detail in Chapter 3.

Sub-Saharan African Populations have higher Age-Standardized Blood Pressures than other Populations

Systematic reviews of blood pressure levels across the world have revealed that age-standardized blood pressures are higher in sub-Saharan Africa compared to that in high-income countries where the incidence of malaria is much lower.^{38,39} There is a lot of heterogeneity in malaria transmission across Africa and if malaria raises blood pressure, it would be expected that blood pressure levels would be higher in parts of Africa that experience more malaria than others. Few studies have examined geographical variation in blood pressure in Africa apart from those looking at the urban-rural differences, which are subject to multiple confounders. Van der Sande *et al* in

the Gambia between 1996-1998 performed a study⁴⁰ where they found that even within rural communities where there is minimal effect of urbanization, there were significant differences in the prevalence of hypertension. These variations were not explained by differences in age or the prevalence of obesity. Apart from the limited number of such studies (of geographical variation in blood pressure), ecological analyses to prove the malaria-high blood pressure link are also limited by the coarse nature of blood pressure measurements. The majority of studies on hypertension in Africa have used the casual method of blood pressure measurement^{6,11}, which as discussed at several points in this thesis has limitations.

Obesity and excessive salt intake are not as common in Kilifi despite high levels of cardiovascular disease

In an analysis of admissions at Kilifi District Hospital for the period from 2007-2012, I found that although infectious diseases dominated by HIV related illnesses were the leading cause of admissions, there was a large burden of cardiovascular disease which ranked second in burden.⁴¹ I had also anecdotally noted that most of the patients I was seeing with hypertension appeared to lack the classical lifestyle related risk factors for the condition such as obesity. This was confirmed in a sub-component of the hypertension prevalence study that I conducted in Kilifi (reported in Chapter 2) where I found that despite having blood pressure levels similar to that found in urban areas of Kenya, the Kilifi population had generally low body mass index and urinary sodium levels.⁴²

Malaria is common in Kilifi and is associated with factors known to cause hypertension

Kilifi has low to moderate malaria transmission.⁴³ Malaria is known to cause low-birth weight, childhood malnutrition and inflammation.⁴⁴⁻⁴⁶ All of these factors have been shown in developed world settings to be associated with the development of non-communicable diseases including hypertension. David .J Barker, whose hypothesis I outline in more detail below⁴⁷, first proposed the link between low-birth weight (LBW) and high blood pressure and cardiovascular disease in adult life. The respective roles of childhood malnutrition and inflammation are discussed in detail in Chapter 3.

The Barker Hypothesis

In a landmark study, Barker et al described an inverse relationship between birth weight and incidence of adult cardiovascular disease.⁴⁷ He and others subsequently demonstrated similarly consistent relationships between low birth weight and the prevalence of hypertension and other traditional cardiovascular risk factors during adulthood.⁴⁸ The explanation for these findings appears to be that of the thrifty phenotype that develops as a result of fetal-maternal conflict as elegantly described by Robert Trivers in 1974.⁴⁹ It is theorized that in an in-utero environment of scarcity as would happen to LBW babies, fetal programming allows the fetus to anticipate the environment that it will encounter at birth. However if this fetus that is programmed for scarcity is born into an environment of excess, the programming is inappropriate and leads to the development of the metabolic syndrome. The effect of intra-uterine programming that is most closely associated with a predisposition to hypertension is the effect of fetal growth retardation on glomerular number

and filtration surface⁵⁰, both of which are reduced, requiring the pressure-natriuresis mechanism to operate in a higher blood pressure range.⁵¹

The High Blood Pressure-Malaria Protection Hypothesis

One potential explanation for the geographical association between malaria and high blood pressure is based on the idea of genetic selection; for example, if genes that led to abnormalities in the RAAS were simultaneously protective against severe forms of malaria disease, the gene frequency would increase in settings with high malaria mortality. This idea was first proposed by Gallego-Delgado et al³⁰ on the basis of a study of 426 adults in India.²⁹ Unfortunately, the malariagen consortium study⁵², which is the largest (n >29,000) study conducted to date examining which genetic variants are protective against malaria did not reveal any polymorphisms involving the renin-angiotensin system suggesting that the result of Dhangadamajhi et al²⁹ in India may have been a false positive.

Despite the malaria-high blood pressure hypothesis being well grounded, 2 major issues on how to test it arose. Both had to do with measurement, which is a critical issue in epidemiology. These issues were how to determine exposure to malaria and how to measure blood pressure (the outcome). I will in the next section discuss the issue of blood pressure measurement followed by a discussion on determining past malaria exposure and how the principle of Mendelian randomization that I used in the study works.

E. Blood pressure measurement

Several authors⁵³⁻⁵⁷ have described the rich history of the evolution of blood pressure measurement, that I paraphrase here as a background to ambulatory blood pressure measurement.

The first recorded instance of the measurement of blood pressure (BP) was in a horse in 1733, performed by the Reverend Stephen Hales in Kent, England.^{54,56} He had entered St Benedicts' College in Cambridge, UK in order to study religion and natural philosophy. After graduating in 1703, he became fascinated by the doctrines of the iatrophysicists, Giovanni-Alfonso Borelli, Robert Boyle and Giorgio Baglivi. The Reverend Hales, together with his friend William Stukeley, a pre-medical student, studied anatomy and repeated some of the experiments that had been conducted by Boyle. In 1733, his manuscript, *Haemastatics*, appeared describing the classical experiments by which blood pressure was first measured:

“ In December, I caused a mare to be tied down alive on her back. A fistula was placed on her withers...having laid open the left crural (femoral) artery about three inches from her belly, I inserted into it a brass pipe whose bore was one sixth of an inch in diameter, and to that, by means of another brass pipe which was fitly adapted to it, I fixed a glass tube, of nearly the same diameter, which was nine feet in length: then untying the ligature of the artery, the blood rose in the tube 8 feet 3 inches perpendicular above the level of the left ventricle of the heart...when it was at its full height, it would rise and fall at and after each pulse 2,3 or 4 inches; ... then I took away the glass tube, and let the blood from the artery mount up in open air, when the greatest height of its jet was not above 2 feet. I measured the blood as it ran out of the artery,

and after each quart was run out, I refixed the glass tube to the artery to see how much force the blood was abated; this I repeated to the 8th quart, and then its force being much abated, I applied the glass tube after each pint had flowed out”.^{54,56}

For the next 150 years, BP measurement technology continued to improve through the efforts of many great physicians such as Poisseuille (1799-1869), Carl Ludwig (1816-1895), Karl von Vierordt (1818-1884) Robert Ellis Dudgeon (1820-1904) and Samuel Siegfried Karl Ritter von Basch (1837-1905).

However, for all their work, all the devices invented were invasive and difficult to use. It is perhaps because of this difficulty in using them that blood pressure measurement was not accepted as a valuable diagnostic tool. The British Medical Journal went as far as mentioning that by using the sphygmomanometer “we pauperize our senses and weaken clinical acuity”.⁵³

Scipione Riva-Rocci (1863-1937) was an Italian internist, pathologist and paediatrician, who in 1896 published four articles in the *Gazzetta Medica di Torino* describing a new sphygmomanometer and the technique of non-invasive BP measurement.⁵⁵ Although his was not the first sphygmomanometer, his ingeniously simple idea of constricting the brachial artery and registering the cuff pressure at which the radial pulse was obliterated as determined by palpation offered medical practitioners an efficient method for obtaining relatively accurate readings.

Harvey Cushing, an American neurosurgeon who visited Riva-Rocci in Pavia in 1901, found Riva-Rocci’s sphygmomanometer a valuable means of reducing mortality from anesthesia. Cushing sketched the mercury sphygmomanometer, and on returning to Baltimore, USA, he introduced blood

pressure measurement of anesthetized patients especially during intra-cranial surgery, thus playing a major role in spreading the use of the instrument.⁵⁸

The palpation technique however did not allow the measurement of diastolic BP. One year after Riva-Rocci described his technique, Hill and Barnard in England reported an apparatus with an arm-encircling inflatable cuff and needle pressure gauge that allowed measurement of diastolic pressure by the oscillatory method.⁵⁸ In 1900, von Recklinghausen, recognizing that Riva-Rocci's device had a significant flaw in accuracy increased the width of the cuff from 5 cm to 13 cm.⁵⁹

Nikolai Sergeyevich Korotkoff, a Russian army physician based at the military hospital in the town of Tsarskoye-Selo, was the first to observe the sounds made by the constriction of an artery in 1905, a discovery that enabled the estimation of diastolic blood pressure.⁵⁷ Korotkoff was working to find indications that would allow a surgeon to predict an outcome of ligation of arteries of traumatised limbs, i.e to predict whether the limb would recover or die after surgery. Korotkoff used a stethoscope and the apparatus proposed by Riva-Rocci in 1896 and established that specific sounds could be heard during the decompression of the arteries. This auscultatory method proved to be more reliable than previous palpation techniques and became the standard practice for over 100 years.^{56,57}

Despite the technology of BP measurement being available, it took several decades for the scientific community to discover and accept that elevated BP was a major risk factor for cardiovascular disease.⁶⁰ The medical care provided to President Franklin D. Roosevelt of the USA (as reviewed by Mahmood *et al*)⁶⁰ who died of heart failure in 1945 serves as a good, if not

painful illustration of the poor understanding of hypertension as a risk factor for cardiovascular disease at that time. In 1932, Roosevelt's campaign office released medical records showing his blood pressure to be 140/100 mm Hg, which did not prompt any medical intervention. The following year the President-elect chose an ear, nose, and throat specialist, Admiral Ross McIntyre, as his personal physician because headaches and sinus problems were predicted to be his main health concern.⁶¹ Between 1935 and 1941, Roosevelt's blood pressure gradually rose from 136/78 mm Hg to 188/105 mm Hg. Despite his rising blood pressure, his personal physician insisted that the President was healthy, and that his blood pressure was "no more than normal for a man of his age". On March 27, 1944, as planning of the Allied landings at Normandy, France, was underway, the President's daughter Anna Roosevelt insisted on a second opinion, and he was admitted to Bethesda Naval Hospital for dyspnoea on exertion, diaphoresis, and abdominal distension. Cardiologist Howard G Bruenn, one of only a few hundred such specialists in the entire country, attended to the President. Bruenn noted that the patient appeared "slightly cyanotic", with "blood pressure 186/108" mm Hg and a chest radiograph showing an "increase in size of the cardiac shadow". Bruenn gave Roosevelt his first diagnosis of "hypertension, hypertensive heart disease, and cardiac failure".^{60,62} Roosevelt died on April 12, 1945, at the age of 63, from cerebral haemorrhage, with a blood pressure of 300/190 mm Hg.⁶²

Epidemiological data obtained from the Framingham Heart Study, established by President Harry Truman who had been President Roosevelt's Vice President eventually led to the medical community accepting that

hypertension was a major cardiovascular risk factor.⁶³ In one of the first of many seminal papers to come from the Framingham Heart Study, Dawber *et al* in 1957 reported that hypertension defined as BP $\geq 160/95$ mmHg was associated with a nearly four-fold increase in incidence of coronary heart disease.⁶³ In 1965, Kannel *et al* reported results of 12 years of observation of 5,106 Framingham participants, finding that high blood pressure was associated with a five-fold increase in the risk of stroke.⁶⁴ These findings have been replicated in many parts of the world including Africa. In the InterStroke study, 3,000 cases (patients with first time stroke) and an equal number of controls were studied in 22 low and middle-income countries. Hypertension (self reported or blood pressure $>160/90$ mm Hg) was associated with a Population Attributable Risk for stroke of 51.8%.¹⁵ In the African InterHeart study, self reported hypertension was associated with a Population Attributable Risk for myocardial infarction of 30%.¹⁷

Accuracy of blood pressure measurement

In Riva-Rocci's seminal paper of 1896 he went to great length to discuss possible sources of error in BP measurement and how to minimize them. He noted that BP variability due to the mental state of the patient could affect BP readings and recommended that one could take 3 measurements in 3 minutes or 5 measurements over a period of 5 minutes to get a more accurate measure. He emphasized the need to have standardized conditions under which the measurements were taken for the procedure to be genuinely useful in clinical practice adding that, "if the procedures are neglected, and the doctor is satisfied with a crude reading, the method will become useless and will be quickly abandoned as a scientists' indulgence." C.P. Donnison whose

paper in the Lancet in 1929 I have referred to earlier¹, described the BP measurement technique in his study as follows:

“The instrument used was an aneroid sphygmomanometer made by Messrs. Down Bros. The rules for taking the readings, described by Halls Daly were followed closely. The first reading was always disregarded, as it was found that the African native, like his white brethren, suffers from apprehension and nervousness which frequently affect his blood pressure; in fact, in a few cases no constant reading could be obtained and no record could be made. After a few minutes a second reading was taken. The pulse rate was then noted, and a third reading taken, and if a constant figure was obtained in the last two readings a record was made.”

It is readily apparent from the above that accuracy of blood pressure measurement had been recognized as a problem from very early on.

Ambulatory Blood Pressure Monitoring

Kain *et al*⁶⁵ in 1964 were among the first to report on the use of ambulatory blood pressure monitors. In the introduction to their paper published in *Circulation* they noted that ‘casual readings of blood pressure recorded in a physician’s office or in an outpatient clinic may not be representative of the patient’s usual blood pressure’, a fact has been confirmed in many other studies.²⁰ Using a semi automatic portable blood pressure recorder that patients wore “with little inconvenience while going about their usual routine” Kain *et al* found significant differences between BP measurements taken in the clinic and those taken over a longer period while subjects underwent their

normal daily activities.⁶⁵ Over the course of the last 50 years numerous technological advances have been made that have resulted in smaller and more convenient ABPM devices, driving their use in more subjects.⁶⁶

Numerous studies have shown that the more precise measurements resulting from repeated inflations and more standardized procedures in ABPM make it a much better predictor of cardiovascular events than other BP measurement methods.²⁰ In the Ohasama study conducted in Japan, 1,332 subjects were followed up for 10.8 years. Ambulatory systolic blood pressure values consistently showed stronger predictive power for cardiovascular mortality risk than did casual blood pressure.⁶⁷ Similar conclusions were drawn from the PAMELA study conducted in Monza, Italy.⁶⁸ Recent studies have, in addition, shown that nighttime BP measurements are more predictive of cardiovascular outcomes than daytime or 24-hour measures.²⁰ Two sets of reasons for this have been postulated:

- a) Those related to improved measurement; better standardization at night of the measurements in terms of physical and mental activity as well as body position, and;
- b) Physiologic reasons, namely alterations in the sympathetic modulation of nighttime blood pressure⁶⁹, disturbed baroreflex sensitivity⁷⁰, sleep apnea⁷¹, or an increased salt sensitivity necessitating a higher blood pressure at night to drive pressure natriuresis^{72,73}.

Apart from obtaining a more accurate measure of a person's usual BP, ABPM use (as well as home BP monitoring) has led to the discovery of 2 previously undescribed BP phenotypes: white coat hypertension, in which office BP is

elevated but out of office BPs are normal; and masked hypertension, in which office BPs are normal but out of office BPs are elevated.

Although numerous studies have demonstrated the advantages of ABPM^{20,66,67,74-79}, the great majority of these have been conducted in developed countries. In addition, apart from the Jackson Heart Study that conducted ABPM on 1,015 African Americans⁸⁰, no large ABPM studies have been done among individuals of African descent. A number of studies utilizing ABPM have been conducted in Africa (Summarized in the Table below), but these have been small and have not been conducted in population-based samples.^{32,81-93} To my knowledge, the studies outlined in this thesis are the first large population-based studies conducted in sub Saharan Africa where ABPM has been used.

Table 1-1: Published studies of ABPM in Africa

First Author	Country	Population studied/Sampling scheme	Sample size
Bhagat ⁸²	Zimbabwe	Hypertensive women	25
Bochud ⁸¹	Seychelles	76 families	314
Borkum ³²	South Africa	HIV positive clinic patients	30
Ikama ⁸⁶	R. Congo	Hypertensive patients	620
Ivy ⁸⁷	Tanzania	≥ 70 year olds	79
Kengne ⁹⁴	Cameroon	Diabetic patients	71
Malan ⁹⁰	South Africa	Male teachers	94
Morar ⁹⁵	South Africa	Medical students (83 were Indian)	154
Nong- Libend ⁹⁶	Cameroon	Diabetic patients	51
Nwafor ⁸⁸	Nigeria	Hypertensive patients	412
Polonia ⁸²	Mozambique	Hypertensive patients	548
Radevski ⁹³	South Africa	Clinical trial in hypertensive patients	42
Schutte ⁸⁵	South Africa	Teachers	409
Skoularigis ⁹¹	South Africa	Clinical trial in hypertensive patients	50
Takah ⁸³	Cameroon	Patients referred to cardiology clinic	500
Thompson ⁸⁴	South Africa	20-30 year olds with normal clinic BP	352

Data obtained from a search of Pubmed.gov using the terms ABPM AND Africa

F. Determining past exposure to malaria

In order to determine whether malaria results in elevated blood pressure, we first need to find a reliable indicator of previous exposure to malaria.

Unfortunately as is the case in many acute infectious diseases, immunological markers indicating past exposure to malaria suffer from several short-comings^{97,98} that limit their use in determining long-term effects of the exposure:

- (i) Both humoral and cellular responses to malaria are poorly understood-they do not develop in everyone exposed to malaria;
- (ii) The duration of humoral and possibly cellular responses may vary according to host factors as well as repeat exposure patterns
- (ii) Responses differ depending on age at exposure and transmission intensity.

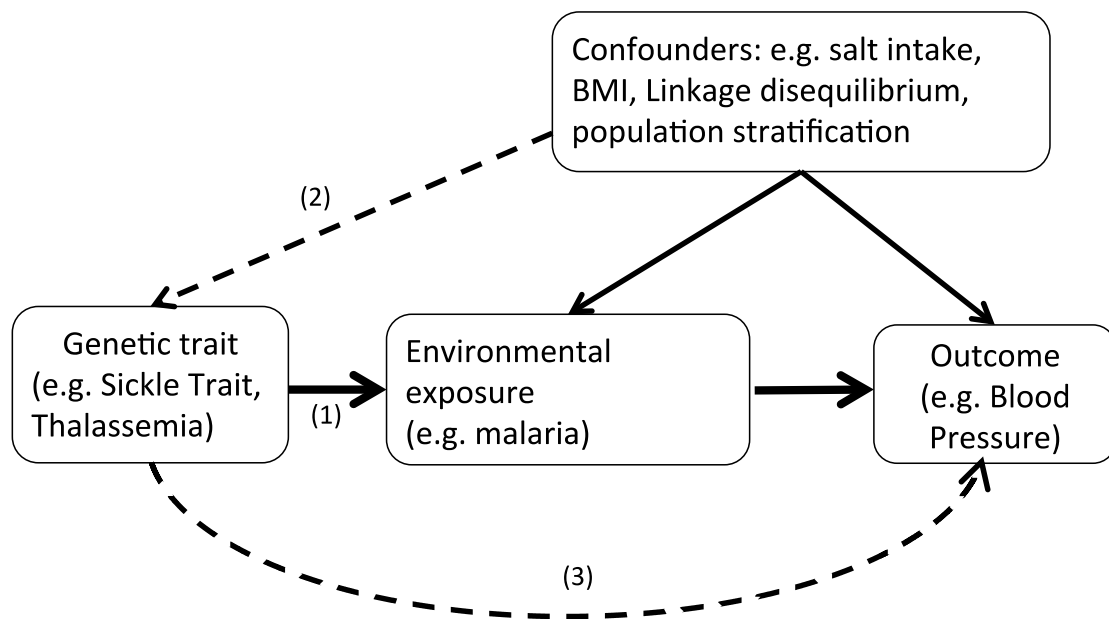
An alternative to using immunological markers for determining past malaria exposure would be to recruit participants who had been part of malaria cohort studies and were known to have experienced malaria during the time that they were being followed up. Such a cohort exists in Kilifi, where children are followed from infancy to about 10 years of age.⁹⁹ However this and other similar cohorts in Africa have been present for not more than 25 years which is below the age at which the incidence of hypertension starts to rise.¹⁰⁰

Several randomized controlled trials of malaria interventions have been conducted in Kilifi e.g. testing the effect of bednets.¹⁰¹ These would provide two groups of individuals that would only differ by malaria exposure and thus I could have tested the hypothesis by comparing blood pressure in these groups. The challenge in using these individuals for the studies again was

that the interventions had only been used for a short period and although they had worked it was likely that the randomized groups had had similar levels of exposure to malaria prior to the trial and after the trials resulting in contamination.

Mendelian Randomization (MR) is a relatively new study design in epidemiology that overcomes the weaknesses of traditional observational studies and also enables inferences to be made in situations where randomized trials are not feasible or are unethical.¹⁰² MR uses genetic variations with known biological effects as instrumental variables to represent an environmental exposure. MR exploits the principle that genotypes are not generally associated with confounders in the population and should be immune to reverse causation bias.¹⁰³ These properties reflect both the fixed nature of genetic variants as well as Mendel's first (segregation of genes) and second (independent assortment) laws of inheritance. After finding genetic polymorphisms to use as proxies, or "instruments," for a target exposure the association of the genetic instrument with the outcome of interest is then tested.¹⁰² An important advantage of MR is that genotypes can be measured with very high accuracy and reflect long-term patterns of exposure. Thus, MR approaches are less susceptible to biases arising from measurement error.¹⁰³ There are three main requirements when conducting MR studies as explained below and in Figure 1-2.¹⁰³ This is followed by a discussion of the considerations that led to the choice of the genetic variants that I used in my studies.

Figure 1-2: Causal diagram showing requirements of MR studies



Absence of arrows between two variables indicates no relationship between them. Numbers (1, 2, 3) represent MR assumptions (explained in the text) and dashed arrows indicate possible violations of these assumptions.

(1) The genetic variant should have a true association with the exposure of interest:

Malaria has exerted the strongest known selective pressure on the human genome.¹⁰⁴ Of all the known malaria associated polymorphisms, sickle cell trait (SCT) provides the highest level of protection and also has the advantage of being relatively common.⁵² SCT has been used in two MR studies in Africa, one by Professor Anthony Scott *et al* showing that malaria is associated with bacteremia¹⁰⁵ and another by Kang *et al* demonstrating increased risk of malnutrition in children who suffered from malaria.⁴⁵ Alpha thalassemia, which is even more common than SCT¹⁰⁶ provides comparatively less protection against malaria episodes¹⁰⁷ and could be used to check for a dose response effect if the malaria-high blood pressure hypothesis is true (provided that neither SCT nor alpha thalassemia violate the other assumptions).

(2) The genetic instrument should not be associated with any confounders of the exposure-outcome relationship

This can be examined by comparing different characteristics in the two genetic groups. It should be noted however that despite the supposedly random distribution of possible confounders between genetic groups, many MR studies (e.g. in Palmer et al¹⁰⁸, Ehret et al¹⁰⁹ and Ferrence et al¹¹⁰) usually adjust for age and sex, which usually have very strong influences on most outcomes. The analysis plan for the MR study (contained in the Appendix) delves further into the issue of adjusting for confounders.

(3) The genetic instrument should not influence the outcome in the absence of the exposure.

This is a special form of confounding, also known as pleiotropy.¹⁰² This meant that in order to use SCT and alpha thalassemia to examine whether malaria influences blood pressure, I would first need to confirm that these genetic variants do not influence blood pressure in the absence of malaria. I checked whether this was the case using the same cohort for alpha thalassemia and sickle cell trait in Nairobi and the results of this investigation are as outlined in Chapters 4-6.

A potential disadvantage of using SCT as a marker for exposure to malaria is that it is a non-specific marker in that it protects against both severe and non-severe malaria. In addition, it's possible effect on maternal malaria is not backed by many studies¹¹¹ and it also does not protect against asymptomatic malaria parasitaemia.¹¹² The implications of this are discussed further in Chapter 7.

G. Background Information on the Study Populations used in this Thesis

The studies in this thesis were conducted among participants derived from the Nairobi Urban Health and Demographic Surveillance System (NUHDSS) and the Kilifi Health and Demographic Surveillance System (KHDSS). Here I briefly describe important characteristics of the populations at these two sites. The NUHDSS (Figure 1-3) is located in Viwandani and Korogocho, two slums in Nairobi and has a total population of ~70,000.¹¹³ The prevalence of hypertension (using clinic BP measurements) in the area is 18% while diabetes is present in 6% of the population.²⁵ Obesity (BMI >25 kg/m²) is present in 42% of women in the area and 16% of men.²⁵ The major causes of death (using verbal autopsy records) are HIV (17%), injuries (21%) and cardiovascular disease (8%).¹¹⁴

Figure 1-3: Location of NUHDSS and Population Structure
(adapted from Beguy et al¹¹³)

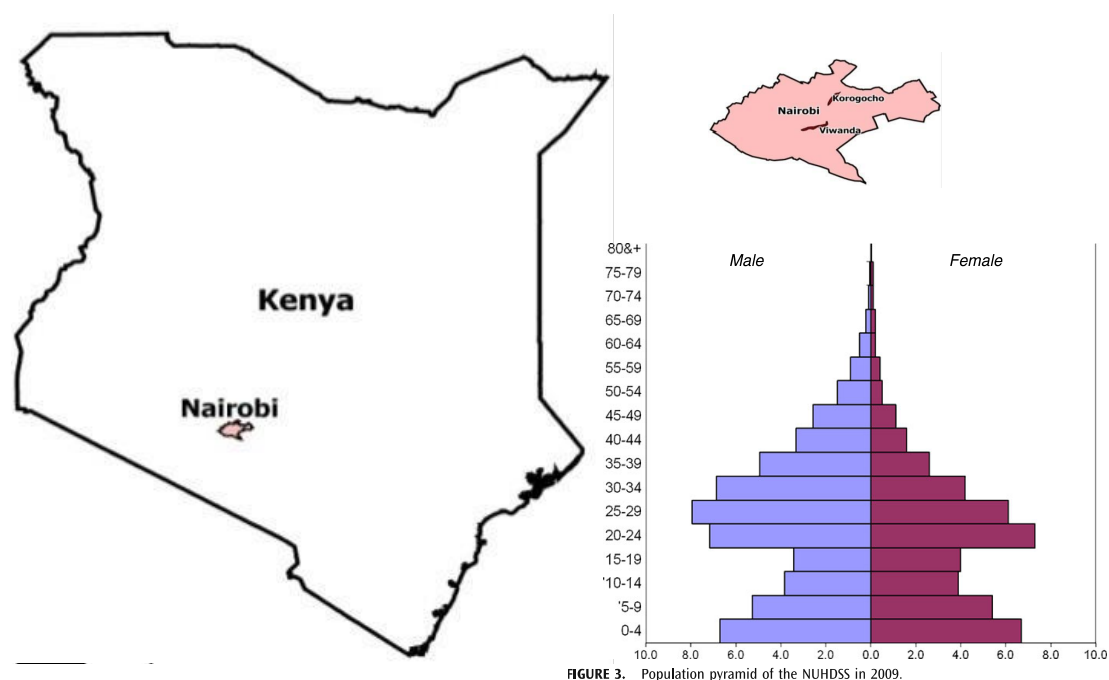
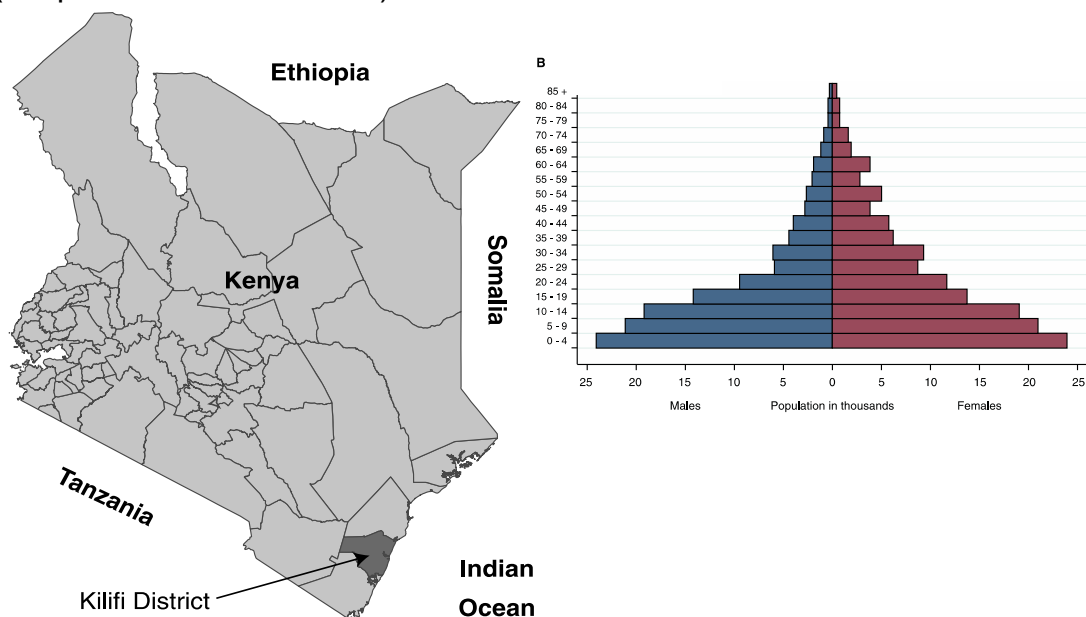


FIGURE 3. Population pyramid of the NUHDSS in 2009.

The KHDSS is located within Kilifi County (Figure 1-4) along the Indian Ocean coast and has a total population of ~300,000.¹¹⁵ The prevalence of hypertension (using clinic BP measurements) in the area is 26% and 17% when using ambulatory blood pressure monitoring.⁴² Body mass index and salt consumption appear to be low in this population.⁴² Diabetes is the third leading cause of Disability Adjusted Life Years lost in the area.⁴¹ The major causes of death in adults (as assessed using verbal autopsy) are HIV, stroke and neoplasms.¹¹⁶

Figure 1-4: Location of KHDSS and Population Structure
(adapted from Scott et al¹¹⁵)



Summary

In this chapter I have reviewed the literature of the epidemiology of high blood pressure in Africa, described the antecedents of the malaria-high blood pressure hypothesis and justified the use of Mendelian Randomization to test the hypothesis using ambulatory blood pressure monitoring. The remainder of the thesis is structured as follows:

- (1) A descriptive population-based study in Kilifi to examine the effect of using ambulatory blood pressure monitoring on population estimates of hypertension (Chapter 2)
- (2) An outline of the epidemiological and pathophysiologic basis for the malaria-high blood pressure hypothesis (Chapter 3)
- (3) Results of the Mendelian Randomization studies testing the hypothesis (Chapters 4-6).
- (4) Discussion of the results of the studies and their implications (Chapter 7).

It is hoped that by providing a better description of the problem of blood pressure elevation and malaria as a possible underlying cause, the studies in this thesis will catalyze an improvement in public health measures to mitigate its consequences.

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Chapter 2. Research paper I: Clinical and Epidemiological Implications of 24-hour Ambulatory Blood Pressure Monitoring for the Diagnosis of Hypertension in Kenyan Adults: A Population Based Study

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Principal Supervisor	Prof Anthony Scott
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Clinical and Epidemiological Implications of 24-hour Ambulatory Blood Pressure Monitoring for the Diagnosis of Hypertension in Kenyan Adults: A Population Based Study

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ABSTRACT

Background

The clinical and epidemiological implications of using ambulatory blood pressure monitoring (ABPM) for the diagnosis of hypertension have not been studied at population level in sub-Saharan Africa. We examined the impact of ABPM use among Kenyan adults.

Methods & Results

We performed a nested case-control study of diagnostic accuracy. We selected an age-stratified random sample of 1,248 adults from the list of residents of the Kilifi Health and Demographic Surveillance System in Kenya. All participants underwent screening blood pressure measurement. All with screening BP $\geq 140/90$ mmHg and a random subset of those with screening BP $< 140/90$ mmHg were invited to undergo ABPM. Based on the two tests, participants were categorized as: sustained hypertensive, masked hypertensive, white-coat hypertensive or normotensive.

Analyses were weighted by probability of undergoing ABPM. Screening BP $\geq 140/90$ mmHg was present in 359/986 individuals, translating to a crude population prevalence of 23.1% (95%CI 16.5-31.5). Age standardized prevalence of screening BP $\geq 140/90$ mmHg was 26.5 (19.3-35.6) %. On ABPM, 186/415 individuals were confirmed hypertensives, crude prevalence 15.6(9.4-23.1)%, age-standardized prevalence 17.1(11.0-24.4) %. Age standardized prevalence of masked and white-coat hypertension were 7.6(2.8-13.7) % and 3.8(1.7-6.1) % respectively. The sensitivity and specificity of screening BP measurements were 80 (73-86) % and 84(79-88) %

respectively. BP indices and validity measures showed strong age related trends.

Conclusions

Screening BP measurement significantly overestimated hypertension prevalence while failing to identify ~50% of true hypertensives diagnosed by ABPM. Our findings suggest significant clinical and epidemiological benefits of ABPM use for diagnosing hypertension in Kenyan adults.

Key words

Ambulatory blood pressure monitoring; Sub-saharan Africa; Hypertension; Diagnostic accuracy; Masked hypertension; White coat hypertension

INTRODUCTION

Across sub Saharan Africa (sSA), numerous studies have been conducted to estimate the prevalence of hypertension in various settings.^{1,2} These data are necessary for planning public health activities, especially given the projected increase in the burden of non-communicable disease in the region.³ There however remains a substantial contribution of communicable diseases, increasing the need for accurate data to guide resource allocation between competing health priorities.⁴

The measurement of blood pressure (BP) is subject to multiple sources of variation⁵ that will have an impact on both the epidemiology and clinical management of hypertension. Measurement error arising from technical problems has been minimized by using validated criteria for BP monitors.⁶ It is possible to minimize observer error in reading BP values by using automated devices and following a defined measurement procedure. Despite such improvements, these protocols only provide a momentary assessment of BP, which can be influenced by a range of environmental and psychological factors.⁷ Ambulatory blood pressure monitoring (ABPM) reduces this limitation and is increasingly used for both clinical and epidemiological purposes.^{7,8} In high-income settings ABPM is a significantly better predictor of future cardiovascular risk than office or home measurement.⁷ It is also the only way to detect abnormal nocturnal dipping patterns that are an independent risk factor for future cardiovascular events⁹ and appear to be more common in populations of African descent.^{10 11} In addition ABPM enables the

measurement of the ambulatory arterial stiffness index (AASI), an independent predictor of cardiovascular outcomes, especially stroke.¹²

The use of ABPM facilitates better prescription patterns among established hypertensive individuals and helps identify masked hypertensives and individuals with abnormal dipping patterns who would otherwise be missed when using casual (standard) BP measurement methods. The increased diagnostic performance of ABPM makes it cost effective in both primary and specialist care in developed world settings.^{13,14} The high initial cost of ABPM devices has, however, precluded their widespread use in low-income settings in sSA and there is little information to assess the potential clinical and public health benefits of ABPM in such settings.

We conducted Shinikizo la Damu (ShinDa), a population-based study in rural coastal Kenya to determine 24-hour BP profiles in adults and compare these with parameters derived from screening BP measurements.

METHODS

This study was conducted from April 2013 to May 2014 in the Kilifi Health and Demographic Surveillance System (KHDSS) located along the coast of Kenya. The 900km² covered by the KHDSS and a population of 300,000 people makes it one of the largest demographic surveillance areas in Africa both in terms of area and population.¹⁵ Within the KHDSS the total fertility rate (4.73), crude birth rate (34.7/1000/year), population growth rate (2.79%/year) and proportion of the population aged <15 years (49%) are similar to those across Kenya. However, the under 5-mortality ratio (41 per 1,000 births) and

HIV prevalence (4.9% among antenatal clinic attendees) are lower than the national averages (74 per 1,000 births and 8.0%, respectively).¹⁶ We have previously documented a double burden of infectious and non-communicable disease in the area, similar to other parts of the developing world.¹⁷

We selected an age-stratified random sample of 1248 adults from the list of KHDSS residents. The sample size was designed to generate a population level estimate of the true prevalence of hypertension with confidence intervals of approximately $\pm 5\%$. We have previously documented a significant distance related bias in presentation to hospital in the study area.¹⁷ In order to minimize this bias, study procedures were carried out at the participants' homesteads. Trained staff visited all subjects who had been selected to participate in the study at their homes. A total of three attempts were made at finding a selected subject before concluding that they could not be found. No replacement was done for participants who could not be found. Women who reported that they were pregnant were excluded from the study.

We used a nested case-control diagnostic accuracy study design in which all subjects with elevated screening BP and a random subset of those with normal screening BP were invited to undergo ABPM.¹⁸ We weighted analyses to account for differential probability of undergoing ABPM based on the result of screening BP measurement¹⁸. We aimed to have 24-hour ABPM performed on equal numbers of subjects with and without elevated screening measurements ($\geq 140/90$ mmHg), assuming that the prevalence of elevated screening BP would be $\sim 30\%$.

All subjects were first asked whether they had had a previous diagnosis of hypertension and whether they were on anti-hypertensive medication. We then took a screening BP measurement using a validated Omron™ M10-IT blood pressure machine. An appropriately sized cuff was placed on the non-dominant arm after the subject had been seated for at least 5 minutes. Three BP measurements were taken over a 5-minute period and the mean of the last 2 measurements was recorded as the screening BP value. All participants whose screening BP was ≥ 140 and/or 90mmHg were invited to undergo 24-hour ABPM. A random sample of 292 (~30%) of individuals with screening BP $< 140/90$ mmHg were invited for ABPM, and analyses were weighted (see statistical analyses section below) to account for this. We used validated Omron™ M24/7 ambulatory blood pressure machines for the 24hr ABPM measurements.¹⁹ The machines were programmed to take BP measurements every 20-30 minutes from 0600-2200 hrs and every 40 minutes from 2200-0600 hrs. Ambulatory blood pressure monitoring was performed within one week of the screening BP measurements in all cases. The same field staff conducted the screening and ABPM measurements and were not blinded to any of the results.

A subset of 200 individuals was selected at random from the original sample of 1248 individuals and requested to participate in additional investigations. We first measured their weight and height using a validated SECA 874™ weighing machine and a portable stadiometer (Seca 213™), respectively. We then requested them to provide a spot urine sample as well as a 24-hour

urine sample for determination of sodium, potassium, albumin and creatinine levels.

Statistical methods

ABPM data were excluded from the analyses if they failed the following criteria, as specified by the European Society of Hypertension: minimum 20 daytime and minimum 7 nighttime readings, where day was defined as 0900-2100 hrs and night as 0100-0600 hrs.⁷ The same time periods were used to determine average daytime and nighttime blood pressures and to evaluate dipping status. Time weighting was applied in calculating average BP values for all time periods.²⁰

We defined screen positives as subjects whose last 2 screening BP measurements had a mean $\geq 140/90$ mmHg and confirmed hypertensives were defined as those who met any of the following three criteria: 24-hr BP average $\geq 130/80$ mmHg; isolated daytime hypertension (daytime BP average $\geq 135/85$ mmHg and nighttime BP average $< 120/70$ mmHg); isolated nocturnal hypertension (nighttime BP average $\geq 120/70$ mmHg and daytime BP average $< 135/85$ mmHg).⁷

Validity measures were computed using data from participants who were not taking anti-hypertensive medications. We categorized these subjects using the combination of screening BP measurements and ABPM into four groups: sustained hypertensives (screen positive and confirmed hypertensive on ABPM); white coat hypertensives (screen positive, not confirmed hypertensive on ABPM); masked hypertensives (screen negative, confirmed hypertensive

on ABPM) or normotensives (screen negative, not confirmed hypertensive on ABPM).²¹ The four categories were used to compute age stratified measures of validity of the screening BP measurements with ABPM as the reference standard.^{22,23} All summary measures were weighted according to the probability of investigation with ABPM in the design.²⁴ Confidence intervals for these measures were obtained using the recommended bootstrap procedure with 1000 replications.²⁴

In subjects who reported that they were taking anti-hypertensive medications, those who met the criteria for white coat hypertension were labeled as having pseudo-resistant hypertension⁷ while those who met the criteria for masked hypertension were labeled as masked uncontrolled hypertensives.⁷

Dipping status was defined using ABPM data only, using previously described methods.²⁵

We computed two additional indices using the ABPM data; 24-hour pulse pressure (the mean of the differences between systolic and diastolic BP values) and the ambulatory arterial stiffness index (AASI) using previously published methods.²⁶

We calculated the local prevalence of each index by weighting the age specific proportions by the age-structure of the Kilifi population. Age standardization for all summary population parameters was performed using weights derived from the WHO standard population.²⁷ Summary statistics computed included means, medians, proportions and rates as appropriate.

All analyses were conducted using Stata™ Version 12 software (College Station, Texas).

The Kenya Medical Research Institute's Ethical Review Committee approved the study and all subjects provided written informed consent.

RESULTS

Of the 1248 subjects selected to participate in the study, 1150 (92%) were found at home and invited to participate in the study. Of these, 986 (86%) gave consent and underwent screening BP measurement (Figure 2-1). Of these, 359 (36%) subjects were screen positive for hypertension (screening BP \geq 140/90 mmHg). All 359 screen positives and 292 screen negatives were invited to undergo ABPM. Of all those invited to undergo ABPM, 477 (73%) actually underwent it. Of the 200 individuals selected to have urine electrolyte and anthropometric measurements, 164 (82%) did so.

Table 1 compares the characteristics of all 986 subjects who had screening BP measurements, the subset of 651 selected to undergo ABPM and the 477 who actually underwent ABPM. The group of subjects that had ABPM performed had higher screening systolic BP (+5mmHg CI 4.2-5.9) and were older (+3.9 years 95% CI 3.4-4.3) compared to the entire group of subjects that was selected for the study. There were no significant differences between those selected to undergo ABPM (n=651) and those who actually underwent the procedure (n=477).

Of the 477 subjects undergoing ABPM 415 (87%) had acceptable readings; unacceptable (<20 daytime and/or <7 nighttime) readings were significantly more common among screen negatives than among screen positives (17% vs. 11%, $p=0.036$). Data from subjects with unacceptable ABPM readings were dropped from further analyses.

Six of the 415 subjects with acceptable ABPM recordings were on anti hypertensive medication.

Relationship between screening BP measurements and ABPM derived measures

Among the 415 subjects who had both screening and 24-hour ABPM measurements, mean screening systolic and diastolic BP were 140mmHg (95% CI 138-143) and 84mmHg (95%CI 83-85) respectively. Corresponding mean 24-hour ABPM systolic and diastolic BPs were 123mmHg (95%CI 121-125) and 72mmHg (95%CI 71-73). The average difference between mean systolic screening and 24 hr ABPM values was 16.8mmHg (95% CI 15-18.6). Mean screening diastolic BPs were 12.2 (95% CI 11.2-13.2) mmHg higher than corresponding 24-hour ABPM values (Figure 2-2).

Age-standardized mean screening systolic and diastolic blood pressures for the population in Kilifi were 128mmHg (95%CI 102-162) and 79mmHg (95%CI 62-101) respectively. 24-hour mean systolic and diastolic blood pressures for the population were 117mmHg (95%CI 114-120) and 70mmHg (95%CI 68-72), respectively.

ABPM-derived mean BPs were lower than those obtained using screening measurement methods for all age groups (Table 2-2). The relationship between 24-hour ABPM derived parameters (except for AASI and diastolic BPs) and age approximated a J-shape. Mean AASI and pulse pressure increased linearly with age (AASI, 0.03 [CI 0.02-0.05] and pulse pressure 3.3 [CI 1.5-5] per 10 year increase in age. The difference between ABPM derived and screening BP values increased with age.

Prevalence of hypertension using casual BP measurement and ABPM

All reported KHDSS population parameters are age standardized unless otherwise specified. The crude prevalence of hypertension in the KHDSS using screening measurements only was 23.1%(95%CI 16.5-31.5). The crude prevalence of hypertension in the KHDSS using ABPM was 15.6% (95%CI 9.4-23.1). Using only screening BP values, the reported age-standardized prevalence of hypertension would have been 26.5% (95%CI 19.3-35.6). The age-standardized prevalence of true hypertension (as determined by ABPM) in the KHDSS was 17.1% (95%CI 11.0-24.4) (Table 2-2). There was a marked increase in prevalence of screen positives and true hypertension with increasing age (Table 2-3).

The prevalence of masked hypertension overall was 7.6% (95% CI 2.8-13.7%) and this was inversely associated with increasing age. White coat hypertension was present in 3.8% (95%CI 1.7-6.1%) of the population and its prevalence increased with age. The non-dipping blood pressure pattern was present in 8.5% (95%CI 3.1-15.3%) of the population, its prevalence being highest in the 70-79 year age band. Figure 2-3 displays the standardized

population prevalences of screen positives, true hypertensives, white coat hypertensives, masked hypertensives and non-dippers.

Two of the six individuals on anti-hypertensive medication who underwent ABPM had pseudo resistant hypertension. No cases of masked uncontrolled hypertension were detected.

Validity of screening BP measurements

The overall sensitivity and specificity of screening BP measurements for diagnosing hypertension in the population were 80% (95% CI 73-86%) and 84% (95%CI 79-88%), respectively. Sensitivity improved with increasing age while specificity decreased (Table 2-4). Overall positive and negative predictive values were 80% (95% CI 74-85%) and 84% (95%CI 79-89%), respectively. Sensitivity and PPV increased with age; specificity and NPV decreased with age. The likelihood ratio (LR) positive was 4.9 (95%CI 3.7-6.8). LR positive was highest in the 30-49 year age groups although the confidence intervals were wide. Likelihood ratio negative overall was 0.2 (95%CI 0.2-0.3). No significant age related trend was observed in the LR negative values. Interval likelihood ratios based on quintiles of the screening systolic and diastolic blood pressures are displayed in Table 2-5. Screening BP measurements performed best in predicting true diagnostic category in individuals with diastolic BPs of less than 80mmHg and systolic BP of 118-129mmHg.

DISCUSSION

This is, to our knowledge, the first population based study that has assessed the validity of screening BP measurements versus ABPM in sub-Saharan Africa. We found a high prevalence of hypertension and poor validity of the screening BP measurements.

As well as inflating the true prevalence of hypertension in the community by 53% (from 17% to 27%), screening BP measurements failed to identify a significant proportion of the population that were identified as having hypertension using ABPM: nearly half of the hypertensive individuals in this study had masked hypertension. Screening BP measurements would have failed to identify this population. In addition, 4% of the population had white coat hypertension and were at risk of being unnecessarily placed on treatment.^{28,29} Overestimating the total number of individuals with hypertension while failing to identify a significant proportion of the population with the condition would lead to inefficient and ineffective use of scarce health resources. Our findings imply that similar to the situation in developed country settings, efforts to control the burden and consequences of hypertension in sub Saharan Africa are likely to be significantly impaired by current screening methods.

The prevailing consensus is that hypertension in developing countries is more prevalent in urban than rural areas.³⁰ However, data from our study, conducted in rural Kenya, indicate that hypertension is not exclusively an urban disease, and this is supported by the previously documented finding of a high burden of stroke and heart failure in the area¹⁷ as well as the recently

published 2015 Kenyan Ministry of Health STEPs survey, which using methods similar to the screening strategy employed here, found a prevalence of raised BP of 25% in rural areas, remarkably similar to what we found on screening in this study.³¹ The prevalence of raised BP in urban areas in the same (STEPS) survey was 21%. Lifestyle habits related to urbanization may therefore have a smaller role in elevating BP in African population than previously thought.

Urinary sodium levels in Kilifi were comparable to those reported by Dahl in Alaskan eskimos in 1958 where the prevalence of hypertension was zero.³² In contrast, results from Nairobi, Kenya in the Intersalt study found lower median 24hr sodium levels of 53mmol/day, with a prevalence of hypertension of 5%³³, although this was not age-standardized. Mean Body Mass Index (BMI) levels in our population were well within the normal ranges. This combination of a high prevalence of hypertension in a rural region with relatively low levels of classical risk factors calls for more detailed study into the pathogenesis of this important condition in tropical Africa.

Previous studies in sSA utilizing ABPM have found a high prevalence of the white coat effect among treated hypertensives.³⁴ Although we found white coat hypertension to be present in 4% of the population, the more significant finding was the high prevalence of masked hypertension at 8% of the population. Given the increased risk of cardiovascular events in this population^{21,35}, strategies aimed at identifying these individuals are urgently needed. The observation that younger age is associated with masked hypertension³⁶ may help direct targeted ABPM at this group. It may also be

possible to identify individuals with masked hypertension by measuring arterial stiffness indices.³⁵

The strengths of this study include its population-based design, the use of reference standard methods for determining BP and utilizing a large and representative age-stratified sample. Limitations include the potential bias introduced by the nested case-control design used despite demonstration that this approach yields essentially similar validity results as compared to full cohort studies.¹⁸ As expected from the sampling strategy used, individuals who underwent ABPM had higher screening systolic BP and were slightly older than the baseline group. Weighting of analyses to correct for the differential probability of undergoing ABPM based on screening results may not have completely eliminated the bias. In addition a larger proportion of screen negatives had poor quality ABPM readings; these limitations mean that although our reported prevalence of masked hypertension was similar to that reported in other studies^{21,37,38}, it may well have been an under-estimate. The fact that BP measurements were done at home and by non-medical personnel could also have reduced our ability to detect the white-coat effect. Taken together, probable underestimation of masked and white-coat hypertension suggest that there could have been more subjects who were misclassified using the screening measurement method, possibly strengthening the case for use of ABPM.

Several issues however need to be considered before a recommendation to adopt ABPM for diagnosis of hypertension in sSA is made; We observed a modest response rate with 27% of those referred for ABPM failing to undergo

the procedure. This suggests that there may be difficulties regarding acceptability of ABPM in the population. Cost effectiveness studies assessing the potential benefit of ABPM in sSA settings are also needed, as are studies to determine whether blood pressure defined by ABPM provides better targeting of blood pressure reduction strategies to reduce vascular morbidity.

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Conflicts of Interest/Disclosures

None of the authors have any conflicts of interest or disclosures to report.

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TABLES

Table 2-1: Characteristics of study subjects

	All (N=986)	Selected to undergo ABPM (N=651)	ABPM performed (N=477)
Women (n, %)	588(60)	400(61)	302(63)
Age in years (mean, sd)	50(19)	53(18)	54(18) [±]
Screening BP in mmHg (mean, sd)			
Systolic	134(26)	142(28)	139(28) [±]
Diastolic	81(13)	84(15)	82(15)
[§] BMI, kg/m ² (median, IQR)	21(19-24)	21(19-24)	21(19-23)
[§] Na excretion mmol/24h(median, IQR)	63(47-92)	62(47-90)	61(47-80)
[§] K excretion mmol/24h (median, IQR)	25 (18-38)	25(19-38)	25(19-38)
[§] Spot Urine Albumin to Creatinine ratio mg/mg (median, IQR)	0.02 (0.01-0.04)	0.02 (0.01-0.04)	0.02 (0.01-0.04)
On medication for hypertension (n, %)	22(2)	16(2)	7(1)

Abbreviations: ABPM, Ambulatory blood pressure monitoring; BMI, Body mass index; IQR, interquartile range; SD, Standard deviation.

[§] BMI, Na, K, UACr ratio based on 164 subjects.

[±] p < 0.001 (Comparison between all screened subjects (N=986) and those that underwent ABPM (N=477)).

Table 2-2: Mean BP, pulse pressure and AASI, by age, derived from screening BP and 24 hour ABPM

Age Group	24 hour ABPM measures									
	Mean Screening BP		Daytime mean		Night time mean		24 hour mean		Mean Stiffness indices	
	Systolic	Diastolic	Systolic	Diastolic	Systolic	Diastolic	Systolic	Diastolic	Pulse Pressure	AASI
18-29	120(102-144)	76(60-92)	118(115-122)	71(69-73)	107(103-112)	60(58-63)	115(111-118)	68(66-70)	47(45-49)	0.31(0.27-0.36)
30-39	120(96-151)	77(61-101)	112(110-115)	71(69-73)	101(98-105)	60(58-63)	109(106-112)	68(66-70)	41(40-43)	0.38(0.34-0.43)
40-49	128(100-164)	81(64-104)	119(116-122)	74(72-76)	106(104-109)	63(61-65)	115(112-118)	70(68-72)	45(43-46)	0.41(0.37-0.45)
50-59	135(100-176)	83(61-106)	125(122-128)	76(74-79)	114(111-117)	66(64-68)	122(119-125)	73(71-75)	49(47-50)	0.41(0.38-0.45)
60-69	147(109-196)	85(66-112)	131(128-134)	77(75-79)	122(119-125)	68(66-71)	127(125-131)	74(72-76)	53(51-56)	0.49(0.46-0.52)
70-79	153(116-196)	84(63-107)	132(128-135)	72(70-74)	129(125-133)	66(64-68)	131(127-134)	70(68-72)	60(58-63)	0.53(0.48-0.57)
80+	156(113-217)	86(66-110)	137(130-143)	75(70-80)	129(123-135)	68(64-71)	134(127-140)	72(68-77)	62(57-66)	0.52(0.47-0.56)

Abbreviations: AASI, Ambulatory arterial stiffness index; ABPM, ambulatory blood pressure monitoring; BP, blood pressure.
Note: Units for all measurements except AASI are in mmHg. AASI is an index with no units. Statistics are mean and 95% confidence interval.

Table 2-3: Age specific prevalence of hypertension using screening measurement and ABPM

Age	Screen Positives	Hypertension [§]	Masked HT [±]	White coat HT [∞]	Non dipping status [¶]
18-29	11.0(6.5-17.3)	10.9(4.3-19.5)	10.9(4.3-19.5)	2.9(1.5-4.4)	6.5(0.0-15.2)
30-39	13.1(8.1-20.1)	12.5(5.7-21.2)	11.5(4.9-19.7)	1.0(0.2-2.0)	9.8(3.3-18.0)
40-49	26.8(19.3-36.2)	11.0(6.5-16.1)	4.5(1.1-9.0)	3.7(1.6-6.2)	5.3(2.0-9.5)
50-59	43.8(34.4-55.0)	20.2(15.6-25.5)	2.8(0.7-5.6)	7.0(3.8-10.3)	6.8(3.6-10.7)
60-69	55.4(44.7-67.8)	33.4(27.7-39.2)	3.1(1.0-5.7)	3.9(1.3-7.1)	12.1(7.7-16.5)
70-79	65.0(51.4-81.1)	47.1(39.7-52.6)	2.7(0.5-4.8)	10.0(5.0-16.0)	24.2(17.4-31.0)
80+	68.8(47.3-96.6)	46.9(35.2-58.3)	3.9(0.0-7.8)	8.6(0.0-17.2)	12.2(4.0-22.4)
All**	26.5(19.3-35.6)	17.1(11.0-24.4)	7.6(2.8-13.7)	3.8(1.7-6.1)	8.5(3.1-15.3)

Abbreviations: ABPM, ambulatory blood pressure monitoring; BP, blood pressure; HT, hypertension.

Notes:

[§]Hypertension defined according to European Society of Hypertension 2013 guidelines¹: 24hr BP > 130/80 mmHg OR day BP > 135/85 mmHg OR nocturnal BP > 120/70 mmHg. Nighttime= 0100-0600 hrs. Daytime = 0900-2100 hrs¹.

[±]Masked hypertension: casual BP <140/90 mmHg but meets criteria for hypertension on ABPM¹. Individuals with masked hypertension are included in the group with true hypertension.

[∞]White coat hypertension: Casual BP >140/90 mmHg, but not meeting criteria for hypertension on ABPM¹.

[¶] Non-dipping status: Ratio of average nighttime BP to average daytime BP ≥1.0.

^{**}Summary prevalences are age standardized to World Health Organization population.

Table 2-4: Validity of measures of casual BP method compared to ABPM

Age category	Sensitivity	Specificity	PPV	NPV	LR positive	LR negative
18-29	0.0(0.0-0.0)	95.0(90.5-98.0)	0.0(0.0-0.0)	83.3(69.7-96.1)	0.0(0.0-0.0)	1.1(1.0-1.1)
30-39	9.7(2.5-24.9)	98.4(96.5-99.6)	55.6(23.6-88.9)	84.1(73.2-93.3)	6.1(1.3-31.5)	0.9(0.8-1.0)
40-49	60.8(35.8-88.7)	91.8(85.4-96.6)	63.6(45.1-83.3)	90.9(80.5-97.7)	7.5(3.4-20.8)	0.4(0.1-0.7)
50-59	87.5(74.8-96.9)	74.8(62.0-86.0)	72.3(59.5-84.5)	88.6(76.1-97.1)	3.4(2.2-6.3)	0.2(0.0-0.3)
60-69	91.2(83.4-97.0)	71.6(53.3-87.4)	86.0(75.9-94.3)	80.6(64.5-92.9)	3.2(1.9-7.0)	0.1(0.0-0.3)
70-79	94.2(88.1-98.9)	24.4(8.5-49.2)	81.7(70.4-90.9)	54.5(25.0-85.7)	1.2(1.0-1.8)	0.2(0.0-0.8)
80+	92.1(81.5-100.0)	31.2(0.0-100.0)	84.2(64.7-100.0)	50.0(0.0-100.0)	1.3(0.9-3.0)	0.2(0.0-1.1)
All[§]	79.9(73.0-86.0)	83.7(79.1-87.9)	79.5(74.0-84.6)	84.2(78.6-89.3)	4.9(3.7-6.8)	0.2(0.2-0.3)

Abbreviations: ABPM, ambulatory blood pressure monitoring; BP, blood pressure; LR, likelihood ratio; NPV, negative predictive value; PPV, positive predictive value.

[§]Summary measures are age standardized to World Health Organization population.

Table 2-5: Interval likelihood ratios for screening systolic and diastolic blood pressures

SBP interval (mmHg)	<u>Systolic BP Interval LRs</u>		DBP interval (mmHg)	<u>Diastolic BP Interval LRs</u>	
	LR positive	LR negative		LR positive	LR negative
<118	0.0(0.0-0.0)	1.0(1.0-1.0)	<73	7.3(2.4-31.4)	0.6(0.4-0.9)
118-129	7.2(1.4-39.0)	0.8(0.6-1.0)	74-80	9.8(4.5-38.7)	0.3(0.1-0.6)
130-142	3.1(1.7-6.1)	0.4(0.2-0.7)	80-85	4.6(2.5-10.9)	0.4(0.2-0.6)
143-159	1.0(1.0-1.0)	-	86-94	1.6(1.1-2.3)	0.3(0.0-0.7)
>160	1.0(1.0-1.0)	-	>95	1.0(1.0-1.0)	-

Abbreviations: LR, likelihood ratio; DBP, diastolic blood pressure; SBP, systolic blood pressure.

Notes:

SBP and DBP intervals were determined by dividing the screening blood pressure values into quintiles.

Empty cells indicate that there was insufficient data to calculate validity measures.

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Figure Legends:

Figure 2-1:

Study recruitment profile

Abbreviations: ABPM, ambulatory blood pressure monitoring; BP, blood pressure; KHDSS, Kilifi Health and Demographic Surveillance System

Figure 2-2: Comparison of screening and ABPM blood pressure distributions

A. Systolic

B. Diastolic

ABPM indicates ambulatory blood pressure monitoring

Figure 2-3: Population prevalences of different BP indices. Data are derived from Table 2-3

HTN indicates hypertension

Figure 2-1: Study recruitment profile

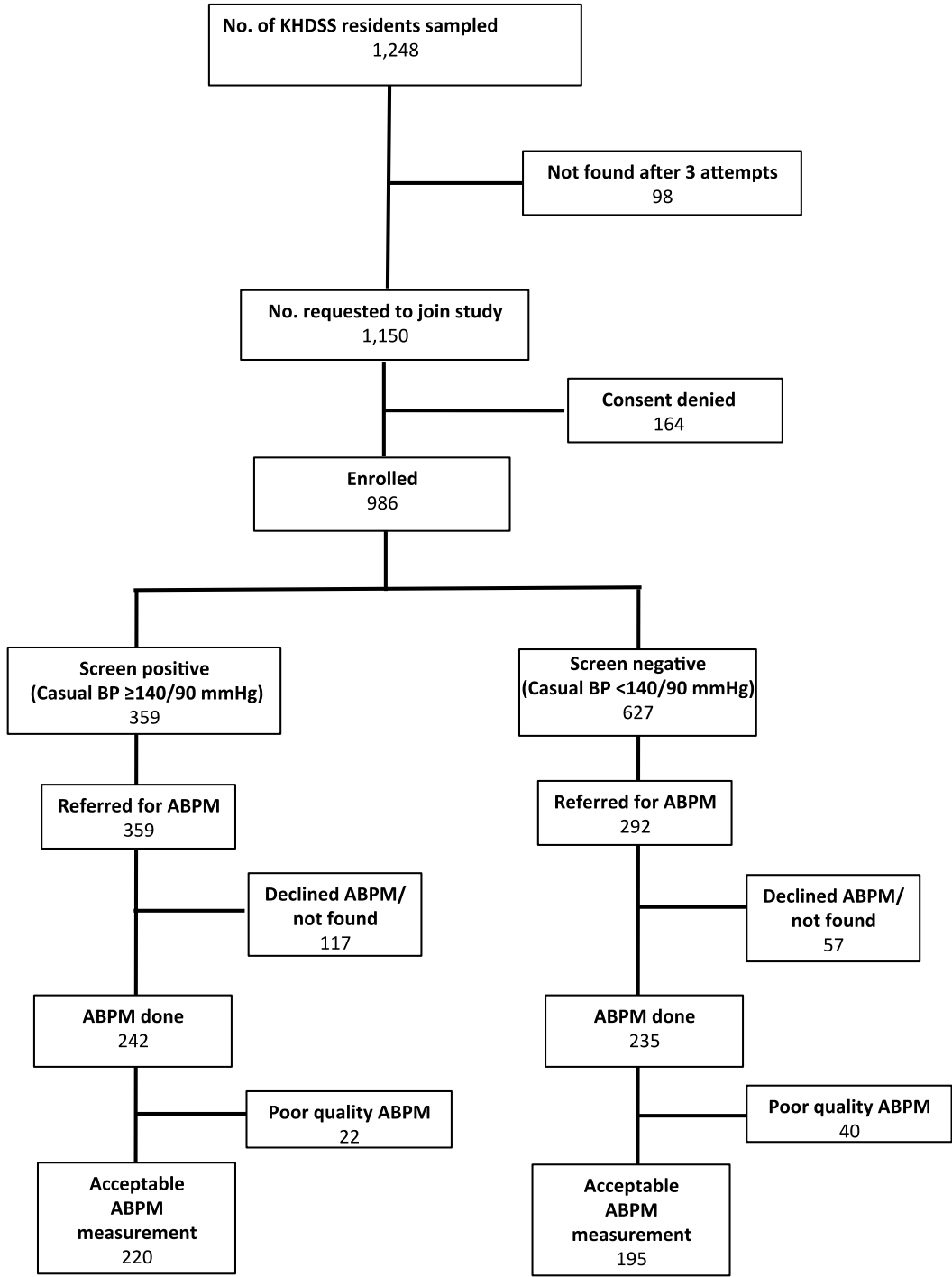
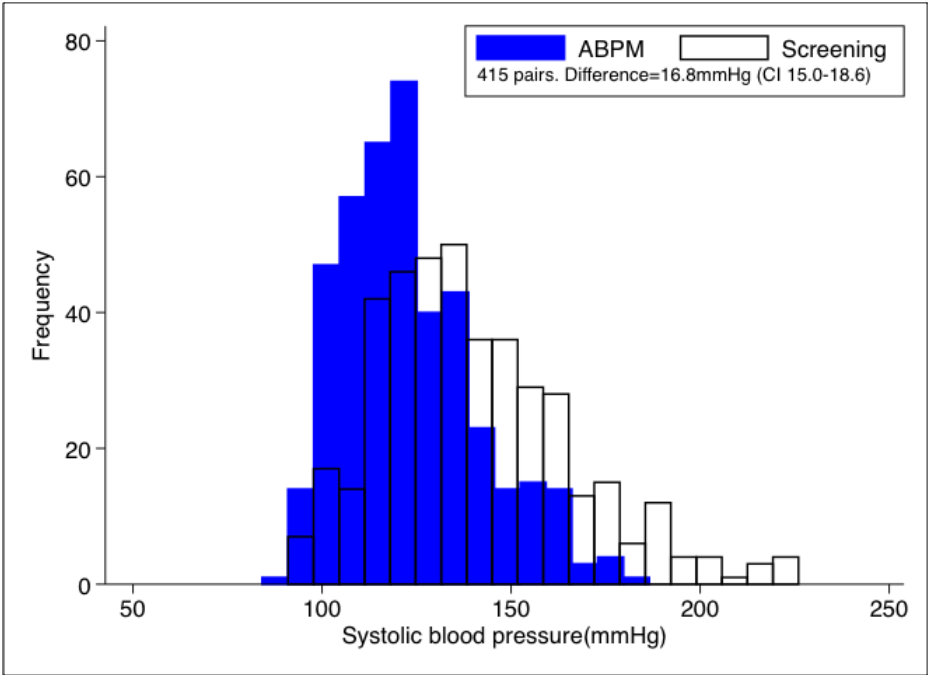


Figure 2-2: Comparison of screening and ABPM blood pressure distributions

A



B

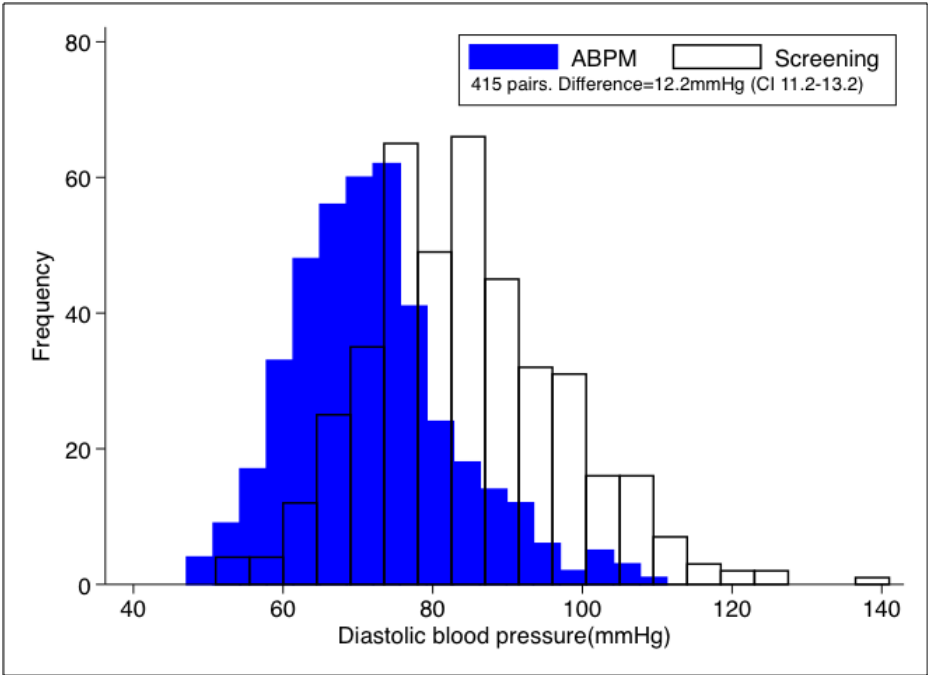
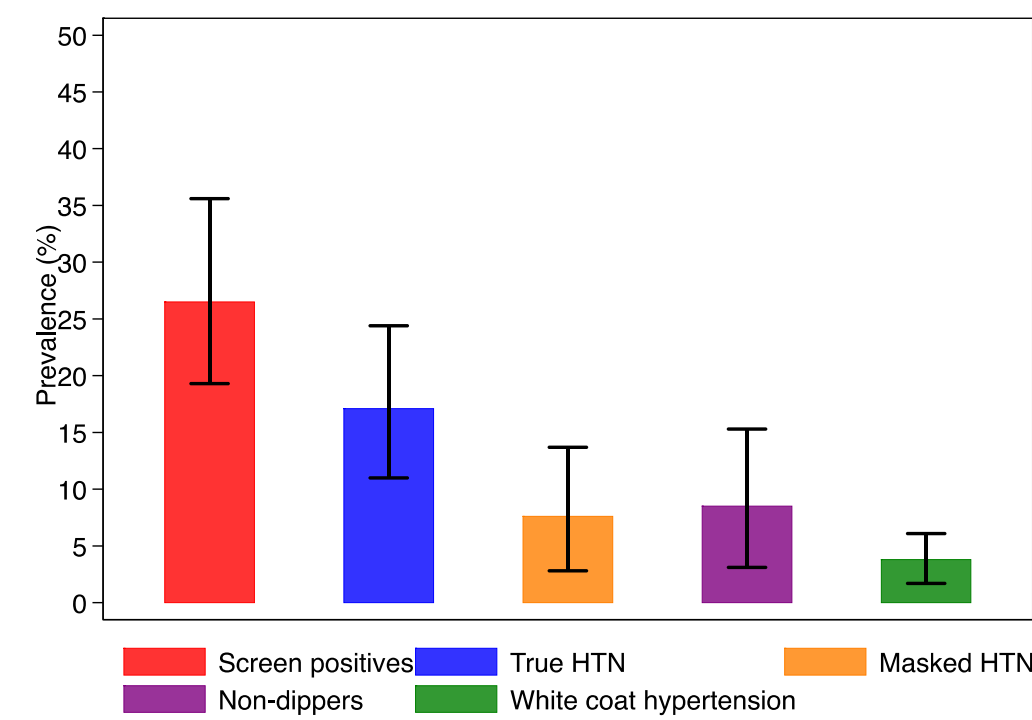


Figure 2-3: Population prevalences of different BP indices



Chapter 3. Research paper II: The Malaria-High Blood Pressure Hypothesis

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Principal Supervisor	Prof Anthony Scott
Thesis Title	Determining the Causal Role of Malaria in Elevating Blood Pressure and Pulse Wave Velocity in Kenyan Adolescents and Adults

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The Malaria-high blood pressure hypothesis

Short title: Malaria-high blood pressure hypothesis

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Abstract

Rationale: Several studies have demonstrated links between infectious diseases and cardiovascular conditions. Malaria and hypertension are widespread in many low and middle-income countries but the possible link between them has not been considered.

Objective: In this article we outline the basis for a possible link between malaria and hypertension, and discuss how the hypothesis could be confirmed or refuted.

Methods and Results: We reviewed published literature on factors associated with hypertension and checked whether any of these were also associated with malaria. We then considered various study designs that could be used to test the hypothesis. Malaria causes low birth weight, malnutrition and inflammation, all of which are associated with hypertension in high-income countries. The hypothetical link between malaria and hypertension can be tested through the use of ecological, cohort or Mendelian randomization studies, each of which poses specific challenges.

Conclusions: Confirmation of the existence of a causative link with malaria would be a paradigm shift in efforts to prevent and control hypertension and would stimulate wider research on the links between infectious and non-communicable disease.

Keywords: Blood pressure; Arterial stiffness; Inflammation; sub-Saharan Africa; Malaria

Nonstandard abbreviations and acronyms

Ang-2: Angiopoietin-2

BP: Blood pressure

HIV: Human Immunodeficiency Virus

LBW: Low birth weight

LMIC: Low and middle-income countries

MR: Mendelian Randomization

SCT: Sickle cell trait

sSA: sub-Saharan Africa

Introduction

Age standardized mean blood pressures (BP) are higher in many parts of Asia and sub-Saharan Africa (sSA) than in high-income countries.¹ Despite the high burden of cardiovascular disease in low and middle-income countries (LMIC), few studies have examined their aetiology, pathophysiology or treatment.^{2,3} While demographic and lifestyle changes including urbanization⁴ contribute substantially to the burden of hypertension in LMICs, examining factors unique to or more prevalent in LMIC settings might reveal new pathophysiological mechanisms that could aid efforts to control the condition. The rise of cardiovascular disease in LMIC is occurring against the background of continuing high burden of infectious diseases.^{5,6} Several studies in more developed settings have reported links between infectious or inflammatory conditions and cardiovascular disease. In this article we outline the hypothesis that hypertension, the leading risk factor for death in LMIC ⁷, could be linked to one of the leading infectious conditions in the same region, malaria.

The malaria hypertension hypothesis

We postulate that malaria contributes to the burden of hypertension in LMIC in the following ways (figure):

(1) Malaria in pregnancy leads to low birth weight (LBW) through pathophysiologically connected mechanisms.⁸ In areas with high malaria endemicity where women are likely to have acquired immunity to prevent most febrile episodes, LBW results from fetal growth restriction which is a consequence of impaired uteroplacental blood flow⁹ and maternal anemia (which is itself due to malaria).^{10,11} Febrile malaria episodes which are more likely among women with low immunity, are thought to induce uterine contractions which are mediated by elevated levels of TNF-alpha leading to preterm birth.^{12,13} Malaria is also associated with hypertensive disorders of pregnancy such as gestational hypertension and preeclampsia in young primigravid women¹⁴⁻¹⁶ and these are risk factors for LBW.¹⁷ Low birth weight children have an increased incidence of hypertension in later life.¹⁸⁻²¹ In a study conducted in Ibadan, Nigeria, infants of mothers who experienced malaria during pregnancy had a higher increase in BP levels during the first year of life compared to those who did not.²² Because BP levels track strongly through to adulthood, such differences could significantly influence the prevalence of adult hypertension.²³⁻²⁵ By virtue of its association with hypertensive disorders of pregnancy that are themselves risk factors for essential hypertension in women^{26,27}, malaria likely contributes to an inter-generational vicious cycle of disease susceptibility as hypertensive parents bear children who develop hypertension more frequently.^{28,29}

(2) Malaria is associated with stunting and malnutrition in childhood^{30,31} which predisposes to the development of hypertension in later life.^{19,23,32}

Although the biological pathways have not been fully characterized, postulated mechanisms involved in the development of hypertension following stunting and chronic malnutrition include reduced nephron numbers¹⁸ and premature senescence in the kidney which is particularly prominent when there is rapid weight gain after growth restriction.³³ In addition, Jamaican survivors of severe acute malnutrition in childhood were found at age 30 years to have markedly smaller left ventricular outflow tracts with reduced cardiac output in the presence of elevated peripheral resistance, a pattern of changes that is likely to lead to hypertension in later life.³⁴

(3) Malaria is a cause of chronic inflammation³⁵ and inflammation predisposes to cardiovascular diseases in high-income countries.³⁶ In a prospective study of 20525 female US health professionals, there was a linear relationship between baseline C-reactive protein levels and incident hypertension.³⁷

Patients with inflammatory bowel disease and rheumatoid arthritis have increased arterial stiffness, which precedes hypertension.³⁸⁻⁴⁰ The link between inflammatory conditions and hypertension may be related to perturbations in the levels of endothelial-based growth factors. Angiopoietin-2 (Ang-2) is a multimeric ligand of the Tie 2 receptor, part of a vascular specific tyrosine kinase signaling pathway that is essential for vessel development and stability.⁴¹ Ang-2 is predominantly secreted by endothelial cells and some smooth muscle cells in many inflammatory and angiogenic states. Ang-2 levels are elevated in children with severe malaria in several different settings^{35,42-45} and in returning travellers infected with malaria⁴⁶. Ang-2 levels

predict cardiovascular disease in children with chronic kidney disease.⁴⁷

Although no causal association has been established, several studies have demonstrated an association between Ang-2 levels and arterial stiffness and BP in adults.^{48,49}

Testing the hypothesis

Observational studies

Ecological studies examining BP levels in relation to malaria incidence are hampered by the lack of finely scaled data on the relevant BP distributions. A worldwide study on BP levels had scarce raw data on BP from sSA where most malaria endemic countries are situated.¹ In contrast there are good epidemiological data on the spatial and temporal distribution of malaria.⁵⁰ Although traditional case-control studies (with malaria as the exposure and hypertension as the outcome) could provide an efficient way to test the hypothesis, they are limited by the non-specificity and non-durability of immunological markers for malaria, a prerequisite for identifying individuals who have previously been exposed to malaria.⁵¹⁻⁵³ This limitation also applies to the potential use of propensity scores⁵⁴ to assemble groups that are comparable in their risk of malaria: in order to generate such scores reliable immunological markers of malaria exposure would be needed.

A life-course epidemiological approach with longitudinal cohorts from the antenatal period or birth would allow the study of many of the postulated pathways through which malaria could be leading to hypertension in LMIC. Studies of pregnant women and children exposed to malaria in demographic surveillance systems with good quality ascertainment of exposure status are necessary. However most demographic surveillance systems in LMIC were

only set up in the last 15 years, and therefore may not have accumulated enough follow-up time to examine these outcomes, assuming that the biases of such retrospective studies can be overcome. Prospective surveillance on the other hand would also require long follow-up and be expensive to conduct.

Randomized intervention studies

Because malaria is of such great public interest, there have been and will continue to be a succession of randomized controlled trials of interventions tested at population level such as vaccines, bed nets and drug treatments. For those interventions that turn out to be effective it might be possible to examine their effect on arterial stiffness and blood pressure. Prospective studies of interventions known to be effective as well as studies of controlled human malaria infection⁵⁵ can not be used here because although they satisfy the criterion of having 2 randomized groups with and without malaria, the fact that the vascular outcomes being tested are potentially irreversible pose an ethical challenge.⁵⁶ As with observational studies, extended follow up might be needed as vascular differences in trial groups due to the effects of malaria may take longer to be apparent compared to the anti-malarial effects of the interventions.

Animal studies are hampered by the fact that murine models of malaria and hypertension are imperfect approximations of their human analogues that have complex pathophysiology.^{57,58}

Genetic studies

Mendelian randomization (MR) studies, where genetic polymorphisms are used as instrumental variables representing malaria exposure, would be

particularly attractive for answering the question as they overcome many of the limitations of observational and intervention studies described above.⁵⁹ Several hemoglobin polymorphisms provide some level of protection against malaria including Hemoglobin C and S, and thalassemia.⁶⁰⁻⁶² A comparison of arterial stiffness indices and BP in subjects with and without the polymorphisms in regions where they have been exposed to malaria in childhood would provide a robust test of the effect of malaria exposure on the development of hypertension.

An important prerequisite for using MR to make causal inferences regarding the effects of environmental exposures, is that the polymorphisms should not display pleiotropic effects, i.e. they should not influence the outcome being studied through a pathway that is independent of the environmental exposure that they are being used as a proxy for.⁵⁹ Some studies suggest that individuals with the sickle cell trait (SCT) are more likely to suffer from cardiovascular events especially under extreme conditions such as military training or athletics.^{63,64} A recent study among African Americans found similar baseline BP in those with and without SCT although on follow up there was an increased incidence of chronic kidney disease in individuals with SCT.⁶⁵ To exclude the possibility of confounding by pleiotropy, it may be necessary to include a control group that has not been exposed to malaria or use additional independent genetic polymorphisms, such as alpha thalassemia. If malaria causes hypertension and there are no pleiotropic effects of SCT, then one would expect to find higher BP in individuals without SCT compared to those with SCT in groups that have been exposed to

malaria. Conversely there would be no difference in BP based on trait status among those who have not been exposed to malaria.

Implications of the hypothesis

Current efforts at understanding hypertension in LMIC have had a narrow focus anchored on traditional risk factors identified among populations in high-income countries. Confirmation of the causative role of malaria in elevating BP would be of immense scientific interest and could lead to a paradigm shift on how to control hypertension in LMIC.

Malaria is only one of many infectious diseases that have a high incidence across LMICs. The inflammatory pathways activated in malaria infection are similar to those of other illnesses.⁶⁶ It is therefore likely that if malaria contributes to the burden of hypertension through inflammation, the same could be true of other chronic infections such as HIV and tuberculosis, providing a novel impetus for the study and control of these infections.

Currently most treatment for infectious illnesses is focused on eliminating the pathogen with little regard for modulating the inflammatory responses that might result in adverse vascular consequences later. Elucidating these inflammatory pathways and their consequences would pave the way for trials of adjunctive therapy such as statins or specific cytokine antagonists to prevent adverse vascular remodeling as a result of infection.

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Novelty and Significance

What is known?

- Malaria and high blood pressure are widespread in low and middle-income countries.
- Malaria is known to cause low birth weight, stunting and inflammation.
- Low birth weight, stunting and inflammation are associated with the development of arterial stiffness and high blood pressure in developed countries.

What new information does this article contribute?

- We outline the likely pathophysiological mechanisms of the hypothesized association between malaria and high blood pressure in low and middle-income countries.
- This hypothesis can be tested through well designed ecological and cohort studies or using Mendelian randomization techniques.

Despite the knowledge that malaria causes low birth weight, inflammation and stunting, all of which have been associated with the development of hypertension, no attempt has been made to link the two conditions. In this article we review the literature in support of the hypothesis that malaria could be contributing to the widespread problem of hypertension in low and middle-income countries. We also outline several ways in which this hypothesis could

be proven or refuted. If proven, this link would be a paradigm shift in efforts to prevent and control high blood pressure in low and middle-income countries.

Figure 3-1: The malaria-high blood pressure hypothesis

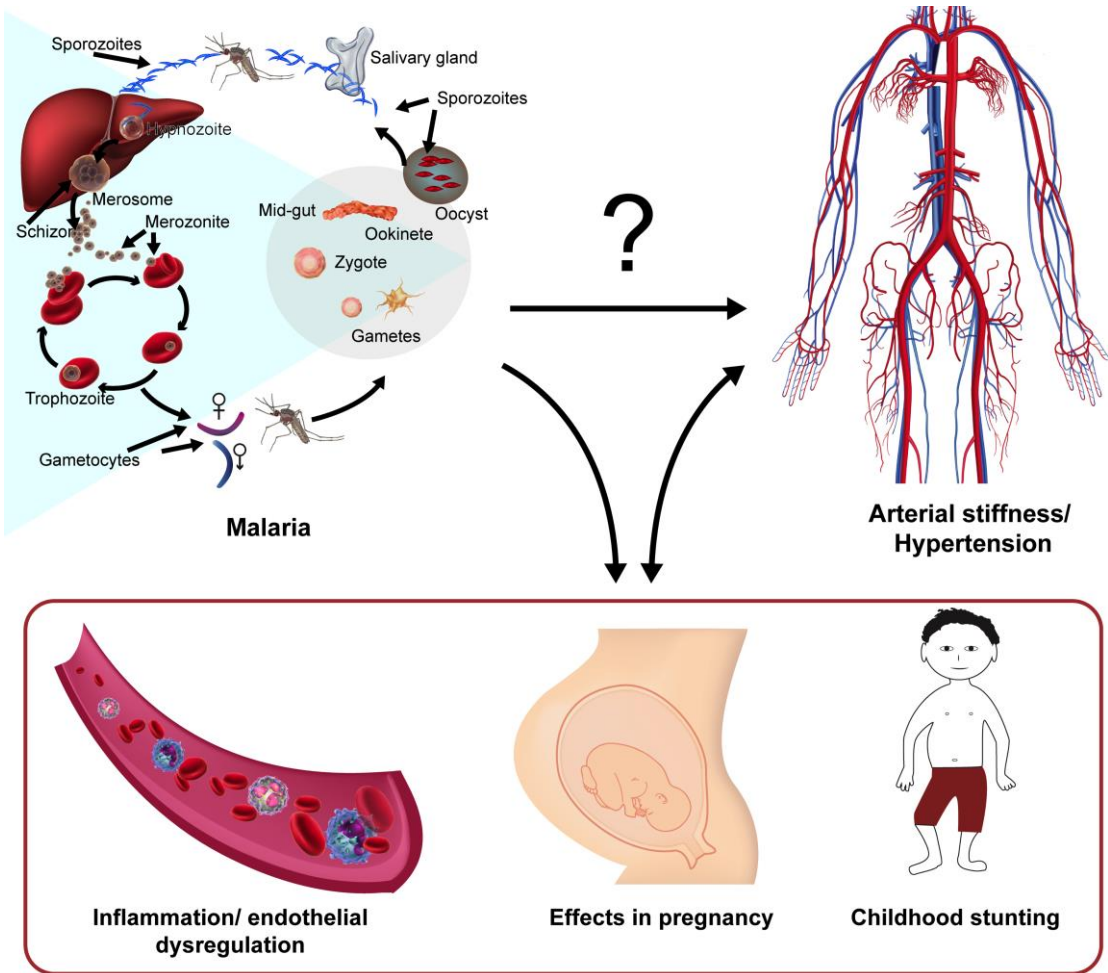


Figure legend: The malaria-high blood pressure hypothesis

Malaria is known to cause low birth weight, inflammation as well as stunting. All these factors have been separately associated with the development of high blood pressure in high-income countries. Studies are needed to confirm whether malaria contributes to the development of high blood pressure in low and middle-income countries.

Chapter 4. Research paper III: Blood Pressure and Arterial Stiffness in Kenyan Adolescents With $\alpha(+)$ Thalassemia.

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Principal Supervisor	Prof Anthony Scott
Thesis Title	Determining the Causal Role of Malaria in Elevating Blood Pressure and Pulse Wave Velocity in Kenyan Adolescents and Adults

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Blood pressure and arterial stiffness in Kenyan adolescents with α^+ thalassemia

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ABSTRACT

Background

Recent studies have discovered that α -globin is expressed in blood vessel walls where it plays a role in regulating vascular tone. We tested the hypothesis that blood pressure might differ between normal individuals and those with α^+ thalassemia, in whom the production of α -globin is reduced.

Methods and Results

The study was conducted in Nairobi, Kenya, among 938 adolescents aged 11-17 years. 24-hour ambulatory blood pressure monitoring (ABPM) and arterial stiffness measurements were performed using an arteriograph device. We genotyped for α^+ thalassemia by PCR. Complete data for analysis were available for 623 subjects; 223 (36%) were heterozygous ($-\alpha/\alpha$) and 47 (8%) were homozygous ($-\alpha/-\alpha$) for α^+ thalassemia while the remaining 353 (55%) subjects were normal ($\alpha\alpha/\alpha\alpha$). Mean 24-hour systolic BP \pm SD was 118 \pm 12 mmHg in $\alpha\alpha/\alpha\alpha$, 117 \pm 11 mmHg in $-\alpha/\alpha$ and 118 \pm 11 mmHg in $-\alpha/-\alpha$ subjects respectively. Mean 24-hour diastolic BP \pm SD in these groups was 64 \pm 8 mmHg, 63 \pm 7 mmHg and 65 \pm 8 mmHg respectively. Mean pulse wave velocity (PWV) \pm SD was 7 \pm 0.8 ms⁻¹, 7 \pm 0.8 ms⁻¹ and 7 \pm 0.7 ms⁻¹ respectively. No differences were observed in PWV and any of the 24-hour ABPM derived measures between those with and without α^+ thalassemia.

Conclusion

These data suggest that the presence of α^+ thalassemia does not affect blood pressure and/or arterial stiffness in Kenyan adolescents.

Keywords

α^+ thalassemia, ambulatory blood pressure monitoring, adolescents

INTRODUCTION

The thalassemias, in which there is disordered or absent production of the α - or β -globin chains that make up normal hemoglobin, are the most common monogenic disorders of humans.¹ The geographical distribution of α^+ thalassemia, in which there is deletion of one or more of the *HBA* genes that encode α -globin ($Hb\alpha$) production, closely mirrors that of malaria transmission² and it has been demonstrated that these deletions confer protection against both severe and non-severe malaria.²⁻⁵

While it has long been believed that $Hb\alpha$ expression is limited to red blood cells, it has recently been demonstrated that $Hb\alpha$ is also expressed in mouse endothelial cells where it plays a role in nitric oxide (NO) signaling, influencing vascular smooth muscle tone in resistance arteries.^{6,7} A macromolecular complex formed by $Hb\alpha$ and endothelial Nitric Oxide Synthase (eNOS), regulates NO signaling at myoendothelial junctions (MEJ).⁸ Disruption of this complex lowers BP in both normotensive and hypertensive mice.⁸ It has also been shown that resistance arteries from mice lacking 2 of the 4 α -globin genes ($-\alpha_2/-\alpha_2$) have reduced contractility after treatment with the vasoconstrictor phenylephrine.⁹ Individuals with α^+ thalassemia have been shown to have higher microvasculature tortuosity.¹⁰ From the foregoing it could be expected that individuals with α^+ thalassemia might have lower BP compared to those with normal hemoglobin. However the few studies conducted in humans have yielded inconsistent results. While one review¹¹ suggested that α^+ thalassemic individuals have moderate hypotension, other investigators have found elevated BPs in subjects with this condition.^{12,13}

These studies were limited by small sample sizes and the failure to use 24-hour ambulatory blood pressure monitoring (ABPM) to measure BP. It is known that one-off office/clinic BP measurements can be influenced by a variety of environmental and psychological factors¹⁴, limitations that are overcome by the use of ABPM, which is considered the reference standard for BP measurement.^{14,15}

If arterial stiffness and/or BP are influenced by Hb α genotype, this would be an important step that could aid the development of compounds either mimicking or antagonizing Hb α as potential therapies for hypertension. In the current study, we have tested the hypothesis that 24-hour BP and arterial stiffness is different in subjects with α^+ thalassemia than in normal individuals.

METHODS

This population-based study was a cross-sectional sample of residents of the Nairobi Urban Health and Demographic Surveillance System (NUHDSS)¹⁶ in Kenya, and was conducted between December 2015 and June 2016. Nairobi, the capital city of Kenya was chosen for this study for 2 reasons. First, Nairobi is located at high altitude (1800 meters above sea-level) and there is no evidence of malaria transmission.¹⁷ This made it possible to study the effect of α^+ thalassemia on BP unconfounded by the presence of malaria, which could potentially influence BP¹⁸ and which α^+ thalassemia protects against. Second, the population of Nairobi is composed of ethnic groups originating from all parts of the country including those whose ancestral lands were endemic for malaria (e.g. Luhya, Luo, Teso, Mijikenda), in whom the frequency of

α^+ thalassemia is significantly higher.² In order to increase our efficiency in recruiting participants with α^+ thalassemia, we limited our recruitment to those who identified themselves as genetically derived from one of these ethnic groups.

The NUHDSS conducts population-wide censuses within the study area 4 times each year.¹⁶ Using NUHDSS data we selected all children currently aged 11-17 years who had a continuous record of residence within the study area since birth. Continuous residency was a requirement in order to minimize potential exposure to malaria as a result of migration. Trained staff visited all subjects who had been selected to participate in the study at their homes. Parents of the children were then asked to bring them to the nearer of two study clinics within the area to undergo study procedures. Up to three attempts were made at finding a selected subject before concluding that they could not be found. Subjects who failed to come to the clinic within 3 months of being invited were considered to have declined to participate in the study.

Recruited subjects first underwent an interview where they answered questions about their past medical history and their socioeconomic status based on the multi-dimensional poverty (MDP) index.¹⁹ Weight and height were measured using a validated SECA 874™ weighing machine and a portable stadiometer (SECA 213™), respectively. Mid-upper-arm circumference (MUAC) was measured in a standardized manner using TALC™ MUAC tapes. We then took a screening BP measurement using a validated Omron™ M10-IT sphygmomanometer. An appropriately sized cuff

was placed on the non-dominant arm after the subject had been seated for at least 5 minutes. Three BP measurements were taken over a 5-minute period and the mean of the last 2 measurements was recorded as the screening BP value. All participants were subsequently fitted with a validated Arteriograph24™ device for 24-hour ABPM as well as pulse wave velocity (PWV) determination.²⁰ These devices were programmed to take measurements every 20 minutes from 0600-2200 hrs and every 40 minutes from 2200-0600 hrs.

As there are no published criteria for acceptable ABPM data in children, we used guidelines for completeness of ABPM data in adults from the International Database of Ambulatory blood pressure in relation to Cardiovascular Outcomes (IDACO) study.²¹ Specifically, ABPM data were considered of acceptable quality if they included a minimum 10 daytime and minimum 5 nighttime readings, where daytime was defined as 1000-2200 hrs and nighttime as 0000-0600 hrs.²¹ The same time periods were used to determine average daytime and nighttime blood pressures and to evaluate dipping status. Time weighting was applied in calculating average BP values for all time periods.²²

We defined screen positives for hypertension as individuals whose mean of the last 2 clinic BP measurements was above the 95th percentile for their age, sex and height.¹⁵ Confirmed hypertensives were those whose 24hr systolic and/or diastolic BP averages respectively were above the 95th percentile for their sex, age and height.¹⁵

We categorized all subjects who were not on anti-hypertensive medication using the combination of clinic BP measurements and ABPM into four categories: sustained hypertensives (screen positive and confirmed hypertensive on ABPM); white coat hypertensives (screen positive, not confirmed hypertensive on ABPM); masked hypertensives (screen negative, confirmed hypertensive on ABPM) or normotensives (screen negative, not confirmed hypertensive on ABPM).²³

Dipping status was defined using ABPM data only, using day and night periods as defined above. Subjects were classified using the following four categories, based on the night/day ratio of mean systolic and/or diastolic BPs: rising or absence of dipping (ratio ≥ 1.0); mild dipping ($0.9 < \text{ratio} \leq 1.0$); dipping ($0.8 < \text{ratio} \leq 0.9$); and extreme dipping (ratio ≤ 0.8).²⁴

Laboratory procedures

We collected 10ml of blood from participants for full blood count, determination of α^+ thalassemia genotype and serum electrolytes. After performing automated full blood counts using an ACT 5™ machine, whole blood samples were frozen at -80°C and then transported to the KEMRI-Wellcome Trust Research Programme laboratories in Kilifi, Kenya for genotyping. DNA was extracted retrospectively from the frozen samples by use of Qiagen™ DNA blood mini-kits (Qiagen, Crawley, United Kingdom) and typed for the common African -3.7kb *HBA* deletion by PCR.²⁵

Serum and urine samples collected from participants were frozen at -80°C within 4 hours of collection and later transported to Kilifi, Kenya for

subsequent analysis. We determined sodium and potassium, urea and creatinine levels in these samples using ion electrophoresis and the Jaffe method, respectively.²⁶ We additionally determined albumin levels in the urine samples by immunoturbidometry using a Quantex™ microalbumin kit.

Estimated glomerular filtration rate (eGFR) was calculated using the Schwarz formula.²⁷

Statistical methods

Based on an expected minimum prevalence for heterozygous α^+ thalassemia ($-\alpha/\alpha$) of 20% in the ethnic groups we were studying, a systolic BP standard deviation of 15 mmHg, and 30% attrition due to poor quality ABPM data, we estimated that a total of 472 participants would provide 80% power to detect 1/3rd of a standard deviation (5 mmHg) difference in 24 hour systolic BP between $-\alpha/\alpha$ and $\alpha\alpha/\alpha\alpha$ individuals.

Summary statistics that were computed included means, medians and proportions as appropriate. We used Student's *t*-test to separately compare continuous variables in $-\alpha/\alpha$ and $-\alpha/-\alpha$ to $\alpha\alpha/\alpha\alpha$ individuals. The χ^2 test was used to compare categorical variables. We conducted multiple regression analyses to determine whether inclusion of α^+ thalassemia genotype predicted 24-hour systolic and/or diastolic BP. Age, sex, BMI, eGFR, and PWV, which have all been previously associated with BP were included as covariates in the base model. To determine whether α^+ thalassemia genotype influenced 24-hour BP, we added it to the base model and used the likelihood ratio test to determine if it improved the fit. We additionally tested for interaction with

the sickle cell trait, as it has previously been associated with cardiovascular and renal events²⁸⁻³¹. All analyses were conducted using Stata™ Version 12 software (College Station, Texas).

The Kenya Medical Research Institute's Ethical Review Committee approved the study. Written informed consent was obtained from parents of study participants. Participating children also provided written assent.

RESULTS

Of the 938 subjects invited to participate in the study, 686 completed enrollment (Figure 4-1). None of the participants were previously aware of their α^+ thalassemia status. The 252 adolescents that were not recruited into the study were 0.6 years (95% CI 0.3-0.9) older than study participants, but with a similar sex distribution (53% female) to those that participated in the study. Data on α^+ thalassemia genotype were available for 664 (97%) participants. 246 (37%) were heterozygous ($-\alpha/\alpha\alpha$) and 49 (7%) were homozygous ($-\alpha/-\alpha$) for α^+ thalassemia while the remaining 369 (56%) of subjects were normal ($\alpha\alpha/\alpha\alpha$). One hundred and three (15.5%) of the adolescents were carriers of the sickle cell trait, distributed equally among the α^+ thalassemia genotypic groups (14%, 17% and 16% in $\alpha\alpha/\alpha\alpha$, $-\alpha/\alpha\alpha$ and $-\alpha/-\alpha$ subjects respectively, $p=0.652$). After excluding those with poor quality ABPM data, 623 (94%) subjects provided quality data for the analysis (Figure 4-1). A slightly lower proportion of $-\alpha/\alpha\alpha$ participants had complete ABPM data (91%) compared to $\alpha\alpha/\alpha\alpha$ (95%) and $-\alpha/-\alpha$ subjects (97%). Mean clinic BP \pm SD among all participants was 98 \pm 11 mmHg systolic and 64 \pm 8 mmHg

diastolic. The mean 24-hour BP \pm SD for all participants was 117 \pm 12 mmHg systolic and 64 \pm 8 mmHg diastolic. Mean 24-hour Pulse Wave Velocity (PWV) \pm SD was 7 \pm 0.8 ms⁻¹. The study had >98% power to detect 1/3rd of a standard deviation (SD) difference in either systolic or diastolic BP (4mmHg and 2.7mmHg respectively) between $\alpha\alpha/\alpha\alpha$ individuals and those with $-\alpha/\alpha\alpha$, and a 0.3 ms⁻¹ (1/3rd SD) difference in PWV between $\alpha\alpha/\alpha\alpha$ individuals and those with $-\alpha/\alpha\alpha$. The study had >90% power to detect differences equivalent to 0.5 SDs in BP and PWV between $\alpha\alpha/\alpha\alpha$ and $-\alpha/-\alpha$ individuals.

Table 4-1 displays the characteristics of study participants according to α^+ thalassemia genotype. As expected, hemoglobin concentrations were significantly lower in $-\alpha/-\alpha$ than in $-\alpha/\alpha\alpha$ or $\alpha\alpha/\alpha\alpha$ subjects. BMI was lower in $-\alpha/\alpha\alpha$ than in $\alpha\alpha/\alpha\alpha$ individuals (18.2 vs 19.2; $p=0.0004$) while mid upper arm circumference was significantly smaller in $-\alpha/\alpha\alpha$ compared to $\alpha\alpha/\alpha\alpha$ individuals. There were no statistically significant differences in the prevalence of masked hypertension, white coat hypertension or in the pattern of non-dipping BP by α^+ thalassemia genotype. PWV was also similar in all 3 groups.

Figure 4-2 displays mean 24-hour, daytime and nighttime blood pressures in study participants by α^+ thalassemia genotype. All measures were similar for all three groups.

The results of regression analyses are displayed in Table 4-2. Age, sex, BMI, eGFR and PWV were all independent predictors of 24-hour systolic BP while

PWV was the only independent predictor of 24-hour diastolic BP. 24-hour BP values were not associated with α^+ thalassemia genotype in any of our regression models and its inclusion in the final model did not improve the fit (likelihood ratio test $p=0.96$ for systolic BP and $p=0.75$ for diastolic BP). Adjustment for proxy markers of hemolysis (hemoglobin level, mean corpuscular volume and mean corpuscular hemoglobin concentration) made no difference to the results, and neither did the inclusion of interaction terms for sex and sickle cell trait status.

DISCUSSION

The α^+ thalassemias are the most common human monogenic disorders¹. Demonstration of altered BP in individuals with any of the mutations would be of immense importance, as it would improve the understanding of BP regulation and aid the development of new drugs. In this detailed study of BP phenotypes and arterial stiffness among adolescents, we did not find any differences between those with and without α^+ thalassemia. Because the exposure measurement was a genetic trait acquired at conception and the participants were ascertained to have remained in the same malaria-free environment since birth we believe that this study suggests that a direct effect of α^+ thalassemia on BP and indices of arterial stiffness within the first 11-17 years of life is highly improbable.

On the face of it, our results do not align with findings from other studies that have suggested the possibility that expression of Hb α might affect blood pressure.^{6,8,11} These studies were either done in-vitro or in mouse models

with very limited sample sizes (N=6).⁸ The review by Butcher *et al*¹¹ that reported an association between α^+ thalassemia and moderate hypotension did not refer to a primary publication. It is possible that the lower BPs observed in subjects with α^+ thalassemia who are relatively protected from malaria could actually be a confirmation that malaria raises blood pressure as we have previously hypothesized.¹⁸ An alternative explanation for similar BP despite the presence of Hb α deletions could be due to canalization, a phenomenon where individuals or organisms develop the same phenotype despite differences in their genetic make up.³² Reddy *et al*³³ have shown that infusion of HbH (levels of which are elevated in α^+ thalassemia) into rats results in elevation of BP as a result of HbH having higher affinity for Nitric Oxide than HbA.³⁴ It is therefore possible that the BP lowering effect of α^+ thalassemia is cancelled out by the opposing effect of elevated levels of HbH. This would also suggest that recently developed molecules that mimic alpha globin³⁵, may have reduced effectiveness in individuals with α^+ thalassemia. Additional studies are needed to fully understand these seemingly contrasting effects and generate a unified model incorporating both environmental conditions and genetic effects.

A major strength of this study was the use of ABPM, which is considered the reference standard for blood pressure measurement in children.¹⁵ The study was well powered to detect very small differences in BP and PWV. Although it could be argued that PWV is predominantly a measure of large conduit arteries which do not express Hb alpha□□□□□□□□□□ integrates the interface between small arteries and resistance vessels-as for instance in

diabetes where small vessel damage is as frequent as large.³⁶ An additional strength of the study is that we used health and demographic surveillance system (HDSS) records that were prospectively collected in order to ascertain residence in a non-malaria zone, there being no better method of doing this in sub-Saharan Africa.

One limitation of this study was the limited age range of subjects recruited, necessitated by the fact that there were no long-term residency records for older individuals. Most HDSSs in sSA were established in the late 1990's to early 2000's.³⁷ Recruiting older individuals would have compromised data on residency status in childhood, the period when malaria risk is highest. While BP differences are likely to be larger at older ages, it is known that differences in adult BP emerge in childhood^{38,39} and that childhood BP levels are predictive of adult BP.⁴⁰ The absence of even a small difference in carefully measured BP and arterial stiffness in our study of adolescents therefore suggests that it is very unlikely such differences would emerge in future.

A second limitation of the study is the fact that we did not measure levels of markers of hemolysis such as HbH and haptoglobin and other potential compensatory mechanisms such as (decreased) eNOS or guanylyl cyclase expression, or increased catecholamine levels among study participants. This would have helped to either confirm or refute the possibility of canalization explaining the lack of an effect of α^+ thalassemia on BP levels. This could form the basis of future studies to better understand the seemingly contrasting findings of experimental and human studies.

It is also important to note that no studies have to date established whether alpha hemoglobin is expressed in endothelial cells of human subjects and if the 3.7kb deletion, the most common defect causing α^+ thalassemia¹ in humans, also results in reduced endothelial expression of alpha hemoglobin. Additional studies are required to determine if there is endothelial expression of alpha hemoglobin in humans, whether the 3.7kb deletion results in reduced endothelial α globin expression and whether other defects resulting in α^+ thalassemia present with the same vascular phenotype that we observed.

In summary, we have demonstrated that there are no differences in BP and arterial stiffness based on α^+ thalassemia genotype in Kenyan adolescents living within a non-malaria-endemic environment. Additional studies are required to explain the apparent contradictory results of experimental studies.

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Conflicts of Interest/Disclosures

None of the authors have any conflicts of interest or disclosures to report.

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Table 4-1 : Characteristics of Study Participants

Characteristic	Normal (α/α) N=353		Heterozygous ($-\alpha/\alpha$) N=223		Homozygous ($-\alpha/-\alpha$) N=47		p-	
	n (%)	mean (SD)	n (%)	mean (SD)	n (%)	mean (SD)	value ¹	value ²
Female	187 (53)		132 (59)		28 (58)		0.3	0.3
White coat hypertension	15 (4)		8 (4)		3 (6)		0.2	0.4
Masked hypertension	25 (7)		21 (9)		7 (15)		1.0	0.073
	n (%)	mean (SD)	n (%)	mean (SD)	n (%)	mean (SD)	p-value ¹	p-value ¹
Age, years	13.4 (2.2)		13.0 (2.2)		13.4 (2.4)		0.0289	0.9
BMI ¹ , kg/m ²	19.2 (3.2)		18.3 (2.6)		19.2 (3.6)		0.0004	0.9
MUAC ² , cm	23.7 (3.9)		22.7 (3.1)		23.2 (4)		0.0007	0.4
Hemoglobin, mg/dL	13.5 (1.5)		13.1 (1.4)		12.2 (1.6)		0.0004	<0.0001
							<0.000	
Mean cell volume, fL	84 (5)		79 (5)		70 (5)		1	<0.0001
Socioeconomic status, MDPi ³ score	2.0 (1.2)		2.3 (1.4)		2.4 (1.1)		0.0126	0.3
Mean corpuscular hemoglobin concentration, g/dL	32 (2)		31 (2)		31 (2)		0.0003	<0.0001
24-hour SBP, mmHg	118 (12)		117 (11)		118 (11)		0.1	1.0
24-hour DBP, mmHg	64 (8)		63 (7)		65 (8)		0.1	0.6
24-hour Pulse wave velocity, ms ⁻¹	7.0 (0.8)		7.0 (0.8)		7.0 (0.7)		0.2	0.5
Systolic Morning BP surge, mmHg								
	9 (12)		8 (12)		11 (10)		0.6	0.2
Augmentation index, %	17 (6)		17 (6)		16 (5)		0.8	0.4
Non dipping BP pattern	24 (7)		7 (3)		2 (4)		0.1	0.9
eGFR ⁴ , ml/min/1.73m ²	109 (15)		111 (14)		110 (13)		0.1	0.6
Log ₁₀ UACr	0.4 (0.6)		0.3 (0.7)		0.2 (1)		0.4	0.1
Urine sodium, mmol/L	137 (82)		130 (53)		128 (51)		0.3	0.5
Urine potassium, mmol/L	48 (32)		46 (29)		42 (20)		0.4	0.2

P-values are for comparisons between normal and heterozygous¹ and normal and homozygous²

¹BMI=Body mass index

²MUAC=mid upper arm circumference ³MDPI=multi-dimensional poverty index ⁴eGFR=estimated glomerular filtration rate

Table 4-2: Regression Analyses Investigating Possible Effect of Thalassemia Status on 24-Hour Systolic and Diastolic BP

	24hr SBP		24 hr- DBP	
	β , 95% CI	p-value	β , 95% CI	p-value
Age (years)	0.6 (0.1 to 1.2)	0.021	0.03(-0.3 to 0.4)	0.9
Male sex	2.6(0.7 to 4.5)	0.009	0.2(-1.1 to 1.4)	0.9
BMI (kg/m ²)	0.6 (0.2 to 1)	0.001	0.2(-0.1 to 0.4)	0.2
PWV (ms ⁻¹)	2.8(1.6 to 4.1)	<0.001	2.7(1.9 to 3.6)	<0.001
eGFR (mls/min/1.73m ²)	0.1(0.03 to 0.2)	0.006	0.02(-0.02 to 0.06)	0.4
α^+ thalassemia genotype	0.04(-1.4 to 1.5)	1.0	0.1(-0.8 to 1.1)	0.8

Likelihood ratio test for models including vs excluding α^+ thalassemia genotype p= 1.0 for SBP and p=0.8 for DBP.

Figures

Figure 4-1: Study Flow Chart

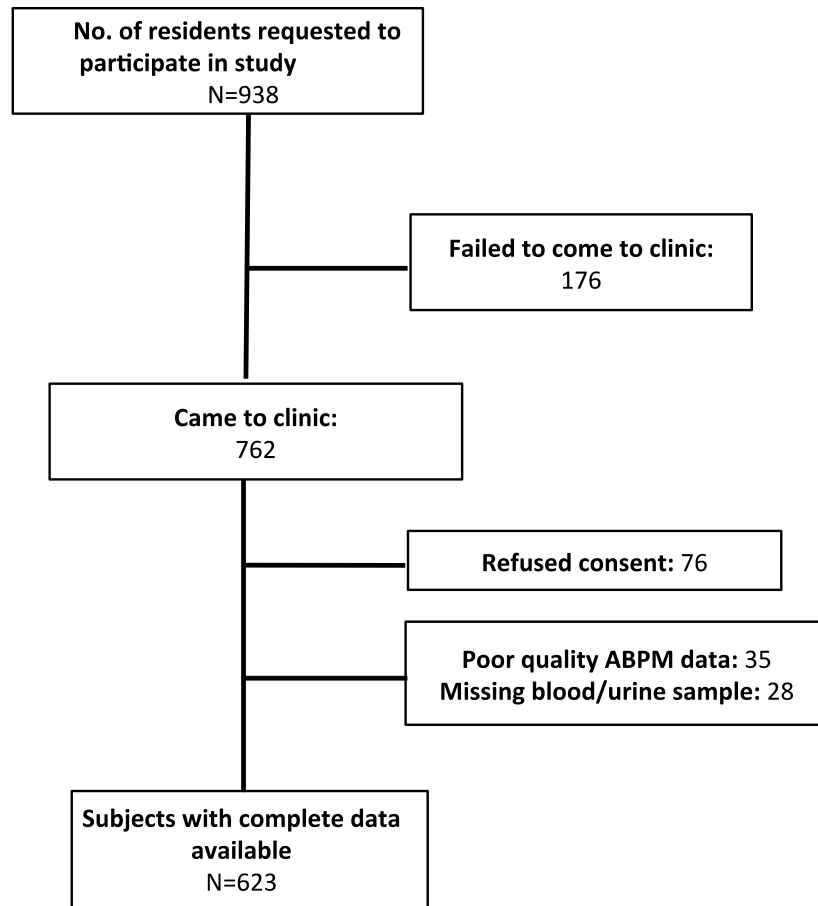
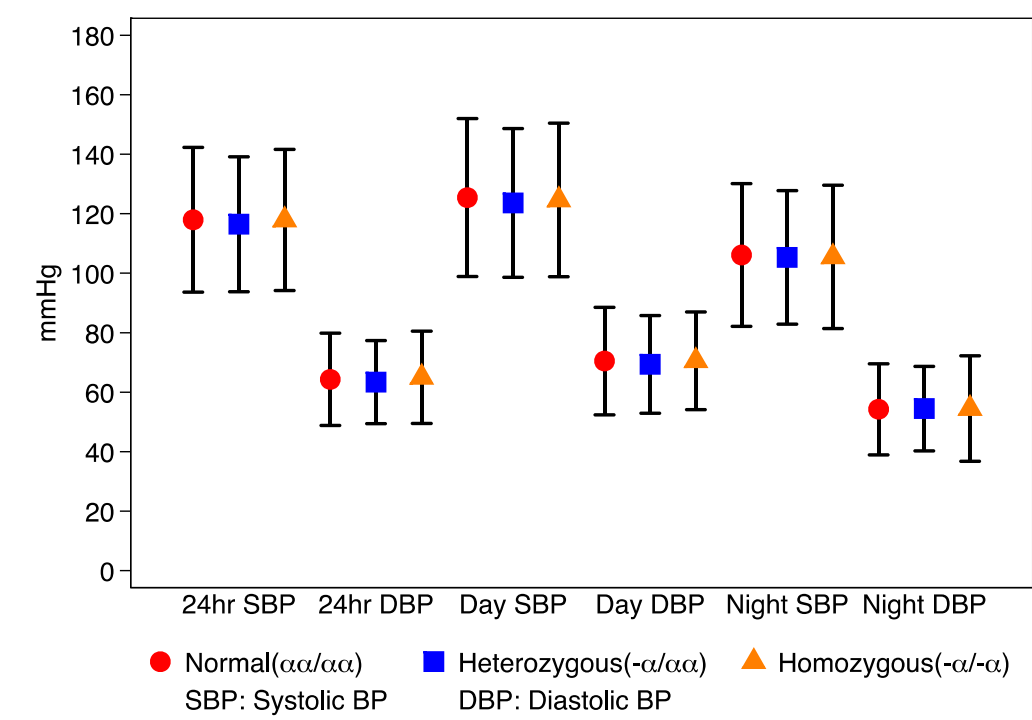


Figure 4-2: Twenty-four hour ABPM measures by alpha thalassemia status



Data are mean and 95% Confidence Intervals

Chapter 5. Research paper IV: Blood Pressure and Arterial Stiffness in Kenyan Adolescents with the Sick Cell Trait

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Student	Anthony Oliwa Etyang
Principal Supervisor	Prof Anthony Scott
Thesis Title	Determining the Causal Role of Malaria in Elevating Blood Pressure and Pulse Wave Velocity in Kenyan Adolescents and Adults

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Blood Pressure and Arterial Stiffness in Kenyan Adolescents with the Sick Cell Trait

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Short title: Ambulatory blood pressure in sickle cell trait

Abbreviations:

ABPM: Ambulatory blood pressure monitoring

BP: Blood pressure

PWV: Pulse wave velocity

SCT: Sickle cell trait

ABSTRACT

The potential association between sickle cell trait (SCT) and increased arterial stiffness/blood pressure (BP) has not been evaluated in detail despite its association with stroke, sudden death and renal disease. We performed 24-hour ambulatory BP monitoring and arterial stiffness measurements in adolescents raised in a malaria free environment in Kenya.

Between December 2015 and June 2016, 938 randomly selected adolescents that had been continuous residents of Nairobi from birth were invited to participate in the study. Standard clinic BP measurement was performed followed by 24-hour ambulatory BP monitoring and arterial stiffness measurement using an Arteriograph device. SCT status was determined using DNA genotyping on contemporaneously collected blood samples. Of 938 invited, 609 (65%) provided complete data for analysis. SCT was present in 103 (17%). Mean 24-hour systolic and diastolic BP, SD was (116, 11.5) and (64, 7) mmHg respectively in SCT; and (117, 11.4) and (64, 6.8) in non-SCT. Mean pulse wave velocity (PWV), SD was (7, 0.8) and (7, 0.8) ms⁻¹ respectively in SCT and non-SCT. No differences were observed in PWV and any clinic or ambulatory BP derived measures between those with and without SCT. These data suggest that SCT does not independently influence BP or PWV.

Keywords

Sickle cell trait; Hypertension; Blood Pressure; Arterial Stiffness

The sickle cell trait (SCT), common among populations of African descent¹ due to the protection it offers against malaria²⁻⁴, has been associated with increased risk of cardiovascular and renal disease.⁵⁻⁸ However the underlying mechanisms of this increased risk have not been elucidated clearly, hampering measures that can be implemented to reduce risk in carriers.

Individuals of African descent have relatively higher blood pressure (BP) compared to other ethnicities⁹, and it is conceivable that increased BP or arterial stiffness, which have been shown to precede clinical events similar to those seen in SCT, could precede the events observed in SCT carriers.^{8,10,11} Alternatively increased arterial stiffness and BP in individuals with SCT could result from the yet to be elucidated mechanisms that lead to cardiovascular and renal events.

Previous studies that assessed BP and/or arterial stiffness in individuals with SCT had several weaknesses; Rossi-Espagnet *et al* in a study conducted in Colombia in 1968 found no difference in BP between individuals with and without SCT.¹² However, BP measurements were performed only once at home visits; there was a poor response rate in men; and the data were susceptible to confounding by malaria, which SCT protects against²⁻⁴ and is possibly related to BP.^{13,14} Bayramoglu *et al* found similar arterial stiffness indices in young Turkish adults with and without SCT, but the sample size was small.¹⁵ Although the studies demonstrating increased cardiovascular and renal disease risk in SCT assessed BP at baseline, the BP measurements were done in the clinic/office, using manual or automated methods.^{7,8} None of these studies utilized 24-hour ambulatory blood pressure

monitoring (ABPM), considered the reference method for BP measurement^{16,17}, raising the possibility that subtle but significant differences in BP could have been missed.¹⁷ ABPM overcomes many of the limitations of office/clinic BP measurement.¹⁷ ABPM also enables detection of masked hypertension (normal clinic BP but elevated 24-hour BP), a cardiovascular risk factor¹⁸ that is more common in populations of African descent¹⁹, the same population that has a high prevalence of SCT.

If arterial stiffness and/or BP are increased in young individuals with SCT they could become the target of interventions aimed at reducing future cardiovascular and renal events. We conducted a population-based study in Nairobi, Kenya to determine whether SCT influences arterial stiffness and BP among adolescents who have had minimal exposure to malaria.

METHODS

The study was a cross-sectional sample of residents of the Nairobi Urban Health and Demographic Surveillance System ²⁰ in Kenya conducted from December 2015 to June 2016. The area has a population of approximately 70,000 and the prevalence of hypertension is high.²¹ Nairobi, the capital city of Kenya was chosen for this study because of 2 reasons: First, Nairobi is located at high altitude (1800 meters above sea-level) and there is no evidence of malaria transmission.²² This made it possible to study the effect of SCT on BP unconfounded by the presence of malaria. This was necessary because malaria could influence BP¹⁴ and at the same time SCT protects against malaria^{3,4}; Second, the population of Nairobi is composed of ethnic groups originating in all parts of the country including those whose ancestral

lands were endemic for malaria (e.g. Luhya, Luo, Teso, Mijikenda). The sickle cell gene frequency is much higher among these ethnic groups.²³ In order to increase our efficiency in recruiting participants with SCT we limited our recruitment to those who identified themselves as genetically derived from one of these ethnic groups.

Population-wide censuses are conducted 4 times a year within the study area.²⁰ Using census data we selected all children currently aged 11-17 years who had a continuous record of residence in the area since birth. Continuous residency was a requirement so as to minimize potential exposure to malaria as a result of migration. Trained staff visited all subjects who had been selected to participate in the study at their homes. Parents of the children were then asked to bring them to the nearer of two study clinics within the area to undergo study procedures. Subjects who failed to come to the clinic within 3 months of being requested to do so were considered to have refused to participate in the study.

Subjects first underwent an interview where they answered questions about their past medical history and their socioeconomic status based on the multi-dimensional poverty index.²⁴ Weight and height were measured using a validated SECA 874TM weighing machine and a portable stadiometer (SECA 213TM) (SECA GMBH, Hamburg, Germany), respectively. Mid-upper arm circumference was measured in a standardized manner using TALCTM (Teaching Aids at Low Cost, Hertfordshire, UK) tapes. We then took a screening BP measurement using a validated automated OmronTM M10-IT (Omron Healthcare Europe B.V, Hoofddorp, The Netherlands) BP machine.

An appropriately sized cuff was placed on the non-dominant arm after the subject had been seated for at least 5 minutes. Three BP measurements were taken over a 5-minute period and the mean of the last 2 measurements was recorded as the screening BP value. All participants were subsequently fitted with an Arteriograph24™ device (Tensiomed Ltd, Budapest, Hungary) for 24-hour ABPM as well as pulse wave velocity (PWV) determination.²⁵ The devices, which have been calibrated in children²⁶, were programmed to take measurements every 20 minutes during daytime hours (0600-2200 hrs) and every 40 minutes at night (2200-0600 hrs).

Laboratory procedures

We collected 10ml of blood from participants for full blood count, determination of sickle hemoglobin status and serum electrolytes. After performing automated full blood counts using an ACT 5™ machine (Beckman Coulter Inc, Brea, CA), whole blood samples were frozen at -80°C and then transported to the KEMRI-Wellcome Trust Research Programme laboratories in Kilifi, Kenya for determination of sickle hemoglobin status. DNA was extracted retrospectively from the frozen samples by use of Qiagen™ DNA blood mini-kits (Qiagen, Crawley, United Kingdom) and typed for sickle hemoglobin using polymerase chain reaction.

Serum and urine samples collected from participants were frozen at -80°C within 4 hours of collection and later transported to Kilifi, Kenya for analysis. We determined sodium and potassium, urea and creatinine levels in these samples using ion electrophoresis and the jaffe method, respectively.²⁷ We additionally determined albumin levels in the urine samples by

immunoturbidometry using a Quantex™ microalbumin kit (Instrumentation Laboratory, Barcelona, Spain).

Statistical methods

Based on an expected minimum prevalence of 10% SCT in the ethnic groups we were studying, a systolic BP standard deviation of 15 mmHg, and 30% attrition due to poor quality ABPM data, we estimated that a total of 550 participants would provide 80% power to detect half of a standard deviation (7.5 mmHg) difference in 24 hour systolic BP between individuals with and without SCT.

As there are no published criteria for acceptable ABPM data in children, we used guidelines for completeness of ABPM data in adults from the International Database of Ambulatory blood pressure in relation to Cardiovascular Outcomes study.²⁸ Specifically, ABPM data were considered of acceptable quality if they met the following criteria: minimum 10 daytime and minimum 5 nighttime readings, where day was defined as 1000-2000 hrs and night as 0000-0600 hrs.²⁸ The same time periods were used to determine average daytime and nighttime blood pressures and to evaluate dipping status. Time weighting was applied in calculating average BP values for all time periods.²⁹

We defined screen positives for hypertension as individuals whose mean of the last 2 clinic BP measurements was above the 95th percentile for their age, sex and height.¹⁶ Confirmed hypertensives were those whose 24hr systolic

and/or diastolic BP averages respectively were above the 95th percentile for their sex, age and height.¹⁶

We categorized all subjects who were not on anti-hypertensive medication using the combination of clinic BP measurements and ABPM into four categories: sustained hypertensives (screen positive and confirmed hypertensive on ABPM); white coat hypertensives (screen positive, not confirmed hypertensive on ABPM); masked hypertensives (screen negative, confirmed hypertensive on ABPM) or normotensives (screen negative, not confirmed hypertensive on ABPM).³⁰

Dipping status was defined using ABPM data only, using day and night periods as defined above. Subjects were classified using the following four categories, based on the night/day ratio of mean systolic and/or diastolic BPs: rising or absence of dipping (ratio ≥ 1.0); mild dipping ($0.9 < \text{ratio} \leq 1.0$); dipping ($0.8 < \text{ratio} \leq 0.9$); and extreme dipping (ratio ≤ 0.8).³¹

Estimated glomerular filtration rate was calculated using the Schwartz formula.³²

Summary statistics computed included means, medians and proportions as appropriate. Comparisons between SCT carriers and non-carriers were made using Student's t-test and χ^2 tests as appropriate. Data that were not normally distributed were log transformed prior to analysis. We compared ABPM and arterial stiffness measures between those with and without the sickle cell trait by Student's t-test. In addition we performed a multivariate regression

analysis testing the effect of sickle carrier status on mean 24-hour systolic and diastolic BP with sex, age, body mass index, pulse wave velocity and estimated glomerular filtration rate as covariates.

All analyses were conducted using Stata™ Version 12 software (College Station, Texas).

The Kenya Medical Research Institute's Ethical Review Committee approved the study. Written informed consent was obtained from parents of study participants. Participating children also provided written assent. The corresponding author had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

RESULTS

Of the 938 subjects requested to participate in the study, 686 (73%) completed enrollment (Figure 5-1). The 252 adolescents that were not recruited into the study were 0.6 years (95% CI 0.3-0.9) older than study participants, but with a similar sex distribution (53% female) to those that participated in the study. Genotype data was available for 644 subjects, 609 (95%) of who had complete ABPM data. Sickle cell trait (SCT) was present in 103 (15%) of participants. The proportion of participants with complete ABPM data did not differ by sickle cell trait (91% vs 90%, $p=0.817$) or any of the other demographic and clinical data collected. One participant had Sickle Cell Disease (Hemoglobin SS) and was dropped from analyses. None of the participants were previously aware of their sickle carrier status.

Mean clinic BP, SD among all participants was (98, 11) mmHg systolic and (64, 8) mmHg diastolic. The mean 24-hour BP, SD for all participants was (117, 11) mmHg systolic and (64, 7) mmHg diastolic. Mean 24-hour Pulse Wave Velocity (PWV), SD was (7, 0.8 ms⁻¹). Based on the data accrued, the study had >98% power to detect a 5 mmHg difference in systolic BP (0.4 standard deviations), a 4mmHg (0.5 standard deviations) difference in diastolic BP and a 0.4 ms⁻¹ (0.5 standard deviations) difference in PWV between those with and those without SCT.

Table 5-1 displays the characteristics of the participants according to SCT carrier status. The mean, SD 24-hour systolic and diastolic BP in subjects with SCT was (116, 11.5) and (64, 7) mmHg. In subjects without SCT the corresponding 24-hour BP values were (117, 11.4) and (64, 6.8) (p=0.8551 and 0.9691 for comparison between SCT carriers and non-carriers). There were no statistically significant between-group differences in estimated glomerular filtration rate, clinic BP, clinic PWV and 24-hour PWV. There were no between-group differences in the prevalence of masked hypertension, white coat hypertension, or non-dipping status. Urinary sodium and potassium were 19.5 mmol/L (95%CI 1.4-37.6, p=0.0352) and 13.5mmol/L (CI 5.8-21.2, p=0.0006) lower in SCT carriers than in non-carriers.

Figure 5-2 displays distribution of 24-hour, daytime and nighttime blood pressures in study participants by SCT carrier status. All measures were similar for SCT carriers and non-carriers.

Table 5-2 displays the results of regression analyses examining whether SCT influenced 24-hour systolic and diastolic BP adjusted for age, sex, body mass index, glomerular filtration rate and pulse wave velocity. While age, sex, body mass index and glomerular filtration rate were all associated with 24-hour systolic BP, there was no association between SCT and 24-hour BP measures. Pulse wave velocity displayed the strongest association with both systolic and diastolic BP. Additional adjustment for urinary sodium and potassium levels made no material difference to the results.

DISCUSSION

It has previously been hypothesized that the excess risk of cardiovascular disease observed in African populations may be result from pleiotropic effects of genetic polymorphisms that protect them from common infections during childhood. An elegant example of this is variants of the *APOL1* gene, which while reducing the risk of trypanosomiasis, increase the risk of hypertension associated chronic kidney disease.³³ Malaria has exerted the strongest known selective pressure on the human genome, SCT being prominent among the polymorphisms under positive selection.³⁴ Given the previously documented excess cardiovascular and renal events observed in both sickle cell disease and SCT, we hypothesized that individuals with SCT would have different BP compared to those without SCT.

In this detailed study of BP phenotypes and arterial stiffness among children who were selected because they had had little exposure to malaria throughout childhood, we did not find any differences between those with and without SCT. Because the exposure measurement was a genetic trait

acquired at conception and the participants were ascertained to have remained in the same malaria-free environment since birth we believe that this study suggests that a direct effect of SCT on BP and indices of arterial stiffness is highly improbable within the first 11-17 years of life.

Estimated glomerular filtration rate and urine albumin to creatinine ratio were the same in SCT carriers and non-carriers in this study. In a study conducted among blacks in the US showing increased renal events in SCT carriers, most participants were recruited at 45 years of age and above⁸, much older than the population we recruited in this study. We however found significantly lower urine electrolyte levels in SCT carriers, which could be attributed to hyposthenuria (impaired urinary concentrating ability) that has previously been described in SCT.³⁵

While we failed to detect any meaningful effect of SCT on BP and arterial stiffness, the results of this study do have important implications; first it seems unlikely that increased BP and arterial stiffness precede or are involved in the pathogenesis of cardiovascular and renal events in individuals with SCT. In view of this, studies of other biomarkers that could predict the development of chronic kidney disease in individuals with SCT are warranted. Because individuals with SCT form a significant proportion of the population in many developing as well as developed countries, early identification of risk factors in this sub-group of individuals could have significant population-wide benefits.

An alternative to the hypothesis that genetic variations protective against infectious diseases predispose to cardiovascular disease is that in some instances the infectious diseases themselves may have long-term consequences in survivors including the development of hypertension.¹⁴ One robust way to test such hypotheses is by utilizing mendelian randomization techniques in which BP is compared in individuals with and without genetic variants that are associated with the infectious disease. An important prerequisite for using these variants is that they should not affect the outcome (BP) in the absence of the infectious disease (malaria). The results of this study suggest that SCT does not influence BP in the absence of malaria and can therefore be used as an instrumental variable in MR studies to test the malaria-high blood pressure hypothesis.¹⁴ SCT is a particularly attractive candidate for such studies as it is relatively common in areas with malaria and displays a very strong protective effect against mild as well as severe malaria^{2,4} thus reducing sample size requirements for such studies. Confirmation of the hypothesis would represent a paradigm shift in understanding the pathogenesis of hypertension in many developing country settings where malaria is endemic.³⁶

A major strength of this population-based study was the use of ABPM, which is considered the reference standard for blood pressure measurement in children.¹⁶ The study was well powered to detect very small differences in BP and PWV. We also used health and demographic surveillance system records that were prospectively collected in order to ascertain residence in a non-

malaria zone, there being no better method of doing this in sub-Saharan Africa.

A potential limitation of this study was the limited age range of subjects recruited, necessitated by the fact that there were no long-term residency records for older individuals. Most demographic surveillance systems in Africa were established in the late 1990's to early 2000's.³⁷ Recruiting older individuals would have compromised data on residency status in childhood, the period when malaria risk is highest. In addition, older subjects would be more likely to have acquired additional risk factors for hypertension, including chronic kidney disease that would have confounded the analyses. While BP differences are likely to be larger at older ages, it is known that differences in adult BP emerge in childhood^{38,39} and that childhood BP levels are predictive of adult BP.⁴⁰ The absence of even a small difference in carefully measured BP and arterial stiffness in our study of adolescents therefore suggests that it is very unlikely such differences would emerge in future.

Although the study was well powered to detect differences in continuous variables such as mean nighttime and daytime BP, we were underpowered to detect differences in the prevalences of categorical variables such as masked hypertension and white coat hypertension. This could form the basis of future studies.

An additional limitation of this study is the fact that as with many BP measurement devices, validation studies for the Arteriograph24™ have only been done in adults.²⁵ It is however unlikely that measurement error

significantly influenced the result, as the oscillometric principle used by the device has been validated in children and is particularly suited for pediatric ambulatory BP studies.^{16,41}

Non-responders in this study were slightly older than those who participated, but the 0.6-year difference is unlikely to have significantly biased our results. We also observed no significant differences between those that provided acceptable ABPM data and those that did not suggesting that the data presented are representative of the larger population of individuals with sickle cell trait.

In summary, we have demonstrated that the presence of sickle cell trait does not influence BP and arterial stiffness in Kenyan adolescents.

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Table 5-1: Characteristics of study subjects according to sickle trait status, Nairobi, Kenya 2015-2016

Characteristic	SCT Non-carrier (N=567)	SCT Carrier (N=103)	p-value
	No (%)	No (%)	
Female sex	323 (57)	49 (48)	0.08
Complete ABPM data	516 (91)	93 (90)	0.82
White coat hypertension ^a	23 (44)	3 (3.2)	0.59
Masked hypertension ^a	37 (7.2)	8 (8.6)	0.63
Non dipping BP pattern ^a	17 (3)	2 (2)	0.55
	Mean (SD)	Mean (SD)	p-value
Age, years	13.2 (2.2)	13.8 (2.3)	0.093
BMI ^b	19 (3)	19 (3)	0.63
MUAC, cm	23.2 (3.6)	23.5 (3.8)	0.41
Hemoglobin, mg/dL	13.2 (1.5)	13.4 (1.6)	0.15
White cell count	5.5 (1.5)	5.6 (1.3)	0.78
Platelet count	310 (87)	305 (95)	0.64
Serum sodium (mmol/L)	139 (6)	139 (6)	0.94
Serum potassium (mmol/L)	4.9 (0.6)	5.0 (0.8)	0.093
Socioeconomic status (MDPI score)	2.2 (1.3)	2.1 (1.3)	0.55
Clinic BP, mmHg			
Systolic	98 (11)	99 (13)	0.31
Diastolic	64 (8)	64 (9)	0.32
Pulse wave velocity (ms ⁻¹)	7 (0.8)	7.1 (0.8)	0.26
eGFR (mls/min/1.73m ²)	110 (15)	108 (14)	0.12
UACr	3.6 (18)	3.5 (8)	0.96
Urine sodium (mmol/L)	136 (73)	119 (45)	0.031
Urine potassium (mmol/L)	48 (31)	36 (23)	0.0001

BMI=Body mass index; eGFR=estimated glomerular filtration rate; MDPI=multi-dimensional poverty index; MUAC=mid upper arm circumference; UACr=Urine albumin to creatinine ratio

^aData on white coat, masked hypertension and non-dipping pattern are based on the 609 individuals that had complete ABPM data

^b BMI is calculated as Weight(kg)/height(m)²

Table 5-2: Predictors of mean 24-hour systolic and diastolic BPs among adolescents in Nairobi, Kenya, 2015-2016

Characteristic	24-hour SBP			24-hour DBP		
	β	95% CI	p-value	β	95% CI	p-value
Age, years	0.6	0.07, 1.1	0.027	0.008	-0.3, 0.3	0.96
Male Sex	2.4	0.4, 4.3	0.016	-0.06	-1.3, 1.2	0.92
BMI ^b	0.6	0.2, 1	0.001	0.2	-0.09, 0.4	0.21
eGFR (mls/min/1.73m ²)	0.08	0.01, 0.15	0.017	0.01	-0.03, 0.05	0.58
PWV (ms ⁻¹)	2.8	1.6, 4.1	<0.001	2.7	1.9, 3.5	<0.001
Sickle carrier status	0.1	-2.4, 2.6	0.923	0.4	-1.1, 2	0.58

SBP=systolic blood pressure; DBP= diastolic blood pressure; BMI=Body mass index; eGFR=estimated glomerular filtration rate; PWV= pulse wave velocity

^aMultivariate analyses were conducted using data from participants that had complete Ambulatory BP Monitoring data (N=609)

^bBMI is calculated as Weight(kg)/height(m)²

Figure 5-1 Legend: Study flow chart of participants in sickle trait–blood pressure study in Nairobi, Kenya 2015-2016.

Figure 5-2 Legend: 24-hour ABPM measures by sickle cell trait (SCT) carrier status in Nairobi, Kenya 2015-2016. For each category of 24-hour, day and night measures, the plots on the left are for systolic blood pressure and those on the right are for diastolic blood pressure. Error bars represent 95% Confidence Intervals.

Figure 5-1: Study Flow Chart

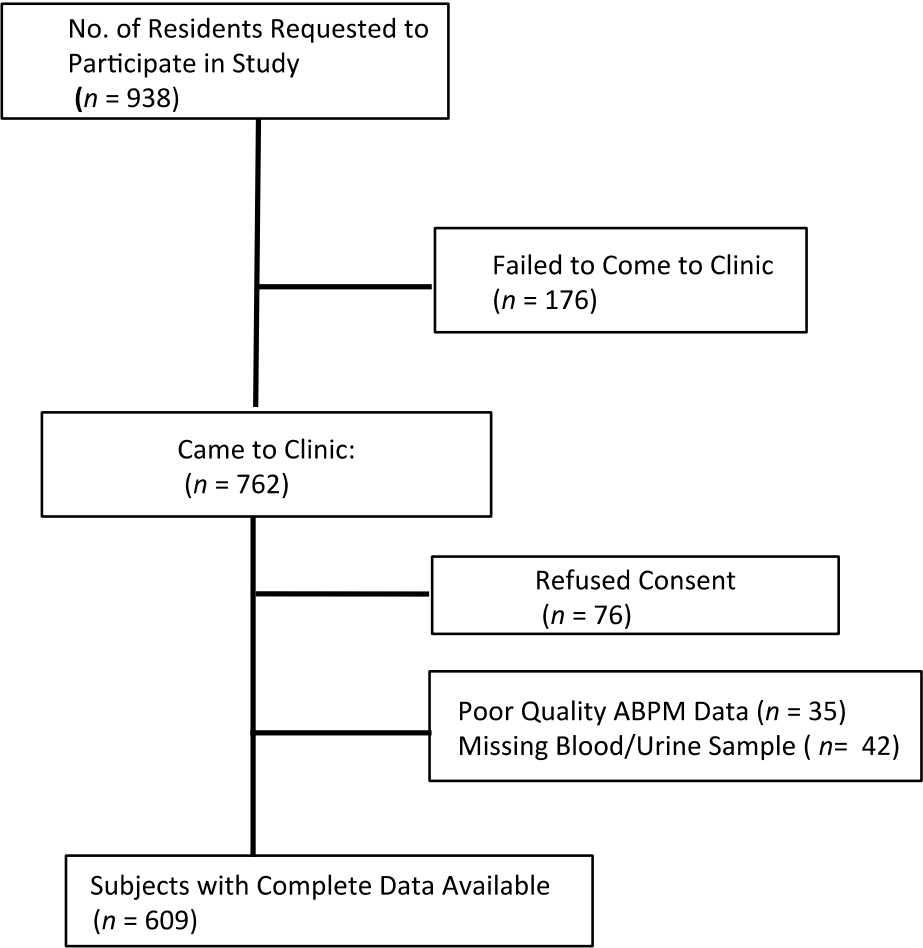
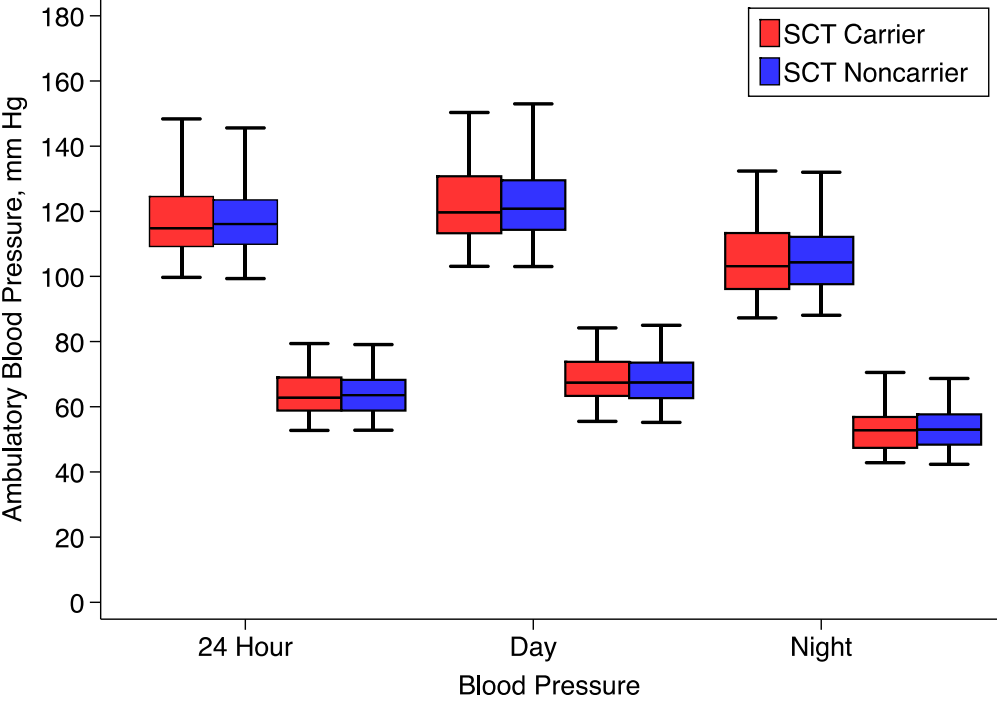


Figure 5-2: Twenty-four hour ABPM measures by sickle cell trait (SCT) carrier status in Nairobi, Kenya 2015-2016



Chapter 6. Research paper V: Relationship between childhood malaria and adult blood pressure

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SECTION A – Student Details

Student	Anthony Oliwa Etyang
Principal Supervisor	Prof Anthony Scott
Thesis Title	Determining the Causal Role of Malaria in Elevating Blood Pressure and Pulse Wave Velocity in Kenyan Adolescents and Adults

If the Research Paper has previously been published please complete Section B, if not please move to Section C

SECTION B – Paper already published

Where was the work published?			
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SECTION C – Prepared for publication, but not yet published

Where is the work intended to be published?	New England Journal of Medicine
Please list the paper's authors in the intended authorship order:	Anthony O Etyang, Sailoki Kapesa, Emily Odipo, Catherine Kyobutungi, Alex Macharia, Thomas N Williams, J Kennedy Cruickshank, Liam Smeeth, J Anthony G Scott
Stage of publication	In preparation

SECTION D – Multi-authored work

For multi-authored work, give full details of your role in the research included in the paper and in the preparation of the paper. (Attach a further sheet if necessary)	Design, data acquisition, data analysis, write up, submission
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Relationship between childhood malaria and adult blood pressure

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ABSTRACT

Background

Childhood malaria may contribute to elevated blood pressure (BP) in adults. We tested this hypothesis by conducting Mendelian randomization studies at 2 sites in Kenya.

Methods

We studied lifelong residents of Nairobi, where there is no malaria transmission; and Kilifi, which has low/moderate transmission. We compared 24-hour ambulatory blood pressure monitoring (ABPM) measures by sickle cell trait (SCT) and α^+ thalassemia, genetic variants that protect against malaria. Primary analyses utilized ABPM data meeting European Society of Hypertension quality criteria.

Results

Complete data were available for 542 of 1026 participants in Nairobi and 1136 of 2371 participants in Kilifi. The frequency of SCT was 15% in Nairobi, 20% in Kilifi. In Kilifi, SCT was associated with -2.4 mm Hg lower 24-hour Systolic BP (95% CI -4.7 to -0.1 , $p=0.037$) and a hypertension odds ratio (OR) of 0.72 (CI 0.58-1, $p=0.05$) in linear and logistic regression models adjusted for age, sex and kidney function. In population-weighted models, SCT was associated with -3.3 mmHg (CI -5.9 to -0.8 , $p=0.01$) lower 24-hour SBP and a hypertension OR of 0.64 (CI 0.45-0.91, $p=0.014$). α^+ thalassemia was associated with statistically insignificant reductions in BP in Kilifi. No differences in ABPM derived measures by SCT/ α^+ thalassemia genotype were observed in Nairobi in any of the regression models.

Conclusion

These data suggest that past malaria exposure influences adult BP.

Elucidation of the underlying mechanisms could yield new therapeutic options for hypertension.

INTRODUCTION

Malaria resulted in more than 600,000 deaths worldwide in 2015, predominantly in children.¹ While malaria's effects on various organ systems have been studied extensively, its potential long-term effects have not gained much attention. We recently hypothesized that through its effects in pregnancy and childhood, falciparum malaria may contribute to the development of high blood pressure (BP) in individuals living in malaria-endemic areas.² In the present analysis we tested whether sickle cell trait (SCT) and α^+ thalassemia, genetic variants that confer protection against malaria^{3,4}, are associated with lower BP among carriers in Kilifi, Kenya.

METHODS

This Mendelian Randomization study was carried out from December 2015 to June 2017 among long-term residents of two sites in Kenya with markedly different levels of malaria transmission: Nairobi, where there is no evidence of malaria transmission⁵, and Kilifi where there has been low to moderate transmission.^{6,7} We utilized SCT and α^+ thalassemia, both of which offer partial protection against malaria to varying degrees both independently and interactively to represent malaria exposure.^{3,8,9} In Nairobi we recruited participants aged ≥ 11 years from the Nairobi Urban Health and Demographic Surveillance System (NUHDSS)¹⁰. The Nairobi sample consisted of predominantly young participants in order to minimize potential misclassification of malaria exposure as residency records were only available for those aged ≤ 16 . In Kilifi we recruited participants age ≥ 11 years that had been lifelong residents of Chonyi and Junju locations within the Kilifi

Health and Demographic Surveillance System (KHDSS).¹¹ Kilifi participants were predominantly of the Chonyi sub-tribe of the Mijikenda ethnic group.

Study procedures have been previously described.^{12,13} Briefly participants were interviewed, followed by measurement of anthropometric indices. We then took a screening BP measurement in the clinic using an Omron™ M10-IT sphygmomanometer followed by 24-hour ABPM using an Arteriograph24™ device. Approximately 10ml of blood was drawn from each participant. All laboratory tests except for full blood counts of subjects recruited in Nairobi were performed at the KEMRI-Wellcome Trust Research Programme laboratories in Kilifi, Kenya. We genotyped for SCT and the common African form of α^+ thalassemia by PCR as described in detail previously.¹⁴

Statistical methods

A detailed pre-specified analytical plan is presented in the supplementary appendix. Separate but identical analyses were conducted for residents of Nairobi and Kilifi. We restricted analysis to subjects with ABPM data that met either of two criteria for completeness as defined by the International Database of Ambulatory blood pressure in relation to Cardiovascular Outcomes (IDACO) study¹⁵ and the European Society of Hypertension¹⁶ respectively. IDACO criteria require ≥ 10 daytime (1000-2000 hrs) and ≥ 5 nighttime (0000-0600 hrs) readings.¹⁵ ESH criteria require ≥ 20 daytime (0900-2100 hrs) and ≥ 7 nighttime (0100-0600 hrs) readings.¹⁶ The respective time periods were used to determine average daytime and nighttime BPs. 24-hour BP averages were calculated using all available readings. We predicted that

the more precise measurements obtained in the smaller group of subjects meeting the more stringent ESH criteria would provide a more accurate estimate of the effect sizes if present, but with lower precision compared to that obtained using the higher numbers of participants meeting IDACO criteria. Prevalent hypertension was diagnosed using previously published ABPM cut-offs.^{16,17}

Summary statistics computed included means, medians and proportions as appropriate. We used χ^2 test and Student's *t*-test as appropriate to compare variables in individuals with and without SCT. Hardy-Weinberg equilibrium was evaluated using χ^2 test. We performed linear regression to determine whether sickle and α^+ thalassemia genotype separately predicted ABPM measures with age, sex and estimated glomerular filtration rate (eGFR) specified as *a priori* covariates. As α^+ thalassemia modifies the protective effect of SCT against malaria⁸, we tested for statistical interaction between these two genes in linear regression models of BP. Logistic regression models with similar covariates to those used in the linear regressions were used to assess whether SCT and α^+ thalassemia influenced the odds of ABPM diagnosed prevalent hypertension. We repeated the above models, applying weights derived from the WHO standard population to compute age-standardized differences. Pre-specified sub group analyses included stratification by sex and 5 age categories and exclusion of participants taking anti-hypertensive medication. All analyses were conducted using StataTM Version 12 software (College Station, Texas). We additionally checked publicly available databases of GWAS studies for any reported associations

(at $p < 0.05$) between SCT and BP among populations that had not been exposed to malaria. AOE and JAGS had access to all the study data. AOE performed all the statistical analyses.

The Kenya Medical Research Institute's Ethical Review Committee approved the study. Written informed consent and assent where appropriate was obtained from study participants.

RESULTS

In Kilifi, we invited 2,790 randomly selected individuals from 9,543 eligible persons to participate in the study (Figure 6-1). Study participants ($N=2,371$) were slightly younger than the eligible population (mean age 39 years vs 40 years, $p=0.03$), with a higher proportion of men (46% versus 38% $p<0.001$). Individuals who did not consent were on average 9 years older (95% CI 6-13), but with a similar sex distribution (54% female) to those that consented. Individuals with homozygous sickle cell disease ($n=5$) were excluded from analyses. Complete data were available for 1986 individuals meeting IDACO criteria and 1136 meeting ESH criteria (Figure 6-1). None of the participants were previously aware of their genetic status. SCT was present in 396 (20%) of subjects while 1337 (67%) subjects carried at least 1 α^+ thalassemia mutation, equally distributed in those with and without SCT (69% vs 67%, $p=0.5$). There was no departure from Hardy-Weinberg equilibrium for both SCT ($p=0.5$) and α^+ thalassemia ($p=0.8$). The proportion of subjects with acceptable ABPM data did not differ by genotype. Participants with acceptable ABPM data were older than those with poor quality data (mean +3

years [CI 0.1–6, $p=0.06$] when using IDACO criteria and +4 years [CI 2-6, $p<0.001$] when using ESH criteria). Age standardized 24-hour BP \pm SD was 125 \pm 15 mmHg systolic and 70 \pm 10 mmHg diastolic. Mean 24-hour Pulse Wave Velocity (PWV) \pm SD was 8.2 \pm 1.4 ms⁻¹.

Table 6-1 displays the characteristics of study participants by SCT. As expected, mean eGFR was significantly lower in participants with SCT in both Kilifi and Nairobi (112 \pm 37 mls/min/1.73m² vs 116 \pm 36, $p=0.027$). Mean HbA1c levels were significantly higher in subjects with SCT, this difference persisted after controlling for age, sex and blood glucose levels (+0.15% in SCT CI 0.09-0.24, $p<0.001$). Similar differences in HbA1c and eGFR were observed when comparing participants with homozygous α^+ thalassemia versus normal (Supplementary Table S1).

Unadjusted summary and age-stratified comparisons of ABPM measures by SCT in Kilifi are presented in Supplementary Tables S16-S21. 24-hour SBP was slightly reduced in SCT overall; 127 \pm 18 mmHg in non-SCT versus 126 \pm 18 in SCT, $p=0.3911$ (Supplementary Table S17), with larger differences observed in women (Supplementary Table S18, S19).

Table 6-2 displays the effects of SCT on ABPM measures in Kilifi and Nairobi separately when adjusting for age, sex and eGFR. In Kilifi, SCT was associated with a –1.4 mm Hg (CI –3.2-0.4, $p=0.1$) reduction in 24-hour SBP (IDACO criteria) and –2.4 mm Hg (CI –4.7to –0.1, $p=0.037$) reduction in 24-hour SBP when using ESH criteria. Inclusion of an interaction term between SCT and α^+ thalassemia did not improve any of the models (LR test $p=0.9$ and

p=0.8 respectively). There was statistically significant interaction with age category (LR test p=0.0003). Age stratified analyses (Table 6-3) revealed a numerically negative relationship between SCT and SBP for nearly all age categories regardless of ABPM quality criteria used, with the 25-39 years and 55-70 years categories displaying the largest reductions when using IDACO and ESH criteria respectively. We did not detect a statistically significant interaction with sex (LR test p=0.21), although sex stratified analyses (supplementary Tables S2, S3) suggested a greater SCT associated BP reduction in women compared to men.

Additional adjustment for HbA1c levels, which were unexpectedly higher in individuals with SCT (Table 6-1), increased the magnitude of BP reduction associated with SCT in Kilifi. (Supplementary Table S14)

SCT in Kilifi was associated with reduced but statistically insignificant pulse wave velocity in a linear regression model adjusted for age, sex and egfr (-0.03 ms^{-1} , CI $-0.14, 0.07$, p=0.5). No such reduction was observed in Nairobi.

Figure 6-2 displays the association between SCT and ABPM measures in age weighted regression analyses adjusting for sex and eGFR in Kilifi and Nairobi. SCT was associated with lowering of all ABPM measures in Kilifi regardless of whether IDACO or ESH criteria were used. Statistically significant differences in the linear regressions were only observed when using data that met ESH criteria; SCT was associated with -3.3 mm Hg lower (CI -5.9 to -0.8 , p=0.01) 24hr SBP and -2.4 mm Hg lower (CI -4.1 to -0.7 , p=0.005) 24-hour DBP. Nighttime measurements revealed greater differences compared

to day measurements, e.g SCT was associated with -4.3mm Hg lower nighttime SBP (CI -7.1 to -1.4 , $p=0.003$) while the effect on daytime SBP was -2.7mm Hg (CI -5.2 to -0.01 , $p=0.049$). The adjusted odds ratio for ABPM diagnosed hypertension in SCT was 0.72 (CI 0.54 - 0.94 , $p=0.018$) when using IDACO criteria and 0.64 (CI 0.45 - 0.91 , $p=0.014$) when using ESH criteria. As with the unweighted regressions, SCT associated BP reduction appeared to be greater in women than in men (Supplementary Figure 1).

No significant differences by SCT/ α^+ thalassemia status were observed among participants in Nairobi in any of the ABPM derived measures (Figure 6-2, Table 6-2, Supplementary Tables S2-S15). We have previously published results restricted to 11-17 year old Nairobi residents showing that SCT and α^+ thalassemia do not directly influence BP.^{12,13}

We checked the phenoscanner database for any reported associations (at $p < 0.1$) between SCT and BP phenotypes from GWAS studies and found none.¹⁸

DISCUSSION

We found that polymorphisms associated with partial protection against malaria were associated with significantly reduced BP and reduced odds of hypertension in Kilifi, an area with malaria transmission, but not in Nairobi, where there is no malaria transmission. We observed significant differences when using SCT, which confers a higher level of protection against malaria episodes than α^+ thalassemia. This suggests that increased risk of malaria in the past is associated with higher BP in the present day.

The magnitude of BP reduction afforded by SCT was roughly similar to that attributed to use of a single anti-hypertensive agent,^{19,20} but several factors suggest that the actual effect of malaria on BP might be greater. Firstly, SCT is only associated with 50% reduction in incidence of non-severe malaria³ and 90% reduction against severe malaria episodes²¹, and in Kilifi this relative protection wanes with age because of the development of immunity in those without SCT.²² Secondly, the protection offered by SCT against malaria is reduced in individuals with concurrent α^+ thalassemia⁸ who comprised 67% of our study sample, but we were not powered to detect the effect of this interaction on BP. In addition Kilifi has low to moderate malaria transmission.⁶ Consequently the estimates obtained in this study likely represent an underestimate of the true effect of malaria on BP.

One strength of this study was the use of ABPM, considered the reference standard for BP measurement in children and adults.²³ By performing multiple repeated measurements ABPM gives more precise estimates of BP¹⁶ and we

observed the largest differences when using the more stringent ESH criteria as well as when using nighttime measurements, which occur under more standardized conditions. Nocturnal BP indices are more predictive of cardiovascular outcomes than daytime or 24-hour values^{24,25}, and nighttime SBP is the single ABP variable most closely related with cardiovascular outcomes.¹⁶ The Mendelian randomization approach using genetic variants that are very strongly associated with malaria is another strength. Study participants in Kilifi were of the same ethnicity, minimizing the possibility of population stratification explaining the differences observed. An additional strength of the study is that we used prospectively collected health and demographic surveillance system (HDSS) records to ascertain residence in malaria/ non-malaria zones.

Although Mendelian Randomization is a well-established method for inferring causality, several limitations need highlighting that could be resolved using longitudinal studies. As we did not have historical malaria data for the participants, we could not determine the number and/or severity of episodes required to chronically elevate BP. Malaria transmission has been changing over time along the Kenyan coast, with a marked reduction observed from the year 2000⁷; longitudinal studies would help explain the interaction that we observed with age by revealing any cohort effects as a result of changing malaria transmission and also determine the age of onset of BP elevation following malaria infection. Lastly, longitudinal studies would also determine the effect of survivor bias due to differential malaria mortality between the genetic groups compared.

We did not study the physiological mechanisms by which malaria could have elevated BP. A potential mechanism would be chronic inflammation induced by malaria.² Inflammation has been associated with the development of hypertension.^{26,27} Malaria also causes stunting and malnutrition, which could influence BP² but anthropometric indices such as BMI and MUAC, were similar in the groups we studied. Mechanistic studies are also required to explain the apparent sex differences that we observed in our study. SCT may protect against malaria in pregnancy²⁸, which has been associated with gestational hypertensive disorders that place women at risk of chronic hypertension. CD4⁺ and CD8⁺ T-cells, which play a role in responses to malaria²⁹ as well as partially explain sex differences in hypertension³⁰, could possibly mediate an interaction with sex. An additional but unlikely mechanism leading to BP elevation would be as a result of anti-malarial treatment given, but none of the commonly used anti-malarial drugs is known to cause long term elevation of BP. Elucidating the mechanisms leading to malaria associated BP elevation could inform the design of novel therapies for the treatment or prevention of hypertension.

While we showed that SCT does not influence BP in the absence of malaria by studying subjects in Nairobi, the study population there was significantly younger than that in Kilifi. Age-standardized comparisons however showed that SCT was associated with lower BP only in Kilifi. Comparisons in the 11-25 year age groups at both sites where we had the best statistical power to detect differences revealed a numerically lower BP associated with SCT in Kilifi with no such signal detected in Nairobi. Two possible explanations for

our inability to detect a statistically significant lower BP associated with SCT in Kilifi in the under 25 age category are: (1) these subjects experienced less malaria than older cohorts, (2) increased BP due to childhood malaria exposure may manifest at older ages. Several other studies that had older participants also strongly suggest that SCT does not directly influence BP. Naik *et al* in a study demonstrating increased risk of kidney disease in SCT among 15,975 African Americans observed no difference in baseline BP based on SCT. Liem *et al* in an analysis of 1995 African Americans followed up for 25 years found no difference in incident hypertension based on SCT.³¹ In addition large GWAS studies conducted in adults have not reported SCT influencing BP at any significance level as confirmed by our query of the PhenoScanner database.^{18,32,33} For our interpretation to be invalidated, SCT or loci in linkage disequilibrium would have to be associated with a large reduction in BP that is only detectable in adult life. This combination of factors is highly unlikely because (1) none of the loci in the Bantu/CAR haplotype that is predominant among individuals with SCT in Kilifi has been associated with BP traits, (2) BP differences e.g. between racial groups are detectable in childhood/adolescence³⁴, and we did not observe this in the malaria unexposed (Nairobi) cohort; and (3) most genetic polymorphisms influencing BP tend to have very small effects, making it unlikely that direct effects SCT could explain the differences that we observed.

In summary, we have demonstrated that exposure to malaria in childhood is associated with higher BP in adult life. One implication is that malaria elimination would lead to health benefits beyond those currently described. In

addition, elucidating the mechanisms leading to BP elevation as a result of malaria infection could yield new therapeutic options for hypertension.

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Conflicts of Interest/Disclosures

None of the authors have any conflicts of interest or disclosures to report.

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Table 6-1: Characteristics of Study Participants by SCT Status

Characteristic	Kilifi N=1986						Nairobi N=814					
	ESH Criteria N=1140			IDACO Criteria N=1986			ESH Criteria N=542			IDACO Criteria N=814		
	SCT n=240	Non-SCT n=900		SCT n=396	Non-SCT n=1590		SCT n=83	Non-SCT n=459		SCT n=121	Non-SCT n=693	
n	%	n	%	n	%	n	%	n	%	n	%	%
Age												
11-24y	95	42	383	43	162	41	717	45	65	78	344	75
25-39y	20	8	64	7	35	9	130	8	7	8	25	5
40-55y	46	19	170	19	76	19	296	19	7	8	47	10
>55y	79	33	283	31	123	31	447	28	4	5	43	9
Female	127	53	532	59	196	49	862	54	40	48	248	54
Smoker	17	7	78	9	34	9	128	8	3	4	8	2
Previously diagnosed hypertensive	37	16	127	14	57	14	216	14	7	8	53	12
On anti-hypertensive medication	9	4	26	3	11	3	36	2	0	0	8	2
Alpha thalassemia present	165	69	603	67	276	70	1061	67	41	50	204	45
successful BP inflations	50	7	50	8	45	10	45	10	53	8	53	6
BMI kg/m ²	21	4	21	4	21	4	21	4	20	4	21	4
HbA1c	5.2	0.6	5.1	0.8	5.2	0.6	5	0.7	5.2	1	5.3	1
eGFR	108	35	115	41	111	39	117	40	108	35	115	41
UACR	4	0.6	3	0.4	3.8	0.4	3	0.3	3.9	0.9	4	1
Urine sodium	104	48	120	63	105	45	121	58	116	42	129	58
Urine potassium	57	42	60	41	62	43	57	39	38	24	50	32

BMI: Body mass index UACR: urine albumin to creatinine ratio eGFR: estimated glomerula filtration rate in mls/min/1.73m² Urine sodium and potassium are in units of mmol/L

Table 6-2: Effect of SCT on Ambulatory blood pressure indices in Kilifi and Nairobi

ABPM measure	ESH Criteria						IDACO Criteria					
	Kilifi (N=1127)			Nairobi (N=527)			Kilifi (N=1965)			Nairobi (N=788)		
	Effect, mm Hg	95% CI	p value	Effect, mm Hg	95% CI	p value	Effect, mm Hg	95% CI	p value	Effect, mm Hg	95% CI	p value
24 hour SBP	-2.4	-4.7, -0.1	0.04	0.5	-2.6, 3.6	0.7	-1.4	-3.1, 0.4	0.1	-0.2	-2.7, 2.4	0.9
24 hour DBP	-1.4	-2.8, 0.1	0.07	0.5	-1.6, 2.6	0.7	-0.7	-1.8, 0.4	0.2	-0.04	-1.7, 1.6	1
Nighttime SBP	-3.2	-5.7, -0.6	0.02	0.7	-2.5, 4.0	0.7	-1.6	-3.5, 0.4	0.1	-0.9	-3.5, 1.8	0.5
Nighttime DBP	-1.8	-3.3, -0.2	0.03	0.6	-1.7, 2.9	0.6	-0.9	-2.1, 0.3	0.1	-0.6	-2.4, 1.1	0.5
Daytime SBP	-1.9	-4.2, 0.4	0.1	-0.1	-3.4, 3.2	1	-0.9	-2.7, 0.9	0.3	-0.5	-3.2, 2.3	0.7
Daytime DBP	-1.0	-2.6, 0.6	0.2	0.1	-2.2, 2.4	0.9	-0.6	-1.8, 0.6	0.4	-0.2	-2.1, 1.7	0.8

SBP: Systolic blood pressure
 DBP: Diastolic blood pressure
 Linear regression models adjusted for age, sex and estimated glomerular filtration rate

Table 6-3: Age specific effects of SCT on BP in Kilifi participants

ESH Criteria	24 hour measures						Night time measures						Daytime measures					
	Age, yr	N	SBP	CI	DBP	CI	SBP	CI	DBP	CI	SBP	CI	DBP	CI				
	11-24	472	-0.5	-2.9,1.8	0.3	-1.3,1.9	-0.4	-2.9,2.1	0.4	-1.3,2.1	-1.3	-3.8,1.3	0.2	-1.6,2.0				
	25-39	82	-5.3	-13.2,2.7	-4.5	-9.8,0.9	-6.4	-15.2,6	-5.5	-11,0.3	-4.3	-12.3,4	-3.4	-8.9,2.1				
	40-54	214	-3.9	-9.5,1.7	-3.9	-7.8,-0.1	-5.7	-12,0.3	-3.7	-7.7,0.3	-2.7	-8.6,3.1	-4.0	-8.1,0.2				
	55-70	233	-4.2	-10.2,1	-2.1	-6.1,1.8	-4.9	-12.2,1	-3.1	-7.2,0.9	-3.1	-9.3,3.1	-1.2	-5.5,3.0				
	>70	126	-1.7	-11.7,5	0.3	-5.1,5.7	-2.9	-14.8,4	-0.6	-6.9,5.6	0.8	-8.3,9.8	1.5	-4.0,7.1				
IDACO Criteria																		
Age, yr	N	SBP	CI	DBP	CI	SBP	CI	DBP	CI	SBP	CI	DBP	CI					
Age, yr																		
11-24	871	-0.4	-2.3,1.5	0.4	-0.8,1.7	0.1	-1.9,2.1	0.6	-0.7,1.9	-1.2	-3.2,0.9	0.2	-1.2,1.8					
25-39	169	-1.5	-13.2,2.7	-0.8	-4.4,2.7	-1.7	-7.5,4.1	-1.1	-5.0,2.8	-1.1	-6.3,4.2	-0.4	-4.2,3.4					
40-54	366	-2.2	-6.3,1.9	-2.2	-5.1,0.7	-3.8	-8.2,0.7	-2.8	-5.9,0.2	-0.5	-4.7,3.8	-2.2	-5.3,0.9					
55-70	382	-3.7	-9.1,6	-1.9	-5.1,1.3	-3.8	-9.7,2.1	-2.3	-5.7,1	-2.6	-7.8,2.6	-1.7	-5.0,1.7					
>70	126	1	-6.5,8.4	0.2	-4.2,4.6	-0.1	-8.6,8.6	-0.4	-5.2,4.4	2.7	-4.8,10	1.2	-3.5,5.9					
SBP: Systolic blood pressure																		
DBP: Diastolic blood pressure																		
Linear regression models adjusted for age, sex and estimated glomerular filtration rate																		

Supplementary tables

S1. Characteristics of study participants by thalassemia status

Characteristic	Kiifi						Nairobi					
	Normal n=654		Heterozygous n=1003		Homozygous n=375		Normal n=457		Heterozygous n=306		Homozygous n=63	
	n	%	n	%	n	%	n	%	n	%	n	%
Age												
11-24y	285	44	449	45	162	33	354	77	236	77	50	81
25-39y	50	8	92	9	28	7	33	7	14	5	1	2
40-55y	134	20	170	17	75	20	30	7	26	9	5	8
>55y	185	28	292	29	110	29	32	7	30	10	5	8
Female	368	56	512	51	205	55	223	49	165	54	33	53
Smoker	55	9	84	8	28	8	11	2	8	3	3	5
Previously diagnosed hypertensive	91	14	127	14	37	16	43	9	30	10	4	6
On anti-hypertensive medication	16	2	25	3	8	2	5	1	6	2	1	2
successful BP inflations	mean 44	sd 10	mean 45	sd 10	mean 45	sd 10	mean 50	sd 9	mean 50	sd 8	mean 52	sd 8
BMI kg/m ²	21	4	21	4	21	4	20	4	20	4	20	4
HbA1c	5.0	0.8	5.1	0.6	5.2	0.6	5.2	1	5.4	1	5.6	1.4
eGFR	117	44	116	38	111	38	117	24	115	24	117	20
UACR	3	5.8	3.9	17	3.9	15	1.5	2.3	1.2	2.3	0.9	1.9
Urine sodium	116	51	117	60	121	53	129	75	126	55	119	50
Urine potassium	57	42	61	41	65	43	48	32	45	30	41	23

BMI: Body mass index UACR: urine albumin to creatinine ratio eGFR: estimated glomerula filtration rate in ml/s/min/1.73m² Urine sodium and potassium are in units of mmol/L

Supplementary tables

S2. Effect of SCT on ABPM indices in Women

ABPM measure	ESH Criteria				IDACO Criteria			
	Kilifi (N=659)		Nairobi (N=275)		Kilifi (N=1046)		Nairobi (N=399)	
	Effect, mm Hg	95% CI p value	Effect, mm Hg	95% CI p value	Effect, mm Hg	95% CI p value	Effect, mm Hg	95% CI p value
24 hour SBP	-3.7	-7.1, -0.4 0.03	-0.2	-4.6, 4.2 0.9	-1.9	-4.5, 0.6 0.1	-1.3	-5.0, 2.3 0.5
24 hour DBP	-1.9	-2.8, 0.1 0.08	0.9	-2.1, 3.8 0.6	-0.7	-2.3, 0.8 0.4	0.1	-2.3, 2.4 1
Nighttime SBP	-4.3	-8.0, -0.6 0.02	0.6	-3.9, 5.2 0.8	-2.0	-4.9, 0.8 0.2	-2.1	-5.8, 1.7 0.3
Nighttime DBP	-2.0	-4.2, 0.1 0.07	1.6	-1.5, 4.7 0.3	-0.7	-2.4, 1.0 0.4	-0.3	-2.8, 2.1 0.8
Daytime SBP	-3.1	-6.4, 0.2 0.07	-1.3	-5.9, 3.4 0.6	-1.4	-3.9, 1.2 0.3	-2.4	-6.3, 1.6 0.2
Daytime DBP	-1.6	-3.9, 0.6 0.2	-0.1	-3.4, 3.2 0.9	-0.8	-2.6, 0.9 0.3	-1.1	-3.8, 1.6 0.4

SBP: Systolic blood pressure

DBP: Diastolic blood pressure

Linear regression models adjusted for age, sex and estimated glomerular filtration rate

Supplementary tables

S3. Effect of SCT on ABPM indices in Men

ESH Criteria				IDACO Criteria								
	Kilifi (N=473)			Nairobi (N=241)			Kilifi (N=919)			Nairobi (N=378)		
ABPM measure	Effect, mm Hg	95% CI	p value	Effect, mm Hg	95% CI	p value	Effect, mm Hg	95% CI	p value	Effect, mm Hg	95% CI	p value
24 hour SBP	-0.7	-3.7, 2.3	0.6	1.8	-2.7, 6.3	0.4	-0.8	-3.2, 1.7	0.5	1.3	-2.2, 5.0	0.5
24 hour DBP	-0.7	-2.7, 1.3	0.5	0.8	-2.3, 3.8	0.6	-0.7	-2.3, 0.8	0.4	0.3	-2.1, 2.6	0.8
Nighttime SBP	-1.6	-5.1, 1.8	0.4	1.4	-3.3, 6.2	0.6	-1.1	-3.7, 1.6	0.4	0.7	-3.1, 4.5	0.7
Nighttime DBP	-1.4	-3.6, 0.8	0.2	0.3	-3.0, 3.7	0.9	-1.1	-2.8, 0.6	0.2	-0.6	-3.1, 2.0	0.6
Daytime SBP	-0.3	-3.4, 2.9	0.9	1.5	-3.3, 6.3	0.5	-0.4	-3.0, 2.2	0.8	1.7	-2.1, 5.6	0.4
Daytime DBP	-0.2	-2.4, 2	0.9	0.9	-2.5, 4.2	0.6	-0.4	-2.1, 1.4	0.7	1.1	-1.6, 3.8	0.4

SBP: Systolic blood pressure

DBP: Diastolic blood pressure

Linear regression models adjusted for age, sex and estimated glomerular filtration rate

Supplementary tables

S4. Effect of Alpha thalassemia on Ambulatory blood pressure indices in Kilifi and Nairobi

ESH Criteria						IDACO Criteria						
ABPM measure	Kilifi (N=1125)			Nairobi (N=514)			Kilifi (N=1961)			Nairobi (N=771)		
	Effect, mm Hg	95% CI	p value	Effect, mm Hg	95% CI	p value	Effect, mm Hg	95% CI	p value	Effect, mm Hg	95% CI	p value
24 hour SBP	-0.5	-1.8, 0.9	0.5	1.6	-0.2, 3.4	0.09	-0.9	-1.9, 0.2	0.1	0.7	-0.7, 2.2	0.3
24 hour DBP	-0.2	-1.1, 0.7	0.7	1.0	-0.2, 2.2	0.1	-0.3	-1.0, 0.3	0.3	0.6	-0.4, 1.5	0.3
Nighttime SBP	-0.5	-2.0, 1.0	0.5	0.7	-2.5, 4.0	0.7	-0.6	-1.8, 0.5	0.3	0.7	-0.8, 2.3	0.3
Nighttime DBP	-0.2	-1.1, 0.7	0.7	0.6	-1.7, 2.9	0.6	-0.3	-1.0, 0.4	0.4	0.7	-0.3, 1.7	0.2
Daytime SBP	-0.4	-1.7, 1.0	0.6	0.8	-1.1, 2.7	0.4	-0.9	-2.0, 0.1	0.08	0.1	-1.5, 1.7	0.9
Daytime DBP	-0.1	-1.0, 0.8	0.8	0.5	-0.9, 1.8	0.5	-0.3	-1.0, 0.4	0.4	0.1	-1.0, 1.2	0.9

SBP: Systolic blood pressure

DBP: Diastolic blood pressure

Linear regression models adjusted for age, sex and estimated glomerular filtration rate

Supplementary tables

S5. Effect of Alpha thalassemia on Ambulatory blood pressure indices in Women in Kilifi and Nairobi

ESH Criteria					IDACO Criteria							
ABPM measure	Kilifi (N=653)			Nairobi (N=275)			Kilifi (N=1044)			Nairobi (N=395)		
	Effect, mm Hg	95% CI	p value	Effect, mm Hg	95% CI	p value	Effect, mm Hg	95% CI	p value	Effect, mm Hg	95% CI	p value
24 hour SBP	-1.0	-2.8, 0.9	0.3	1.1	-1.3, 3.5	0.4	-1.2	-2.6, 0.2	0.08	0.7	-1.4, 2.7	0.5
24 hour DBP	-0.7	-1.8, 0.5	0.3	0.9	-0.7, 2.5	0.3	-0.6	-1.5, 0.2	0.2	0.3	-1.0, 1.7	0.6
Nighttime SBP	-0.9	-2.9, 1.2	0.4	0.7	-1.8, 3.2	0.6	-0.6	-1.8, 0.5	0.3	0.7	-0.8, 2.3	0.3
Nighttime DBP	-0.5	-1.7, 0.7	0.4	0.9	-0.9, 2.6	0.3	-0.3	-1.0, 0.4	0.4	0.7	-0.3, 1.7	0.2
Daytime SBP	-0.9	-2.8, 1.0	0.3	0.3	-2.2, 2.8	0.8	-1.2	-2.5, 0.2	0.1	-0.04	-2.2, 2.2	1
Daytime DBP	-0.6	-1.8, 0.7	0.4	0.5	-1.3, 2.3	0.6	-0.5	-1.4, 0.4	0.3	-0.1	-1.6, 1.4	0.9

SBP: Systolic blood pressure

DBP: Diastolic blood pressure

Linear regression models adjusted for age, sex and estimated glomerular filtration rate

Supplementary tables

S6. Effect of Alpha thalassemia on Ambulatory blood pressure indices in Men in Kilifi and Nairobi

ABPM measure	ESH Criteria						IDACO Criteria					
	Kilifi (N=472)			Nairobi (N=239)			Kilifi (N=917)			Nairobi (N=376)		
	Effect, mm Hg	95% CI	p value	Effect, mm Hg	95% CI	p value	Effect, mm Hg	95% CI	p value	Effect, mm Hg	95% CI	p value
24 hour SBP	0.3	-1.6, 2.2	0.8	2.2	-0.5, 4.9	0.1	-0.4	-1.9, 1.1	0.6	0.7	-1.4, 2.9	0.5
24 hour DBP	0.5	-0.7, 1.7	0.4	1.2	-0.6, 3.0	0.2	-0.01	-0.9, 0.9	1	0.7	-0.6, 2.1	0.3
Nighttime SBP	0.03	-2.1, 2.2	1	3.0	0.2, 5.9	0.04	-0.1	-1.7, 1.5	0.9	0.7	-0.8, 2.3	0.3
Nighttime DBP	0.2	-1.2, 1.6	0.8	2.3	0.3, 4.3	0.03	-0.01	-1.0, 1.0	1	0.7	-0.3, 1.7	0.2
Daytime SBP	0.4	-1.6, 2.3	0.7	1.3	-1.6, 4.2	0.4	-0.7	-2.2, 0.9	0.4	0.2	-2.1, 2.4	0.8
Daytime DBP	0.6	-0.8, 1.9	0.4	0.5	-1.5, 2.5	0.6	-0.09	-1.1, 1.0	0.9	0.3	-1.3, 1.8	0.8

SBP: Systolic blood pressure

DBP: Diastolic blood pressure

Linear regression models adjusted for age, sex and estimated glomerular filtration rate

Supplementary tables
S7. Age specific effects of SCT on BP in Nairobi participants

ESH Criteria	24 hour measures					Night time measures					Daytime measures				
	Age, yr	N	SBP	CI	DBP	CI	SBP	CI	DBP	CI	SBP	CI	DBP	CI	
11-24	389	0.4	-2.5, 3.4	0.7	-1.2, 2.6	1.1	-1.9, 4	0.8	-1.2, 2.8	-0.5	-3.8, 2.7	0.1	-2.2, 2.3		
25-39	31	9.7	-3.6, 23	-1.1	-9.3, 7.1	3.1	-9.2, 16	2.1	-6.2, 11	0.2	-11, 12	-3.2	-13, 6.0		
40-54	50	3.8	-12, 20	-3.3	-14, 7.3	-1.8	-21, 17	-5.1	-18, 8	6.9	-9.3, 23	2.7	-9.2, 15		
>55	46	5.8	-18, 30	6.7	-9.0, 22	9.6	-15, 34	12	-5.3, 29	4.5	-19, 28	5.0	-11, 21		
IDACO Criteria															
Age, yr															
Age, yr															
11-24	607	-0.5	-2.9, 2.0	0.1	-1.5, 1.6	-1	-3.4, 1.5	-0.6	-2.3, 1.1	-0.9	-3.7, 1.8	-0.3	-2.1, 1.6		
25-39	42	-2.9	-14, 8.6	-2.9	-11, 5.0	-1.3	-13, 11	-0.3	-8.4, 7.8	-3.8	-16, 8.4	-4.7	-14, 4.6		
40-54	65	2.2	-10, 15	-2.1	-11, 6.7	-2.1	-16, 12	-5.4	-15, 4.0	4.6	-8.0, 17	1.1	-8.5, 11		
>55	63	4.6	-14, 23	4.1	-7.5, 16	8.2	-11, 27	7.7	-4.4, 20	1.1	-18, 20	1.8	-10, 14		
SBP: Systolic blood pressure															
DBP: Diastolic blood pressure															
Linear regression models adjusted for age, sex and estimated glomerular filtration rate															

Supplementary tables

S8. Effect of SCT on Ambulatory blood pressure indices in Kilifi and Nairobi-population weighted analyses

ESH Criteria					IDACO Criteria							
	Kilifi (N=1127)			Nairobi (N=527)			Kilifi (N=1965)			Nairobi (N=788)		
ABPM measure	Effect, mm Hg	95% CI	p value	Effect, mm Hg	95% CI	p value	Effect, mm Hg	95% CI	p value	Effect, mm Hg	95% CI	p value
24 hour SBP	-3.3	-5.9, -0.8	0.01	1.7	-4.5, 7.9	0.6	-1.8	-3.8, 0.2	0.08	0.3	-4.7, 5.3	0.9
24 hour DBP	-2.4	-4.1, -0.7	0.01	1.2	-2.9, 5.3	0.6	-1.1	-2.4, 0.2	0.1	-0.3	-3.5, 3.0	0.9
Nighttime SBP	-4.3	-7.1, -1.4	0.003	1.7	-4.7, 8.1	0.6	-2.1	-4.3, 0.1	0.06	-0.2	-5.6, 5.2	0.9
Nighttime DBP	-2.9	-4.7, -1.1	0.002	1.9	-2.6, 6.5	0.4	-1.3	-2.7, 0.2	0.08	-0.2	-4, 3.6	0.9
Daytime SBP	-2.6	-5.2, -0.01	0.05	1.4	-5.0, 7.9	1	-1.2	-3.2, 0.8	0.2	0.1	-5, 5.1	1
Daytime DBP	-1.9	-3.7, -0.1	0.04	0.9	-3.4, 5.1	0.7	-0.9	-2.3, 0.5	0.2	-0.3	-3.7, 3.1	0.9

SBP: Systolic blood pressure
DBP: Diastolic blood pressure
WHO standard population weighted linear regression models adjusted for sex and estimated glomerular filtration rate

Supplementary tables

S9. Effect of SCT on Ambulatory blood pressure indices in Women in Kilifi and Nairobi-population weighted analyses

ESH Criteria					IDACO Criteria							
ABPM measure	Kilifi (N=659)			Nairobi (N=275)			Kilifi (N=1046)			Nairobi (N=399)		
	Effect, mm Hg	95% CI	p value	Effect, mm Hg	95% CI	p value	Effect, mm Hg	95% CI	p value	Effect, mm Hg	95% CI	p value
24 hour SBP	-4.5	-8.2, -0.9	0.02	2.8	-6.9, 13	0.6	-2.0	-4.7, 0.7	0.3	1.6	-7.5, 11	0.7
24 hour DBP	-2.8	-5.1, -0.4	0.02	3.2	-3.3, 9.6	0.3	-0.9	-2.7, 0.9	0.3	1.6	-4.2, 7.4	0.6
Nighttime SBP	-5.3	-9.3, -1.3	0.01	3.5	-6.4, 13	0.5	-2.1	-5.1, 1	0.2	1.4	-7.7, 11	0.8
Nighttime DBP	-3.3	-5.8, -0.8	0.01	4.2	-2.7, 11	0.2	-1	-3.0, 1.0	0.3	2	-4.0, 7.9	0.5
Daytime SBP	-3.7	-7.3, -0.1	0.05	1.5	-8.6, 12	0.8	-1.7	-4.3, 1.0	0.2	0.4	-9.0, 9.9	0.9
Daytime DBP	-2.3	-4.7, 0.2	0.07	1.7	-5, 8.4	0.6	-1.0	-2.8, 0.8	0.3	0.4	-6.0, 6.8	0.9

SBP: Systolic blood pressure

DBP: Diastolic blood pressure

WHO standard population weighted linear regression models adjusted for sex and estimated glomerular filtration rate

Supplementary tables

S10. Effect of SCT on Ambulatory blood pressure indices in Men in Kilifi and Nairobi-population weighted analyses

ESH Criteria					IDACO Criteria										
	Kilifi (N=473)				Nairobi (N=241)				Kilifi (N=919)				Nairobi (N=378)		
ABPM measure	Effect, mm Hg	95% CI	p value	Effect, mm Hg	95% CI	p value	Effect, mm Hg	95% CI	p value	Effect, mm Hg	95% CI	p value	Effect, mm Hg	95% CI	p value
24 hour SBP	-1.6	-5.0, 1.9	0.4	3.6	-2.9, 10	0.3	-1.5	-4.1, 1.1	0.3	3.0	-2.2, 8.1	0.3			
24 hour DBP	-1.8	-4.2, 0.7	0.2	2.3	-2.3, 6.9	0.3	-1.4	-3.2, 0.4	0.1	1.7	-3.0, 6.4	0.5			
Nighttime SBP	-3.3	-7.7, 1.0	0.1	1.9	-4.9, 8.8	0.6	-2.6	-5.6, 0.4	0.09	1.2	-4.1, 6.5	0.7			
Nighttime DBP	-2.6	-5.5, 0.3	0.08	2.7	-3.0, 8.5	0.3	-2.1	-4.1, -0.2	0.03	1.1	-4.5, 6.7	0.7			
Daytime SBP	-0.5	-4.2, 3.2	0.8	4.9	-1.9, 12	0.2	-0.4	-3.2, 2.5	0.8	4.3	-1.7, 10	0.2			
Daytime DBP	-1.1	-3.8, 1.6	0.4	2.9	-1.1, 7.0	0.2	-0.6	-2.8, 1.6	0.6	2.7	-1.7, 7	0.3			

SBP: Systolic blood pressure
DBP: Diastolic blood pressure
WHO standard population weighted linear regression models adjusted for sex and estimated glomerular filtration rate

Supplementary tables

S11. Effect of Alpha thalassemia on Ambulatory blood pressure indices in Kilifi and Nairobi-population weighted analyses

ESH Criteria					IDACO Criteria											
ABPM measure	Kilifi (N=1125)			p value	Nairobi (N=514)			p value	Kilifi (N=1961)			p value	Nairobi (N=771)			p value
	Effect, mm Hg	95% CI			Effect, mm Hg	95% CI			Effect, mm Hg	95% CI			Effect, mm Hg	95% CI		
24 hour SBP	-0.9	-2.3, 0.7	0.3		2.4	-3.1, 7.9	0.4		-1.4	-2.5, -0.3	0.01		0.7	-4.3, 5.6	0.8	
24 hour DBP	-0.3	-1.4, 0.7	0.6		1.6	-2.1, 5.3	0.4		-0.6	-1.4, 0.1	0.1		0.3	-2.9, 3.5	0.9	
Nighttime SBP	-1.0	-2.7, 0.7	0.2		2.0	-4.2, 8.1	0.5		-1.4	-2.7, -0.2	0.03		0.5	-5.0, 6	0.9	
Nighttime DBP	-0.7	-1.8, 0.5	0.2		1.9	-2.1, 6.0	0.4		-0.8	-1.6, 0.04	0.06		0.5	-2.9, 4.0	0.8	
Daytime SBP	-0.8	-2.2, 0.8	0.3		1.8	-3.2, 6.9	0.5		-1.5	-2.7, -0.4	0.01		-0.01	-4.7, 4.7	1	
Daytime DBP	-0.1	-1.2, 1.0	0.9		1.1	-2.4, 4.7	0.5		-0.5	-1.4, 0.3	0.2		-0.3	-3.5, 3.0	0.9	

SBP: Systolic blood pressure

DBP: Diastolic blood pressure

WHO standard population weighted linear regression models adjusted for sex and estimated glomerular filtration rate

Supplementary tables
S12. Effect of Alpha thalassemia on Ambulatory blood pressure indices in Women in Kilifi and Nairobi-population
weighted analyses

ESH Criteria					IDACO Criteria										
	Kilifi (N=653)				Nairobi (N=275)				Kilifi (N=1044)				Nairobi (N=395)		
ABPM measure	Effect, mm Hg	95% CI	p value		Effect, mm Hg	95% CI	p value		Effect, mm Hg	95% CI	p value		Effect, mm Hg	95% CI	p value
24 hour SBP	-0.8	-2.8, 1.3	0.5		2.0	-5.4, 9.5	0.6		-1.5	-3.0, 0.04	0.06		0.7	-6, 7.3	0.8
24 hour DBP	-0.5	-1.8, 0.8	0.4		1.4	-3.5, 6.3	0.6		-0.8	-1.7, 0.2	0.1		-0.3	-4.5, 4.0	0.9
Nighttime SBP	-0.5	-2.6, 1.6	0.7		0.7	-7.5, 8.9	0.9		-1.5	-3.2, 0.2	0.08		-0.1	-7.3, 7.0	1
Nighttime DBP	-0.4	-1.8, 1	0.5		1.2	-4.2, 6.7	0.7		-0.8	-1.9, 0.2	0.1		-0.1	-4.7, 4.5	1
Daytime SBP	-0.9	-3.0, 1.1	0.4		1.1	-5.8, 7.9	0.8		-1.5	-3, -0.004	0.05		-0.6	-7.0, 5.7	0.9
Daytime DBP	-0.4	-1.8, 0.9	0.5		0.8	-4.0, 5.5	0.7		-0.6	-1.6, 0.4	0.2		-1.2	-5.5, 3.1	0.6

SBP: Systolic blood pressure
 DBP: Diastolic blood pressure
 WHO standard population weighted linear regression models adjusted for sex and estimated glomerular filtration rate

Supplementary tables
S13. Effect of Alpha thalassemia on Ambulatory blood pressure indices in Men in Kilifi and Nairobi-population weighted analyses

ABPM measure	ESH Criteria				IDACO Criteria							
	Kilifi (N=472)			Nairobi (N=235)			Kilifi (N=917)			Nairobi (N=371)		
	Effect, mm Hg	95% CI	p value	Effect, mm Hg	95% CI	p value	Effect, mm Hg	95% CI	p value	Effect, mm Hg	95% CI	p value
24 hour SBP	-0.7	-2.8, 1.5	0.6	4.1	-0.1, 8.3	0.06	-1.5	-3.1, 0.2	0.09	2.1	-1.7, 5.9	0.3
24 hour DBP	0.3	-1.5, 2.0	0.8	2.6	-0.4, 5.7	0.09	-0.4	-1.7, 0.9	0.5	1.4	-1.3, 4.1	0.3
Nighttime SBP	-1.7	-4.5, 1.1	0.2	4.1	-0.8, 8.9	0.1	-1.9	-3.9, 0.05	0.06	2.3	-2.1, 6.7	0.3
Nighttime DBP	-0.9	-2.9, 1.2	0.4	3.4	-0.06, 6.9	0.05	-1.1	-2.5, 0.4	0.1	1.7	-1.4, 4.8	0.3
Daytime SBP	-0.1	-2.3, 2.1	0.9	3.9	-0.4, 8.1	0.08	-1.4	-3.2, 0.4	0.1	2.2	-1.5, 5.9	0.2
Daytime DBP	0.8	-1, 2.5	0.4	2.0	-1.2, 5.2	0.2	-0.3	-1.7, 1.1	0.7	1.2	-1.6, 4.0	0.4

SBP: Systolic blood pressure
 DBP: Diastolic blood pressure
 WHO standard population weighted linear regression models adjusted for sex and estimated glomerular filtration rate

Supplementary tables

S14. Effect of SCT on ABPM measures in Nairobi and Kilifi (additional adjustment for HbA1c)

ESH Criteria					IDACO Criteria							
ABPM measure	Kilifi (N=992)			Nairobi (N=516)			Kilifi (N=1702)			Nairobi (N=775)		
	Effect, mm Hg	95% CI	p value	Effect, mm Hg	95% CI	p value	Effect, mm Hg	95% CI	p value	Effect, mm Hg	95% CI	p value
24 hour SBP	-3.0	-5.4, -0.6	0.01	0.5	-2.6, 3.6	0.7	-2.1	-4.0, -0.2	0.03	-0.2	-2.8, 2.4	0.9
24 hour DBP	-1.9	-3.4, -0.3	0.02	0.5	-1.6, 2.6	0.7	-1.1	-2.3, 0.06	0.06	-0.05	-1.7, 1.6	1
Nighttime SBP	-3.6	-6.2, -0.9	0.008	0.7	-2.5, 4.0	0.7	-2.2	-4.2, -0.2	0.03	-0.9	-3.6, 1.7	0.5
Nighttime DBP	-2.4	-4.0, -0.7	0.005	0.6	-1.7, 2.8	0.6	-1.4	-2.6, -0.1	0.04	-0.6	-2.4, 1.1	0.5
Daytime SBP	-2.7	-5.1, -0.2	0.03	-0.1	-3.4, 3.2	1	-1.8	-3.8, 0.1	0.07	-0.5	-3.2, 2.3	0.7
Daytime DBP	-1.5	-3.1, 0.2	0.08	0.1	-2.2, 2.4	0.9	-1.0	-2.3, 0.3	0.1	-0.2	-2.1, 1.7	0.8

SBP: Systolic blood pressure

DBP: Diastolic blood pressure

Linear regression models adjusted for age, sex and estimated glomerular filtration rate

Supplementary tables

S15. Effect of SCT on ABPM measures in Kilifi and Nairobi (excluding participants on anti-hypertensive medications)

ABPM measure	ESH Criteria						IDACO Criteria					
	Kilifi (N=1092)			Nairobi (N=508)			Kilifi (N=1918)			Nairobi (N=765)		
	Effect, mm Hg	95% CI	p value	Effect, mm Hg	95% CI	p value	Effect, mm Hg	95% CI	p value	Effect, mm Hg	95% CI	p value
24 hour SBP	-2.5	-4.8, -0.2	0.03	0.6	-2.4, 3.7	0.7	-1.3	-3.1, 0.5	0.2	-0.3	-2.8, 2.3	0.8
24 hour DBP	-1.4	-2.8, 0.1	0.07	0.6	-1.5, 2.6	0.8	-0.7	-1.8, 0.5	0.3	-0.02	-1.7, 1.6	1
Nighttime SBP	-3.1	-5.7, -0.6	0.02	0.9	-2.3, 4.1	0.6	-1.5	-3.4, 0.5	0.1	-0.9	-3.5, 1.7	0.5
Nighttime DBP	-1.8	-3.3, -0.2	0.03	0.7	-1.6, 2.9	0.6	-0.9	-2.0, 0.3	0.2	-0.6	-2.4, 1.2	0.5
Daytime SBP	-2.0	-4.3, 0.4	0.1	-0.01	-3.3, 3.3	1	-0.9	-2.7, 0.9	0.3	-0.5	-3.3, 2.2	0.7
Daytime DBP	-1.0	-2.6, 0.6	0.2	0.1	-2.2, 2.5	0.9	-0.6	-1.8, 0.7	0.4	-0.2	-2.1, 1.7	0.9

SBP: Systolic blood pressure

DBP: Diastolic blood pressure

Linear regression models adjusted for age, sex and estimated glomerular filtration rate

Supplementary tables
S16. Unadjusted age specific Systolic and Diastolic BP by SCT in Kilifi (IDACO Criteria)

A. Age specific systolic BP by SCT in Kilifi all (IDACO)

Age group	N	%	24-hour Systolic BP (SD)			Nighttime Systolic BP (SD)			Daytime systolic BP (SD)		
			SCT	Non-SCT	p-value	SCT	Non-SCT	p-value	SCT	Non-SCT	p-value
11-24	879	18	119(12)	119(13)	0.8	109(12)	109(13)	0.6	127(13)	127(14)	0.7
25-39	165	21	123(14)	120(13)	0.3	114(15)	112(14)	0.5	130(15)	128(13)	0.4
40-54	372	20	125(17)	124(14)	0.7	118(18)	115(15)	0.3	130(18)	131(15)	0.7
55-69	385	20	136(21)	133(21)	0.3	130(24)	127(23)	0.4	140(21)	139(20)	0.6
≥70	185	24	143(21)	145(23)	0.6	140(24)	141(27)	0.9	145(22)	149(23)	0.3
All	1986	20	126(18)	126(18)	0.9	118(20)	118(21)	1	132(18)	133(18)	0.6

B. Age specific diastolic BP by SCT in Kilifi all (IDACO)

Age group	N	%	24-hour Diastolic BP (SD)			Nighttime Diastolic BP (SD)			Daytime Diastolic BP (SD)		
			SCT	Non-SCT	p-value	SCT	Non-SCT	p-value	SCT	Non-SCT	p-value
11-24	879	18	64(7)	65(8)	0.3	57(8)	58(8)	0.3	70(9)	70(9)	0.5
25-39	165	21	70(10)	68(8)	0.4	64(10)	63(9)	0.6	74(11)	73(9)	0.5
40-54	372	20	72(12)	71(10)	0.3	68(12)	66(11)	0.2	76(13)	75(11)	0.4
55-69	385	20	76(13)	75(13)	0.4	72(13)	70(14)	0.2	80(14)	79(13)	0.6
≥70	185	24	78(13)	79(15)	0.6	75(13)	75(16)	1	80(14)	82(15)	0.3
All	1986	20	70(12)	70(12)	0.7	64(13)	64(13)	1	74(12)	74(12)	0.7

Supplementary tables
S17. Unadjusted age specific Systolic and Diastolic BP by SCT in Kilifi (ESH Criteria)

A. Age specific systolic BP by SCT in Kilifi all (ESH)

Age group	N	%	24-hour Systolic BP (SD)		p-value	Nighttime Systolic BP (SD)		p-value	Daytime systolic BP (SD)		p-value
			Non-SCT	SCT		Non-SCT	SCT		Non-SCT	SCT	
11-24	478	20	119(11)	119(13)	0.7	109(11)	110(13)	0.6	126(12)	126(13)	0.9
25-39	84	24	124(16)	119(12)	0.2	117(18)	110(13)	0.1	130(16)	125(13)	0.3
40-54	216	21	126(18)	123(15)	0.2	120(19)	115(16)	0.1	131(19)	129(16)	0.5
55-69	236	21	134(20)	132(20)	0.4	130(23)	126(21)	0.3	139(20)	137(21)	0.6
≥70	126	23	145(21)	144(22)	0.9	143(27)	141(26)	0.7	147(21)	149(21)	0.7
All	1140	21	127(18)	126(18)	0.4	120(21)	118(20)	0.3	132(18)	132(18)	0.6

B. Age specific diastolic BP by SCT in Kilifi all (ESH)

Age group	N	%	24-hour Diastolic BP (SD)		p-value	Nighttime Diastolic BP (SD)		p-value	Daytime Diastolic BP (SD)		p-value
			Non-SCT	SCT		Non-SCT	SCT		Non-SCT	SCT	
11-24	478	20	64(7)	65(8)	0.3	58(7)	59(8)	0.3	69(8)	70(9)	0.4
25-39	84	24	72(11)	67(7)	0.1	67(12)	61(8)	0.05	75(12)	72(8)	0.2
40-54	216	21	74(12)	71(10)	0.07	70(12)	67(11)	0.1	77(13)	73(11)	0.08
55-69	236	21	76(13)	75(13)	0.4	73(13)	70(12)	0.2	79(13)	79(13)	0.8
≥70	126	23	78(12)	80(14)	0.6	76(14)	76(16)	1	81(13)	83(13)	0.3
All	1140	21	71(12)	70(11)	0.5	66(13)	65(12)	0.3	75(12)	74(12)	0.9

Supplementary tables

S18. Unadjusted age specific Systolic and Diastolic BP by SCT in Women in Kilifi (IDACO Criteria)

A. Age specific systolic BP by SCT in Kilifi women (IDACO)

Age group	N	%	24-hour Systolic BP (SD)			Nighttime Systolic BP (SD)			Daytime systolic BP (SD)		
			SCT	Non-SCT	p-value	SCT	Non-SCT	p-value	SCT	Non-SCT	p-value
11-24	338	16	115(9)	115(10)	0.6	106(10)	105(11)	0.6	123(10)	122(11)	0.4
25-39	94	26	120(15)	118(14)	0.6	112(16)	111(16)	0.8	126(15)	124(13)	0.5
40-54	279	19	123(17)	122(14)	0.7	116(19)	114(14)	0.5	129(18)	129(15)	0.9
55-69	250	18	134(20)	131(22)	0.4	129(23)	126(24)	0.4	138(19)	137(21)	0.6
≥70	97	21	143(23)	143(21)	1	141(25)	141(22)	0.9	144(23)	146(23)	0.9
All	1058	19	125(19)	124(18)	0.6	118(21)	117(20)	0.6	130(18)	130(18)	0.8

B. Age specific diastolic BP by SCT in Kilifi women (IDACO)

Age group	N	%	24-hour Diastolic BP (SD)			Nighttime Diastolic BP (SD)			Daytime Diastolic BP (SD)		
			SCT	Non-SCT	p-value	SCT	Non-SCT	p-value	SCT	Non-SCT	p-value
11-24	338	16	63(7)	63(8)	0.8	57(7)	57(8)	0.9	68(8)	69(8)	0.9
25-39	94	26	68(10)	67(9)	0.6	64(11)	63(11)	0.7	72(11)	71(9)	0.6
40-54	279	19	71(12)	70(9)	0.4	67(12)	66(10)	0.4	74(12)	73(9)	0.3
55-69	250	18	75(12)	75(14)	0.7	72(13)	71(14)	0.5	78(13)	78(14)	0.9
≥70	97	21	77(12)	78(14)	0.8	74(12)	76(13)	0.6	79(14)	80(15)	0.8
All	1058	19	70(12)	70(12)	0.9	65(13)	65(13)	1	74(12)	73(12)	0.8

Supplementary tables

S19. Unadjusted age specific Systolic and Diastolic BP by SCT in women in Kilifi (ESH Criteria)

A. Age specific systolic BP by SCT in Kilifi women (ESH)

Age group	N	%	24-hour Systolic BP (SD)		Nighttime Systolic BP (SD)		Daytime systolic BP (SD)	
			SCT	Non-SCT	SCT	Non-SCT	SCT	Non-SCT
11-24	215	17	115(9)	114(8)	0.6	106(10)	106(11)	0.9
25-39	49	24	122(18)	114(13)	0.2	116(20)	106(14)	0.1
40-54	168	23	125(19)	122(15)	0.4	119(20)	115(15)	0.2
55-69	155	19	135(21)	130(23)	0.3	130(23)	124(23)	0.2
≥70	72	15	145(24)	145(18)	1	143(28)	144(22)	0.9
All	659	19	126(20)	123(18)	0.1	120(23)	116(20)	0.1

B. Age specific diastolic BP by SCT in Kilifi women (ESH)

Age group	N	%	24-hour Diastolic BP (SD)		Nighttime Diastolic BP (SD)		Daytime Diastolic BP (SD)	
			SCT	Non-SCT	SCT	Non-SCT	SCT	Non-SCT
11-24	215	17	63(6)	63(7)	0.9	57(7)	58(8)	0.7
25-39	49	24	70(12)	65(8)	0.2	67(12)	60(9)	0.06
40-54	168	23	73(13)	69(10)	0.1	70(12)	67(10)	0.2
55-69	155	19	76(13)	74(15)	0.5	74(13)	70(14)	0.2
≥70	72	15	77(12)	80(10)	0.6	76(13)	79(12)	0.5
All	659	19	71(12)	69(12)	0.2	67(13)	66(12)	0.3

Supplementary tables

S20. Unadjusted age specific Systolic and Diastolic BP by SCT in Men in Kilifi (IDACO Criteria)

A. Age specific systolic BP by SCT in Kilifi men (IDACO)

Age group	N	%	24-hour Systolic BP (SD)		Nighttime Systolic BP (SD)		Daytime systolic BP (SD)	
			SCT	Non-SCT	SCT	Non-SCT	SCT	Non-SCT
					p-value			p-value
11-24	541	20	121(13)	121(14)	1	111(13)	111(14)	0.6
25-39	71	15	127(12)	126(6)	0.8	117(14)	114(8)	0.5
40-54	93	25	130(15)	128(13)	0.6	122(16)	117(16)	0.2
55-69	135	24	138(23)	136(20)	0.6	131(25)	129(22)	0.6
≥70	88	28	144(19)	147(26)	0.5	140(23)	141(31)	0.9
All	928	22	127(17)	128(19)	0.5	118(19)	119(21)	0.6

B. Age specific diastolic BP by SCT in Kilifi men (IDACO)

Age group	N	%	24-hour Diastolic BP (SD)		Nighttime Diastolic BP (SD)		Daytime Diastolic BP (SD)	
			SCT	Non-SCT	SCT	Non-SCT	SCT	Non-SCT
					p-value			p-value
11-24	541	20	65(8)	65(8)	0.4	57(8)	58(9)	0.3
25-39	71	15	72(9)	71(3)	0.9	64(10)	63(5)	0.7
40-54	93	25	76(12)	73(12)	0.3	71(12)	66(13)	0.1
55-69	135	24	78(14)	76(11)	0.3	73(14)	70(13)	0.3
≥70	88	28	79(13)	80(16)	0.8	76(15)	75(18)	0.7
All	928	22	69(12)	70(12)	0.6	63(13)	63(13)	0.8

S21. Unadjusted age specific Systolic and Diastolic BP by SCT in Men in Kilifi (ESH Criteria)

A. Age specific systolic BP men (ESH)

Age group	N	%	24-hour Systolic BP (SD)		p-value	Nighttime Systolic BP (SD)		p-value	Daytime systolic BP (SD)		p-value
			Non-SCT	SCT		Non-SCT	SCT		Non-SCT	SCT	
11-24	263	22	122(12)	122(14)	0.8	112(12)	112(14)	0.9	130(13)	129(14)	0.8
25-39	35	23	128(13)	127(6)	0.8	118(17)	116(10)	0.7	135(12)	134(9)	0.8
40-54	48	17	130(14)	127(13)	0.6	123(15)	115(18)	0.2	136(15)	137(14)	0.8
55-69	81	25	134(18)	134(16)	0.9	128(22)	128(16)	1	140(18)	139(17)	0.9
≥70	54	33	146(17)	144(25)	0.8	144(25)	140(29)	0.5	148(16)	150(23)	0.7
All	481	23	128(16)	129(18)	0.6	119(19)	120(20)	0.8	134(16)	135(18)	0.6

B. Age specific diastolic BP men (ESH)

Age group	N	%	24-hour Diastolic BP (SD)		p-value	Nighttime Diastolic BP (SD)		p-value	Daytime Diastolic BP (SD)		p-value
			Non-SCT	SCT		Non-SCT	SCT		Non-SCT	SCT	
11-24	263	22	65(7)	66(9)	0.4	58(7)	59(9)	0.4	71(9)	72(10)	0.5
25-39	35	23	73(10)	71(3)	0.5	66(12)	63(5)	0.4	78(10)	76(6)	0.6
40-54	48	17	76(11)	74(10)	0.5	72(12)	68(14)	0.4	80(12)	79(10)	0.8
55-69	81	25	76(12)	75(8)	0.7	72(13)	70(8)	0.6	80(13)	80(9)	0.8
≥70	54	33	80(12)	80(16)	0.9	77(16)	75(18)	0.6	82(12)	85(15)	0.6
All	481	23	70(11)	71(11)	0.7	65(13)	65(12)	1	75(11)	76(11)	0.5

Figure 6-1: Study Flow Chart

A. Kilifi B. Nairobi

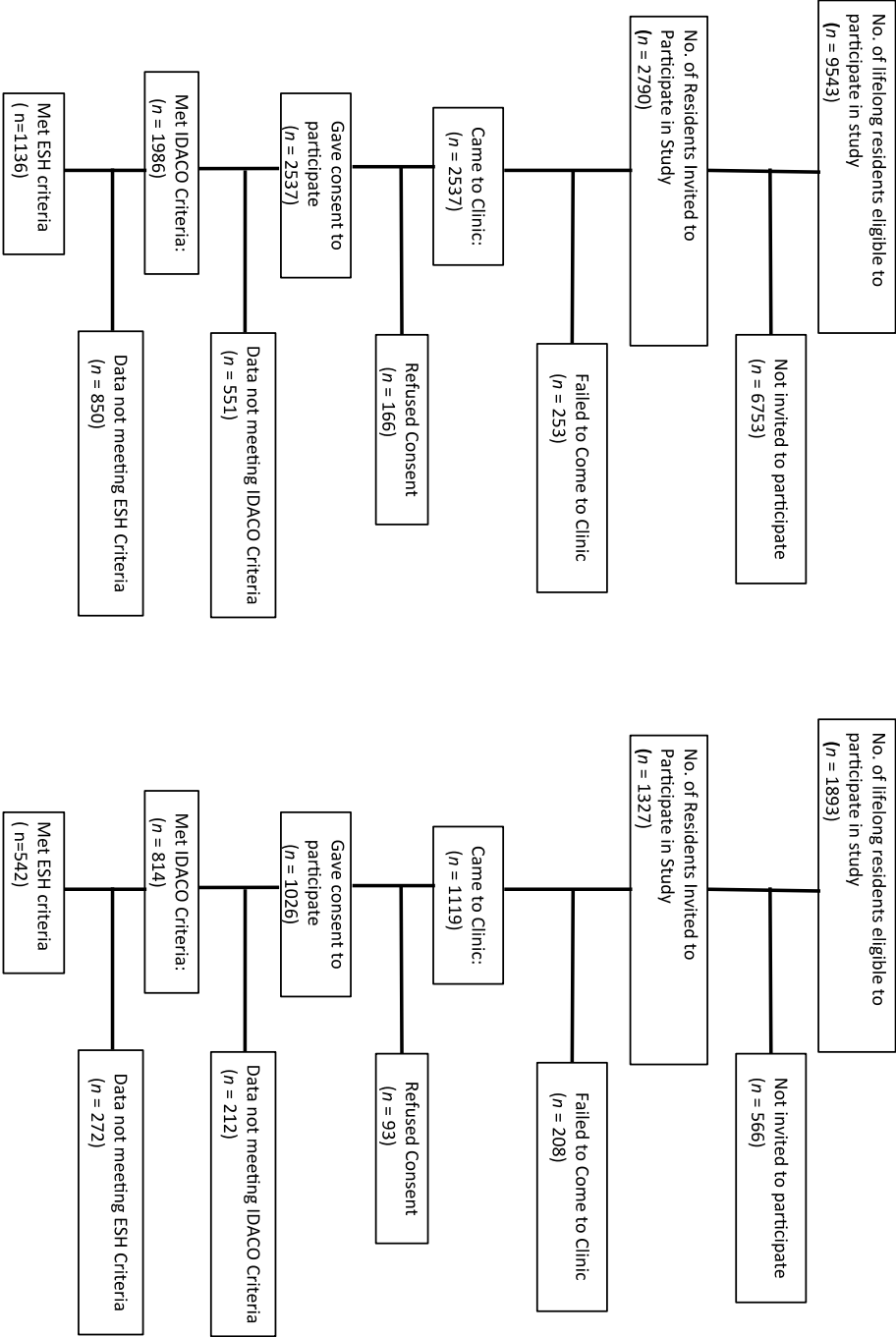
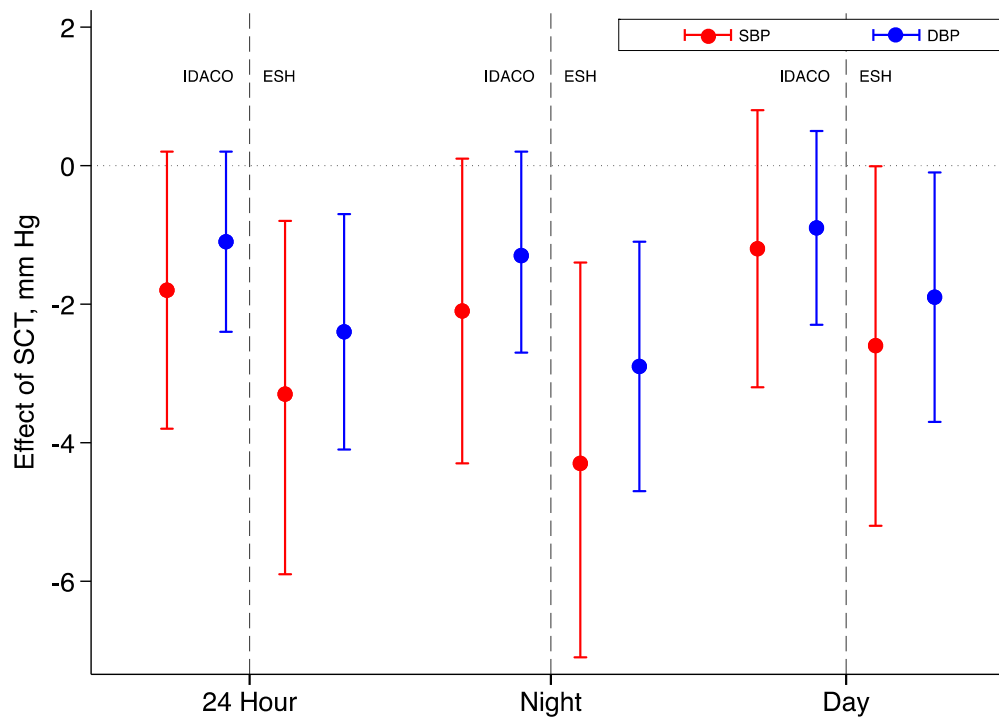
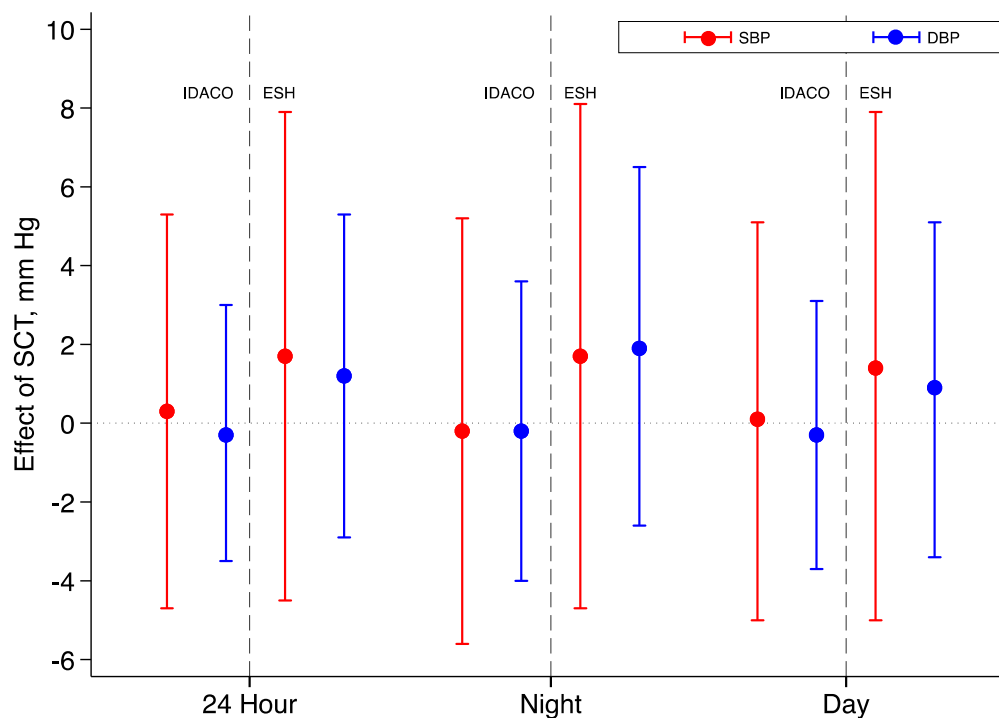


Figure 6-2: Age Standardized[±] Effect of SCT on ABPM Indices in Kilifi and Nairobi

A: Kilifi



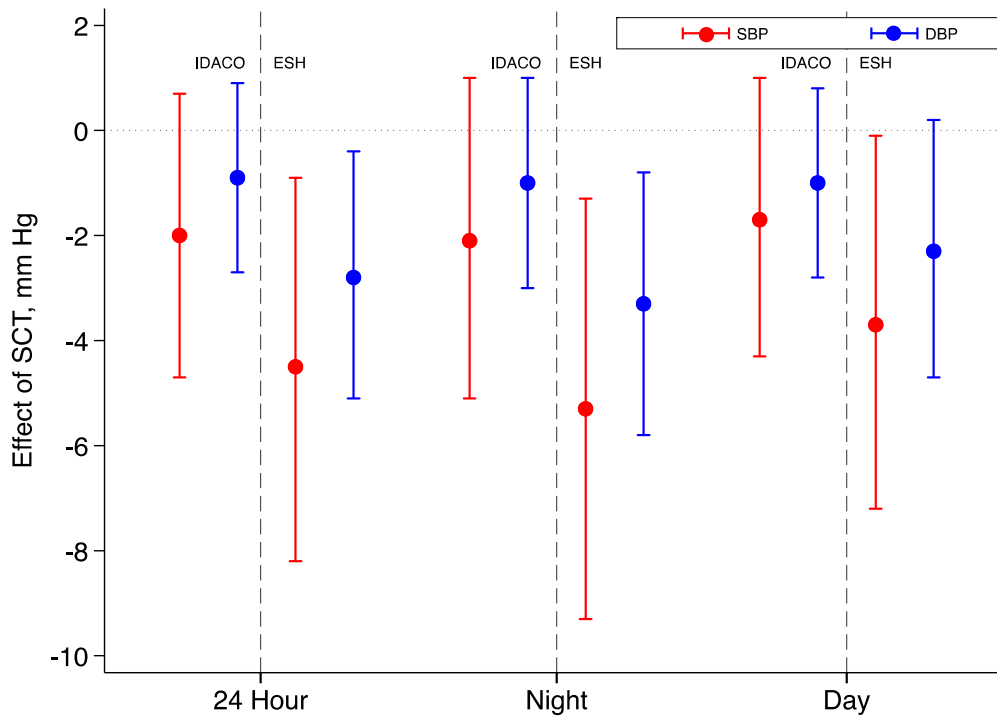
B. Nairobi



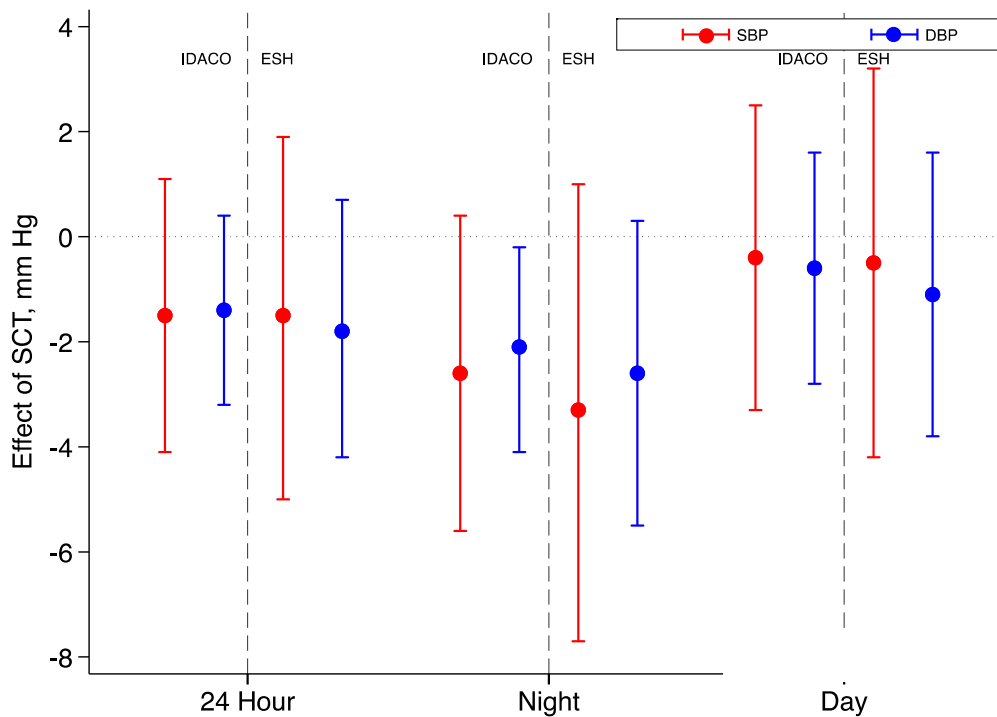
^aWHO population weighted linear regression models with adjustment for sex and estimated glomerular filtration rate. Plots to the left of the vertical dashed lines refer to participants who met IDACO criteria. Plots to the right of the vertical dashed lines refer to participants who met ESH criteria. SBP: Systolic blood pressure; DBP: Diastolic blood pressure; IDACO: International Database for Ambulatory blood pressure in relation to Cardiovascular Outcomes; ESH: European Society of Hypertension

Supplementary Figure 1: Age Standardized[‡] Effect of SCT on ABPM indices by sex in Kilifi

A: Women (N=1058 IDACO, 659 ESH)



B: Men (N=928 IDACO, 481 ESH)



[‡]WHO population weighted linear regression models with adjustment for sex and estimated glomerular filtration rate. Plots to the left of the vertical dashed lines refer to participants who met IDACO criteria. Plots to the right of the vertical dashed lines refer to participants who met ESH criteria.

SBP: Systolic blood pressure; DBP: Diastolic blood pressure; IDACO: International Database for Ambulatory blood pressure in relation to Cardiovascular Outcomes; ESH: European Society of Hypertension

Chapter 7. Discussion and Conclusion

In this chapter I summarize the main findings of this body of work, its strengths and limitations, its implications, and my opinion of what direction future studies should take.

A. Increased risk of malaria is associated with increased blood pressure and increased odds of hypertension- *replication and mechanistic studies needed*

The main finding was that of an association between increased risk of malaria and increased blood pressure. The Mendelian Randomization approach that I used is a particular strength because genetic variants are acquired at conception, eliminating the possibility of reverse causation. Genetic variants are also generally not associated with other potential confounding variables. The main finding in the work presented has the potential to radically improve current knowledge about the development of hypertension if it can be confirmed through additional studies. This would strengthen the argument outlined in Chapter 1 that environmental factors play a role in the development of hypertension in this setting. Viral, parasitic, fungal and bacterial infections are very common in sub-Saharan Africa. Existing data showing that HIV increases risk of hypertension as a result of inflammation as well as due to the body fat abnormalities induced by anti retroviral medications.¹⁻⁴ The findings in this thesis suggest that another common infection, malaria, may also be responsible for blood pressure elevation. It is quite likely that other infections contribute to increased cardiovascular risk in sub-Saharan Africa but this is yet to be clearly demonstrated. While the work in this thesis failed to show any evidence of genetic factors in driving the

incidence of hypertension, I only looked at 2 polymorphisms (sickle cell trait and alpha thalassemia). This work does not therefore contradict the prevailing opinion that a combination of genetic and environmental factors contributes to the burden of hypertension in sub-Saharan Africa.

As previously mentioned (discussion section of Chapter 6), the contribution of malaria to elevated blood pressure might be considerably higher than that demonstrable in the studies in this thesis. This is because (a) Kilifi is a low-moderate transmission area and therefore participants in this study will have been exposed to lower levels of malaria infection than in other parts of the continent; (b) the instrumental variables used to represent malaria risk do not completely protect against malaria; I would expect that with complete protection from malaria the difference in blood pressure between the two groups would have been greater.

It is worth reviewing at this point some important limitations about the study when considering the findings:

- There was a significant difference in the age distributions of the cohorts in Kilifi and Nairobi respectively. Because Kilifi participants were older than those in Nairobi, the possibility that sickle cell trait could directly influence BP in older individuals was not ruled out. However as mentioned in the discussion section of Chapter 6, based on studies conducted in other parts of the world where there is no malaria transmission, the possibility that sickle cell trait could directly influence blood pressure is very small.
- Given that sickle cell trait and alpha thalassemia are protective against a major childhood killer (malaria) in Kilifi, the potential for survivor bias

exists in the analyses performed. It is possible that malaria had a different effect on blood pressure among children who died compared to those who survived the infection but the study design did not allow this comparison to be made

- The mechanism for blood pressure elevation due to malaria was not elucidated. I had hypothesized that malaria would result in increased arterial stiffness as the mechanism leading to increased blood pressure.⁵ Data from the Framingham Heart Study showed that increased arterial stiffness precedes the development of hypertension.⁶ However in my studies I found numerically lower pulse wave velocity in individuals with sickle cell trait in Kilifi and this was not statistically significant despite having sufficient statistical power for that outcome.
- In addition, the specific exposure leading to BP elevation could not be ascertained because SCT, which I used as an instrumental variable representing malaria exposure protects against both severe and non-severe malaria, while not protecting against symptomless parasitaemia.⁷ SCT may also protect against malaria in pregnancy and this could have explained the differences observed.
- The sample size was insufficient for interaction analyses for example to ascertain if there was an interaction with alpha thalassemia.

Definitive linkage of malaria infection to elevated blood pressure would require the following in order to satisfy the Bradford Hill criteria⁸:

i) Replication at other sites of malaria transmission

To improve efficiency these sites would need to have malaria transmission at levels higher than that documented in Kilifi. Settings that

meet this criterion include Eastern Uganda, Northwestern Tanzania, the Democratic Republic of the Congo and Nigeria.⁹ The populations in these settings also have high frequencies of other malaria-protective polymorphisms, which would enable the use of alternative instrumental variables if a Mendelian randomization approach is used. One disadvantage though of using alternative polymorphisms is that none of the malaria protective polymorphisms discovered to date provides more protection and/or is more common than sickle cell trait¹⁰ meaning that much larger sample sizes will be required.

ii) Use of alternative study designs

In the introduction to this thesis (Chapter 1) as well as the hypothesis paper (Chapter 3), I discussed some of the alternative study designs that can be used to test the hypothesis. Given the findings of the MR study that I conducted it is worth articulating these alternative study designs in detail, and the extent to which they could be useful in confirming or refuting the hypothesis. These include:

C. Long term observational studies

There are several cohort studies enrolling children with malaria in sub-Saharan Africa and Asia. These can be used to examine and confirm if the hypothesis is true in various ways:

- A classical cohort analysis could be performed comparing blood pressure in those known to have suffered malaria during follow up versus those who did not
- In areas of the world where complete elimination of malaria has been achieved recently, comparison of blood pressure in individuals born before

and after the date of elimination could add to the evidence of malaria influencing blood pressure. One such country is Sri Lanka, which was certified by the World Health Organization as being malaria free in September 2016.

- Randomized studies

If blood pressure can be included as an outcome measure in clinical trials of anti-malarial drugs, differential effects on blood pressure according to randomization arm could be shown for interventions that are superior to the control. One such trial where blood pressure as an outcome could be included is the planned phase IV cluster randomized trial of the RTS,S vaccine targeting a population of over 1 million that will be conducted in Ghana, Kenya and Malawi.

Studies of controlled human infection with malaria have recently begun to be conducted in Africa.¹¹ While the exposure to malaria in these studies is very brief, they can be used to determine the short-term effects of malaria on blood pressure as well as examine possible pathophysiologic mechanisms for any blood pressure changes observed.

iii) Elucidation of the mechanisms leading to blood pressure elevation.

Malaria is known to cause inflammation, and inflammation has been linked to the development of hypertension. One likely candidate inflammatory marker that could result in hypertension from malaria is angiotensin-2 (Ang-2) as discussed in the hypothesis paper (Chapter 3). Recently published studies have also linked other related angiotensin like proteins to the development of coronary artery disease, additionally demonstrating clinical efficacy of monoclonal antibodies targeting them.^{12,13} Similar

studies are required in the case of malaria and these could yield new drugs to help control high blood pressure and reduce the burden of cardiovascular disease in Africa. If the mechanism of blood pressure elevation involves inflammatory cytokines, then trials of anti-inflammatory agents including statins during or before malaria infections could provide a method of preventing the adverse cardiovascular consequences of malaria infection. As an example, patients who were on statin therapy before suffering from pneumonia had better outcomes than those who were not on the drugs. ¹⁴

Additional implications if malaria does cause hypertension are as follows:

(i) The cost benefit ratio of malaria prevention/elimination strategies will be modified. Trials of malaria vaccines have so far been only modestly successful in preventing malaria in children¹⁵, leading to questions as to whether they are a worthwhile investment. If it can be confirmed that malaria has an additional adverse cardiovascular consequence on the health of populations exposed to the parasite, then the benefits of even a modest reduction in incidence would increase, improving the justification for use of vaccines that may not have 100% efficacy.

(ii) The arbitrary division of tropical diseases into “infectious diseases” and “non-communicable diseases” is reductionist and a wider perception of health as the product of genes, environment including exposure to infection should be considered.

B. Ambulatory blood pressure monitoring can be performed in an African setting- *but challenges exist*

As demonstrated in the 4 papers that report primary data, I was able to successfully perform ambulatory blood pressure monitoring (ABPM) in both rural and urban-based participants of various ages. There has been limited work utilizing ambulatory blood pressure monitoring in an African setting. The studies in this thesis are a significant addition to the data so far available and can be put to many more additional uses in future. One important use will be in determining the prognostic ability of ABPM in predicting cardiovascular outcomes as well as what thresholds to use for diagnosing hypertension. This also includes determining whether blood pressure measurements conducted in childhood/adolescence tracks into adult life as this has not yet been demonstrated in African populations. Ambulatory blood pressure monitoring has been shown to be superior in predicting cardiovascular outcomes mainly in Western populations. A recent study from the US found that ABPM thresholds for diagnosing hypertension developed using data from European, Japanese and South American populations do not predict cardiovascular risk accurately in African Americans.¹⁶

Several patterns were observed that are in keeping with what has been seen in other parts of the world where ABPM has been adopted on a larger scale:

- ABPM measurements were as expected more precise than one-off measurements performed at home or in the clinic. This was evident from the narrower confidence intervals observed when using ABPM measures compared to screening/office BP measurements. A similar pattern of results was seen in the seminal PAMELA study¹⁷, a Danish

study¹⁸ and a large Spanish study (N=104,639)¹⁹. The precision afforded by ABPM enabled me to detect a small but significant difference in BP by sickle cell trait in Kilifi, which would have required a much larger sample size to detect using screening measurement methods.

- The relationship between screening BP measurements and ABPM values differed by age. Younger participants in general had higher ABPM derived BP values compared to their clinic BP values while in older participants this pattern was reversed. As a result of this masked hypertension was more common in younger participants while white coat hypertension was more common in older participants, as has been observed in studies conducted in developed countries.²⁰ It is thought that the higher ambulatory values in younger participants could be related to the fact that they are more physically active than older individuals and this would directly affect ambulatory BP values especially the daytime components. One way to determine if increased physical activity is responsible for these differences would be to perform actigraphy where participants' motor activity and position are recorded simultaneously with ABPM. Apart from elucidating the reasons behind the age-related patterns, the clinical relevance of white coat hypertension and masked hypertension in an African setting needs to be established. Studies conducted in western populations demonstrated a progressive increase in cardiovascular risk when moving from normotension (normal clinic and ambulatory blood pressure) to white-coat hypertension (elevated clinic BP but normal

ambulatory BP) to masked hypertension (normal clinic BP but elevated ambulatory BP) and finally sustained hypertension (elevated clinic and ambulatory BP).²¹

Apart from the need for local evidence about its superiority in predicting cardiovascular outcomes, several difficulties that I encountered during my studies will need to be considered before widespread adoption of the technique can be recommended in Africa.

- Expense- Purchasing the ABPM devices took a significant proportion of the study budget (~£ 50,000) despite the fact that I obtained discounts for bulk purchases. Until cost-effectiveness studies unequivocally demonstrate a clear advantage to using ABPM for diagnosing and managing hypertension, the high cost of the devices will likely prevent the technique from being widely adopted.
- Participant discomfort- although I did not formally study participants' reactions about undergoing ABPM, a significant number reported that the repeated inflations were discomforting and that they were unlikely to accept to undergo repeat measurements. Whether the discomfort could have interfered with sleep patterns and therefore influenced the results reported is difficult to ascertain. It is also possible that discomfort resulted in the significant number of measurement errors observed as evidenced by the proportion of participants providing data that met neither of the 2 recognized quality control criteria. It must be mentioned however that both sets of criteria were arbitrarily decided upon and no outcome data are available to determine the minimum number of ABPM readings needed to predict cardiovascular outcomes

within reasonable limits. In the Mendelian Randomization study in Kilifi, no statistically significant effects were observed when using the less stringent quality control criteria, suggesting that more accurate measurements resulting from a higher number of inflations are likely to be more predictive of cardiovascular outcomes. Advances in clinic BP measurement could also mean that reliance on ABPM is no longer necessary. In the SPRINT trial²², participants underwent Automated Office BP (AOBP) measurement²³ where they were left alone in a quiet room for 5 minutes after which the BP machine automatically took 3 BP readings. However, the relationship between AOBP and ABPM has not been clearly elucidated and a comparison of ABPM measures done midway through the SPRINT trial with clinic BP values was markedly different from that found in other studies.^{24,25} Another unknown issue about ABPM measurements is their reliability, i.e. consistency of measurements in the same person. It is possible that a person might behave differently on repeat measurement, given previous experience and discomfort to the extent that radically different readings are obtained. This possibility has however not been studied. Another cause of lack of reliability in ABPM measurements could be lack of standardization of the software used for calculating the various outputs from the device as was classically demonstrated by Rossen *et al* using the popular Spacelabs™ ABPM device.²⁶

Recommendations on use of ABPM in Research Studies and Clinical Practice

Based on the findings in this thesis there are clear advantages to using ABPM in epidemiological research and I would recommend that it be used more

widely for this purpose. However, there are insufficient data to suggest that it is both cost-effective and acceptable in clinical practice and I would recommend additional studies on these two aspects before ABPM can be adopted in routine clinical practice in sub-Saharan Africa.

C. Sickle cell trait and alpha thalassemia do not directly influence blood pressure- *unlikely to contribute to ethnic differences in cardiovascular disease*

The Nairobi component of my study was designed specifically to determine whether Sickle Cell Trait and alpha thalassemia would be appropriate genetic instruments to use as a proxy for determining whether malaria risk is associated with changes in blood pressure in Kilifi. These studies however brought up an important topic worth discussing: are ethnic differences in the risk of cardiovascular disease related to intrinsic biologic differences between the groups? To answer this question, the effects of any of these biologic variations need to be studied within the specific ethnic groups in which they are highly prevalent. Sickle cell trait and alpha thalassemia are among the most common polymorphisms in populations of African descent due to the protection they provide against malaria, and the more severe forms of these polymorphisms (sickle cell disease and thalassemia major) are associated with significant cardiovascular disease. So does this selective advantage against malaria come at the cost of increased cardiovascular risk?

Biomedical essentialism, the concept that intrinsic biologic differences explain racial (or other) disparities in health had been in a period of decline until the turn of the century.²⁷ However with the advent of molecular epidemiology and the Human Genome Project, this concept has again assumed widespread

importance.²⁸ However, as has been pointed out by several authors^{27,29} this concept suffers from one major flaw: there is no scientific consensus on the definition of race or ethnic group. It is now recognized that the concept of ethnicity has been largely shaped by cultural and political considerations, and that the generation of scientific theories and hypotheses as well as the conduct and reporting of research are often influenced by cultural, social and economic factors.^{28,30} With no clear biological definition of ethnicity, it follows that any attempt to ascribe differences in the prevalence of cardiovascular disease to ethnicity would encounter serious difficulty. Despite the huge effort that has been put into genome wide association studies to find genetic causes of cardiovascular disease, the majority of studies have either reported negative findings or variants with very small effects that on average only explain ~1% of the phenotypic variance in cardiovascular related traits.³¹ The negative findings in the studies that I performed in Nairobi add to the evidence against there being a large contribution of genetics to the development of cardiovascular disease. One limitation though of the Nairobi studies was that I examined for differences in a young population where the risk of hypertension is low, although as argued in the discussion sections of Chapters 4-6, differences in BP can usually be detected during childhood and adolescence. The corollary of there being minimal contribution of intrinsic factors underlying the development of cardiovascular disease is that it is likely that environmental factors are the main players. The advantage of this is that environmental factors are more amenable to modification in order to reduce the risk of cardiovascular disease (although it should be noted that with the

advent of gene editing technology, the potential for modifying intrinsic genetic causes of disease will soon be available).

D. How do we interpret these findings in view of previous studies demonstrating that factors related to urbanization are responsible for increased blood pressure?

Malaria has been present in Africa for thousands of years. How then do we reconcile the apparently contradictory findings of an almost complete absence of hypertension in Africa prior to urbanization and the finding in my studies of an increased risk of hypertension associated with malaria? I would like to propose two possible explanations:

- Hypertension causation is multi-factorial

It is now well known that the hypertension phenotype results from multiple factors, genetic and environmental operating within and between generations. Most etiological studies have looked at a single risk factor rather than combinations of risk factors, due to the complex study designs required to study combinations of risk factors. We therefore do not know which permutations of risk factors and at what dose are required to result in hypertension. It is possible that malaria only raises blood pressure in the presence of other dietary and lifestyle factors that are present now but were not there previously. This can be examined by conducting larger scale studies with measurement of these factors. However as argued by Geoffrey Rose, such an approach will only enable the detection of differences within the current range of variation in these factors in the population.³² If as it is

likely, previous populations had a markedly different distribution of these factors, it will be difficult to make any comparisons with previous studies.

- Traditional methods for measuring blood pressure were inaccurate

It is quite evident now that casual methods of blood pressure measurement suffer from many shortcomings leading to increasing use of more standardized methods such as ambulatory blood pressure monitoring and automated office blood pressure measurement. Random misclassification arising from the methods used to measure blood pressure may have accounted for the failure to detect differences in blood pressure based on exposure to malaria.

A final explanation for the apparent contradiction is that my interpretation that malaria raises blood pressure could be wrong. This will be borne out by future studies.

E. Public Health Relevance of the Findings in this Thesis and Potential Interventions

In summary, the major public health implications of the findings in this thesis are as follows:

- Hypertension is at least as common in Kilifi as it is in urban parts of Kenya. Interventions to reduce blood pressure levels in the Kilifi population are likely to reduce the high burden of cardiovascular disease in the area.
- The findings of the Mendelian Randomization study present a reasonably robust case for an association that is likely to have profound effects on health in Africa. The implications are

i) It is necessary to confirm the finding and obtain more precise estimates of the effect size and relationship with age, sex and other malaria protective genes

ii) It is necessary to tease out the mechanism of action by which malaria leads to blood pressure elevation in order to develop new therapeutic agents
If confirmed as outlined above the findings will add further scientific and economic weight to the case for malaria control and elimination.

F. Proposed Future Work

In this final section I briefly outline a summary of what my next research projects would be based on the findings in this thesis. These are of course dependent upon obtaining funding to carry out the research.

- Confirmation of, and elucidating the pathophysiological basis of the malaria-high blood pressure hypothesis by repeating Mendelian Randomization studies in a different site with higher malaria transmission intensity; and by including blood pressure as an outcome in the forthcoming multi-site RTSS malaria vaccine trial. Proteomic techniques in humans as well as animal models of malaria infection could be used to elucidate mechanisms of blood pressure elevation.

- Additional studies on the clinical utility of ambulatory blood pressure monitoring compared to existing and newer methods for screening blood pressure measurement.

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Appendix

1. Analysis plan for Mendelian Randomization Study
2. Standard Operating Procedures used in data collection

Analysis Plan Contents

<i>Reporting format</i>	206
<i>Outcome measures</i>	207
<i>Sample size estimation</i>	208
<i>Causal diagram, confounders and model building</i>	209
<i>b) Model building</i>	216
<i>c) Pleiotropy</i>	216
<i>d) Testing for Cohort effects</i>	216
POTENTIAL SOURCES OF BIAS	217
WHAT DATA TO INCLUDE/EXCLUDE IN THE ANALYSES	218
REFERENCES	219

Reporting format

While there are no specific guidelines for reporting Mendelian randomization (MR) studies, the principles outlined in the Strengthening the Reporting of OBservational studies (STROBE)¹ guidelines as well as the STROBE Extension for Genetic Association studies (STREGA)² will be used. In addition Boef et al published a review of the quality of reporting of MR studies³ and I will also be guided by recommendations that were made that paper.

Appendix 1: Analysis Plan for Mendelian Randomization Study

Outcome measures

The primary outcome measures will be linear regressions to determine the age, sex and estimated glomerular filtration rate (eGFR) adjusted effect of SCT on 24-hour, daytime and nighttime systolic blood pressures obtained by ambulatory blood pressure monitoring using the arteriograph device. Numerous studies have shown that the more precise measurements resulting from repeated inflations and more standardized procedures in ABPM (as outlined in chapter 1) make it a much better predictor of cardiovascular events than other BP measurement methods.⁴ The justification for adjusting for age, sex and eGFR is given in section 4 on confounders and model building.

Secondary outcome measures will be:

- a) Comparison of the primary outcome measures using alpha thalassemia rather than sickle trait to represent malaria exposure
- b) Comparison of the diastolic components of the primary outcome measures
- c) Estimation of the odds ratio for prevalent hypertension in those with and without SCT and those with and without alpha thalassemia, using ABPM cutoffs. Hypertension is diagnosed by any one of the following criteria in individuals aged ≥ 16 years: ^{4,5}
 - i) 24-hours systolic BP ≥ 130 mmHg and/or 24-hour diastolic BP ≥ 90 mm Hg
 - ii) Daytime systolic BP ≥ 135 mm Hg and/or daytime diastolic BP ≥ 85 mm Hg
 - iii) Nighttime systolic BP ≥ 120 mm Hg and/or nighttime diastolic BP ≥ 70 mm Hg
- d) Weighted regression analyses of the primary and secondary outcomes using WHO standard population weights⁶ adjusting for sex and eGFR

Appendix 1: Analysis Plan for Mendelian Randomization Study

Adjustment for multiple testing will not be necessary in this scenario of a limited number of clinically relevant pre-specified tests (e.g. compared to GWAS studies)⁷

Sample size estimation

The initial sample size calculation outlined at the start of the study was based on a two-sample t-test comparing mean 24-hour systolic blood pressure in those with and without the sickle cell trait (SCT). The following assumptions were made:

- That the prevalence of SCT would be $\geq 15\%$
- That the standard deviation of 24-hour systolic BP would be ≤ 20 mm Hg
- That 85% of subjects selected to participate in the study would consent to do so
- That 85% of those consenting to participate would provide data that met quality control criteria (see section 2 below)

Based on these assumptions I calculated that I would need to approach 1084 participants (resulting in ~783 subjects with complete data) at each of 2 sites in Kilifi in order to detect a statistically 5mm Hg difference in 24-hour systolic BP and a 0.5ms^{-1} difference in aortic pulse wave velocity between individuals with and without the sickle cell trait. The 2 sites were chosen so as to enable the examination of a dose-response effect in BP as they have traditionally had differing malaria transmission intensities.

An interim analysis at the mid-point of the study (December 2016) revealed an issue that had not been previously considered; there was a high prevalence of thalassemia mutations with only ~25% of recruited subjects not having any thalassemia mutations. Coinheritance of SCT and alpha thalassemia may negate the protective effect of SCT against malaria episodes.⁸ We considered that this might reduce the power of the study to determine the main outcome.

In order to increase power to detect an effect it was agreed to limit recruitment to one site in Kilifi (the one with higher malaria transmission) and double the recruitment

Appendix 1: Analysis Plan for Mendelian Randomization Study

target for that site to 2168 individuals. Any dose-response effect would be examined by looking for differential effect sizes based on sickle and thalassemia status, which provide different levels of protection against malaria.

No formal sample size estimation was made for linear regression analyses (see section on confounders below). The literature suggests that the major consideration for sample size calculations in linear regression models is to ensure that there are at least 2-50 subjects per variable in the model⁹, a requirement that would almost certainly be achieved if most of the assumptions stated above held true.

If the final results of the study differ from the assumptions made at the outset, I will conduct a post hoc analysis to determine what power there was to detect statistically significant differences based on the data accumulated.

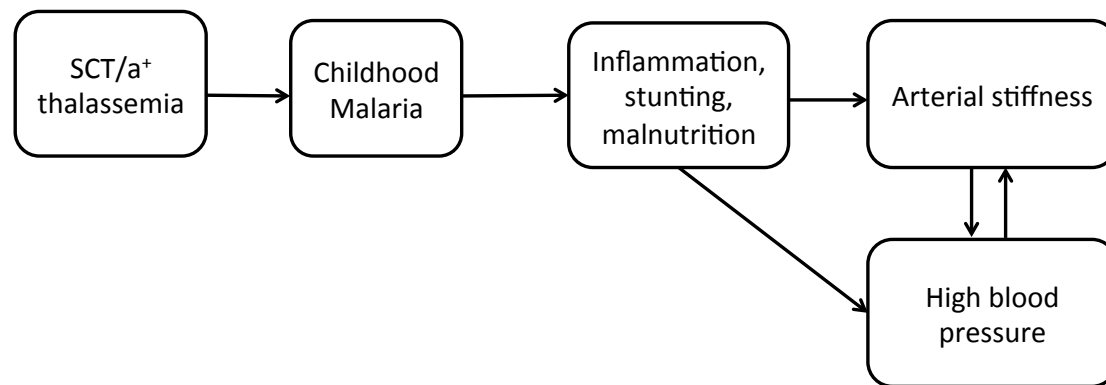
Causal diagram, confounders and model building

The theoretical basis for the malaria-high blood pressure hypothesis has been published previously.¹⁰ Briefly, the primary hypothesis to be tested is that individuals exposed to more malaria disease in their childhood (represented by those having haemoglobin AA) will have higher 24-hour systolic blood pressure than those who were exposed to less malaria disease (represented by those having haemoglobin AS [SCT]). Additional analyses related to the primary hypothesis will examine the effect of alpha thalassemia mutations, which on their own confer less protection against malaria compared to the SCT, and when inherited in combination with the SCT result in a reduction of the protective effect of SCT against malaria.^{8,11-13}

The proposed causal diagram, drawn purely for purposes of informing the analytical plan is as below.

Figure 1. Causal diagram for the malaria high blood pressure hypothesis

Appendix 1: Analysis Plan for Mendelian Randomization Study



In this directed acyclic graph (DAG) the arrows can be interpreted as causal relations. The term acyclic is somewhat misplaced here because the relationship between arterial stiffness and high blood pressure is bidirectional i.e. arterial stiffness can lead to high blood pressure¹⁴ and high blood pressure can lead to increased arterial stiffness^{15,16}. The bidirectional nature of this particular relationship appears to be partially related to age but as this is not the subject of this study there will be limited examination of the issue. For purposes of this analysis however it is important to note that both increased arterial stiffness and blood pressure are hypothesized outcomes of malaria. Arterial stiffness and blood pressure are also on each other's causal pathway, therefore it would be wrong to adjust analyses assessing the relationship between malaria and either outcome for either blood pressure or arterial stiffness. Similarly, because malnutrition and stunting are on the causal pathway from malaria to either outcome, adjustment for body mass index (BMI) and other anthropometric indices (e.g mid upper arm circumference) would be inappropriate.

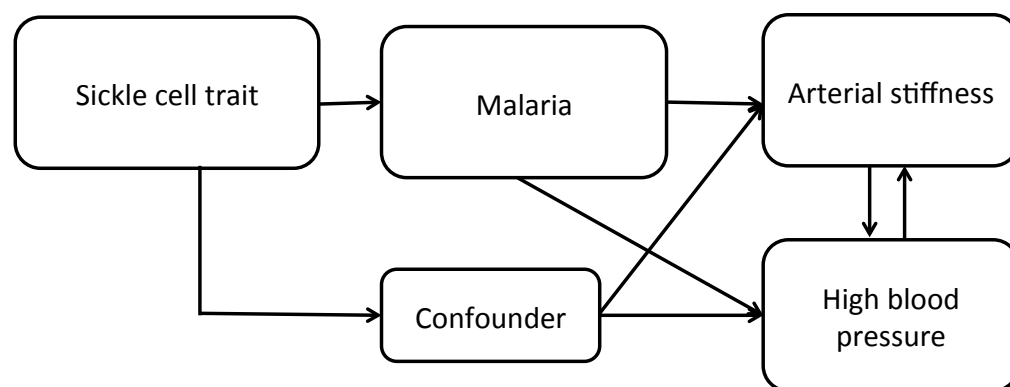
a) Confounders

The principle of Mendelian randomization holds that because comparisons are based on genetic traits acquired at conception, any relationships between the genetic trait and the outcome are unlikely to be confounded by other exposures as these will be randomly distributed between carriers and non-carriers of the trait.¹⁷

Appendix 1: Analysis Plan for Mendelian Randomization Study

However age, sex, and BMI are known to have a very strong influence on BP and other cardiovascular diseases¹⁸, and are usually adjusted for as ‘fixed covariates’ in MR/Genome wide association studies e.g in Palmer et al¹⁹, Ehret et al²⁰ and Ferrence et al²¹. I have outlined above why it would be inappropriate to adjust for BMI. Confounding can also occur if the genetic trait influences the outcome through a pathway that is independent of the exposure (pleiotropy) as illustrated in figure 2 below.

Figure 2: Illustrating confounding due to pleiotropy.



Apart from adjustment for age and sex I will perform adjustment for 2 additional covariates that also have a strong influence on BP as justified below:

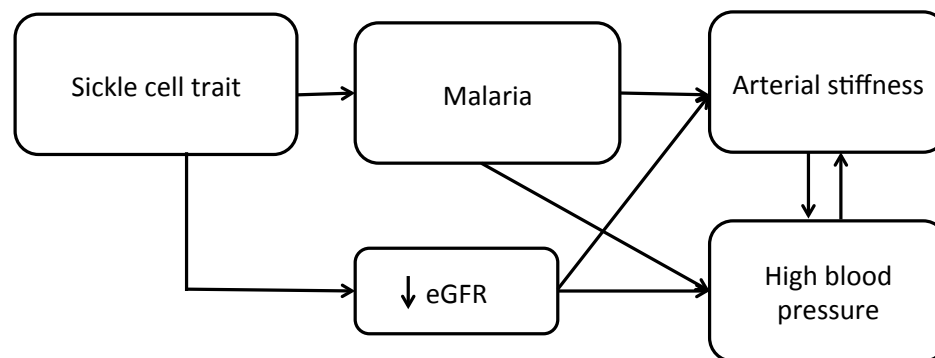
i. Kidney function

Sickle cell trait has been associated with impaired kidney function as measured by decline in estimated glomerular filtration rate (eGFR) and albuminuria.²² This association was independent of blood pressure elevation. Another abnormality that appears in individuals with SCT is decreased urinary concentrating ability (hyposthenuria) that leads to lower than normal urinary sodium levels.²³ We confirmed the presence of hyposthenuria among adolescents with sickle cell trait who had been continuous residents of Nairobi, Kenya where they were not exposed to malaria.²⁴ Impaired kidney function is associated with elevations in blood pressure as a result of sodium retention²⁵, increased activity of the renin-angiotensin system²⁶,

Appendix 1: Analysis Plan for Mendelian Randomization Study

increased sympathetic activity²⁷, secondary hyperparathyroidism²⁸, impaired nitric oxide synthesis²⁹ and increased prevalence of nocturnal non-dipping BP pattern.³⁰ It is also possible that kidney disease could arise from hypertension.³¹ The direction of the relationship between BP and kidney function, like that of arterial stiffness and BP, has been the subject of debate.³² However, evidence from genetic studies suggests that the relationship between decreased renal function and blood pressure elevation is more likely to operate in the direction of decreased renal function resulting in high blood pressure. In a large (n>200,000) genome wide association study (GWAS), loci that were associated with BP elevation and cardiovascular disease showed no association with kidney disease or kidney function.²⁰ If SCT compromises renal function and this in turn leads to elevated BP, this would result in a bias toward a null result when using SCT as a proxy for testing the malaria-high blood pressure hypothesis. As can be seen in Figure 3, impaired kidney function is associated with both the exposure and the outcome, but is not on the causal pathway from malaria to the outcome. Kidney function is therefore a confounder that should be adjusted for in regression analyses.

Figure 3. Illustrating confounding effect of eGFR in subjects with SCT



In the primary analysis I will use eGFR as the measure of Kidney function. I will check to see that eGFR is associated with SCT and with BP in a univariate linear regression model and after adjusting for age and sex. If no association is present between SCT and eGFR then it will not be included as a predictor variable in the model examining the effect of malaria (SCT) on BP. eGFR will be calculated using the Schwartz formula in children and the CKD-EPI formula in adults.^{33,34} While the CKD-EPI formula was derived from a general population of adults, no study has been conducted to generate a formula for GFR from children free of chronic kidney disease. The Schwartz formula was derived from children with chronic kidney disease, and this may result in a distortion of the eGFR in children participating in this study. In sensitivity analyses (described further down), I will assess the effect of additional adjustment for urine albumin levels, which have been causally associated with increased BP when eGFR is normal³⁵, also checking to see that there is no collinearity with eGFR.

Acute renal failure is a known presentation of severe malaria and repeated episodes could result in chronic pyelonephritis and elevated BP. However, in a study conducted in Kilifi, renal failure was a very rare outcome among children admitted

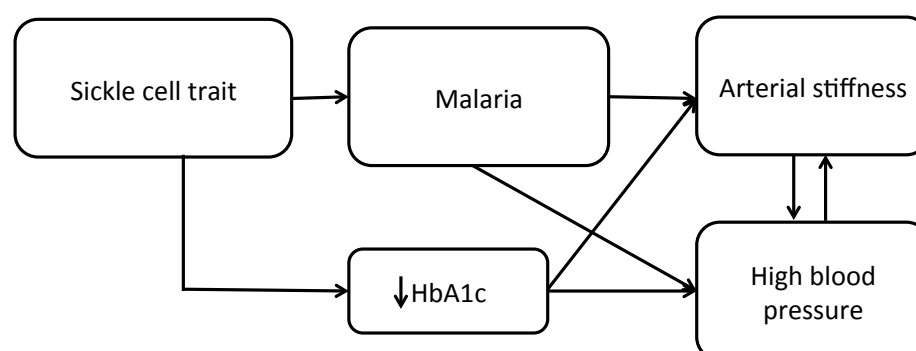
Appendix 1: Analysis Plan for Mendelian Randomization Study

with malaria (2 out of 1,844).³⁶ As this falls on the causal pathway between malaria and high blood pressure, it does not qualify as a confounder.

ii. Glucometabolic status

Glucometabolic status, measured using glycosylated haemoglobin (HbA1c) is strongly associated with increased arterial stiffness, blood pressure and cardiovascular and renal disease in both diabetic and non-diabetic subjects.^{18,37-40} This association is independent of body mass index.^{18,38} Because HbA1c levels like BP track over time i.e they tend to remain constant over time in an individual, it is possible to predict individuals at risk of developing hypertension based on their blood sugar levels taken as many as 18 years earlier⁴¹. This also reduces the problem of time-varying confounding. At the same time, SCT has been associated with reduced HbA1c levels, even when using assay methods that have been standardized to ensure accurate measurement of HbA1c in subjects with hemoglobinopathies.⁴² It is not clear whether this association is a laboratory artefact or real. Based on this HbA1c, as a marker for glucometabolic status, is a potential confounder of the relationship between SCT and BP/arterial stiffness as shown in Figure 3. As with kidney function, I will perform univariate and adjusted analyses to confirm the existence of an association between SCT and HbA1c and before including it as covariate in the main regression model.

Figure 3. Confounding role of HbA1c



b) Effect modifier: Thalassemia

Alpha thalassemia, in which there is reduction in the amount of alpha hemoglobin, is common in regions where malaria transmission occurs. Williams et al⁸ have demonstrated a negative epistatic effect when alpha thalassemia is coinherited with SCT. The effect of coinheritance of the mutations is to reduce the malaria protective effect of SCT to about 27% (from 50%) for uncomplicated malaria and to 44% (from 80%) for severe malaria.⁸ Put simply, the presence of alpha thalassemia reduces the protective effect of SCT against both uncomplicated and severe malaria by about half. The frequency of any thalassemia mutation (i.e homozygous or heterozygous) is ~70% in the Kilifi population. If the malaria-high blood pressure hypothesis is true, then the largest BP difference based on SCT will be seen in individuals without any alpha thalassemia mutations. However, because of the high frequency of alpha thalassemia mutations, there will be very few individuals for this stratified analysis resulting in loss of statistical power. I will instead include alpha thalassemia as an interaction term (interacting with SCT) in the main regression model and examine whether its inclusion changes the effect estimate for SCT in predicting BP. From this model, I will also examine the effect estimates for SCT in those with and without alpha thalassemia mutations. I expect these to be different from those obtained in the model that does not include thalassemia as an interaction term.

In a related analysis, I will run a linear regression model examining the effect of alpha thalassemia on BP with the same covariates used in the main analysis for SCT. Because alpha thalassemia confers less protection against malaria than SCT, it is expected that the effect estimate in this model will be lower than that of SCT.

Appendix 1: Analysis Plan for Mendelian Randomization Study

b) Model building

I will conduct forward stepwise regression, sequentially adding the specified covariates (age, sex, eGFR and hbA1c) in the model. The criteria for determining whether a candidate covariate improves the model will be a likelihood ratio of ≤ 0.05 or a change of $\geq 10\%$ in the effect estimates (regression coefficient for SCT) when comparing the model with and without the candidate covariate.

c) Pleiotropy

In order to make valid conclusions that malaria affects/does not affect BP, the regression models described above shall be applied to data obtained from lifelong residents of Nairobi where there is no malaria transmission.

I will in addition examine the published literature on genome association studies conducted in malaria free regions and that include populations that have a high prevalence of SCT (e.g African Americans) to find out whether any of these studies have found an association between SCT and BP.

d) Testing for Cohort effects

Malaria incidence in Kilifi has been changing over time and this may influence results obtained. Data on the changing levels of transmission go back to 1990 and they show that a significant drop in transmission in Kilifi began around 1999-2000.⁴³ In addition, because blood pressure rises with age, it is possible that the effects of malaria on outcome measures may be more apparent later in life. While it will be impossible to determine the individual contributions of changing malaria exposure and aging to any differences observed in outcome measures, I will attempt to display these differences by performing comparisons of the outcomes by sickle trait in 5-7 age categories.

Appendix 1: Analysis Plan for Mendelian Randomization Study

Potential Sources of Bias

a) Subjects aware of their exposure status

Screening for sickle cell trait is not routinely done in Kilifi. It is, however, possible that first degree relatives of individuals with homozygous (HbSS) sickle cell disease, which almost always has severe clinical manifestations, would infer that they have at least a 50% probability of having SCT if they are siblings of the affected individual and nearly 100% probability of having SCT if they are parents of the affected individual. If these individuals then altered their behaviour with regard to avoiding malaria (e.g less likely to use bed nets or seek treatment for possible malaria) then this could bias the result toward the null. We will have no way of measuring this possibility, but given the 2% prevalence of sickle cell disease in the population and the multiple probabilities involved in the pathway to increased malaria exposure, the magnitude of misclassification bias due to this factor is likely to be minute.

b) Subjects aware of their outcome status

On the other hand, many more individuals are likely to have had their blood pressure measured and possibly diagnosed as being hypertensive. Management of hypertension involves lifestyle and diet modification, and drugs. As these will be applied equally in those with and without SCT, their effect would be to bias the result of the study towards the null. We have only one measure that we can use to determine the existence of this bias, urinary sodium levels. I will compare urinary sodium excretion in individuals previously diagnosed as hypertensive with that of those not previously diagnosed as hypertensive. Significantly lower levels of urinary sodium in those previously diagnosed as hypertensive (and not on medication e.g diuretics) after controlling for eGFR, will suggest that they could have decreased their salt consumption. Consideration will be given to excluding individuals previously

Appendix 1: Analysis Plan for Mendelian Randomization Study

diagnosed as hypertensive from the regression analyses. Including these individuals in the analyses and then adjusting for (or stratifying by) urinary sodium, drug intake, or previous diagnosis of hypertension would lead to collider bias⁴⁵ as the so-called confounder would be a common effect of the exposure (malaria) and the outcome (BP).

What data to include/exclude in the analyses

There are 2 internationally recognized quality control criteria used for ABPM data which are based on completeness of observations. The International Database of Ambulatory blood pressure in relation to Cardiovascular Outcomes (IDACO) study⁴⁶ defined ABPM data as acceptable if they include ≥ 10 daytime and ≥ 5 nighttime readings, where daytime is defined as 1000-2000 hrs and nighttime as 0000-0600 hrs.⁴⁶ The guidelines from the European Society of Hypertension (ESH) are more stringent; they require ≥ 20 daytime and ≥ 7 nighttime readings where daytime is defined as 0900 to 2100 hrs and nighttime as 0100 to 0600 hours.⁴ It is important to note that these criteria were arbitrarily set by experts and were not based on outcome studies. As this study is taking place in a rural African setting, where the practical complexities of ABPM are increased, it is likely that the more stringent criteria will exclude a large proportion of the data obtained, The ESH criteria are however likely to yield higher quality data with less (random) misclassification bias. For these reasons, I propose a primary analysis that uses data evaluated by the ESH criteria and a secondary analysis that uses data evaluated by the IDACO criteria.

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**KENYA MEDICAL RESEARCH INSTITUTE /
 WELLCOME TRUST RESEARCH PROGRAMME**
(Center for Geographic Medicine Research – Coast)



Study Specific Procedure		SSP No: SSP SHINDA-001	
		Version: Original Supersedes: None Effective Date: 31/10/2015	
Long title: Study Specific Procedure (SSP) for informed consent for Shinikizo la Damu (ShinDa) 2: Investigating the role of malaria in elevating blood pressure and pulse wave velocity in Kenyan children and adults Short title: SHInikizo lA DAmu (SHINDA II) study			
PREPARER	NAME	SIGNATURE	DATE
REVIEWING AUTHORITY	Anthony Etyang		
QA UNIT AUTHORITY			
APPROVAL AUTHORITY	Anthony Etyang		

1. GOALS AND SCOPE/RESPONSIBILITY:

This SOP outlines the process of obtaining consent from participants in the SHINDA II study. Consistent and appropriate use of the SOP will increase the quality of the study by ensuring compliance to the ethical standard requiring that participants provide informed consent to take part in the study.

1.1. Overall goals

- This SSP aims to ensure that voluntary informed consent is obtained from each participant in accordance with national e.g. PPB, institutional (KEMRI ERC and KEMRI Wellcome Trust Research Programme), ICH-GCP and international guidelines for ethical conduct of research. Towards this goal, the SSP provides information on the development of the informed consent process, training and monitoring of staff involved, administration of consent and (where applicable) materials used to support consent.
- This SSP describes the way the consent process will be administered, with adequate supportive information, to ensure the following:
 - All relevant information is DISCLOSED in an appropriate language
 - The potential participant COMPREHENDS the information and understands how their involvement in the study differs from normal clinical care
 - The potential participant makes a VOLUNTARY DECISION about whether or not to participate and understands that he/she can agree and later withdraw, without influence from forms of undue inducement or coercion
- Since the design of and information and consent form (ICF) is part of a wider consent process, the SSP aims to guide study teams in key elements of the consenting process, in addition to the development and use of ICFs

1.2. Scope/Responsibility

- This SSP describes the process to be followed for obtaining written or verbal informed consent from subjects/guardians taking part in the research study SHINDA.
- This study is under the direction of Anthony Etyang and the lay summary is appended
- This SSP applied to the following study staff that have been delegated the task of obtaining informed consent appropriately from participants in research studies:
 - Trained field workers:
 - Study clinicians:
 - Study Nurses:
- Monitoring of adherence to this SSP is the responsibility of Sailoki Kapesa and Christopher Wandabwa or other designees of the PI Anthony Etyang

1.3. Acronyms and terms used in this document

CCC – Communication and Consent Committee

ICF – Information and Consent Form

GCP – Good Clinical Practice

ICH - International Conference on

224 Harmonization

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PI – Principal Investigator

CSC - Centre Scientific Committee

SOP – Standard Operation Procedure: detailed written instructions to achieve uniformity of the performance of study specific consent procedures

SSP – Study Specific Procedures

SERU – Scientific and Ethical Review Unit (KEMRI Nairobi, under the National Council for Science and Technology or NCST)

CLG – Community Liaison Group: a group of community liaison offices who coordinate community engagement activities in Kilifi within the KEMRI-Wellcome Trust Programme

CAST groups – Community engagement Advisory groups for Studies: a group set up by the CLG manager including community liaison officers and members of HSSR (Health Systems and Social Research group) who work on research ethics. The group will support planning an implementation of community engagement activities when projects are ready to start, including community consultation, outreach, communicating with participants, informed consent, resolving emerging community issues in studies and feedback of research findings at the end of a study.

SHINDA II - SHinikizo IAmu study II: short title for Investigating the role of malaria in elevating blood pressure and pulse wave velocity in Kenyan children and adults

ASSENT: An affirmative agreement of a child/minor (less than 18 years of age) or an individual with impaired consent capacity to participate in research. Mere failure to object i.e. absence of affirmative agreement should not be construed as assent

DESIGNEE: A person designated by another individual to act on the latter's behalf

INFORMED CONSENT: The permission, voluntary given by an individual who understands the purpose and nature of the study, what participation in the study requires of the individual, the nature of the risks and what benefits are intended to result from participation in the study

INFORMATION AND CONSENT FORM: a document comprising: 1) An information sheet containing all the information required for a potential subject/guardian to make an informed decision on whether to participate in a study, and 2) A form on which informed consent is documented by the dated signature of a subject/guardian, investigator/designees and witness (where applicable). In the KEMRI Wellcome Trust Research Programme, obtaining a signature from those who do not agree to research is not recommended given risks of perceived coercion in this community

ICF AMENDMENT – Changes to the ICF, which have implications for the study participants or for data analysis and which need prior approval of the CCC and by KEMRI CSC and SERU e.g. strengthened translations

WITNESS – A person who attends the entire informed consent process if the subject or the subject's guardian cannot read and/or write. The witness should be the study investigator or a member of the study team or any other person whose main professional responsibilities are involved in the conduct of the study.

2. DEVELOPMENT OF THE ICF AND CONSENT PROCESS:

The ICFs to be used in the Shinda II study ²²⁵ were developed by the study investigators

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with guidance from the Communications and Consent Committee. The KEMRI Scientific and Ethical Review Unit has approved all the documents that will be used in the study.

3. TRAINING OF STAFF INVLED IN THE INFORMED CONSENT PROCESS AND MONITORING AND EVALUATION OF THE CONSENT PROCESS

- 3.1. **Trainees:** Field workers, study nurses and study clinicians will perform the informed consent process for the study. These recruiters will be trained in the informed consent process.
- 3.2. **Trainers:** Communication for informed consent and study specific informed consent training will be run in conjunction with CLG with support from Anthony Etyang and his designees Sailoki Kapesa and Christopher Wandabwa
- 3.3. **Content:** the training objectives for the communication for informed consent training, the study specific informed consent process training process and training curriculum to be used are detailed in the appendix. Included is the training curriculum for recruiters (health centre workers and health action leaders).
- 3.4. **Training evaluation:** The training will be evaluated by a person designated by the CLG through appropriate role-plays and dry runs prior to study initiation.
- 3.5. **Monitoring:** Sailoki Kapesa and Christopher Wandabwa and community facilitators attached to the study through supportive supervision will monitor the informed consent process regularly. Monitoring will be performed in the following way:
 - 3.5.1. Through fortnightly review of completed ICFs by Sailoki Kapesa and Christopher Wandabwa or other designee
- 3.6. **Documentation of training:** Completing a post-training evaluation and signing of a personal training log will document training activities.

4.0 ADMINISTRATION OF INFORMED CONSENT

- Informed consent in the Shinda II study shall be obtained at the participants' homes (Kilifi) and in the study clinic (Nairobi)
- The recruiter shall approach the randomly selected participants and explain:
 - a. the purpose of the research
 - b. the procedures that the study will entail
 - c. the fact that participation is voluntary
 - d. the fact that a participant can withdraw from the study at any time that they feel like
- The participants shall be given a copy of the information and consent form to read. If they agree to participate, both the participant and recruiter will be required to sign the consent document in duplicate. One copy will remain with the participant and the other will be retained by the study team.

5.0 ENSURING THE QUALITY OF THE CONSENT PROCESS

- The study team will ensure that the consent process is being adhered to by ensuring the consent forms are properly signed and by obtaining feedback from participants regarding the consenting process. The PI/designee will oversee this process.
- The study quality assurance mechanisms will ensure that checking adherence to this SSP during the monitoring process adheres to the procedures described in this document.

- Understanding of the participant/parent/guardian/legally acceptable representative will be continuously assessed during follow up visits by checking that the participant understand that they are participating in research and remember what this entails
- The consent process for this study will be supported by and overlap with a broader community engagement strategy that is being carried out by the CAST.
- The investigator/designee will document the informed consent process in the participant's source documents and comment about nay issues that may influence decisions to allow participants to continue participating in research

6.0 MATERIALS

DOCUMENT CHANGE HISTORY

Version Table:

Original: Title: Study Specific Procedure (SSP) for informed consent for SHINDA	Dated: 15/10/2015	SSP No.: SSP SHINDA-001	No. Pages: 8
Version 1: Title: Study Specific Procedure (SSP) for informed consent for SHINDA	Dated: 14 Oct 2015	SSP No.: 001	No. Pages:8
Version 2: Title:	Dated:	SSP No.:	No. Pages:
Version 3: Title:	Dated:	SSP No.:	No. Pages:
Version 4: Title	Dated:	SSP No.:	No. Pages:
Version 5: Title	Dated:	SSP No.:	No. Pages:

SSP Review and Updating Logs

DATE	NAME OF REVIEWER	SIGNATURE	REASON FOR REVIEW
14 Oct 2015	Anthony Etyang		Update

DOCUMENT COPY CONTROL

SSP DISTRIBUTION

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Training Documentation Log for SOP Files

Kenya Medical Research Institute Wellcome Trust Research Laboratory Standard Operating Procedure				Copy Number ___ of ___	SOP No: Version: Supersedes: Effective Date:
Title:					
P. NO.	DATE	NAME	SIGNATURE	TRAINER	

Reviewed By Documentation:

Name: _____ Signature: _____ Date: _____

APPENDICES:

APPENDIX 1

Objectives for Communication for Informed Consent Training and Study Specific

Informed Consent Training:

At the end of the training, participants will be equipped with knowledge and skills to

- Communicate effectively about KEMRI's core values and its roles
- Address common rumors in the community about research, KEMRI and its roles
- Explain the difference between medical research and treatment
- Appreciate the importance of research in development of new knowledge
- Understand key principles of research ethics and how to apply them
- Undertake a true informed consent process
- Understand the general community entry approaches as defined in the KWTRP's community engagement strategy

APPENDIX 2

Training curriculum for informed consent process for SHINDA study

Appendix 2: Standard Operating Procedures used in data collection

SOP Title: SHINDA 2 – Blood sample collection

SOP No:



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welcometrust

Standard Operating Procedure		SOP No: Version: 1 Supersedes: None Effective Date:	
Title: SHINDA 2 – BLOOD SAMPLES – FIELD COLLECTION			
	NAME	SIGNATURE	DATE
PREPARED BY	Anthony Etyang		03 rd Nov 2015
REVIEWED BY			
QA AUTHORITY			
APPROVAL AUTHORITY			

1.0 PURPOSE / INTRODUCTION:

This SOP outlines the protocol for collecting blood samples from participants in the SHINDA 2 study in the Kilifi and Nairobi Health and Demographic Surveillance System area.

2.0 SCOPE / RESPONSIBILITY:

2.1 Scope:

The SOP will apply to the SHINDA II study.

2.2 Responsibility:

- 1.1.1 SHINDA study field workers
- 1.1.2 SHINDA study investigators

3.0 SAFETY/RISK ASSESSMENT:

3.1 Handling blood specimens:

- 3.1.1 There is risk of blood borne infections especially from needle stick injuries when handling blood samples.
- 3.1.2 To minimize risk, non-sterile gloves will be provided for field workers

4.0 DEFINITIONS: Provide definitions for all important words and abbreviations referenced in this procedure.

- 4.1 BP = Blood pressure
- 4.2 PWV= pulse wave velocity
- 4.3 SHINDA = SHINikizo la DAMu, study of hypertension in Nairobi and Kilifi
- 4.4 KHDSS = Kilifi Health and Demographic Surveillance System
- 4.5 NUHDSS= Nairobi Urban Health and Demographic Surveillance System
- 4.6 KEMRI/WT = Kenya Medical Research Institute/ Wellcome Trust
- 4.7 APHRC= African Population and Health Research Center

5.0 SPECIMEN: Provide the specifics of the specimen that will be required in carrying out this procedure.

- 5.1 Blood sample
 - 5.1.1 EDTA and serum samples

6.0 EQUIPMENT / MATERIALS/ REAGENTS:

6.1 Equipments

- 6.1.1 Sample carrier container
- 6.1.2 Blood collection bottles (cryovials, EDTA and SST bottles)
- 6.1.3 Non-sterile gloves

6.2 Materials

- 6.2.1 Consent form
- 6.2.2 Study information form

Appendix 2: Standard Operating Procedures used in data collection

SOP Title: SHINDA 2 – Blood sample collection

SOP No:

- 6.2.3 24 hour ABPM/PWV information sheet
- 6.2.4 Visit Data collection forms (BP, Urine, blood)
- 6.2.5 Sample labels

6.3 Reagents

- 6.3.1 NONE

7.0 METHODOLOGY:

7.1 Principle

- 7.1.1 Hypertension is increasingly becoming a major health issue in Sub-Saharan Africa
- 7.1.2 Inflammation is thought to be a leading risk factor for developing hypertension
- 7.1.3 In this study we aim to determine whether past exposure to malaria affects blood pressure.

7.2 Timetable

All study participants to receive 2 visits, where the following activities should be performed in the following order:

First visit:

1. Explanation of aims of study and requirements of participation
2. Consent procedure
3. Blood pressure measurement
4. Complete first questionnaire (Visit 1 Data Collection Form)
5. Spot urine collection
6. Blood collection
7. Weight measurement
8. Height measurement
9. Mid upper arm circumference (MUAC) measurement

At the end of the day, return all samples and paperwork to the laboratory

FIRST VISIT

7.3 Consent form

- 7.3.1 Complete in accordance with Study Specific Procedure (SSP) for informed consent for ShinDa II

7.4 Blood pressure/Pulse Wave Velocity

- 7.4.1 Complete in accordance with SOP – Shinda – Blood Pressure/Pulse Wave Velocity Measurement
- 7.4.2 This should be completed for ALL participants

7.5 Completing the Visit 1 Data Collection Form

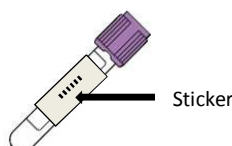
- 7.5.1 This is to be completed on the first visit, by a trained field worker, using the “Visit 1 Data Collection Form”. Complete ALL fields on the form.

Appendix 2: Standard Operating Procedures used in data collection

SOP Title: SHINDA 2 – Blood sample collection

SOP No:

- 7.5.2 Date of birth and age – give best estimate of date of birth/age if not known. Write ‘estimated’ on the form if exact information is not available
- 7.5.3 For female participants:
 - 7.5.3.1 Last menstrual period/Pregnancy. Because sodium levels can be affected by pregnancy, this question is important.
 - 7.5.3.2 If participants are pregnant, they must be excluded from the study – no further procedures should be undertaken.
- 7.6 **Blood collection**
 - 7.6.1 Participant should have given informed consent prior to procedure
 - 7.6.2 Explain procedure to participant
 - 7.6.3 Document the time that the blood sample was taken on the data collection form AND on the participant’s 24 hour BP/PWV leaflet
 - 7.6.4 Barcode labels are to be placed on each tube during collection as shown below



- 7.6.5 Please ensure that the barcodes match on the tubes and the questionnaire. One purple topped 4ml, and one 6ml yellow capped tubes will be collected for a total of 10ml blood.
- 7.6.6 **Purple capped tubes (EDTA).** These are for full blood count and DNA genotyping
 - 7.6.6.1 One 4 ml purple capped tube is to be collected from each participant
 - 7.6.6.2 Purple top tubes should be 50-60% full. Do not overfill tubes.
 - 7.6.6.3 Gently mix specimen by inverting 5-10 times and place it on a rocker if available for up to 30 minutes, then refrigerate at 2-8°C or place in cool box.
 - 7.6.6.4 Refrigerated EDTA blood is stable for full blood count (FBC) and genotyping for up to 24 hours. Clotted or hemolyzed specimens are unacceptable. Check for clots by using a clean wooden applicator stick and gently swirling blood in tube.
 - 7.6.6.5 EDTA microtainers must be shaken 10-15 times to overcome the surface tension within the tube
 - 7.6.6.6 EDTA samples are to reach the lab within 6 hours of collection. Once in the KEMRI CCR lab, 1-1.5ml from the EDTA tube is to be aliquoted into each of two barcoded cryovials (2ml) that are to be refrigerated at negative 80°C as soon as possible, awaiting shipment to Kilifi for genotyping and HbA1c testing. The samples should be processed within 1 hour of delivery to the lab.
 - 7.6.6.7 The remaining sample in the EDTA tube (approximately 1ml) will be for running a full blood count test at CCR lab.

Appendix 2: Standard Operating Procedures used in data collection

SOP Title: SHINDA 2 – Blood sample collection

SOP No:

- 7.6.7 **Yellow capped tubes (SST).** These are for serum electrolytes. Part of these samples will be stored for future analyses. One 6ml SST tube will be used to collect at least 3ml of blood from each participant.
- 7.6.7.1 Immediately after collection, mix SST tubes by inverting 8-10 times
- 7.6.7.2 For clot formation to occur, tubes must be mixed well.
- 7.6.7.3 Avoid hemolysis of the specimen during collection and mixing
- 7.6.7.4 Allow blood to clot by placing tube vertically in a rack at room temperature until the separation is visible.
- 7.6.7.5 Store in refrigerator at 2-8°C awaiting transfer to CCR lab
- 7.6.7.6 Transfer to lab within 4 hours of collection
- 7.6.7.7 At the lab the sample will be centrifuged and 2 aliquots, each 500 µl to be harvested and placed in barcoded labeled cryovial
- 7.6.7.8 Cryovials are to be transferred to -80°C freezer as soon as possible to await shipment to Kilifi
- 7.6.7.9 Remaining sample in SST tube for serum electrolyte testing

Notes:

- It is the responsibility of the laboratory technician/manager in charge of the processing to ensure the participant cryovial barcodes correctly matches the Vacutainers barcode for the participant whose sample is being aliquoted for cryopreservation.
- For the CCR- Nairobi printed results, the participant identifier barcode must reflect on the result sheet for unique identity and any back reference desired at any point in the course of the study.

Please complete checklist at bottom of Data Collection Form 1, to ensure that all required actions are complete.

SECOND VISIT

(This should take place as close as possible to 24 hours after the time ABPM/PWV was started on the first day)

7.7 **Completing the Visit 2 Data Collection Form**

- 7.7.1 This is to be completed on the SECOND visit, by a trained field worker, using the “Visit 2 Data Collection Form”. Complete ALL fields on the form.
- 7.7.2 The purpose of the questionnaire is to determine how complete the 24 BP/PWV measurement was and sleeping and waking times.

Please complete checklist at bottom of Data Collection Form 2, to ensure that all required actions are complete.

7.8 **Returning to Lab at end of day**

- 7.8.1 At the end of the day, return all samples to the KEMRI-WT top lab (Kilifi)/ CCR lab (Nairobi)
- 7.8.2 Please ensure that all samples are labelled and forms are appropriately filled out

Appendix 2: Standard Operating Procedures used in data collection

SOP Title: SHINDA 2 – Blood sample collection

SOP No:

- 7.8.3 Document the date and time that samples arrive in the lab in both:
 - 7.8.3.1 The main laboratory logbook
 - 7.8.3.2 The sample request form
- 7.8.4 Please ensure that all paperwork is signed
- 7.8.5 Please file all paperwork to remain as hard copy evidence even after inputting data into computer database

8.0 APPENDICES: This section is to be used for inserting figures, tables, diagrams or forms referenced in the SOP.

Include copies of the Data Collection forms 1 and 2, and the lab request form

9.0 REFERENCES:

Provide a list of the guidance documents, cross-references, higher-level procedures or publications that have contributed to the generation of this procedure.
Start typing here...

10.0 SOP CHANGE HISTORY:

10.1 Version Table:

Original: Title:	Dated:	SOP No.:	No. Pages:
Version 1: Title:	Dated:	SOP No.:	No. Pages:
Version 2: Title:	Dated:	SOP No.:	No. Pages:
Version 3:	Dated:	SOP No.:	No. Pages:
Version 4: Title:	Dated:	SOP No.:	No. Pages:
Version 5: Title:	Dated:	SOP No.:	No. Pages:
Version 6: Title:	Dated:	SOP No.:	No. Pages:
Version 7: Title:	Dated:	SOP No.:	No. Pages:

10.2 PERIODIC SOP REVIEW LOG

Appendix 2: Standard Operating Procedures used in data collection

SOP Title: SHINDA 2 – Blood sample collection

SOP No:

DATE	NAME OF REVIEWER	COMMENTS	SIGNATURE

10.3 SOP Revision and Updating Logs

DATE	NAME OF REVISOR	CHANGES MADE

10.4 SOP COPY CONTROL

SOP DISTRIBUTION

<u>DATE</u>	<u>SECTION</u>	<u>RECEIVED BY</u>

Appendix 2: Standard Operating Procedures used in data collection

SOP Title: SHINDA 2 – Blood sample collection

SOP No:

10.5

SOP AWARENESS LOG

I, the undersigned below, hereby confirm that I have read the accompanying SOP in existence from the date stated herein and that I shall keep abreast with the current and subsequent SOP versions in fulfilment of Good Clinical Laboratory Practice (GCLP).

Kenya Medical Research Institute Wellcome Trust Research Laboratory Standard Operating Procedure	SOP No: Version: Supersedes: Effective Date:
Title:	

#	Names	Signature	Date



**KENYA MEDICAL RESEARCH INSTITUTE /
WELLCOME TRUST RESEARCH PROGRAMME**
(Center for Geographic Medicine Research – Coast)



Standard Operating Procedure		SOP No: Version: 1 Supersedes: None Effective Date:	
Title: SHINDA 2 – URINE SAMPLES – FIELD COLLECTION			
	NAME	SIGNATURE	DATE
PREPARED BY	Anthony Etyang		11 th Sept 2015
REVIEWED BY			
QA AUTHORITY			
APPROVAL AUTHORITY			

10.0 PURPOSE / INTRODUCTION: This SOP outlines the protocol for collecting urine samples and anthropometric data in the field from participants in the SHINDA 2 study in the Kilifi and Nairobi Health and Demographic Surveillance System area.

11.0 SCOPE / RESPONSIBILITY:

2.3 Scope:

The SOP will apply to the SHINDA II study.

2.4 Responsibility:

- 1.1.3 SHINDA study field workers
- 1.1.4 SHINDA study investigators

12.0 SAFETY/RISK ASSESSMENT:

12.1 Handling urine specimens:

- 12.1.1 There is minimal risk to field workers of handling urine bottles whose exteriors have been contaminated with urine.
- 12.1.2 To minimize risk, non-sterile gloves will be provided for field workers
- 12.1.3 All urine samples are to be transported in sealed sample containers from the time of sample collection to being logged at the laboratory

13.0 DEFINITIONS:

- 13.1 BP = Blood pressure
- 13.2 SHINDA = SHINikizo la DAMu, study of hypertension in Nairobi and Kilifi
- 13.3 KHDSS = Kilifi Health and Demographic Surveillance System
- 13.4 MUAC= Mid upper arm circumference
- 13.5 NUHDSS= Nairobi Urban Health and Demographic Surveillance System
- 13.6 KEMRI/WT = Kenya Medical Research Institute/ Wellcome Trust
- 13.7 ‘Spot’ urine sample = urine sample collected as one-off, small sample

14.0 SPECIMEN:

- 14.1 ‘Spot’ urine sample
 - 14.1.1 Small (<30 ml) urine sample

15.0 EQUIPMENT / MATERIALS/ REAGENTS:

15.1 Equipment

- 15.1.1 Sample carrier container
- 15.1.2 Spot urine collection bottle
- 15.1.3 Non-sterile gloves
- 15.1.4 Omron M10-IT digital automatic blood pressure monitor
- 15.1.5 4x AA batteries for above blood pressure monitor

- 15.1.6 Weighing scales
- 15.1.7 Seca 213 Stadiometer (height measuring equipment)
- 15.1.8 MUAC tape

15.2 **Materials**

- 15.2.1 Consent form
- 15.2.2 Study information form
- 15.2.3 24 hour ABPM/PWV information sheet
- 15.2.4 Visit 1 Data collection form
- 15.2.5 Visit 2 Data collection form
- 15.2.6 Sample labels

15.3 **Reagents**

- 15.3.1 NONE

16.0 METHODOLOGY:

16.1 **Principle**

- 16.1.1 Hypertension is increasingly becoming a major health issue in Sub-Saharan Africa
- 16.1.2 Salt intake is thought to be a leading risk factor for developing hypertension
- 16.1.3 In this study we aim to determine whether salt intake as measured in the urine affects blood pressure.

16.2 **Timetable**

All study participants to receive 2 visits, where the following activities should be performed in the following order:

First visit:

- 10. Explanation of aims of study and requirements of participation
- 11. Consent procedure
- 12. Blood pressure measurement
- 13. Complete first questionnaire (Visit 1 Data Collection Form)
- 14. Spot urine collection
- 15. Weight measurement
- 16. Height measurement
- 17. Mid upper arm circumference (MUAC) measurement

At the end of the day, return spot urine samples to laboratory

At the end of the day, return all samples and paperwork to the laboratory

FIRST VISIT

16.3 **Consent form**

- 16.3.1 Complete in accordance with Study Specific Procedure (SSP) for informed consent for ShinDa II

-
- 16.4 **Blood pressure/Pulse Wave Velocity**
- 16.4.1 Complete in accordance with SOP – Shinda – Blood Pressure/Pulse Wave Velocity Measurement
- 16.4.2 This should be completed for ALL participants
- 16.5 **Completing the Visit 1 Data Collection Form**
- 16.5.1 This is to be completed on the first visit, by a trained field worker, using the “Visit 1 Data Collection Form”. Complete ALL fields on the form.
- 16.5.2 Date of birth and age – give best estimate of date of birth/age if not known. Write ‘estimated’ on the form if exact information is not available
- 16.5.3 For female participants:
- 16.5.3.1 Last menstrual period/Pregnancy. Because potassium level in the urine could be affected by presence of blood, and sodium levels can be affected by pregnancy, this question is important.
- 16.5.3.2 If participants are pregnant or during the menstrual phase of their period, they must be excluded from the study – no further procedures should be undertaken.
- 16.6 **Spot urine collection**
- 16.6.1 Participant should have given informed consent prior to procedure
- 16.6.2 Explain procedure to participant
- 16.6.3 Ensure that this is NOT the first urination of the day
- 16.6.3.1 If it is, ask the participant to void immediately. You can then collect a urine sample after 30-60 minutes
- 16.6.4 Affix the participant's specimen label, marked onto one of the small spot urine containers. Use spot urine container with a lid, to avoid spillage. Ask the participant to produce a urine sample into the container, so that it is around $\frac{3}{4}$ full. Any remaining urine is discarded in the participant's usual manner.
- 16.6.4.1 **Female collection**
- 16.6.4.2 Wash hands thoroughly with soap and water. Dry with a paper towel
- 16.6.4.3 With one hand, gently spread genital skin folds apart
- 16.6.4.4 Using an antiseptic skin towelette, wash vulva, wiping from front to back and discard towelette in the sanitary bin
- 16.6.4.5 Repeat, wiping from front to back
- 16.6.4.6 Void the first portion of urine into toilet.
- 16.6.4.7 Void the midstream portion into a 30ml sterile container provided, being careful not to touch the inside of the container
- 16.6.4.8 Stop collection when container is about half full and void the remaining urine stream into the toilet
- 16.6.4.9 Screw cap tightly and wash hands. Give the filled container to the designated staff member
- 16.6.4.10 **Male collection**
- 16.6.4.11 Wash hands thoroughly with soap and water. Dry with a paper towel

-
- 16.6.4.12 Gently pull back foreskin, (if uncircumcised) and cleanse glans penis with an antiseptic skin towelette
 - 16.6.4.13 Void first portion of urine into the toilet, and then void the midstream portion into the urine container, being careful not to touch the inside of the container
 - 16.6.4.14 Stop collection when the container is about halfway to 3-quarters full. Complete void into the toilet
 - 16.6.4.15 Screw the cap onto the container and wash hands
 - 16.6.4.16 Give the filled container to the designated staff member
 - 16.6.5 **Processing of urine samples**
 - 16.6.6 Handle the urine sample with non-sterile gloves. Place the sample in the sample collection coolbox or refrigerator. Ensure that sample lid is firmly screwed on before placing in coolbox/refrigerator.
 - 16.6.7 Ensure that the coolbox has ice packs in it
 - 16.6.8 Document the time that the spot urine sample was produced on the data collection form AND on the participant's 24 hour BP/PWV leaflet.
 - 16.6.9 If a participant is unable to void for the "spot" urine, ask the participant to take some fluids (e.g. 2 glasses of water), and wait until the participant is able to try again. Proceed with the remainder of the visit until the participant indicates that he or she is able to void.
 - 16.7 **Weight**
 - 16.7.1 Only to be measured at the first visit
 - 16.7.2 Participant should have given informed consent prior to procedure
 - 16.7.3 Explain procedure to participant
 - 16.7.4 Remove participant's shoes and minimise heavy clothing
 - 16.7.5 The floor surface on which the scale rests must be hard, flat, and must not be carpeted or have other soft materials.
 - 16.7.6 Participant should be asked to stand still on centre of scale, as standing off-centre may affect the measurement
 - 16.7.7 Measure weight to nearest 0.1 kg
 - 16.7.7.1 For scales weighing to 2 decimal points: if 0.05kg & above round up; if <0.05kg round down
 - 16.7.8 Say weight out loud and write it down immediately on the data collection form (check that order of digits is not inverted)
 - 16.7.9 Show weight to participant
 - 16.7.10 Self-reported weights are not acceptable in ambulatory participants. Refusals to be weighed should be recorded as refusals. Only participants who are not ambulatory (e.g., amputees) may self-report their weight. Be sure to note this on the form. The reading of the scales is to be done by the field worker.
 - 16.8 **Height**
 - 16.8.1 Only to be measured at the first visit
 - 16.8.2 Participant should have given informed consent prior to procedure
 - 16.8.3 Explain procedure to participant

- 16.8.4 Construct Seca stadiometer. Ensure that all pieces are firmly in place to prevent errors
- 16.8.5 The floor surface must be hard, flat, and should not be carpeted or have other soft materials.
- 16.8.6 Remove participant's shoes/socks and heavy garments; remove hair ornaments; compress braids
- 16.8.7 Participant stands upright in centre of board in the feet positions indicated, with heels together touching the back plate; hands by side
- 16.8.8 Back of participant's head, shoulders, buttocks, calves & heels must touch the backboard
- 16.8.9 Participants must look forward; direction of gaze is at 90 degrees to vertical plane
- 16.8.10 Ask the participant to take in a deep breath for full height
- 16.8.11 Lower head-board onto top of participant's head; read and call out height to nearest 0.1cm
- 16.8.12 Record height as soon as possible on the data collection form.
- 16.8.13 Show height to participant
- 16.8.14 Disassemble the Seca stadiometer. Please ensure that at the end of the day, the stadiometer is completely disassembled (using instructions on bottom of foot plate) and returned to its carry-case.
- 16.8.15 Self-reported heights are not acceptable in ambulatory participants and should not be recorded. Only persons who are not ambulatory (e.g., amputees) may self-report their heights. Be sure to note this on the form.

16.9 MUAC measurement

- 16.9.1 Only to be measured at the first visit
- 16.9.2 Measure the upper arm length as below:
- 16.9.3 Position the subject: Direct the subject to turn away from you. Ask him or her to stand upright with the weight evenly distributed on both feet, the right arm bent 90° at the elbow, and the right palm facing up. Demonstrate the correct position if necessary.
- 16.9.4 Mark the measurement site: Locate the end of the spine of the right scapula by following the scapula out to the arm until it makes a sharp V-turn to the front of the body. Using a biro pen, make a horizontal line on the uppermost edge of the posterior border of the spine extending from the acromion process (see picture 16.9.4 below).



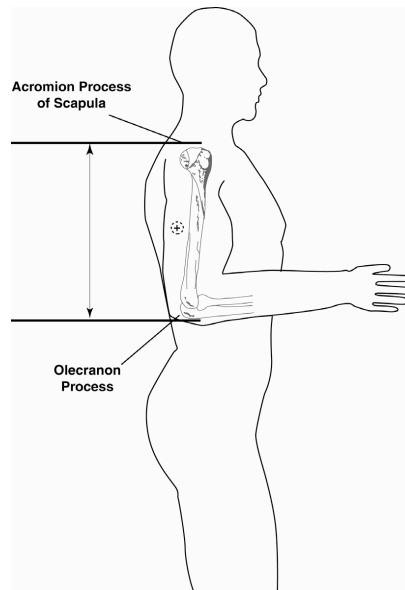
16.9.4 Marking spine extending from acromion process

- 16.9.5 Take the measurement: Hold the zero end of the measuring tape at this mark and extend the tape down the posterior surface of the arm to the tip of the olecranon process, the bony part of the mid-elbow (picture 16.9.5a). Take the measurement to the nearest 0.1 cm. IMPORTANT: The tape must be centered on the posterior surface of the arm. Exhibit 16.9.5b shows the correct placement of the measuring tape centered on the posterior surface of the arm; whereas Exhibit 16.9.5c shows the measuring tape placed incorrectly.

Appendix 2: Standard Operating Procedures used in data collection

SOP Title: SHINDA 2 – Urine sample collection and anthropometry

SOP No:



16.9.5a Subject position for upper arm length and midpoint



16.9.5b ✓ Correct tape placement for upper arm length



X16.9.5c Incorrect tape placement for upper arm length

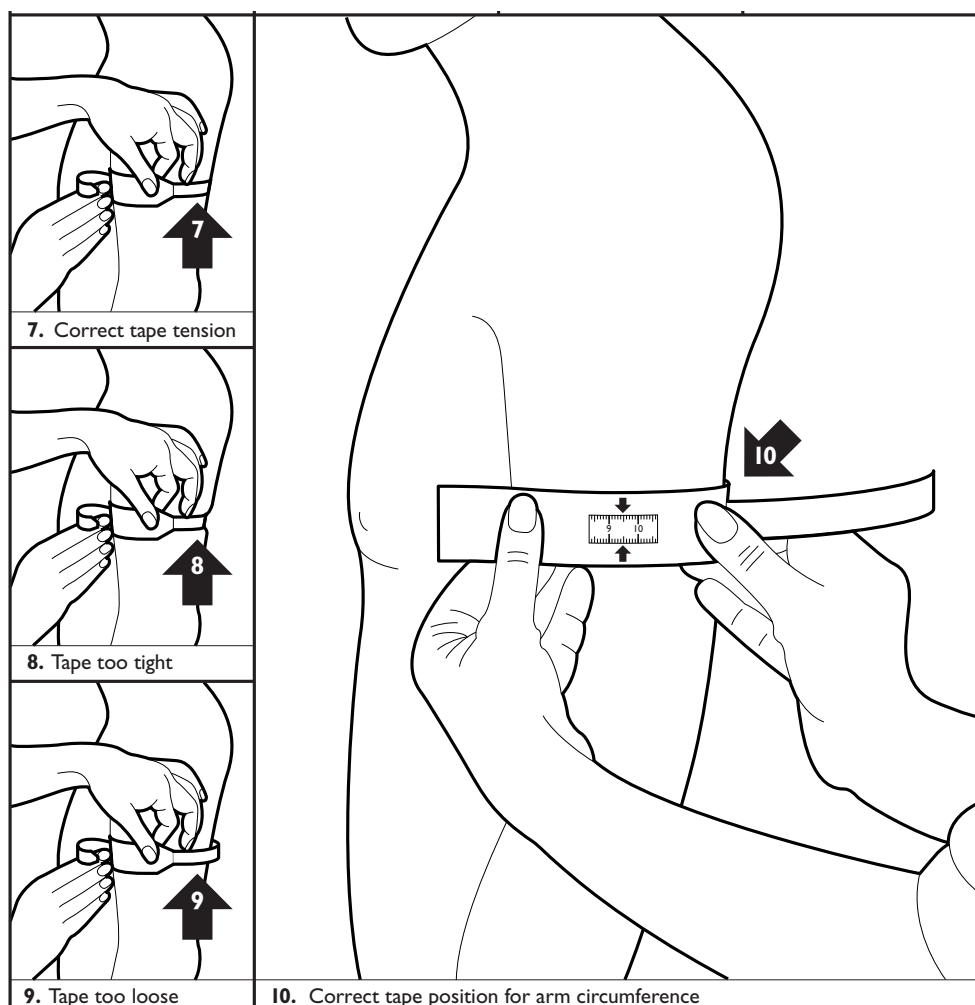
16.9.6 Note down this result. Keep the measuring tape in position.

16.9.7 Mark the midpoint: While holding the tape in place, make a horizontal mark at the midpoint and cross this mark with a perpendicular line (Exhibit 16.9.7). You may need to remove the tape in order to complete the cross mark (+). IMPORTANT: The vertical line must be centered on the posterior surface of the arm. This mark defines the site at which both the arm circumference will be measured. Finally, tell the SP to relax the right arm. Proceed to the arm circumference measure.



16.9.7 Marking upper arm length mid point

- 16.9.8 Take the MUAC measurement: Continue to stand facing the right side of the subject. Do not stand behind the subject for this measurement. Wrap the MUAC tape around the arm at the level of the upper arm mid-point mark. Make sure the numbers are the right side up. Make sure the tape is flat around the skin and that it is not too tight or too loose. When the tape is in the correct position read and call out the measurement to the nearest 0.1cm. Write down this measurement in the questionnaire.



Please complete checklist at bottom of Data Collection Form 1, to ensure that all required actions are complete.

SECOND VISIT

(This should take place as close as possible to 24 hours after the time of the spot urine on previous day)

16.10 Completing the Visit 2 Data Collection Form

16.10.1 This is to be completed on the SECOND visit, by a trained field worker, using the “Visit 2 Data Collection Form”. Complete ALL fields on the form.

16.10.2 The purpose of the questionnaire is to determine how complete the 24 BP/PWV measurement was and sleeping and waking times.

Please complete checklist at bottom of Data Collection Form 2, to ensure that all required actions are complete.

16.11 Returning to Lab at end of day

16.11.1 Nairobi: All samples are to be shipped to CCR lab at KEMRI offices in Mbagathi. Motorcycle rider will be on hand to transport the samples

16.11.2 Kilifi: At the end of the day, return all samples to the KEMRI-WT top lab

16.11.3 Please ensure that all samples are labelled and forms are appropriately filled out

16.11.4 Document the date and time that samples arrive in the lab in both:

16.11.4.1 The main laboratory logbook

16.11.4.2 The sample request form

16.11.5 Please ensure that all paperwork is signed

16.11.6 At the end of the day input all of the paperwork onto the database

17.0 APPENDICES: This section is to be used for inserting figures, tables, diagrams or forms referenced in the SOP.

Include copies of the Data Collection forms 1 and 2, and the lab request form

18.0 REFERENCES:

Provide a list of the guidance documents, cross-references, higher-level procedures or publications that have contributed to the generation of this procedure.
Start typing here...

1. Elliott P, Stamler R. Manual of operations for "INTERSALT", an international cooperative study on the relation of sodium and potassium to blood pressure. Controlled clinical trials. 1988;9(2 Suppl):1S-117S. Epub 1988/06/01.

10.0 SOP CHANGE HISTORY:

10.1 Version Table:

Original: Title:	Dated:	SOP No.:	No. Pages:
Version 1: Title:	Dated:	SOP No.:	No. Pages:
Version 2: Title:	Dated:	SOP No.:	No. Pages:
Version 3:	Dated:	SOP No.:	No. Pages:

Appendix 2: Standard Operating Procedures used in data collection
SOP Title: SHINDA 2 – Urine sample collection

SOP No:
Version: Original

SSP SHINDA-001

Version 4: Title:	Dated:	SOP No.:	No. Pages:
Version 5: Title:	Dated:	SOP No.:	No. Pages:
Version 6: Title:	Dated:	SOP No.:	No. Pages:
Version 7: Title:	Dated:	SOP No.:	No. Pages:

**Kenya Medical
Research Institute**



wellcometrust

Standard Operating Procedure			SOP No: Version No: Supersedes: Effective Date:
Title: Ambulatory Blood Pressure Monitoring for the SHINDA study			
	NAME	SIGNATURE	DATE
PREPARER	Anthony Etyang		12 Sept 2015
REVIEWING AUTHORITY			
APPROVING AUTHORITY			

2.0 PURPOSE / INTRODUCTION:

Shinda is a project whose objective is to determine pulse wave velocities and ambulatory blood pressure values in individuals exposed and not exposed to malaria. This document outlines the procedure for conducting 24hr PWV and ABPM to ensure that the results obtained are valid.

3.0 SCOPE / RESPONSIBILITY:

This applies to all clinically trained staff involved in 24 hr ABPM/PWV measurement as part of the Shinda study.

4.0 DEFINITIONS:

Blood pressure
Ambulatory blood pressure monitoring
Pulse wave velocity
Standard blood pressure measurement
Subject/Patient

5.0 EQUIPMENT / MATERIALS/ REAGENTS:

1. Computer/Tablet/netbook running windows 7 or later and Microsoft office
2. Arteriograph 24 PWV/ABPM machine
3. 4 fully charged AA size alkaline batteries
4. Crepe bandage for securing BP cuff on arm

6.0 METHODOLOGY:

Indications for conducting 24h ABPM/PWV.

- a) 24h ABPM/PWV is to be conducted on all individuals who have been selected to participate in the Shinda II study
- b) Preparing for arteriograph measurement
 - i. Take height and weight of the subject as described in SOP for taking anthropometric measurements
 - ii. Measure also the mid upper arm circumference (MUAC)
- c) Setting up the Arteriograph monitor
- d) Open the software icon “24 TensioWin” on the computer/tablet. There is no password
- e) Click on New to open the “Patient details” interface.
- f) Enter the subject’s names, date of birth, height, weight, MUAC, ID and sex into the computer software. Press OK to return to previous interface

- g) Click on the “set up protocol and device” button and select a program. Daytime measurements should be taken every 20 minutes while nighttime measurements should be taken every 40 minutes.
- h) Click “send” to transfer the subject’s information to the device. At this point a window will appear with the cuff size based on the arm circumference.
 - while the transfer is proceeding the word ‘connect’ will appear on the arteriograph screen
 - at the bottom right corner of the computer/tablet screen, the serial number of the device being programmed will appear. As there might be more than one device within range in the room, please confirm that the right device is being programmed by confirming the serial number located on the back of the device
- i) Wrap the indicated cuff around the bare arm or on light clothing.
- j) Place the device close to the computer and ensure that the device is connected via Bluetooth.
- k) Inform the subject that the measurement will begin in 5 minutes time and should sit/lie calmly. The subject should relax the arm and avoid any arm movement once the cuff starts to inflate.

Please ensure that your computer time and date is accurate as this is the time that will be recorded on the arteriograph and it will be synchronized with that of the computer

- a. Attaching monitor on patient
 - I. Ensure that you have a **fully charged** set of AA batteries for every examination. Please check battery voltage will be shown on the display as soon as you install all the batteries. If the voltage measurement is less than 3.5V replace the batteries with a new set and repeat the check.
 - II. Explain procedure to patient verbally. In addition give them the 24h ABPM/PWV information sheet as well as diary.
 - III. Place the appropriate sized cuff on the patient’s non-dominant arm. Unlike standard blood pressure monitors, the air tube is made to face up rather than down overlying the brachial artery. This is done so as to facilitate the air tube going under the patient’s clothes. The patient may wear some light clothing (e.g a thin shirt) under the cuff to prevent itching/sweat. Secure the cuff using a crepe bandage to prevent it from slipping downward during the course of the measurement.
 - IV. Connect the airtube to the monitor and secure the monitor on the patient using the carry pouch and waist/shoulder straps.
 - V. Demonstrate to the patient how to remove and reattach the device/cuff when they need to take a bath.
 - VI. The patient should not operate any buttons on the device.
 - VII. Ensure that the monitor is switched on and that the first inflation is

successful before leaving the patient.

b. Removing monitor from patient

Disconnect the airtube from the monitor. Remove the crepe bandage and cuff from the patient's arm. Remove the carry pouch as well as the monitor from the patient. Switch off the monitor to avoid it attempting to take measurements when not connected. DO this by pressing the START button 4 times when you will see 'OFF' on the LCD display.

c. Downloading data from monitor to computer

Open the software icon "24 TensioWin" on the computer/tablet. Follow the instructions to transfer the data to the computer.

Select the individual patient and then follow these instructions in order to export a report of the 24h ABPM/PWV:

- i. On the right side of the window you will see the name of the participant and details of when the test started and ended.

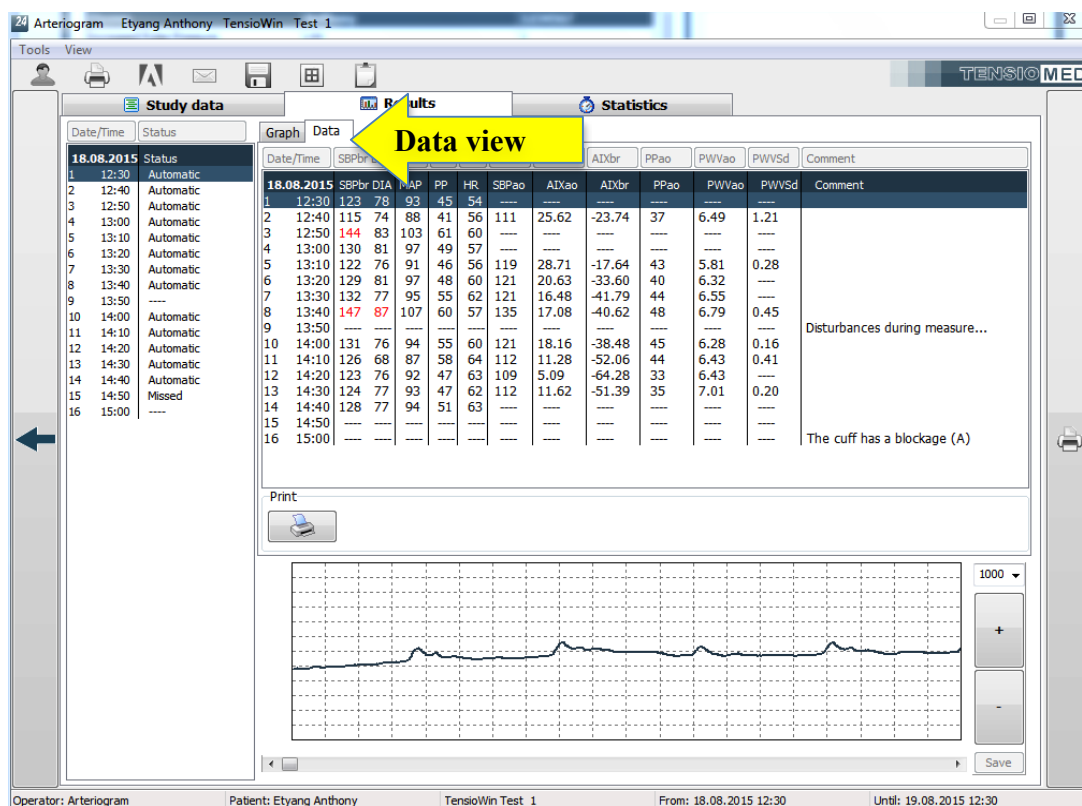


- ii. Click on the "Graph view" tab

- iii. This will show you a graph of the subjects BP measurements

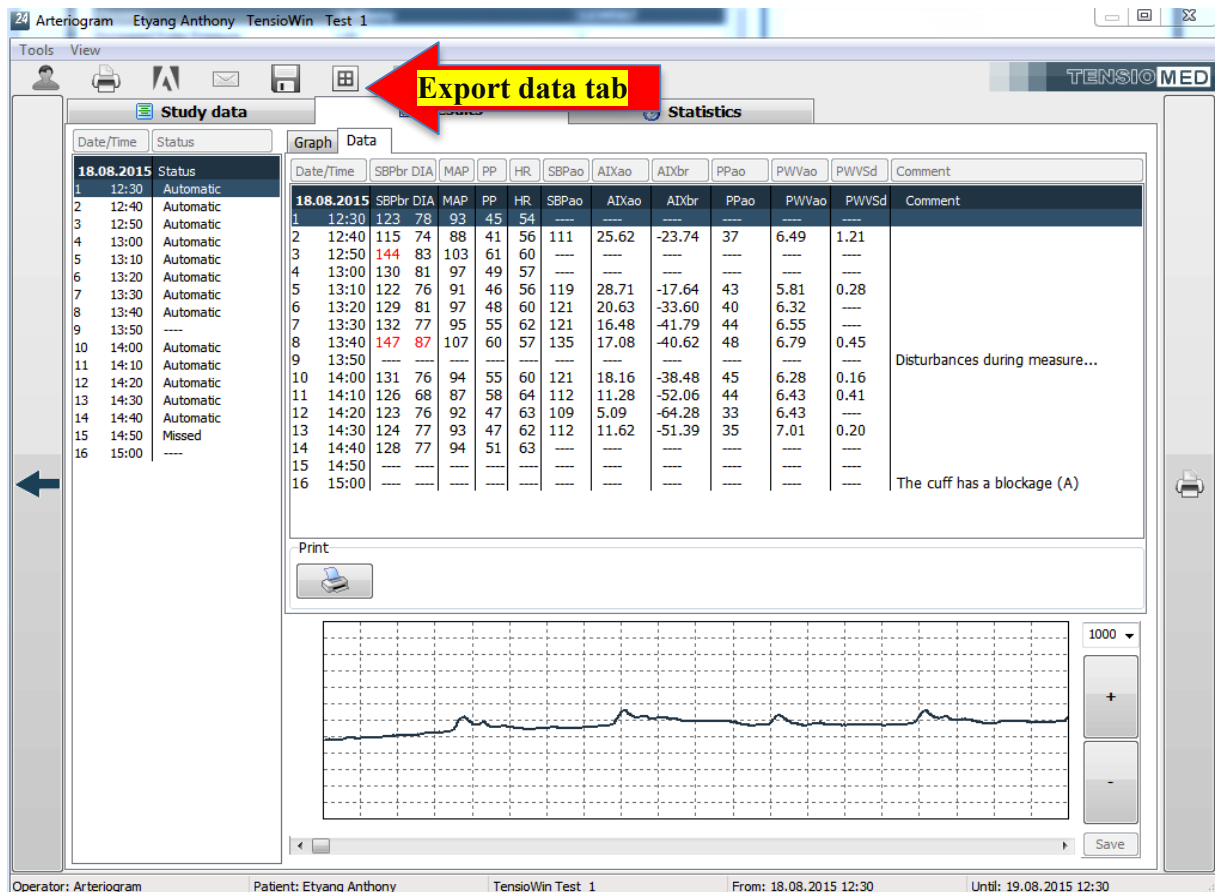
- iv. Click on the "Data" tab at the top of this window as shown below. This will display the subjects individual readings that were taken over the 24 hour period as shown below

Appendix 2: Standard Operating Procedures used in data collection
SOP Title: Standard Operating Procedure (SOP) for ABPM/PWV measurement for SHINDA II
Version: Original **SSP SHINDA-001**



NOTE: This is a CONTROLLED document. Any SOPs that are not stamped in red “APPROVED” are not controlled. Anyone using an uncontrolled copy is responsible for checking that they have the latest revision of the SOP prior to use.

- v. Click on the export data tab at the top of this window as shown below



- vi. Save the report using the patient's PID as the file name. It will be saved in Microsoft word TXT format
- vii.
- d. Entering 24 hr ABPM data into database
- Open the patient's 24h ABPM report saved in the previous step in TXT format. At the same time open the blank excel spreadsheet that has the same name (PID) as the TXT file that you have opened. Select the table of BP readings in the TXT file. Make sure you have selected the entire table to include all the readings for the period. The columns in the table will consist of the following: Time; T SBPbr DBP pulse PP MAP SBPao AIXao PPao PWVao PWVsd
 - Copy this table into the excel spreadsheet. Confirm once more that the excel file has the same name (PID) as the TXT file you have copied from. Save the excel file. Then transfer this file into the study database.

Enter any additional information present on the participant diary under the 'Note' column

7.0 APPENDICES: This section is to be used for inserting figures, tables, diagrams or forms referenced in the document.

8.0 REFERENCES:

Please review the instruction manuals for the Arteriograph 24 for more details.

9.0 DOCUMENT CHANGE HISTORY

This section is to be completed by the Quality Management or designee

Version Table:

Version 1: Title: Ambulatory Blood Pressure/PWV Monitoring for the SHINDA II study	Dated: 12 October 2015	SOP No.:	No. Pages:
Version 2: Title:	Dated:	SOP No.:	No. Pages:
Version 3: Title:	Dated:	SOP No.:	No. Pages:

SOP Review and Updating Logs

DATE	NAME OF REVIEWER	SIGNATURE	REASON FOR REVIEW

DOCUMENT COPY CONTROL

SOP DISTRIBUTION

DATE	SECTION	RECEIVED BY

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