**UNIVERSITY OF NAIROBI**

# CLINICAL PREDICTORS AND GENETIC DETERMINANTS OF RESPONSE TO WARFARIN THERAPY IN KENYAN PATIENTS


## DAVID GITONGA NYAMU,

**BPharm (UoN), MPharm (UoN), Fellow, Implementation Science (UoN/UMB)**

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| **Name of Student:** | **David Gitonga Nyamu** |
| **Registration Number:** | U80/52841/2018 |
| **College:** | College of Health Sciences |
| **School:** | School of Pharmacy |
| **Department:** | Pharmaceutics and Pharmacy Practice |
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**DAVID GITONGA NYAMU,**

BPharm, MPharm (UoN), Fellow-Implementation Science (UoN/UMB)

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## APPROVAL BY SUPERVISORS

This Thesis has been submitted for examination with our approval as the Research Supervisors.

**Professor Anastasia N. Guantai, PhD**

Professor of Pharmacology & Therapeutics
Department of Pharmacology & Pharmacognosy
University of Nairobi

Signature ..... Date 27th November 2019.

**Professor George O. Osanjo, PhD**

Associate Professor of Pharmacology & Therapeutics
Department of Pharmacology & Pharmacognosy
University of Nairobi

Signature ..... Date 27/11/2019

**Professor Eleni Aklillu, PhD**
Division of Clinical Pharmacology
Karolinska University Hospital - Huddinge
Karolinska Institutet
Stockholm, Sweden

Signature ..... Date 27/11/19



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# DEDICATION

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# ABBREVIATIONS AND ACRONYMS

**ADRs:** Adverse Drug Reactions

**AiBST:** African Institute for Biomedical Science and Technology

**AJ**: Ashkenazi Jewish

**AOR**: Adjusted Odds Ratio

**ANOVA**: Analysis of Variance

**ASO**: Allele specific oligonucleotide

**BMI**: Body Mass Index

**BSA**: Body Surface Area

***CYP 2C9***: Cytochrome P450 2C9

***CYP 4F2***: Cytochrome P450 4F2

**DDI**: Drug-drug interaction

**ddNTP**: dideoxynucleotide triphosphate

**DNA:** Deoxyribonucleic Acid

**dNTP:** deoxy nucleotide triphosphate

**DVT**: Deep Vein Thrombosis

**EDTA**: Ethylene Diamine Tetra-acetate

**FDA**: Food and Drug Administration

**FWS:** Fetal warfarin syndrome

**GCP**: Good Clinical Practice

**gDNA**: genomic DNA

***GGCX***: Gamma Glutamyl Carboxylase Enzyme

**GIT**: Gastrointestinal

**GLP**: Good Laboratory Practice

**GWAS**: Genome-wide Association Study

**HIT**: Heparin Induced Thrombocytopaenia

**HPLC**: High Performance Liquid Chromatography

**HR:** Hazard Ratio

**HWE**: Hardy-Weinburg Equilibrium

**ICTH**: International Committee on Thrombosis and Hemostasis

**IBM SPSS**: IBM Statistical Package for Social Sciences

**INR**: International Normalized Ratio

**ISO**: International Standards of Organization

**KEMRI**: Kenya Medical Research Institute

**KMTC**: Kenya Medical Training College

**KNH**: Kenyatta National Hospital

**KNH/UoN-ERC**: Kenyatta National Hospital/University of Nairobi Ethics and Research committee

**LMIC**: Lower and middle income countries

**MAF**: Minor allele frequency

**MALDITOF**: Matrix-assisted laser desorption ionization-time-of-flight

**OR**: Odds Ratio

**PCR**: Polymerase Chain Reaction

**PE**: Pulmonary Embolism

**PI**: Principle Investigator

**PIVKAs**: Proteins Induced [by] Vitamin K absence/antagonism

**PT**: Prothrombin Time

**RPharmS**: Royal Pharmaceutical Society of Great Britain

**SAP**: Shrimp Alkaline Phosphatase

**SBE**: Single Base Extension

**SNPs**: Single-nucleotide polymorphisms

**TTR**: Time-to-Therapeutic Range

**UK**: United Kingdom

**UoN-CHS**: University of Nairobi-College of Health Sciences

**USA**: United States of America

**VKOR**: Vitamin K Epoxide Reductase

***VKORC1***: Vitamin K [Ep] oxide Reductase complex subunit 1

**VTE:** Venous Thromboembolism

**WHO**: World Health Organization

# DEFINITION OF TERMS

**Adverse Drug Reaction (ADR):** The WHO defines an ADR as "a response to a drug which is noxious and unintended and which occurs at doses normally used in man for prophylaxis, diagnosis, or therapy of disease or for the modification of physiologic function."

**An allele:** Is an alternative form of a gene (one member of a pair) that is located at a specific position on a specific chromosome.

**Anticoagulation:** The prevention or hindering of the formation of blood clots, especially by an anticoagulant drug such as warfarin. The practice reduces the likelihood of the formation of blood clots but increases the risk of bleeding.

**Black Kenya patient**: A Kenyan citizen by birth and whose both parents are of any of the Kenya's racial group having dark skin colouring, typically of sub-Saharan African descent.

**Blood clot**: The gel-like mass formed by platelets and fibrin in the blood to stop bleeding.

**Clone**: A unique segment of DNA (which contains a particular gene) that is generated *in vitro*, usually through the use of restriction enzymes, and reproduced *in vivo* (in *Escherichia coli*).

**DNA sequencing**: The process of determining the exact order of chemical building blocks (called bases) that makes up the DNA of different human chromosomes.

**Gene Probe:** A clone specific sequence of DNA in which nucleotides are radiolabelled.

**Gene**: A sequence of chromosomal DNA that encodes for a single protein.

**Genetic Polymorphisms:** Is the occurrence in the same population of two or more alleles at one locus, each with appreciable frequency, where the minimum frequency is typically taken as 1%.

**Genotype:** The part of the genetic makeup of an individual which determines one of his/her characteristics

**Genotyping**: The process of determining the genotype of an individual by the use of biological assays. Current methods of doing this include PCR, DNA sequencing, ASO probes, and hybridization to DNA microarrays or beads.

**Haplotype:** Is a group of alleles in an individual that are inherited together from a single parent.

**Hybridization**: The process whereby fragments of DNA bind specifically to their complementary DNA sequences.

**International normalized ratio (INR):** A standardized method established by the WHO and the ICTH for reporting the results of blood coagulation tests. The therapeutic range for INR during warfarin therapy is 2 to 3 but in few cases, the target range may be as high as 2.5–3.5 if more intense anticoagulation is required.

# PUBLICATIONS FROM THESIS WORK

1. **Nyamu, D.G**., Guantai, A.N., Osanjo, G.O., Aklillu, E., (2019). Trends of anticoagulation control among adult outpatients on long-term Warfarin therapy in a Tertiary Teaching and Referral Hospital in Kenya. *East Afr. Med. J.* **95** (7): 1705–1709.

2. **Nyamu, D.G**., Guantai, A.N., Osanjo, Aklillu E., Tele A.K. (2019). Prevalence of Nutritional and Herbal Medicine use and its Impact on Warfarin Dose and Response in a Tertiary Referral Hospital in Kenya. *PJK*. **24**(1):7-14.

3. **Nyamu, D.G**., Guantai, A.N., Osanjo, G.O., Mwatha, E., Gitonga, I., Kanyiri, M.L. (2017). Predictors of Adequate Ambulatory Anticoagulation among Adult Patients in a Tertiary Teaching and Referral Hospital in Kenya. *Afr. J. Pharmacol. Ther*. **6(**1): 20-26

# ABSTRACT

**Background**: Warfarin is the most extensively used long-term oral anticoagulant in most parts of the world. However, sociodemographics, patients' clinical characteristics and individual's genetics contribute to inter-patient variation in warfarin dose and response. Sociodemographics have been shown to contribute to almost a fifth of inter-patient variability in warfarin dose requirements among individuals from the western communities. In addition, the most important genes that determine warfarin dose and response include Vitamin K Epoxide Reductase Complex Subunit 1(*VKORC1*), Cytochrome P450 2C9 *(CYP 2C9)* and Cytochrome P450 4F2 *(CYP 4F2)* which account for ~ 30%, ~ 10% and ~2 % of the inter-patient warfarin dose variation in the western countries, respectively. There is paucity of Kenyan data on the clinical predictors and genetic variability on warfarin dose and response.

**Study Objective**: To characterize the clinical predictors and genetic determinants of response to warfarin therapy in order to develop a model for safe warfarin dosing among black Kenyan patients.

**Methods:** The study areas were the medical records department and three anticoagulation clinics of the leading teaching and referral hospital in Kenya. Black Kenyan patients aged ≥18 years, who were on long term warfarin therapy (≥ 28 days), were recruited. Two study designs were used: Prospective longitudinal study design (180 patients) which characterized the clinical determinants of warfarin response and cross-sectional study (40 participants) which characterized the genetic variability among the patients. Sociodemographics, ethnicities, dietary habits, nutritional and herbal medicine utilization, duration of warfarin use and adverse drug reactions (ADRs) to warfarin were obtained through face-to-face patient interviews. Data on clinical indications of anticoagulation, concomitant medications and concurrent diseases were acquired through review of the medical records.

Venous blood samples were drawn for the determination INRs and genetic testing. Patients were followed up for six consecutive clinic visits while determining the INRs whose therapeutic levels were set at 2-3 in accordance with the established international guidelines. DNA extraction was conducted using the Quick-DNA$^{TM}$ Miniprep plus kit in accordance with manufacturer's

instructions and the purity was assessed by comparing $A_{260}$ and $A_{280}$ ratio using the NanoDrop technology. *CYP 2C9 (*2, *3, *4, *5, *6, *8, *11* and **13), VKORC1 rs9923231* as well as *CYP 4F2 [*3(1347C>T)* and *rs2189784;G>A* were analyzed for single nucleotide polymorphisms (SNPs) using MassARRAY Compact Mass Spectrometer and Agena real-time detection platform, using primers.

**Statistical Analyses**: Data analyses were conducted onto IBM Statistical Package for Social Sciences version 23 at 95% confidence limit with values having $p \leq 0.05$ being considered statistically significant. Associations between the predictors (socio-demographics, patients' clinical characteristics and genetic polymorphisms) and outcomes (warfarin doses, INRs and ADRs) were computed. Regression analyses were achieved using an identity link and logit link to determine independent predictors of outcome variables. $R^2$ and Nagelkerke's $R^2$ regression models were used to estimate the relative contribution of individual predictor variables to the inter-patient variability in warfarin maintenance doses, ADRs and INRs observed.

**Results**: There was female preponderance (77.0 %). Patients had a mean age 43.4 (±13.2) years where majority were overweight/obese (>60.0%) and married (65.6%). The median duration of warfarin therapy was 24.8 (range 1.1-375.9) months. The mean warfarin maintenance dose was 6.2±2.8mg per day. Venous thromboembolic (VTE) (56.6%) and cardioembolic (33.3%) events were the main indications of warfarin anticoagulation. Higher warfarin dose requirement was found among males, participants aged ≤ 50 years, obese/overweight and with spouses ($p$=0.05). Although a diagnosis of VTE was the independent predictor of higher warfarin dose requirement [β=0.72, CI: 0.07, 1.36; $p$=0.030], patients with cardioembolic events had better INRs than those with VTEs. Furthermore, significant proportion of males were better anticoagulated than females (51.3% vs. 30.4%; $p$=0.040). Gender [β=-3.38, 95% CI: -5.38 to -1.38, $p$=0.001] and patients' age (β=-0.06, 95 %CI: -50.13 to -0.00, $p$=0.050) independently predicted warfarin maintenance doses that would give therapeutic INRs. The most common ADR to warfarin therapy was haemorrhage (27.8%) which was significantly found among patients without spouses ($p$=0.008) and cardiac diseases ($p$=0.050). However, the independent predictor of ADRs was living without spouses (β=0.76; 95% CI: 1.13, 4.06; A.O.R=2.14, $p$=0.019). The patients' clinical data explained approximately 14.0%, 20.0% and 19.0% of the variability in inter-patient warfarin

doses, INRs and ADRs, respectively with body surface area (BSA) contributing the greatest variability to warfarin dose (6.92%) and INRs (4.54%).

There were two ethnolinguistic groups of Africa captured for the genetic study: Bantu and Nilotes. *CYP 4F2* gene was the most polymorphic, with *CYP 4F2 (rs2189784; G>A)* at 60% and *CYP 4F2 *3(1347C>T)* (17.5 %), followed by *VKORC1 rs9923231* (12.5 %). Unlike *CYP 2C9*8* and **11* where **8* showed heterozygousity (*GA*) genotypes at 17.5% while *11 revealed heterozygousity (*CT*) genotypes at 2.5%, polymorphisms of *CYP 2C9*2, *3, *4 *5 *6* and **13* were not detected in the population. The wild type *CYP 2C9 *8* and **11*, variant *VKORC1* and *CYP4F2 (1347C>T) (CT*) genotypes required clinically higher warfarin maintenance doses. However, *CYP 4F2 (rs2189784; G> A*) wild type (*GG*) genotype [β =3.01, 95%CI: 1.36-4.65, $p$<0.001] and *VKORC1 (rs9923231)* wild type (*CC*) genotype [β =3.77, 95%CI: 1.51-6.03, $p$=0.001] were the independent genetic determinants for the warfarin maintenance doses which could achieve therapeutic INRs. The genetic data accounted for approximately 21.0 %, 38.0 % and 31.0 % of the warfarin dose variability, INR responses and ADRs among the patients in the two ethnolinguistic groups studied. *CYP 4F2* polymorphisms contributed the greatest in variability (9.6%) of inter-patient warfarin dose requirements. The clinical and genetic information led to derivation of the model for safe warfarin dosing to produce INR 2-3 as: **Safe Warfarin Dose (Mg) =-3.38(Male) +3.01 *CYP 4F2 (rs2189784*; wild type *GG*) + 3.77 *VKORC1 (rs9923231*; wild type *CC*).**

**Conclusions:** Higher warfarin doses among the Kenyan patients on anticoagulation may be necessitated by male gender, younger age, obesity, diagnosis of VTE, *VKORC1* and *CYP 4F2* polymorphisms. BSA and *CYP 4F2* polymorphisms contributed the greatest variability of inter-patient warfarin doses and may guide dosing of warfarin in long-term anticoagulation. Males and patients with heart diseases were better anticoagulated; but the latter had more ADRs suggesting that intensification of warfarin anticoagulation should be done among females as well as for patients with cardioembolic disorders. Future studies should correlate health facility, prescriber-related and pharmacoeconomic factors with anticoagulation outcomes. Studies exploring the role of other genes involved in warfarin pharmacokinetics and pharmacodynamics are also recommended.

# CHAPTER ONE: INTRODUCTION

## 1.1: Background to the Research

In early 1900s, there was a report of an occurrence of uncommon disease in cattle in some parts of Northern America and Canada. The clinical features of this disease included fatal haemorrhage, either naturally or from slight bruises and mouldy fodder from sweet clover was implicated (Holbrook *et al*., 2005; Pirmohamed, 2006). Roderick, in North Dakota discovered that the sweet clover had some chemical that inhibited prothrombin (Holbrook *et al*., 2005). In 1940s, Carl Link and his investigators in Wisconsin, revealed that the haemorrhagic factor was 4-hydroxycoumarin (Ramachandran and Pitchai, 2018). Further laboratory work led by Link discovered and synthesized warfarin, a vitamin K antagonist, in 1948. This drug was approved for killing rodents in the USA four years later. As such, coumarin led to the synthesis of warfarin. Coumarin occurs naturally in some plants such as woodruff (*Gallium odoratum*), lavender and liquorice (Ansell *et al*., 2004).

Coumarins act as anticoagulants by inhibiting the enzyme Vitamin K epoxide Reductase Complex (VKORC). The enzyme participates in the conversion of oxidized vitamin K to reduced form after carboxylation of blood clotting factors II, VII, IX, X as well as protein S, C and Z (Ansell *et al*., 2004).

Warfarin has been used clinically for almost seventy years (Ageno *et al*., 2012) and it is one of the most extensively used oral anticoagulant in Kenya (Ogendo, 2000) and other places in the world (Pham and Pham, 2007).Warfarin has been proven to be very effective through many clinical trials conducted for the prophylaxis of venous thromboembolic events (Lutsey *et al*., 2018). Evidence has shown that warfarin is one of the most commonly used anticoagulation therapies because it reduces morbidity and mortality associated with thromboembolism (Lowery *et al*., 2005). More so, it is cheap and readily available (Lowery *et al*., 2005). Furthermore, it has been shown that warfarin can prevent thromboembolic events in people with mechanical heart valves, atrial fibrillation, acute or recurrent myocardial infarction as well as prevention of death in patients suffering from ischaemic heart disease (Ageno *et al*., 2012; Lutsey *et al*., 2018).

Anticoagulation therapy using vitamin K antagonists is an essential component of treatment for patients suffering from thrombotic disorders (Lowery *et al*., 2005). Furthermore, studies have demonstrated that venous thromboembolic events (VTE), which comprise deep venous thrombosis (DVT) as well as pulmonary embolism (PE) (Zierler, 2004), are the commonest indication of warfarin therapy (Kibiru, 2012; Maina *et al*., 2013; Mariita *et al*., 2016). Local studies done in Kenya on profiles of patients using warfarin showed a prevalence of VTE at 78% (Mariita *et al*., 2016) and 80% (Maina *et al*., 2013) and these individuals are on long term warfarin treatment.

In related studies across the world, warfarin has been shown to prevent thrombosis in people suffering from atrial fibrillation (De Caterina *et al*., 2012) and after major surgeries including hip or knee replacement (Ogendo, 2000) as well as prevention of blood clotting after heart valve repair (Ogendo, 2001) or ischaemic stroke in predisposed patients (Aguilar and Hart, 2005).

Long term management of thromboembolic events with warfarin has several limitations because of the intra-individual and inter-individual dose variation. A number of factors have been known to impact on the response to warfarin's activity. Factors such as patients' sociodemographic characteristics (Mariita *et al*., 2016), knowledge (Iqbal, 2017) and food-drug interactions (Holbrook *et al*., 2005) have been implicated. In addition, genetic variability in warfarin metabolism has been cited to contribute to about a two-fifths of dose variation among Caucasian patients (Scott *et al*., 2008). However, there is paucity of African data on the clinical variables as well as genetic variability and their contribution to warfarin dose and response.

### 1.2: Demographics and Doses of Warfarin among Patients

Warfarin is used across all age groups but mostly in the elderly. Studies from the Western countries have revealed that its use is commonest among elderly patients suffering from atrial fibrillation (Karamichalakis *et al*., 2015). However, some Kenyan studies have shown that its use is commonest among the young adults of less than 30 years (Ogendo, 2001)while some have indicated 40 years but less in the elderly (Kibiru, 2012; Mariita *et al*., 2016).This variance is attributed to the differences in the clinical indications of warfarin anticoagulation between the developing and developed countries.

Dosing of warfarin is patient specific and varies from patient to patient depending on the desired intensity of anticoagulation and response (Gage *et al*., 2000). For most of the patients, commencing treatment at 5 mg warfarin daily dose and titrating the dose based on INR response has been shown to yield therapeutic INR in less than one week (Gage *et al*., 2000). However, lower doses or higher values may be necessary depending on individual host factors such as nutritional status, hepatic function, thyroid function and concurrent medication that influence warfarin effect (Ansell *et al*., 2004). Maintenance doses of warfarin is usually based on constant monitoring of clinical and laboratory data due to intra-patient and inter-patient variability (Gage *et al*., 2000).

The internationally recommended initial daily dose of warfarin is 10 mg (Ageno *et al*., 2012) while the maintenance dose varies depending on patient clinical status. The average warfarin dose per week is between 25 mg and 55 mg (Gage *et al*., 2000). Local studies have documented a mean daily maintenance dose of less than 10 mg. For instance, a study done on pattern of anticoagulation control showed a mean daily dose of approximately 7.0±2.67 mg (Ogendo, 2000). This was slightly higher than other countries, especially among the Chinese population whose warfarin requirement was 3.10 ± 0.97 mg per day (Liu *et al*., 2017).

### 1.3: Monitoring of Response to Warfarin Therapy

Despite its effectiveness, use of warfarin has several limitations owing to its narrow therapeutic window. As such, optimal titration of warfarin dose is complicated as it interacts with many conventional drugs and even some foods (Holbrook *et al*., 2012). The interactions may augment or diminish the anticoagulation effect of warfarin and therefore, in order to improve the beneficial effect of the drug without risking dangerous side effects, the level of anticoagulation must be monitored closely (Hirsh *et al*., 2003) by regular blood assessment for the international normalized ratios (INRs) (Ansell *et al*., 2004; Nutescu *et al*., 2011). INR measurement is the most commonly used method for monitoring warfarin therapy and compares the patient prothrombin test (PT) to the mean normal PT (Favaloro, 2017).

The World Health Organization (WHO) introduced INR as a way of standardizing the monitoring of warfarin therapy in 1980s due to varying hospital protocols and variation in the

available thromboplastins (Van den Besselaar and van der Meer, 1992). Kenyatta National Hospital (KNH) started routine INR testing some ten years later and up to today the facility has not devised new methods. An INR of 2-3 is termed therapeutic because studies have indicated that INR which is above 3 increases the risk of haemorrhage, while that which is < 2 increases the chances thromboembolic events (Jones *et al*., 2005).

Although INR monitoring forms an integral part of warfarin monitoring, it has a number of challenges including direct costs and underreporting (Nyamu *et al*., 2017). Additionally, studies have revealed that only less than 10% of the patients are within the recommended INR ranges for more than half of the follow-up time (Ogendo, 2000) and it is suggested that better monitoring and dosing methods are necessary. Owing to the challenges encountered with INR testing, studies have recommended other methods of monitoring of warfarin therapy including checking for clinical signs and symptoms of adverse effects such as bleeding (Ogendo, 2001), fractures (Caraballo *et al*., 1999; Talmadge and Spyropoulos, 2003) and purple toes (Talmadge and Spyropoulos, 2003) although the latter two are uncommon.

### 1.4: Factors Impacting on Warfarin Dose Variations among Patients

The inter-patient variability in warfarin response, its narrow therapeutic index and a high probability of stroke in patients restricts its medical use and maintenance doses prescribed (Kamali, 2006; Loebstein *et al*., 2001; Sconce and Kamali, 2006). For instance, among the Chinese population, the most important determinant of response and dose was age, with statistically significant lower warfarin requirements decreasing with increasing age (Yu *et al*., 1996).

In the local setting, African patients undergoing double valve replacement surgeries were found to receive a statistically significant lower dose than other indications (Ogendo, 2000). Additionally, some regional studies have demonstrated that the body surface area and body mass index (Ogunsua *et al*., 2015) would predict warfarin maintenance dose while studies in the Western countries have indicated that individual's genetic composition would determine warfarin maintenance dose (Sconce *et al*., 2005; Takeuchi *et al*., 2009).

Some research has indicated that the activity of warfarin is determined partly by genetic constitution of an individual (Wadelius *et al*., 2005). Food and Drug Administration (FDA) emphasized this as a prospect for healthcare workers to use genetic testing to advance anticoagulation by providing a reasonable initial estimate of warfarin dose for individual patients (Nutescu *et al*., 2011). In this respect, genetic polymorphisms in some genes were found to play a central role in optimization of anticoagulation and determining the response to warfarin's activity. These genes included Vitamin K Epoxide Reductase subunit 1 (*VKORC1*) , Cytochrome P450 2C9 (*CYP 2C9*) and Cytochrome P450 4F2 (*CYP 4F2*) (Wadelius *et al*., 2005).

*VKORC1* polymorphisms explain approximately a third of the inter-individual dose variation (Wadelius *et al*., 2005) with some mutations making it less susceptible to suppression by warfarin (Rost *et al*., 2004). The group A which are the low-dose haplotype and group B or the high-dose haplotype of *VKORC1* may explain 25 % of inter-patient warfarin dose variation (Rieder *et al*., 2005). As such, studies have indicated that polymorphisms in *VKORC1* group A lead to a quicker attainment of a therapeutic INR and shorten the time to over-anticoagulation, which may cause fatal haemorrhage (Schwarz *et al*., 2008). Some studies have documented that *VKORC1* variants may explain why African-Americans require higher warfarin doses owing to the large fraction of haplotypes of group B while the Asian-Americans largely require lower warfarin doses because they have larger proportion of *VKORC1* group A (Rieder *et al*., 2005).

*CYP 2C9* polymorphisms have been found to explain approximately 10 % of the dose variations between Caucasian patients (Wadelius *et al*., 2005) . However, studies have revealed that *CYP 2C9* polymorphisms are infrequent in African-American and most of the Asian people (Sanderson *et al*., 2005). The *CYP 2C9* polymorphisms do not impact on the time to therapeutic INR unlike the *VKORC1*polymorphisms, but they shorten the time to reaching INR greater than 4 (Schwarz *et al*., 2008). On the other hand, polymorphisms in *CYP 4F2* have been indicated to impact on the requirements of warfarin doses and may account for about 2 % of dose variation between patients, mainly African-Americans (Takeuchi *et al*., 2009).

Studies have also revealed that there are some types of foods and medicines which may interact with warfarin (Pham and Pham, 2007). Besides the metabolic interactions between other

medicines and warfarin , drugs which are highly bound to albumin may displace warfarin from binding thereby necessitating a downward titration of warfarin dose but frequent INR monitoring (Gage *et al*., 2000).Therefore, when commencing the treatment with a drug that will possibly interact with warfarin, monitoring of the response is intensified or dosages of warfarin are titrated until a reasonable and safe dose is instituted.

The current study was aimed at characterizing the determinants of response to warfarin anticoagulation. The main outcome variable was the response to warfarin's activity as measured by INRs, ADRs and doses prescribed. The research had binary outputs which were investigated. The first output was to describe the pattern of use of warfarin among the Kenyan patients on anticoagulation therapy. This output also explored clinical predictors associated with the doses prescribed as well as the response to therapy. The second output was to characterize the polymorphisms of genes impacting on the activity of warfarin and correlating the findings with the response as well as the dose.

Characterization of the relevant SNPS was prompted by the fact that Western studies have revealed that genotyping for *CYP 2C9*, *VKORC1* and *CYP 4F2* variants in a population coupled with use of patients' clinical data could predict the initial dose of warfarin rather than the trial and error method using the INR checks (Burmester *et al*., 2011).

## 1.5: Statement of the Research Problem

Warfarin is one of the most worldwide prescribed oral anticoagulant (Hirsh *et al*., 2003; Holbrook *et al*., 2012). The upsurge in its use over the years has been attributed to enormous evidence of its activity in the prophylaxis and treatment of venous and cardiac thromboembolic events (Ageno *et al*., 2012; Aguilar and Hart, 2005). In Kenya, it is also the most widely available vitamin K antagonist (Ogendo, 2001) that is used for long term management of VTEs (Mariita *et al*., 2016), atrial fibrillation as well as prevention of blood clotting after cardiac valve surgery (Ogendo, 2000).

Some Kenyan studies on anticoagulation have dwelt on adequacy of anticoagulation control among patients on warfarin therapy (Kibiru, 2012; Ogendo, 2000) while others have looked into patients' knowledge on anticoagulation management (Mariita *et al*., 2016). The pattern of anticoagulation control among heart valve patients has also been studied (Ogendo, 2000) where a single patient population with only valvular abnormalities was followed at the cardiothoracic clinic in a tertiary teaching and referral hospital (Ogendo, 2000). The available local anticoagulation studies have also looked at adequacy of INR (Kibiru, 2012) without considering the various dosages that the patients are taking and other anticoagulation monitoring parameters. Therefore, factors impacting on the warfarin doses prescribed as well as response as measured by INR and ADRs, remained to be explored. Furthermore, there is scant published local literature on the pattern of warfarin therapy pertaining to the patient profiles, clinical indications, details of warfarin therapy and monitoring parameters.

Warfarin is ranked third among the medications that cause admissions to the hospitals owing to their deleterious consequences on their utilization (Pirmohamed *et al*., 2004). Several studies have reported that the main side effect of warfarin is haemorrhage (Linkins *et al*., 2003; Nyamu *et al*., 2017) which may be fatal. From the a meta-analysis of over thirty studies, serious and lethal haemorrhagic episodes occurred at rates of approximately 7.0 and 1.5 per100 patient-years, respectively (Linkins *et al*., 2003) suggesting that there is need to conduct research aimed at minimization of the adverse effects. These studies may only be feasible if the factors impacting on warfarin response are characterized as this would indicate the targets of interest. Furthermore, rigorous studies on patients taking warfarin due to atrial fibrillation indicated that

for almost 50% of the follow up time, the INR was not within the 2-3 therapeutic range (Boulanger *et al*., 2006). These problems are further compounded by the fact that patient's warfarin dosage requirements differ broadly between and within individuals. Therefore, factors which correlate the warfarin response and dose need to be considered before prescribing.

Previous and related studies in Kenya on anticoagulation control revealed that 7% of patients are able to maintain adequate anticoagulation for more than half of their follow up time (Ogendo, 2000) and suggested better ways of anticoagulation practice to be instituted. It seems that characterization of the determinants impacting on warfarin response, particularly the genetic differences between patients, had not been previously studied in the black Kenyan population.

## 1.6: Research Questions

1. What are the profiles of black adult Kenyan patients on long-term warfarin therapy at Kenyatta National Hospital?
2. What are the clinical indications, dosing and monitoring parameters of warfarin therapy among black adult Kenyan patients attending anticoagulation clinics of Kenyatta National Hospital?
3. What are the non-genetic factors associated with warfarin dosing and response among black adult Kenyan patients attending anticoagulation clinics of Kenyatta National Hospital?
4. What are the genetic determinants of the variability in the inter-individual warfarin dose and response among black adult Kenyan patients on anticoagulation treatment at Kenyatta National Hospital?

## 1.7: Objectives of the Study

### 1.7.1: General Objective

To characterize the clinical predictors as well as genetic determinants of response to warfarin therapy among black Kenyan patients on long-term anticoagulation in order to develop a model for safe drug dosing.

### 1.7.2: Specific Objectives

1. To describe the profiles of black adult Kenyan patients on long-term warfarin therapy at Kenyatta National Hospital (KNH) anticoagulation clinics.
2. To describe the indications, dosing and monitoring patterns of warfarin therapy among black adult Kenyan patients attending anticoagulation clinics of KNH.
3. To characterize non-genetic factors associated with warfarin dosing and response among black adult Kenyan patients attending anticoagulation clinics of KNH.
4. To determine the prevalence of *CYP 2C9*, *VKORC1* and *CYP4F2* single nucleotide polymorphisms (SNPs) in the black Kenyan adult patients on long term warfarin anticoagulation at KNH.

## 1.8: Research Hypothesis

### Null Hypothesis

Patients' clinical characteristics and genetic variability in *CYP 2C9*, *VKORC1*, and *CYP 4F2* may affect warfarin dose and response among black Kenyan patients.

### Alternate Hypothesis

Patients' clinical characteristics and genetic variability in *CYP 2C9*, *VKORC1*, and *CYP 4F2* may not affect warfarin dose and response among black Kenyan patients.

## 1.9: Justification of the Study

To the best of my knowledge, there is limited published data on the patients' profiles, clinical indications, patterns of dosing, monitoring and the factors associated with warfarin dosing and response. Therefore, part of this study generated the data for the population characteristics of patients taking warfarin, indications, the doses prescribed and monitoring parameters. The study also characterized the factors associated with the warfarin doses prescribed as well as level of anticoagulation before embarking into the genetic variability and its contribution to response of the drug in black Kenyan population.

Several factors have been found to impact on the dose requirements for the warfarin among patients' in Europe, North America and China (Lam and Cheung, 2012). Studies have shown that the polymorphic *CYP 2C9* and *VKORC1* genes, vitamin K intake, co- medications, patients' gender, age and surface area of the body could contribute to individual's requirements for warfarin dose (Lindh *et al*., 2005; Takahashi and Echizen, 2003; Wadelius *et al*., 2009).

The presently identified environmental and genetic factors explain about 50 % of the inter-individual warfarin dose variation (Carlquist *et al*., 2006; Kamali, 2006; Wadelius and Pirmohamed, 2007; Yin and Miyata, 2007; Zhu *et al*., 2007). Importantly, dosing models that combine many of the clinical factors and the genotypes of *CYP 2C9, CYP 4F2* as well as *VKORC1* have been demonstrated in the western communities (Scott *et al*., 2008; Takeuchi *et al*., 2009; Tham *et al*., 2006). The variant *CYP 2C9* *2 and *3 polymorphisms have been found in patients who are warfarin ''sensitive'' and need lower mean warfarin maintenance doses (Kirchheiner and Brockmoller, 2005; Yin and Miyata, 2007) whereas *VKORC1* polymorphisms have been found in individuals who are resistant to warfarin and are prescribed higher mean warfarin maintenance doses (D'ambrosio *et al*., 2007; Harrington *et al*., 2004; Li *et al*., 2004; Loebstein *et al*., 2001; Rost *et al*., 2004). Therefore, to enable the use of *CYP 2C9*, *VKORC1* and *CYP 4F2* genetic testing in anticoagulation management, it is vital to characterize the SNPs and frequencies of alleles in specific populations because of ethnic differences (Scott *et al*., 2008). However, reports of allele frequencies in some communities in Africa have found rare variants of *CYP 2C9* *2 and *3 (Matimba *et al*., 2009).

Kenyan studies on the management of thromboembolic disorders in patients with mechanical heart valves have showed that anticoagulation control still needs improvements in better dosage adjustments to achieve therapeutic INR values (Ogendo, 2000). One such method for improving the anticoagulation services would be to characterize the clinical and genetic data that is likely to affect warfarin's activity because some genetic variants exhibiting large effect on warfarin response have been found in some ethnic groups and not others (Lam and Cheung, 2012).

Some studies have indicated the need to explore the gene-dose as well as gene-response associations for vital *CYPs* that are involved in drug metabolisms (Zhou *et al*., 2009) . Moreover, studies have suggested that an individualized strategy for optimal anticoagulation using warfarin for Africans should not only take into consideration their diverse pharmacogenomics but also the clinical factors (Suarez-Kurtz and Botton, 2013). To the best of my knowledge, this is the first population study in Kenya exploring the role of *CYP 2C9*, *VKORC1* and *CYP 4F2* genes and clinical predictors in determining warfarin dose and response.

# CHAPTER TWO: LITERATURE REVIEW

## 2.1: Perspectives of Literature Review

This chapter appraises the literature on the use of vitamin K antagonists. It commences by exploring the types of thrombotic events and the burden of thromboembolic events requiring long term warfarin therapy. It also examines the risk factors associated with development of disorders as well as highlighting on the available pharmacotherapeutic options. The section highlights on the clinical profiles of patients using warfarin, the clinical indications and the dosing patterns in reference to the doses prescribed and duration of use. Explored in the chapter are also the patterns of laboratory monitoring of the drug such as the frequency of INR testing as well as the clinical monitoring including the ADRs. Factors which impact on the response to warfarin, including the clinical factors and genetic variability have been appraised. The chapter summarizes by identifying the gaps in the studies which were the motivation for the present research.

## 2.2: Types of Thrombotic Disorders

Thromboembolic events are broadly classified into two: arterial and venous thrombotic diseases. These disorders more often manifest in the large blood vessels such as the coronary arteries or deep veins around the calf muscles. The arterial thrombi are composed primarily of platelets. They also contain fibrin and infrequently leucocytes. They occur in arteries which have rapid blood flow and are initiated by natural or mechanical rupture of atherosclerotic plaques (Hallett *et al*., 2001).

Thrombotic events which occur in the large veins are referred to as venous thromboembolic (VTE) disorders. These diseases chiefly include the PE and DVT. Venous thrombi are principally found in venous circulation and are composed entirely of fibrin and erythrocytes. They form due to either venous stasis or vascular injury after trauma or surgery. Venous blood flow is slow especially in the large veins of the legs. Damage of venous valves as well as prolonged periods of immobility encourages stasis (Hallett *et al*., 2001).

Thrombotic disorders may complicate other cardiovascular disorders such as stroke or embolization in the heart due to atrial fibrillation or lesions in the endocardium and heart valves. As such, embolization in the heart is likely to occur in patients with arrhythmias and

abnormalities of the endocardium including patients with mechanical heart valves or anomalies of the native valves due to conditions such as rheumatic heart disease. The abnormalities of the valves may also contribute to the development of aortic regurgitation, aortic and mitral stenosis, among others, which create areas of non-laminar blood blow and hence coagulation (Camm *et al*., 2010).

## 2.3: Epidemiology of Thromboembolic events

Studies have documented that proportions of thromboembolic events worldwide have risen over the past 40 years despite improved prophylaxis (Heit *et al*., 2001; Schuman, 1965). In the USA for instance, the incidence of VTE exceeded 100 per 100,000 cases (White, 2003) and over 200,000 new cases occurred every year where a third of these patients suffered from mortality (Heit *et al*., 2001; White, 2003). In Western Europe, Australia, and Argentina , the annual incidences of VTEs has been reported to range from 75 to 269 per 100,000 individuals in the population and is almost four-fold among the elderly (Raskob *et al*., 2014). In Japan, epidemiological studies of VTEs revealed that almost 70.0% patients presented with DVT, and 17.0% had PE alone while 14.4% had both (Nakamura *et al*., 2014).

Epidemiological studies of VTEs in African population are scarce. Nevertheless, most of the Africa is comprised of lower and middle income countries (LMIC) where the risk factors for the development of VTEs are predominant. Available literature, however, indicated that the incidence of VTE is on the increase among hospitalized patients. In Egypt, for example, the incidence of DVT among the hospitalized patients with stroke was almost 10 % and atrial fibrillations as well as intracranial haemorrhage were the independent predictors (Abdel-Aziz and Elfawwal, 2015). In Kenya, the prevalence of DVT has been reported to be 36% among the hospitalized patients (Ogeng'o *et al*., 2011) and in other related studies, this condition has been documented to occur in almost 60% of the patients (Pastakia *et al*., 2010).

## 2.4: Risk factors for Development of Thromboembolism

Studies on VTE have revealed that there are three primary factors that influence the formation of pathologic clots. These are described in the Virchow's Triad and include: abnormalities of blood flow such as venous and intracardiac stasis, abnormalities of blood vessel walls and hypercoagulability as well as abnormalities in clotting factors (Watson *et al*., 2009). Additionally, studies have revealed that the independent predictors for the development of VTEs include advancing age of male gender, major surgery, surgical operations or trauma, prolonged immobility, malignancies, neurologic conditions which may cause paresis, central catheterization or pacemaker and varicose veins (Heit *et al*., 2001). Among women, the risk factors that have been documented include pregnancy, use of family planning "pill" and hormone replacement therapy (White, 2003). However, the independent predictors for recurrence of VTEs include being elderly or obese, malignancies, and paresis of extremities (Watson *et al*., 2009).

## 2.5: Long Term Management of Thromboembolic events

Once formed, the clot may remain asymptomatic, spontaneously lyse or obstruct blood flow. The thrombus may also disseminate into proximal veins or embolize. Therefore, patients who have been diagnosed with VTEs are required to undertake immediate treatment to dislodge the clot after which they are prescribed long term anticoagulant therapy (Hallett *et al*., 2001).

Several drugs may be used to achieve optimal anticoagulation including unfractionated heparins, low molecular weight heparins such as enoxaparin and fondaparinux. Patients may also be prescribed the directly acting thrombin inhibitors such as lepirudin, desirudin, bivalirudin and argatroban or the oral formulation such as dabigatran etexilate and rivaroxaban. Furthermore, in preventing VTEs especially in high risk patients, a combination of pharmacologic and non-pharmacologic interventions should be used (Ansell and Bergqvist, 2004).

The choice of anticoagulant drug should be based on patients' risk of thromboembolism, risk of bleeding, clinical condition, cost, route of drug administration and availability of drugs (Kearon *et al*., 2012). The orally available anticoagulants include warfarin, rivaroxaban and direct acting thrombin inhibitors such as dabigatran and ximelagatran (Ansell and Bergqvist, 2004). Ximelagatran was withdrawn due to liver toxicity (Agnelli *et al*., 2009). Rivaroxaban is rarely used because of the exorbitant cost (Lee *et al*., 2012). As such, for long-term anticoagulation

management, warfarin remains the drug of choice, especially in low resource settings because it is readily available as a cheap oral formulation which can be used for the prevention as well as therapy for VTE and other thromboembolic-associated complications including those caused by atrial fibrillation, prosthetic valves and myocardial infarction (Hirsh *et al*., 2003). Furthermore, warfarin and its related compounds are the pharmacological agents of choice for the management and prevention of thromboembolic events in many settings (Kearon *et al*., 2012).

Research on the comparative effectiveness of warfarin and other newer and expensive anticoagulants has also proved that the coumarins are superior. For instance, in a study done to compare warfarin and the orally available direct thrombin inhibitors, dabigatran, researchers found that patients with prosthetic valves who used dabigatran had increased rates of thromboembolic as well as haemorrhagic episodes and as such there was no benefit but an excess risk (Eikelboom *et al*., 2013). Furthermore, the trial on over 250 patients was terminated prematurely owing to those complications among the participants receiving the direct thrombin inhibitor. Additionally, in the intention to treat analysis, dose titrations or stoppages of dabigatran was made in 30% of patients. Stroke followed in less than 10% of participants in the dabigatran group and none in the warfarin group while major haemorrhage occurred in 4% and 2%, respectively (Eikelboom *et al*., 2013).

## 2.6: Mechanisms of Action of Warfarin

Warfarin comprises of a racemic mixture of duo active enantiomers. These are R- and S- forms, with S-form having almost ×5 the activity of the R-enantiomer in regard to vitamin K antagonism. Each of the isomers is metabolized by different pathways (Hirsh *et al*., 2003). When taken orally, warfarin is quickly and extensively absorbed into the systemic circulation, reaching highest concentration in about 90 minutes. The oral bioavailability is >90% but it is extensively bound to albumin (Hirsh, 1991).

Warfarin prevents the action of factors II, VII, IX and X which depend on vitamin K for activation. The drug also inhibits the action of protein C, protein S, and protein Z which regulate the blood clotting (Hirsh, 1991). Osteocalcin and matrix Gla protein, which do not participate in blood coagulation are also be inhibited (Gage *et al*., 2006).The precursors of these elements require carboxylation of their terminal glutamate residues for the coagulation factors to attach to phospholipids on the endothelium of blood vessels. The enzyme which controls the

carboxylation reaction is gamma-glutamyl carboxylase (*GGCX*). The carboxylation reaction occurs only if the enzyme is capable of converting reduced form of vitamin K to the oxidized form simultaneously (Hirsh, 1991). The oxidized vitamin K is then converted to vitamin K and its hydroquinone form by VKOR enzyme (Hirsh, 1991). Warfarin inhibits the epoxide reductase (Whitlon *et al*., 1978), specifically the *VKORC1* subunit from achieving its target (Li *et al*., 2004; Rost *et al*., 2004), thereby decreasing the available active vitamin K as well as vitamin K hydroquinone in blood, which further inhibits the glutamic acid carboxylation by *GGCX*. When this happens, the clotting factors are no longer carboxylated and consequently are incapable of binding to the endothelial walls of the blood vessels, and become biologically inactivated (Hirsh, 1991). As such, the body's stores get depleted of active clotting factors over some days and these are substituted by inactive factors. When this happens, the anticoagulation outcome becomes apparent over 2-3 days. The consequence of warfarin therapy, then, is to reduce blood coagulation in the patient (Whitlon *et al*., 1978).

## 2.7: Clinical Uses and Doses of Warfarin

Warfarin has been proved to be effective for preventing coagulation and embolism in many VTEs. It was licensed for VTE therapy in the early 1950s. Ever since, it has remained the standard therapy for many VTE disorders especially in low resource countries. Warfarin is the most extensively prescribed oral anticoagulant medicine in North America (Pham and Pham, 2007) and the rest of the world (Pirmohamed, 2006). Warfarin treatment can help prevent thrombosis and reduce risks of embolism (Geerts *et al*., 2008).

The commonest clinical indications of warfarin use include VTEs, atrial fibrillation, antiphospholipid syndrome as well as coronary artery thrombosis which may precipitate myocardial infarction (Hirsh *et al*., 2003). In Kenya, the commonest clinical indications of warfarin include VTEs and prevention of thromboembolism after heart surgery (Ogendo, 2000) where majority of patients start therapy at 5mg daily dose. Warfarin doses are adjusted based on response so as to produce therapeutic effect in 4-5 days. Studies have also revealed that the weekly warfarin doses ranges from 25 mg to 55 mg although some patient parameters, such as advancing age, are associated with lower doses. However, lower or higher warfarin doses may be accepted subject to individual host factors (Gallus *et al*., 2000).

Dosing of warfarin is individualized and depends on the desired level of anticoagulation as well as response from the patient (Gallus *et al*., 2000). However, the dose must be based on continued monitoring of clinical signs as well as laboratory assessment of INRs due to intra-patient/inter-patient variability. It is recommended that while on warfarin therapy, patient's response should be measured and monitored every 3-5 days until they are stable because anticoagulation effect may need up to two weeks to be achieved (Kucher *et al*., 2004). Nevertheless, warfarin dose titrations are not required to be made unless after every three days. The doses should be adjusted by estimating the weekly dose and titrating upwards or downwards by 5-25%. However, the outcome of dose alteration may not be apparent until 5-7 days have elapsed (Gallus *et al*., 2000).

## 2.8: Practical Uses of Warfarin

In practice, patients are usually prescribed heparin combined with warfarin with the heparin being given for the first 1-2 days and thereafter withdrawn (Ageno *et al*., 2012; Gallus *et al*., 2000). This overlap is necessary for several reasons. Firstly, warfarin is slower-acting than heparin because its half –life is 2.5 days. Therefore, it takes a number of days to achieve anticoagulation effect. On the hand, heparin is fast acting and its antithrombotic effects are usually demonstrated within a short period of time. Secondly, heparin can precipitate a prothrombotic disorder referred to as heparin-induced thrombocytopenia (HIT), which is an antibody-mediated sudden reduction in platelet count. This increases the risk of thrombosis which may be countered by giving warfarin (Ageno *et al*., 2012).

Lastly, the combination is necessary to combat warfarin necrosis, an infrequent but serious complication from warfarin and occurs more often after commencing treatment in individuals who lack protein C, which is an innate anticoagulant. The activation of this protein requires carboxylation which is mediated by vitamin K. Warfarin firstly decreases protein C concentrations faster than the clotting factors and consequently it can unexpectedly increase the coagulation when treatment is commenced leading to substantial thrombosis accompanied with necrosis of skin and gangrene of lower limbs (Ad-El *et al*., 2000).

Warfarin should cautiously be used in patients with peptic ulcer disease, uncontrolled hypertension and renal impairment (Tadros and Shakib, 2010). Additionally, warfarin passes through the placental barrier and is, therefore, contraindicated in pregnancy because it may cause bleeding in *utero,* spontaneous abortion, preterm birth or neonatal death (Loftus, 1995; Schardein

and Macina, 2006). Warfarin is also teratogenic and may cause a collection of congenital anomalies referred to as fetal warfarin syndrome (FWS), which is characterized by bone abnormalities, including nasal hypoplasia and scoliosis as well as calcifications in the vertebral column, femur, finger, toes and heel bone (Schardein and Macina, 2006). FWS may also be may also be associated with low birth weight and developmental disabilities (Loftus, 1995; Schardein and Macina, 2006). The predominant congenital defects associated with warfarin therapy in third trimester are central nervous system effects, including spasticity, seizures and eye defects (Hall *et al*., 1980).

## 2.9: Adverse Effects of Warfarin

### Haemorrhage

This is the commonest adverse effect of warfarin. Although the risk of severe haemorrhage may be small, it is definite and has a median rate of 90 to 270 per 10, 000 persons per year (Horton and Bushwick, 1999). Locally, the prevalence of bleeding has been found to occur in almost a third of patients receiving warfarin (Mariita *et al*., 2016) where haemorrhage occurred in the nostrils, gums or gastrointestinal system. The risks of bleeding are increased when warfarin is given concomitantly with other drugs that interfere with platelet aggregation such as ticlopidine, aspirin, prasugrel and clopidogrel as well as non-steroidal anti-inflammatory drugs (Delaney *et al*., 2007). The risk may also be increased in elderly individuals (Hylek *et al*., 2007) as well as in patients undergoing haemodialysis sessions (Elliott *et al*., 2007).

### Osteoporosis

In a study of over 500 women using warfarin for DVT, the risk of rib as well as vertebral fracture was increased but other fracture forms were infrequent (Caraballo *et al*., 1999). In addition, a retrospective study of almost 14,500 participants revealed that continued warfarin therapy for more than one year was associated with over 50% increased risk of osteoporotic fractures in men (Gage *et al*., 2006). However, a study on randomly selected 1523 patients with osteoporotic fracture found no association of warfarin use with increased risk of fracture when

compared to the controls. In addition, on stratification on the duration of warfarin use, there was no significant trend revealed towards the development of fractures (Pilon *et al*., 2004).

**Purple toe syndrome**

This is a rare complication that may occur within 1-2 months of warfarin use and may require discontinuation of the drug. The condition is thought to result from cholesterol micro-embolism into the superficial capillaries of the lower limbs. This cholesterol causes some bluish to purple colour, especially on the big toe (Talmadge and Spyropoulos, 2003) and it is very painful. The micro-embolism may also involve the other parts of lower limb.

## 2.10: Drug Interactions among Patients Receiving Warfarin Therapy

Drug-drug interaction (DDI) with warfarin is likely to occur when other drugs are co-administered and the pharmacokinetic or pharmacodynamics effects change (Reis and Cassiani, 2011). These interactions may cause harmful effects ranging from minor morbidities to fatal effects such as increased risk of gastrointestinal haemorrhage (Delaney *et al*., 2007). Information on the DDIs and their potentially harmful outcomes is, therefore, a relevant clinical concern because it would decrease risk of adverse drug events as well as alterations in dose requirements in the patient. This would reduce healthcare costs that occur in the course of the management of the disease (Moura *et al*., 2011).

Patients on anticoagulation therapy are likely to be also having polypharmacy because of other comorbidities. In addition, the presence of comorbidities such as cardiovascular disorders and advancing age are also likely to contribute to the use of many drugs, further increasing this risk (Shah and Hajjar, 2012). Drug related problems such as drug interactions in the management of thromboembolism increase morbidity and mortality (Moura *et al*., 2011) but there are limited published data to characterize them especially among the black Kenyan population.

Warfarin has a high propensity to cause drug-drug and drug-food interactions (Juurlink, 2007) hence patient monitoring and education is required. The drug may cause toxicity on slight dose changes and hence patients should be screened for use of other drugs before initiating therapy. For instance, at each visit patient drug and diet history, especially vitamin K intake should be

evaluated because foods containing vitamin K, if taken repetitively and in large amounts inhibit warfarin activity and as such moderation is important (Khan *et al*., 2004).

### 2.11: Warfarin interactions with other diseases, drugs and herbs

Warfarin may interact with several commonly used conventional medicines and some foods (Holbrook *et al*., 2005). For instance, warfarin is 99% bound to serum albumin and therefore, highly albumin bound medications can dislodge it from the binding sites to cause serious side effects such as bleeding (Gage *et al*., 2000). However, these changes are transient in individuals with normal hepatic function but may be prolonged in persons with liver dysfunction. Similarly, drugs that alter haemostasis, platelet function or clearance of clotting factors, can increase bleeding risk. This makes it difficult to find the correct warfarin dosage and therefore, when there is concurrent use of a drug that interacts with warfarin, regular monitoring is important to avoid serious consequences (Shalansky *et al*., 2007).

Many commonly used antibiotics reduce the metabolism of warfarin and as such augment its anticoagulant effect (Holbrook *et al*., 2005). Furthermore, use of broad-spectrum antibiotics, such as ceftriaxone, will decrease the level of the normal flora found in the GIT. The normal bacterial flora synthesizes substantial amounts of vitamin K. When their amount is low, the effect of warfarin is potentiated (Juurlink, 2007). Additionally, food types containing huge amounts of vitamin K such as green leafy vegetables reduce the anticoagulant effects of warfarin (Holbrook *et al*., 2005).

The disease-drug interaction is also important. For instance, hyperthyroidism seems to impact on warfarin dosing requirements by boosting the anticoagulant effect (Chute *et al*., 1997; Kurnik *et al*., 2004) while hypothyroidism diminishes sensitivity to warfarin therapy (Stephens *et al*., 1989). These observations have be thought to arise due to alterations in the degree of metabolism of coagulation factors and warfarin (Kellett *et al*., 1986; Kurnik *et al*., 2004). Excessive use of alcohol decreases the metabolism of warfarin and can cause haemorrhage (Weathermon and Crabb, 1999).

Warfarin interacts with many herbs (Lininger, 1999) including Ginkgo, Ginseng, Ginger and Garlic, when used as dietary supplements, which may increase bleeding episodes in patients taking the oral anticoagulant. Starflower oil and St. John's Wort have revealed comparable

effects (Shalansky *et al*., 2007) as well as cranberry juice (Aston *et al*., 2006; Suvarna *et al*., 2003). Therefore, studies have indicated that healthcare workers and users of warfarin be aware of these interactions for optimal coagulation control (Pham and Pham, 2007).

## 2.12: Monitoring of the Therapeutic Response to Warfarin

Drug interactions may alter the anticoagulation outcomes of warfarin (Holbrook *et al*., 2005) and therefore, frequent laboratory monitoring is recommended in order to optimize dose and prevent serious side effects such as bleeding. Despite this, local studies have shown that almost 70% of the laboratory monitoring are underreported by attending clinicians (Nyamu *et al*., 2017).

Monitoring of warfarin's activity is usually done through measurement INRs which requires comparison of a patient's prothrombin time with the control (Hirsh *et al*., 2003). The target INR is based on therapeutic indication but generally, it is 2 – 3. The exception is for patients with artificial heart valves where it is between 2.5 and 3.5 (Baglin *et al*., 2006). Once the patient's dose- response status is recognized, the laboratory measurement of INR should be made every 1-2 weeks until it stabilises, then every 28 days afterwards. Patients' education on anticoagulation is critical at each visit as studies have document that knowledge of the effects warfarin among them is lacking (Mariita *et al*., 2016).

## 2.13: Management of Excessive Anticoagulation with warfarin

The strategies employed for the management of excessive anticoagulation depends on the level of INR. For patients with mildly elevated INR (3-5), withholding few doses or reducing the dose manages bleeding. However, when a fast achievement of therapeutic INR is desired, vitamin $K_1$ may be provided either parenterally or orally (Ageno *et al*., 2012).

If INR is ranging from 5-9, warfarin dose should be withheld and the patient should receive oral vitamin K1 ≤5mg as well. On the other hand, INR>9 requires 5mg of oral vitamin K1. High doses of 10mg vitamin K may cause prolonged resistance to warfarin and even thromboembolic problems. However, in cases of life threatening bleeding, intravenous vitamin K should be given together with frozen plasma, clotting factor concentrates or recombinant factor VII.

## 2.14: Pharmacogenomics of Warfarin

Warfarin is metabolized in the liver mainly by the *CYP 2C9* enzyme (Kaminsky and Zhang, 1997). In addition, the anticoagulant action of warfarin is facilitated by the inhibition of

*VKORC1* enzyme. The end result of this reaction is decreased plasma levels of activated coagulation factors II, VII, IX and X thereby generating therapeutic anticoagulation.

There is wide inter-individual variability between genes encoding for *VKORC1* and *CYP 2C9*. This suggests that variations in the genetic factors encoding for responses to warfarin action are particularly important. This is the reason as to why the therapeutic response to warfarin's activity is explained partially by genetic constitution of an individual. As such, studies have highlighted the importance of patients' genetic information to approximate the first warfarin doses so as to individualize therapy (Johnson *et al*., 2011). Presently, there are three widely studied genes; *VKORC1*, *CYP 4F2* and *CYP 2C9*.

### 2.14.1: Vitamin K Epoxide Reductase Complex (*VKORC1*)

The *VKORC1* gene encodes the VKORC1 protein, which is a key enzyme in the pharmacodynamics of vitamin K and hence warfarin's action (Owen *et al*., 2010). VKORC1 is a 163 amino acid integral membrane protein associated with the endoplasmic reticulum and VKORC1 mRNA is broadly expressed in many different tissues (Oldenburg *et al*., 2006). The enzyme is responsible for the conversion of vitamin K epoxide to vitamin K, which is the rate-limiting step in the physiological process of vitamin K cycle (Wallin *et al*., 2008). Reduced vitamin K is of particular importance for several coagulation factors, including factor II, factor VII, factor IX, and factor X as well as protein C, protein S, protein Z and matrix Gla protein (Stafford, 2005) . *VKORC1* is of therapeutic interest both for its role in contributing to interpatient variability in warfarin dose requirements (Owen *et al*., 2010).

*VKORC1* genetic polymorphisms account for a third of the inter-patient warfarin dose variation (Johnson *et al*., 2011; Rost *et al*., 2004; Wadelius *et al*., 2005). *VKORC1* polymorphisms occur in two main forms. These include the low-dose group A as well as a high-dose group B haplotypes (Rieder *et al*., 2005). Unlike group B, the low-dose form not only attain therapeutic anticoagulation level rapidly but also have a shorter time to achieve an INR greater than 4, which is linked to the ARDs such as bleeding (Sanderson *et al*., 2005).

African-Americans mainly have higher proportion of the group B haplotypes. As such, *VKORC1* genetic polymorphisms may explain why they are relatively resistant to warfarin's action whereas the Asian-Americans are usually more sensitive to warfarin because they have a larger

proportion of the low dose haplotypes (Rieder *et al*., 2005). Polymorphisms of *VKORC1* gene has allowed the investigation on the impact of SNPs on sensitivity to warfarin among the African Americans, Chinese and Asians (Li *et al*., 2004; Rost *et al*., 2004).

### 2.14.2: Cytochrome P450 2C9 (*CYP 2C9*)

This forms ~20% of the cytochrome P450 protein in liver microsomes and metabolizes approximately 100 conventional drugs , including warfarin as well as acenocoumarol, and some nonsteroidal anti-inflammatory drugs (Rettie and Jones, 2005). Studies have shown that CYP 2C9 hydroxylates almost a fifth of the medicines that are currently in use, including S-warfarin. CYP 2C9 hydoxylates S-warfarin to inactive products (Hallett *et al*., 2001). The extrahepatic *CYP 2C9* metabolizes polyunsaturated fatty acids to some active biological products (Spector and Kim, 2015).

*CYP2C9* gene is highly polymorphic and > 50 SNPs have been described in the regulatory and coding regions of the CYP2C9 gene. Some of them are associated with reduced enzyme activity compared with wild type (Jarrar and Lee, 2014). The impairment in *CYP 2C9* metabolic action might cause complications in warfarin dose titrations and adverse effects (Schwarz, 2003). Studies have shown that the adverse drug reactions often result from unexpected alterations in *CYP2C9* enzyme activity due to genetic polymorphisms. Furthermore, *CYP2C9* substrates such as warfarin have been shown to have diminished metabolic capacity because of genetic polymorphisms and drug-drug interactions can lead to toxicity at normal therapeutic doses (Van Booven *et al*., 2010).

Single-nucleotide polymorphisms of the *CYP 2C9* gene have gradually been documented as predictors that underlie interindividual and ethnic differences (Schwarz, 2003) and it has been found that its polymorphisms account for a tenth of the warfarin dose variation between patients (Rost *et al*., 2004; Verhoef *et al*., 2014). *CYP 2C9* has been extensively studied among the Caucasians population (Rost *et al*., 2004). Additionally, studies have documented the prevalence of *CYP 2C9* genetic polymorphisms among the Ashkenazi Jewish (AJ) healthy persons (Scott *et al*., 2007). However, SNPs of *CYP 2C9* are uncommon in African-American and majority of the Asian populations (Sanderson *et al*., 2005). Furthermore, the *CYP 2C9* genetic polymorphisms

do not affect the time to therapeutic INR although they do shorten the time to reach INR greater than 4 (Sanderson *et al*., 2005).

Both *VKORC1* and *CYP 2C9* genes contribute to the warfarin activity, with genetic polymorphisms of the *VKORC1* (c.-1639G>A) and *CYP 2C9* *2 and *3 (c.430C>T and c.1075A>C, respectively) making an individual more sensitive to warfarin and explaining a third to half of the inter-patient variability in the requirements of warfarin dose among the some Western populations (Wadelius *et al*., 2009).

### 2.14.3: Cytochrome P450 4F2 (*CYP 4F2*)

The *CYP4F2* gene on chromosome 19 encodes for the CYP4F2 protein, which has been shown to catalyze hydroxylation of vitamin $K_1$ into its inactive hydroxylated form (Kim *et al*., 2018). This suggests that individuals with low activity of CYP 4F2 may inefficiently convert vitamin K to a more active form thereby increasing the activity of warfarin which requires active vitamin K (Kim *et al*., 2018). CYP4F2 localizes to the endoplasmic reticulum of cells, principally in the liver and kidneys (Alvarellos *et al*., 2015).Warfarin acts by inhibiting the activity of vitamin K in hepatocytes, which leads to diminished action of a number of coagulation factors (Wadelius and Pirmohamed, 2007).

The *CYP4F2* rs2108622 variant, which involves a V433M missense mutation with downstream reduced CYP4F2 activity and reduced vitamin K1 metabolism (McDonald *et al*., 2009), has been associated with an increased warfarin dose requirement due to elevated levels of active vitamin K, thereby necessitating higher warfarin dosage to elicit an anticoagulant response (Borgiani *et al*., 2009). In addition, rs2108622 has been reported to account for a modest additional 1% - 4% of warfarin dosage variability (Alvarellos *et al*., 2015). Table 2.1 shows the distribution and clinical relevance of genetic polymorphisms of *CYP 2C9, VKORC1* and *CYP 4F2.*

**Table 2.1: Distribution and Clinical Relevance of Genetic Polymorphisms of *CYP 2C9, CYP 4F2* and *VKORC1***

| Gene | Protein | Allelic Variants | Effect on Activity of Protein | SNPs | Allelic Frequency in Populations | Clinical Relevance in Warfarin Dosing |
|---|---|---|---|---|---|---|
| *CYP 2C9* | CYP 2C9 Enzyme | *CYP 2C9*1* | Normal | Wild-type | General Population | - |
| | | *CYP 2C9*2* | ↓ | R144C (3608C→T) | 12.8% European Descent; 1.3% African Americans | Adjust Dose upwards |
| | | *CYP 2C9*3* | ↓ | I359L (42614A→C) | 6.3% European Descent; 1.9% African Americans; 2-4% Asians | Adjust Dose upwards |
| | | *CYP 2C9*5* | ↓ | D360E (42619C→G) | 0.9-1.8% African Americans; | Adjust Dose upwards |
| | | *CYP 2C9*6* | Null | 10601delA (818delA) | 0.1-0.75% African Americans | No dose Change |
| | | *CYP 2C9*11* | ↓ | R335W (1003C→T) | 1.5% African American | Adjust Dose upwards |
| *CYP 4F2* | CYP 4F2 enzyme | *CYP 4F2 *2* | ↓ | rs2189784 (G→A) | | Adjust Dose upwards |
| | | *CYP 4F2 *3* | ↓ | rs2108622 (1347C→T) | | Adjust Dose upwards |
| *VKORC1* | VKORC1 Enzyme | *VKORC1*2* | ↓ | rs 9923231 -1639 G→A (3673 G→A) | 13 % American, 92% Asian, and 40% Caucasian | Adjust Dose upwards |
| | | *VKORC1 (rs61742245)* | ↓ | (D36Y) | | Adjust Dose upwards |

**Key**: C = cystine, D = aspartate, E = glutamate, I = isoleucine, L = leucine, R = arginine, W = tryptophan; letters in (parenthesis) represent the single nucleotides that make up the DNA sequence and codons to code for an amino acid (A = adenine, C = cytosine, G = guanine, T = thymine), SNPs-Single Nucleotide Polymorphisms, ↓-reduced.

## 2.15: Pharmacogenomic Studies Characterizing Warfarin Metabolizing Enzymes

Knowledge on pharmacogenomics is useful in optimizing drug therapy while minimizing adverse events especially for drugs, such as warfarin, which have narrow therapeutic index as well as extensive inter-patient variability in response (Yin and Miyata, 2007). As such, there is rising awareness in whether the study of pharmacogenetics can precisely estimate initial doses of warfarin because more than 30 genes are associated with the mechanism of its action (Wadelius and Pirmohamed, 2007). Several studies and reviews have, however, indicated that pharmacokinetics and pharmacodynamics of warfarin are mainly influenced by polymorphisms of *CYP 2C9* and *VKORC1* (Ingelman-Sundberg, 2004; Johnson *et al*., 2011; Wadelius and Pirmohamed, 2007). For instance, once warfarin has gone into the systemic circulation it is metabolized by *CYP 2C9* enzyme to hydroxylated warfarin while *VKORC1* is involved in activation of Vitamin K (Kaminsky and Zhang, 1997).

Warfarin acts by inhibiting vitamin K in the liver (Hildebrandt and Suttie, 1982; Whitlon *et al*., 1978). As such, enzymes that alter the activity of vitamin K will influence warfarin's action. In that respect, *CYP 4F2* is an enzyme that metabolizes vitamin K into an inactive form suggesting that individuals with low activity of *CYP 4F2* may inefficiently convert vitamin K to a more inactive form (Kim *et al*., 2018) thereby decreasing the activity of warfarin which requires active vitamin K. *CYP 4F2* has been shown to impact on activity of warfarin in genomic studies ( Takeuchi *et al*., 2009).

Studies have revealed that the most significant genetic marker in the pharmacokinetics of coumarins is the *CYP 2C9*, while the focal gene in its pharmacodynamics is *VKORC1* (Li *et al*., 2009; Wadelius and Pirmohamed, 2007).These two genes (Takahashi and Echizen, 2003) coupled with environmental and patient factors (White, 2010), partially account for the inter-patient variability in the dose requirements for warfarin. Furthermore, the prevalence of genetic polymorphisms in the two genes differ greatly between Caucasians and Chinese individuals and, mainly explain the difference in the required warfarin doses among those populations (Lam and Cheung, 2012).

### 2.15.1: Population Distribution of Allelic Frequencies of Warfarin Metabolizing Genes

The rates of the polymorphisms of pharmacogenetic importance differ across populations. Many different SNPS in *CYP 2C9* that differ according to ethnicity as well as their functional status have been documented (Carlquist *et al*., 2006; Lindh *et al*., 2005; Matimba *et al*., 2009; Takahashi and Echizen, 2003). Furthermore, *CYP 2C9* and *VKORC1* alleles have been widely characterized in Japan, China, India and Europe where they have revealed to largely affect the warfarin dose and response (Carlquist *et al*., 2006; Wadelius and Pirmohamed, 2007).

Scott *et al*., (2008) studied the frequencies of *CYP 2C9* and *VKORC1* genotypes among Ashkenazi (AJ) and Sephardi (SJ) Jewish participants. They found that the ‘‘sensitive’’ *CYP 2C9* *2 and *3 alleles were significantly higher among the SJ compared to AJ individuals, 0.19 and 0.14 versus 0.13 and 0.08, respectively. Contrary, the *VKORC1* p.D36Y SNPs, which predicted resistance to warfarin had a statistically significantly higher proportion among the AJ than in SJ participants, 0.04 versus 0.01, respectively .The findings showed that close to 85% of AJ individuals and about 90% of SJ participants had either ≥one ‘‘sensitive’’ marker (*CYP 2C9* *2, *3, *VKORC1* g.-1639G/A) or ‘‘resistant’’ allele which was *VKORC1* p.D36Y (Scott *et al*., 2008).

In an effort to determine the frequencies of various SNPs influencing warfarin metabolism among the various races and ethnicities , some researchers genotyped blood from Caucasians, Asians, African-Americans, Ashkenazi Jewish (AJ) and Hispanics for *CYP 2C9* Variants *CYP 4F2* (*3 [p.V433M] and rs2189784) as well as *VKORC1* (g.-1639G>A) (Scott *et al*., 2010). The researchers revealed that the combined frequencies of variant *CYP 2C9* frequencies for the alleles were 0.212, 0.078, 0.133, 0.212 and 0.178 among Caucasians, Asians, African-Americans, Ashkenazi Jewish (AJ) and Hispanics, respectively. In addition, almost half of the African-Americans and approximately 90% of Caucasians, Asians, Ashkenazi Jewish (AJ) and Hispanics had a variant *VKORC, CYP 2C9* and/or *CYP 4F2* *3 alleles. Furthermore, *CYP 4F2* *3 frequencies were less prevalent among the African- Americans but significantly more common among Caucasians, Asians, Ashkenazi Jewish (AJ) and Hispanics. In relationship with other races investigated, less than 2% of African-Americans exhibited *CYP 4F2* *3 homozygousity, suggesting that these individuals may not benefit from warfarin dosing models that incorporate this variant (Scott *et al*., 2010).

In one Chinese study, there was no difference in prevalence of SNPs in the studied genes in three ethnicities of Bai, Tibetan, and Han Chinese (Zeng *et al*., 2012). For example, the frequency of A-allele of *VKORC1* 3673G > A was 92.8%, 90.2%, 90.8% in Bai, Tibetan and Han Chinese, respectively, although Bai Chinese had statistically higher A-allele frequency of *VKORC1* 3673G > A than Han Chinese . Furthermore, the frequency of *CYP 2C9* *3 was low in Bai (4.5%), Tibetan (2.8%) and Han Chinese (4.6%) while a quarter of each of the ethnics had the mutant T-allele of *CYP 4F2* rs108622 (Zeng *et al*., 2012).

African populations have been shown to portray the greatest genetic heterogeneity compared to the Caucasians for the frequencies of SNPS influencing warfarin response. For instance, studies have revealed that the distribution of the defective of *CYP 2C9* *2 and *3 polymorphisms among sub-Saharan population is absent or rare (Suarez-Kurtz and Botton, 2013). In a South African Study, twenty six novel single nucleotide polymorphisms, 7 *CYP 2C9* variants and 3 *VKORC1* SNPs were identified. Additionally, eleven variants of the *CYP 2C9* and 2 variants of the *VKORC1* had high rates to influence the requirements of warfarin doses (Mitchell *et al*., 2011). In Benin, studies on the relative frequencies of *VKORC1* revealed that there were rare SNPs of *VKORC1* 1173C>T variant (Allabi *et al*., 2012). Besides, Ethiopian studies have revealed that p.Asp36Tyr in *VKORC1* occurs in less than 2% of a heterogeneous population and it is associated with warfarin resistance (Shuen *et al*., 2012). These diverse differences in allelic frequencies in Sub-Saharan Africa suggests that generalization of results among African ethnicities to individualize warfarin dose should be done with caution (Dandara *et al*., 2011).

Characterization of *VKORC1* Asp36Tyr SNP has also been carried out in some African countries. This SNP was found to be most common among the Sudanese and Kenyans exhibiting a minor allele frequency (MAF) of less than 10%. Egyptians and Saudi Arabians showed a MAF of 2.5 and 3%, respectively. However, this SNP was not detected among West Africans and in a large cohort of African Americans. Additionally, *VKORC1* Asp36Tyr which is associated with resistance to warfarin seemed to be limited to some participants from Middle-Eastern and north-eastern Africa (Shahin *et al*., 2013). In Ethiopia, the prevalence of *VKORC1* Asp36Tyr was found to be 4%. However, Asp36Tyr was found in high frequencies among the Jews of

Ethiopian descent with allele frequency of 15% and to smaller degree in AJ at 4% (Aklillu *et al*., 2008).

### 2.15.2: Genetic Factors affecting the Response to Warfarin Therapy

Warfarin is an old drug but continues to be widely used for long-term management and prevention of several thrombotic disorders. There is presently unparalleled awareness in the pharmacogenetics of warfarin, particularly in the European and Asian countries, owing to a general awareness in whether pharmacokinetic and pharmacodynamics studies can optimize anticoagulation as well as prevent serious side effects such as bleeding. As such, the impact of genetic differences on warfarin response has been the focus of several pharmacogenomic studies (Daly and King, 2003; Kirchheiner and Brockmöller, 2005; Linder, 2001; Lindh *et al*., 2005; Takahashi and Echizen, 2003).

Several studies have investigated and documented the importance of *CYP 2C9* as well as *VKORC1* polymorphisms in warfarin dose requirements (D'ambrosio *et al*., 2007; Li *et al*., 2009). Furthermore, numerous studies have shown that pharmacogenetic analysis of the two genes, *CYP 2C9* and *VKORC1*, affect warfarin maintenance dose (Carlquist *et al*., 2006; Schwarz, 2003; Wadelius *et al*., 2005). Additionally, studies have indicated that individuals exhibiting the polymorphisms in rs2108622 of *CYP 4F2* have high levels of Vitamin K1 in the liver, requiring a higher dose of warfarin to produce the similar anticoagulation effect (McDonald *et al*., 2009;Takeuchi *et al*., 2009). These revelations suggest that genetic polymorphisms in *VKORC1*, *CYP 2C9*, *CYP 4F2* contribute significantly to the inter-patient variability in the dose of warfarin dose and hence response.

In another study involving 1,053 Swedish participants in determining the significance of polymorphisms of the genes that altered therapeutic doses of warfarin, it was found that Caucasians varied 20 times in the required warfarin to achieve therapeutic levels of anticoagulation (Takeuchi *et al*., 2009). Prior study indicated that a third of the warfarin dose variability may be accounted by SNPs of *VKORC1* gene, while an extra 12 % could be explained by *CYP 2C9* (*2, *3). Further study on multiple regression adjusting for *VKORC1*, *CYP 2C9*, age and gender, which affect warfarin dose revealed *CYP 4F2 rs2108622* SNP that was involved in the protein coding of the *CYP 4F2* (Takeuchi *et al*., 2009). The study confirmed this

finding in a smaller subset of 588 Swedish patients. Another group of researchers revealed independent findings from examination of genes that metabolize warfarin. The study therefore, found a novel gene variant, *CYP 4F2*, accounting for ≥1.5% of the warfarin dose variation between patients (Takeuchi *et al*., 2009).These GWAS results provided further incentive for assessing patient benefit from genotyping to predict warfarin dose.

A study done by Scott *et al* (Scott *et al*., 2008) revealed that 11.3 % of the participants from AJ origin who were projected to be *CYP 2C9* extensive metabolizers as well as 8.7% of those expected to be slow and intermediate metabolizers of the drug expressed *VKORC1* p.D36Y and required higher doses of warfarin to elicit therapeutic anticoagulation. In that study, almost 10 % of all individuals who were AJ would have been mis-classified if only genotyping was done for the *VKORC1* g.-1639G/A and *CYP 2C9 (**2, *3), highlighting the significance of genotyping for p.D36Y before commencing long term warfarin therapy in AJ patients. The findings suggested that ethnicities are distinct in regard to pharmacogenetics of warfarin and it would be of value to perform genetic testing in order to predict initial warfarin doses that are safe(Scott *et al*., 2008; Verhoef *et al*., 2014).

In a study to characterize the quantitative impact of *VKORC1* and *CYP 2C9* variants on warfarin dose requirements among the Turkish individuals, slightly more than 200 patients who had been taking warfarin for >2months were genotyped. The researchers explored the association of the polymorphisms and the average requirements in warfarin doses. They found that the *VKORC1* polymorphisms of 3673 G>A significantly impacted on weekly mean warfarin doses. Furthermore, weekly mean warfarin doses were 43.18mg, 33.78mg and 25.83mg for *GG*, *GA* and *AA* genotypes, respectively. In addition, participants who exhibited *VKORC1* and *CYP 2C9* polymorphisms required a two-fifths reduction on weekly mean doses of warfarin in relation to the wild types. They identified the factors that significantly necessitate lower warfarin doses among the participants as advancing age, one or two *CYP 2C9* variants and *VKORC1* 3673 *AA* or *GA* genotype as well as other clinical indications for warfarin therapy apart from VTE (P = 0.002). This study concluded that among the Turkish individuals, SNPs in *VKORC1* and *CYP 2C9* genes were paramount in determination of the doses of warfarin for optimal anticoagulation therapy (Ozgon *et al*., 2008).

Scientific reviews have shown that the genotypes of *VKORC1* and *CYP 2C9* have been strongly and constantly impacting on the required warfarin doses in many studies (Johnson and Cavallari, 2015).In addition, one Chinese study was not only consistent with these findings but also revealed that *CYP 4F2* polymorphisms accounted for approximately 4% inter-patient variability in the warfarin dose needed for anticoagulation (Cen *et al*., 2010). Another similar study revealed that participants exhibiting *CYP 4F2 CT* or *TT* allele required a significantly higher dose of warfarin when matched with carriers of *CC* (3.4 ± 0.1mg/day vs. 2.8 ± 0.1 mg/day). Thus *CYP 4F2* rs2108622 had a minor but important correlation with stable doses of warfarin among the Han-Chinese patients (Liang *et al*., 2012). As such, warfarin dosing models combining clinical and genetic data have been documented to be important in establishing stable warfarin maintenance doses in patients undergoing anticoagulation (Johnson and Cavallari, 2015).

Studies among the children, though rare, have yielded similar findings to adults. For instance, in a study to determine whether *CYP 2C9* and *VKORC1* predicted warfarin dose requirements, the researchers enrolled 93 children (<18 years) for genotyping and determination of warfarin treatment outcomes. They found that three-quarters of inter-individual variability in warfarin dose was accounted by weight, clinical indication of warfarin therapy, *CYP 2C9* *2/*3 as well as *VKORC1*-1639G/A, with the genotypes identified explaining a fifth of variability. In addition, *VKORC1* polymorphisms had an important influence on the time to therapeutic level of anticoagulation as well as over-anticoagulation (INR greater than 4) when commencing treatment. Furthermore, participants who exhibited *CYP 2C9* *3 were eleven-fold at a higher risk of severe haemorrhage while on warfarin therapy. The researchers also found an extra variant in *CYP 2C9* (rs7089580) which was considerably associated with the requirements of the doses of warfarin (Shaw *et al*., 2014).

Several studies on genetic factors affecting warfarin adverse response have been done among the Chinese population. In one prospective cohort study, *CYP 2C9* *3 variant was associated with a heightened risk of all and minor bleeding. Additionally, *CYP 2C9* *3 was associated with a risk of patients having INR>4 (HR 2.9, 95 % CI: 1.1-7.9). However, *VKORC1* -1639 G/A, and *CYP 4F2* rs2108622 did not reveal any significant increase in risk of bleeding or getting INR associated with haemorrhage. The duration to bleeding episodes was statistically significantly

smaller for individuals carrying *CYP 2C9* *3 genotype compared to the ones who exhibited none, but not in persons having *CYP 4F2* rs2108622 or *VKORC1* -1639 *G/A* genotype. This study concluded that *CYP 2C9* *3 may be the central genetic determinant in bleeding complications among the Chinese individuals on Vitamin K antagonist therapy (Ma *et al*., 2012).

Similar studies by Liang *et al* (2012), among the Han-Chinese patients confirmed that *CYP 2C9* *3 polymorphism was significantly associated with lower doses of warfarin compared to wild type. Additionally, *VKORC1*-1639 *AG* carriers required significantly higher daily maintenance warfarin doses than in patients exhibiting the *AA* variant. In this study, the logistic regression modeling comprising *VKORC1*-1639G>A, *CYP 2C9, CYP 4F2* and participants demographics could explain about two-fifths of the observed variability in the maintenance doses of warfarin (Liang *et al*., 2012).

Studies done in Indonesia have also found similar results. For instance, Rusdiana *et al* (2013) conducted a genetic study which involved genotyping of *VKORC1*, *CYP 2C9* and *CYP 4F2* among persons managed with 1-2 mg warfarin dose per day, using INR as a surrogate marker for warfarin response. They found that patients managed with 1mg warfarin daily had higher INR which had an association with *VKORC1*-1639 *AA* genotyped than with the *GA* ($p < 0.01$) and *GG* ($p < 0.01$) polymorphisms, and with *CYP 2C9* *1/*3 compared to *1/*1 ($p < 0.05$). However, the genotype variations of *CYP 4F2* were not significantly associated with the level of anticoagulation as measured by the INRs (Rusdiana *et al*., 2013). These findings were contradictory among the Japanese population where related genetic studies done by Nakamura *et al* (2012) indicated that warfarin maintenance doses were significantly higher among *CYP 4F2* 1347 *CT* carriers (3·60±1·80 mg/d) compared to those with *CYP 4F2 CC* variant (2·90±1·00 $mgd^{-1}$). In addition, the effect of *CYP 4F2* V433M genetic polymorphisms on the maintenance doses of warfarin dose was identified but was somewhat minor in relation to the effects observed with *CYP 2C9* and *VKORC1* polymorphisms (Nakamura *et al*., 2012).

Similar studies have shown that genetic polymorphisms in the *VKORC1* and *CYP 2C9* highly impact on the warfarin's steady state dose. *VKORC1* and *CYP 2C9* polymorphisms also impact

on the initial INR values as well during commencement of warfarin therapy. For instance, in a study of 214 patients (Li *et al*., 2009) starting warfarin therapy and being monitored with INR measurements, it was determined whether *VKORC1* and *CYP 2C9* polymorphisms had significant effect on the early measures of sensitivity to warfarin. In this study, the outcome of interest included time to INR ≥ 2 and > 4 as well as the initial warfarin doses which had been stabilized. It was found that the initial INRs were statistically significantly related to all measured variables and were more helpful than the genetic information. For the time to INR ≥2, adding either initial INRs or genetic data to the baseline model improved the goodness-of-fit. For the initial doses of warfarin which had been stabilized, adding either initial INRs or genetic information to the model improved the goodness-of-fit as well (Li *et al*., 2009).

African genetic studies on genetic variability and warfarin response have mixed findings. A South African study demonstrated that *CYP 2C9* *8 and two novel *CYP 2C9* SNPs (g.16179 and g.46028) significantly necessitated a reduction in the dose of warfarin but two known *VKORC1* polymorphisms (rs7200749 and rs7294) required an upward adjustment of warfarin dose (Mitchell *et al*., 2011). The pooled effect of all *CYP 2C9* variants in this South African black cohort study was stated to account for about a fifth of the inter-patient variance in the required dose of warfarin (Suarez-Kurtz and Botton, 2013). However, the *VKORC1*, *CYP 2C9* polymorphisms and a minor subclass of environmental predictors explained nearly 45% of the variance in warfarin dose among the black South Africans (Mitchell *et al*., 2011).

In a study assessing 203 warfarin-treated patients from Sudan and the genetic factors associated with warfarin response, it was found that carriers of *CYP 2C9* *2,*5,*6 and *11 variants needed warfarin dosage which was a fifth lower compared to those with *CYP 2C9* *1/*1. In addition, *CYP 2C9* variants in *2, *5, *6, *11; *VKORC1* variants including rs7199949, rs8050984 and rs7294, INR targeted, patient's body weight as well as concomitant drugs could account for approximately 37% of inter-individual dose variance. This finding suggests that the *VKORC1* and *CYP 2C9* variants are significant determinants impacting on the required dose of warfarin among Sudanese individuals and consequently, incorporation of relevant polymorphisms could advance estimations of the optimal warfarin doses (Shrif *et al*., 2011). In contrast, pharmacodynamic response to acenocoumarol was found to be highly variable between the participants and. the variability was associated with *CYP 2C9* *5/*8 and *9/*11 polymorphisms

as well as demographic factors, principally patients weight and age, among the Benin populations (Allabi *et al*., 2012).

Studies on the impact of SNPs of *VKORC1* have indicated that Egyptians who exhibit *VKORC1* Tyr36 polymorphisms needed a higher dose of warfarin (nearly 57.0 ± 29.0 $mgweek^{-1}$ ) compared to patients carrying Asp36Asp variants (approximately 36.0 ± 17.0 $mgweek^{-1}$). Additionally, the SNP was revealed to improve the warfarin dose variability among the Egyptians. On the other hand, the warfarin resistant *VKORC1* Asp36Tyr was rare among the Egyptians but in communities which exhibited it, there was a substantial impact on the required dose of warfarin (Shahin *et al*., 2013). In Ethiopia, *VKORC1* Asp36Tyr was present among populations and predicted higher doses of warfarin that the patients required (> 70 mg/week) (Aklillu *et al*., 2008).

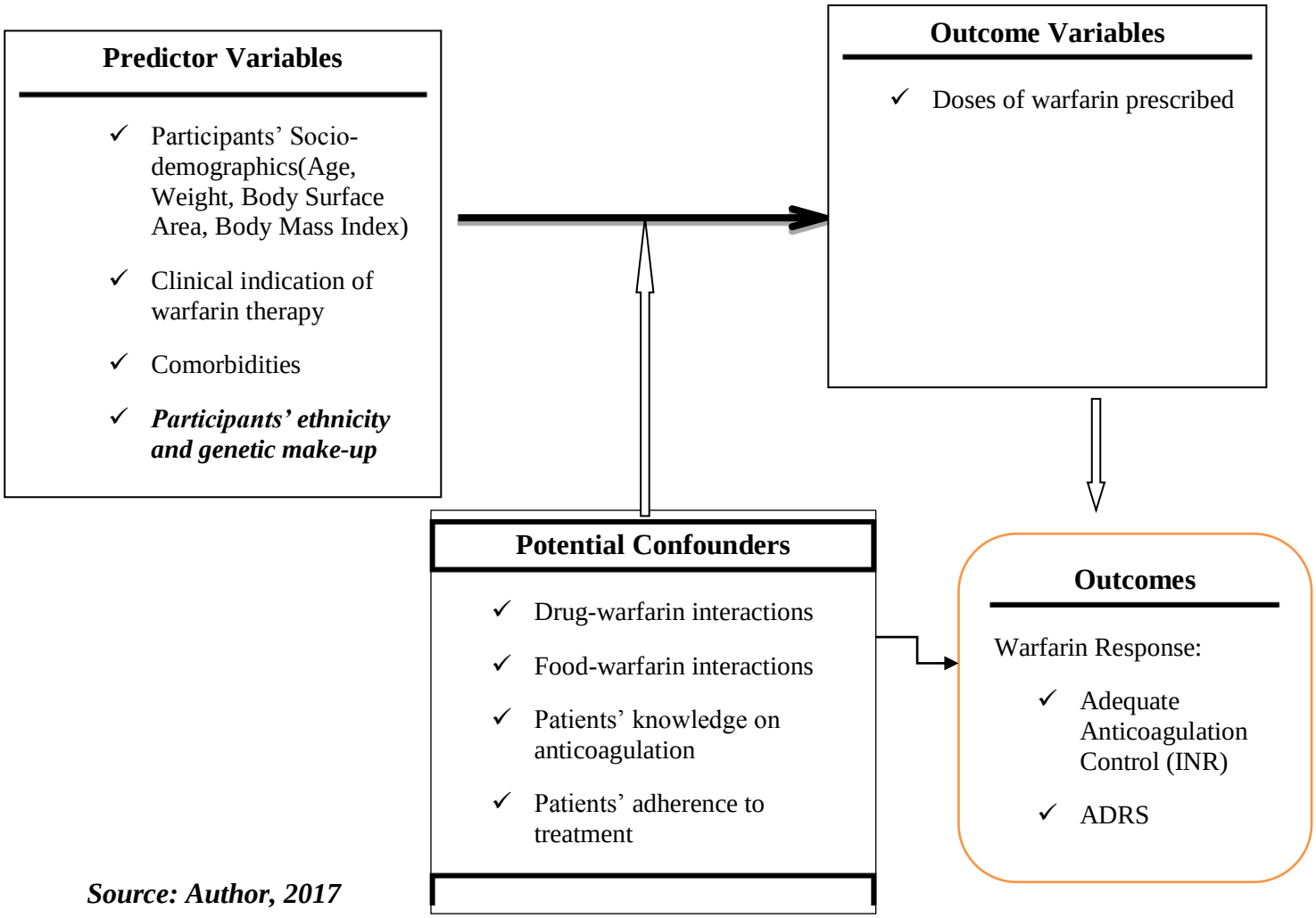
## 2.16: Gaps in Literature

Published literature on anticoagulation management among the Kenyan patients remains scarce. Furthermore, there are limited studies on the frequencies of genetic variants of important warfarin metabolizing enzymes among the Kenyan population, except for the prevalence of *VKORC1* polymorphisms (Shahin *et al*., 2013). In addition, the most widely studied genetic variations have been undertaken among the Asian communities and include polymorphisms in *CYP 2C9* and *VKORC1*. Few studies done on the prevalence of *CYP 2C9* allele in some African communities have found rare variants of these SNPs (Matimba *et al*., 2009). Among European populations, genotyping of *CYP2 C9 *2* and **3* alleles is recommended before initiation of warfarin anticoagulation (Johnson *et al*., 2011) but data in Kenyan population is unavailable.

Studies have revealed that patient's warfarin maintenance dose is related to the clinical factors and genetic variations (Verhoef *et al*., 2014; Yin and Miyata, 2007; Zhu *et al*., 2007). As such, several dose-prediction algorithms have been developed incorporating both genetic and patients' clinical factors among the western populations (Lee and Klein, 2013). Furthermore, studies have revealed that genotyping could be used to individualize therapy in about a fifth of all medications required by the patient to improve the efficacy of medication (Ingelman-Sundberg, 2004).

In order to enable utilization of *VKORC1* and *CYP 2C9 (*2, *3, *3*, *4, *5, *6, *8, *11* and **13)* genetic testing in improving the practice of anticoagulation in Kenya, it is imperative to describe the allele variants that occur among individuals. Additionally, no studies have been undertaken in the Kenyan population to establish the distribution of *VKORC1*, *CYP 2C9* and *CYP 4F2* polymorphisms despite the fact that warfarin dosing algorithms incorporating pharmacogenetics have been demonstrated in Western communities (Pink *et al*., 2014). Pharmacogenetic dosing methods have also been shown to improve the efficacy of medication as well as general health (Ingelman-Sundberg, 2004). This study is the first Kenyan population study in exploring the role *CYP 2C9*, *VKORC1* and *CYP 4F2* genes as well as the clinical factors in determining warfarin dose requirements and its response (Ozgon *et al*., 2008).

## 2.17: Conceptual Framework for the Present Study




***Source: Author, 2017***

**Figure 2.1: Conceptualized study framework on factors affecting warfarin dose and response**

The conceptual framework illustrates the interaction between the predictor, outcome variables and potential confounders in the study.

The schematic diagram (**Figure 2.1**) highlights the various determinants of the response to warfarin therapy. The main outcome of interest in this study was response to warfarin therapy, which was measured by INR and ADRs. The INRs measured may be dependent on the prescribed warfarin doses. As such, warfarin doses were also assessed as outcome variables.

There were several predictor variables which impacted on the outcomes. The participants' sociodemographics and clinical characteristics may impact on the initial and maintenance doses of warfarin that were prescribed. Furthermore, the dosages of warfarin may be determined by factors such as the clinical indication, patients' age, gender, body surface area, body mass index and weight. For instance, clinicians are likely to prescribe a slightly lower dose to elderly as compared to the young patients owing to decreased metabolic and excretory function in the older age group. Similarly, individuals having a higher body mass index would probably receive a higher warfarin dose owing to the extensive volume of distribution.

Whereas the recommended target INR range is 2-3, clinical indications like prevention of blood clotting after heart valve surgery may require a higher value of 2.5-3.5 (Ageno *et al*., 2012). This will consequently necessitate an upward titration of warfarin dose compared to other indications such as atrial fibrillation and VTEs. This suggests that the clinical indication of warfarin therapy should be taken into account before initiating the treatment.

Patients' comorbidities may impact on the dose of warfarin prescribed. For instance, comorbidities such as renal impairment, liver and heart failure may necessitate reduction in warfarin dose while comorbidities which promote thromboembolism such as HIV infection, sepsis and malignancies will necessitate an increased dose of warfarin to attain therapeutic anticoagulation (Chan *et al*., 2008).

Studies have revealed that an individual's genetic make-up largely contributes to the response of warfarin and therefore, the dose prescribed (Wadelius, 2014). It has been revealed a third of inter-patient dose variation may be explained by genetic polymorphism, which is attributable to the ethnicity. Additionally, genetic polymorphism in *CYP 2C9*, *VKORC1* and *CYP 4F2* have been identified among Caucasians (AL-Eitan *et al*., 2018; Borgiani *et al*., 2009; Sanderson *et al*., 2005).

Although the predictor variables can clearly be established, it was important to take into account the various potential confounders. Some of the potential confounders may directly impact on the level of anticoagulation control by influencing the INR. For example, some foods and drugs are

known to interact with warfarin thereby increasing or decreasing its effects depending on the type of interaction (Nutescu *et al*., 2011). Substances which inhibit warfarin metabolism would promote the response whereas those which promote blood clotting will decrease its effects. These effects may not be seen at the outset and hence they form potential confounders of warfarin response.

Patients' knowledge on anticoagulation (Iqbal, 2017) and adherence to the medication have been documented to impact on the level of anticoagulation control (Mariita *et al*., 2016). Some studies have shown that patients with adequate knowledge on anticoagulation have better time-to-therapeutic range (TTR) than those without (Karuri, 2016). However, studies on treatment adherence level and anticoagulation control have reported conflicting findings (Mariita *et al*., 2015). Some studies have shown that adequate adherence will improve anticoagulation control while others have revealed a net effect (Kneeland and Fang, 2010). Assessment of patients' knowledge and adherence to anticoagulation therapy were beyond the scope of the present study.

# CHAPTER THREE: MATERIALS AND METHODS

## 3.1: Context of Research Methodology

Chapter Three describes the theoretical and conceptual structure of the research as well as how the various study designs were employed. The study area and the sample sizes as well as the sampling technique are also illustrated. The chapter also describes how the participants were enrolled, how the data was collected and the ethical considerations that were undertaken during the study. The techniques that were used for the data capture, analysis and determination of the strengths of statistical associations as well as detailed laboratory procedures have employed.

## 3.2: Study Designs

Two study designs were used for this research: Prospective longitudinal and cross-sectional. A tertiary hospital-based prospective longitudinal study design was used to characterize the clinical predictors while the cross-sectional design was employed for the genetic determinants.

Prospective longitudinal study design was used because it was a cost-effective approach that provided sufficient descriptive snapshots of the black Kenyan adult population undergoing warfarin anticoagulation at the time. Adult patients who were aged ≥18 years and receiving care at the three anticoagulation clinics of KNH were recruited into the study. These patients were followed up for the six consecutive clinic visits to determine the level of anticoagulation (INRs) and the corresponding warfarin doses they were receiving.

In order to characterize the genetic determinants of warfarin response, a cross-sectional study was also carried out at KNH. Adult patients on warfarin were recruited and their blood samples genotyped for *CYP 2C9*, *CYP 4F2* and *VKORC1* variants. This information was correlated with the warfarin doses as well the clinical response, as measured by the INRs and ADRs.

## 3.3: Study Area and Site

The two studies were conducted at the anticoagulation clinics of KNH. KNH is a tertiary care hospital located in Kenya's capital, Nairobi. It is the leading referral and teaching health facility in East and Central Africa, located on 45.7 hectares of land space. It harbors several government agencies such as the Wellcome Trust as well as the Kenya Medical Research Institute (KEMRI) and the Kenya Government Chemist. As a teaching facility, it serves both the University of

Nairobi-College of Health Sciences (UoN-CHS) and the Kenya Medical Training College (KMTC), Nairobi campus. It has a bed capacity of about 1800 with the average annual outpatient and inpatient attendance of over 600,000 and 90,000 patients, respectively. The hospital has 50 wards and 22 specialized outpatient facilities, including the anticoagulation clinics.

The anticoagulation clinics are located in the main hospital and they form the main entry point for patients requiring anticoagulation such as those with VTEs, atrial fibrillations, open heart surgeries, and prosthetic valves. These are patients requiring long term warfarin therapy to prevent thromboembolic events. KNH has three specialized anticoagulation clinics; the cardiothoracic, hemato-oncology and the cardiac clinic. The cardiac clinic serves patients with cardiovascular disorders such as heart failure, cardiomyopathies, valvular heart disease and those with atrial fibrillation who are usually on prophylactic anticoagulation therapy. The hemato-oncology clinic serves patients on treatment for VTE and those at high risk of developing thrombosis such as patients with malignancies. The cardiothoracic clinic serves patients who have undergone heart valve corrective surgery and are therefore on prophylactic anticoagulation therapy. An average of about twenty patients on anticoagulation therapy is seen on each clinic day.

Patients who are admitted in the wards and requiring long term anticoagulation are usually discharged through these clinics. In other instances, the walk-in anticoagulation patients from the Nairobi suburbs, who are unable to be managed at the KNH regular outpatients' clinics, are referred here. Additionally, anticoagulation patients are referred from the peripheral health facilities across the country to KNH anticoagulation clinics or are treated in the wards. Once admitted patients recover, they are discharged through anticoagulation clinics and enrolled for long term thromboembolic management. Furthermore, KNH has a well-equipped Central Health Records and Information office where all the documentation for her patients is systematically stored and can be availed to the research personnel upon request through structured systems including the Ethical Review Committee and Chair of Department.

### 3.4: Study Population

The study was carried out among patients who were taking warfarin for various indications, including VTEs, atrial fibrillation, valvular heart disease and prevention of thromboembolism after stroke, heart valve surgery or other major surgeries. Either gender was eligible to participate

as long as the person was aged ≥18 years and on long term warfarin anticoagulation. The prospective patients were recruited for the study during their clinic appointments. Only freely consenting patients participated in the study.

According to the available records, approximately 300 patients are diagnosed with various forms of VTEs, atrial fibrillation or had undergone corrective heart valve surgeries every year. Those who survived had to be on long term anticoagulation therapy for at least three months.

## 3.5: Eligibility Criteria

### 3.5.1: Inclusion criteria

Patients involved in the research were those that met the following criteria:

1. Black Kenyan adults of either gender (aged≥18 years), diagnosed with a medical condition requiring warfarin therapy.
2. Consistently on warfarin treatment for at least one month to allow for adequate dose titrations as well as to make the patients get accustomed to their medical conditions and their management.
3. Underwent follow-up for anticoagulation at KNH clinics
4. Gave voluntary informed consent and signed consent declaration form.

### 3.5.2: Exclusion criteria

The participants excluded from the study were:

1. Healthy volunteers.
2. Patients less than 18 years of old
3. Patients who were not on warfarin therapy at the time of study either due to non-adherence or treatment alteration based on clinical judgment of attending physician.
4. Patients who declined to give consent.
5. Pregnant women because of the pharmacokinetic changes in pregnancy as well as the ethical issues related to studies in this population.
6. Patients with uncontrolled hypertension, peptic ulcer disease or inherited coagulation disorders or any other bleeding disorder.
7. Non-black adult Kenyan patients on long-term warfarin therapy.

## 3.6: Sample Size

### 3.6.1: Sample Size Estimation for the Prospective Longitudinal Study

In this study, the principal outcome variable of interest was adequacy of anticoagulation control among the study participants. Reports on proportions of patients who are adequately anticoagulated with vitamin K antagonists vary in different set-ups. One related study revealed that approximately 14 % of the patients were able to maintain good anticoagulation control (Davis *et al*., 2005). Another study indicated that adequate anticoagulation control for patients using warfarin was maintained by approximately 10 % of the patients studied (Gladstone *et al*., 2009). Therefore, the Cochran formula below, which is used for such epidemiological surveys (Barlett *et al*., 2001) was applied for sample calculation.

**Equation 1: Sample Size Estimation for Prospective Longitudinal Study**

$$\mathrm{N} = \frac{\mathrm{Z}^2\ P(1-\mathrm{P})^2}{(\mu_\mathrm{d})^2}$$

*Where;*

*N* = the minimum sample that is needed for the present study.

Z = the average normal deviate value at 95% confidence interval for a population >120, which is 1.96.

P=the proportion of patient using warfarin, who are adequately anticoagulated which is 12%, an average of 10% (Gladstone *et al*., 2009) and 14% (Davis *et al*., 2005).

$\mu_d$ is the acceptable margin of error (0.05). This is the desired precision for this study which is generally the expected margin of error for descriptive scientific research studies.

Substituting for the values, we get;

$$N = \frac{1.96^2 \times 0.12\ (1\text{-}0.12)}{0.05^2}$$

***N=162***

The minimum sample size for this first part of the study was one hundred and sixty-two participants.

To cater for data losses, a 10% was added to the calculated sample size to make 178 participants. Therefore, a round sample size of 180 patients on warfarin therapy was used for this prospective longitudinal study which followed patients for six clinic visits.

### 3.6.2: Sample Size Estimation for Cross-sectional Study

The main primary outcome of interest for this part of the study was the frequencies of alleles in the genes impacting on warfarin response. These frequencies have been reported to differ across populations. For instance, among the Chinese populations, frequencies of *CYP 2C9* and *VKORC1* variants ranged from 2.8-4.6% and 90.2-90.8% , respectively (Zeng *et al*., 2012) while *CYP 4F2* have been reported at approximately 2% in some studies (Scott *et al*., 2010). Some research from African populations have revealed that the frequencies of *CYP 2C9* variants are less than 1% (Shuen *et al*., 2012), although in Ethiopia the frequency was reported at 4% (Aklillu *et al*., 2008). In Kenya, prevalence of *VKORC1* polymorphisms have been found to be 6% (Shahin *et al*., 2013).

In view of the above, Cochran (2007) developed the formula below to yield a representative sample for proportions for these kinds of studies.

**Equation 2: Sample Size Estimation for Cross-sectional Study**

$$\boldsymbol{n = \frac{(z^2)P(1-P)}{(m)^2}}$$

Where;

n=Minimum sample Size.

Z=1.96, which the value of Z corresponding to 95% confidence level.

P, Frequencies of *CYP 2C9*= 4.6% (Zeng *et al*., 2012), *VKORC1*=6% (Shahin *et al*., 2013) and *CYP 4F2*=2% (Scott *et al*., 2010) in populations.

m=precision, which was taken as 0.05.

By substituting z, p and m, we get 67, 87 and 30 participants for genotyping *CYP 2C9*, *VKORC1* and *CYP 4F2* variants, respectively. Since each participant was to be genotyped for the three genes the value giving the highest sample size (87) was taken into consideration. To cater for the data losses, a 20% was added to make approximately 110 participants. Therefore, blood from 110 participants from various ethnicities was collected for genetic testing of *CYP 2C9*, *VKORC1* and *CYP 4F2* variants in black Kenyan population.

However, DNA extraction was done for 105 study participants because five samples were spoilt. Genetic testing was conducted 40 participants owing to the high costs genotyping and lack of adequate funding. The selection of the 40 participants for genetic testing was done through clusters. The105 participants were placed in their respective ethnolinguistic groups and proportionate sampling done to ensure each group was adequately represented. There were ten tribes representing two ethnolinguistic groups (Bantus and Nilotes). Further selection of the 40 participants was dictated by the yields of DNA obtained (**Appendix IX**). Participants with very low DNA yields were excluded to avoid loss of data during SNP identification.

### 3.7: Piloting of the Study

Pilot study was done at anticoagulation clinics where the actual study was carried out. Piloting of the study involved 15 patients. This number was approximately 10 % of the estimated sample size for the prospective study. This study was done to test the applicability, relevance, comprehensiveness and simplicity of administration of the data collection tools. This study was also necessary for validation of data collection tools and establishment of database in IBM Statistical Package for Social Sciences (SPSS) version 23 computer software. The database was used for regular data entry after abstraction into the questionnaire.

On the day of the clinic, the PI review the patient files to identify patients who met the inclusion criteria using the eligibility checklist form (Appendix II). Outpatient files that met the inclusion criteria were kept aside and tagged with a red sticker for ease of identification. Using the files, eligible participants were invited for the pilot study before they saw the attending clinicians.

They were taken through the informed consent process explaining to them the purpose of the piloting and those who signed the consent document were administered the questionnaire.

The attending clinicians were also requested to send the identified participants to the PI for inclusion into the pilot study. These patients were also taken through the consenting process and questionnaire administration. The information which they could not provide was retrieved from their files and medication charts. This procedure continued until a target of 15 participants was achieved. Where the statements in the data collection tools seemed unclear, ambiguous or difficult, amendments were done to improve on the quality and clarity of the documents. In addition; modification of the database was done accordingly.

Participants who had no INR tests results were also referred to the bleeding centre for the phlebotomy services. In this centre, the trained and experienced phlebotomist drew approximately 2-4millitres of venous blood from the participants, while observing good laboratory practice (GLP) and good clinical practice (GCP). The blood samples were immediately put it into the vacutainers laced with citrate for INR determination. These blood samples were taken to UoN Department of Haematology and Blood Transfusion laboratory for INR tests. The results of the tests were availed to the attending clinicians for warfarin dose adjustments and patients' education. Blood samples for genotyping were not drawn at piloting phase as they were not required.

### 3.8: Sampling Method

On the early morning of the clinic day, the files for booked patients which were retrieved from the Central Health Records and Information Office of KNH were perused by the Principal Researcher to identify patients who met the inclusion conditions. A list of the file numbers and names of patients that met the study inclusion criteria was made. These files were then tagged for ease of identification and tracking so as to avoid duplication of data. Tagging was done with small pink stickers.

On each of the clinic days, there were approximately 3-9 eligible participants. Simple random selection of the participants was made by tossing the coin such that only the patients who got the

tails were recruited into the study. This sampling procedure continued until the targeted minimum sample size was achieved.

### 3.9: Participants' Recruitment and Consenting Process

Two methods of recruiting the study participants were applied. Firstly, files were perused through as the patients were waiting to be attended by the clinicians during the clinic days. These files were assessed for eligibility whereby the eligible patients' files were kept aside. Eligibility for the participants was determined using the checklist form (Appendix IIA). Any patient who answered a 'no' to any of the inclusion criteria or a 'yes' to any exclusion criteria was not invited to participate into the study. The Principal Investigator (PI) would toss the coin against the files isolated and participants who got the tails were invited to participate in the study. Once the patient presented themselves, a rapport was created first and eligible participants were taken through the informed consent process. Participants who wished to participate signed the consent form before proceeding to the next level of completing the questionnaire. Those who declined to participate for various reasons were excused and excluded from the study.

Secondly, the patients who were called in to be attended by the clinicians before the face-face-interview were later followed up. The attending clinicians were advised to send them to the PI for inclusion in the study. Once they presented themselves for the study, a rapport was first created. The patients were thereafter screened for eligibility using the checklist form and then taken through the informed consent process. Patients who agreed to participate in the research were invited to sign the declaration form for the consent. They were thereafter taken through the questionnaire by the PI. The two processes of participants' recruitment were carried out until the estimated minimum sample sizes for the study were achieved.

### 3.10: Research Instruments and Data Collection

Data were collected through a researcher administered semi-structured questionnaire in a face-to- face interview with the participant. The questionnaire had eight parts. Part one comprised of socio-demographic data including gender, age, marital status, level of education, place of birth, place of residence, ethnicity and information on participants' parents' ethnic origin. The second part comprised of details of drug use such as duration of warfarin use, dose, adverse drug reactions, and the levels of INRs at development of ADRs. The third section gathered details of concomitant medications that the participant was using; while the fourth and fifth parts captured

clinical indications of warfarin as well as maintenance doses and participants' comorbidities, respectively.

Information about the frequency of consumption of various food types, use of herbal products and nutritional supplements was captured in section six. Participants' smoking status and history of alcohol use were gathered in section seven while the last part contained information on INRs values obtained as well as the results on genetic testing.

Participants who signed the consent declaration form and successfully taken through the questionnaire were escorted to the KNH bleeding centre for the blood sample collection by trained and experienced phlebotomists where 8mL of venous blood was drawn aseptically from each participant. Approximately 4ml of blood sample was put in 12mm x 75 mm citrate laced bottles (Chengdu Rich Science Industry Co. Ltd, Germany) for the determination of INR values and another similar amount was collected in 12mm x 75 mm EDTA laced vacutainers (Chengdu Rich Science Industry Co. Ltd, Germany) for genetic testing. The exceptions for drawing the 8mL blood samples were for the patients who had already done their INRs that particular day whereby only one blood sample of 4mL for genetic analysis was collected in EDTA laced vacutainers.

Blood samples for INR determinations were immediately taken to University of Nairobi (UoN), Department of Hematology and Blood Transfusion Laboratory. The blood samples for genetic testing were taken to UoN-African institute for Biomedical Science and Technology (UoN-AiBST) laboratory located at the Department of Pharmacology & Pharmacognosy, School of Pharmacy, College of Health Sciences, KNH campus, Nairobi, where they were stored at -20$^{o}$C awaiting DNA extraction, quantification and genetic testing. Genomic DNA extraction from the whole blood was carried out at UoN-AiBST using the Quick- DNA™ Miniprep Plus Kit (Zymo Research, USA) procured from Inqaba Biotec Africa Limited.

### 3.11: Medical Record and Medication Chart Review

Review of the participants' medical files and charts was done to capture the information that the patient could not provide through face to face interview. This information included the diagnosis such as DVT, PE, atrial fibrillations and valvular abnormalities and predisposing conditions. In addition, other medications that the participant was using as well as their indications were also

extracted from the files. Data relating to the medication therapy that were abstracted into the data collection tools included details such as name(s) of the drug(s), pharmacologic class and duration of therapy.

### 3.12: Laboratory Methods

#### 3.12.1: INR Determination

INR test measured the clotting time of plasma in the presence of thromboplastin, indicating the efficiency of the extrinsic coagulation system which was a proxy measure of the response to warfarin treatment.

The blood was mixed and then centrifuged to separate blood cells from plasma which is used to measure prothrombin time. Approximately 0.1ml of plasma was put into a glass test tube and incubated at 37$^{o}$C (nominal approximation of human body temperature) for one minute. Then 0.2ml of thromboplastin reagent (Helena Laboratories Limited, Beaumont, Texas), which is commercially available thromboplastin mixed calcium chloride, was added as the stop watch was started simultaneously. The thromboplastin reagent contained an excess of calcium in a phospholipid suspension. Excess calcium reversed the effects of citrate thus enabling the blood to clot again. Thromboplastin (Tissue factor III) was added in order to activate the extrinsic clotting cascade. The tube was shaken gently at somewhat horizontal position at quick moments. The time to clotting was noted in seconds. The tests were run in duplicate and the control plasma sample likewise.

To ensure quality control, there were regular checks on the stability of the reagents as well as blood samples and the tests were done at 37$^{o}$C. Grossly hemolysed and clotted samples were not used as they could affect the results. In addition, blood samples were not kept longer than two hours at room temperature or more than 4 hours at 2-8$^{o}$C before INRs were determined.

The results were read as mean of duplicate test readings and expressed as prothrombin time (PT) in seconds. The PT index was computed as the prothrombin time for a patient sample divided by the result for control plasma and multiplied by a hundred. The INR was the ratio of a patient's prothrombin time to a control sample, raised to the power of the International Sensitivity Index

(ISI) value for the analytical system being used. INRs were reported as absolute values without units.

The ISI value indicates how a particular batch of thromboplastin reagent containing the tissue factor compares to an international reference tissue factor. The ISI is usually between 0.94 and 1.4 for more sensitive and 2.0-3.0 for less sensitive thromboplastins.

### 3.12.2: DNA Extraction

Extraction of DNA from nucleated blood was done using the DNA extraction kit in accordance with manufacturer's instructions (Zymo Research Group, USA*)*. Approximately 200 µl of nucleated blood was added to Bio Fluid & Cell Buffer (Red), 200 µl, Proteinase K, 20 µl and DNA elution Buffer (or TE Solution), 200 µl. This was mixed thoroughly by vortexing at 12,000 revolutions per minute and then incubated at $55^{o}$C for 20 minutes. The samples were allowed to digest and become homogenous after which 620 µl of Genomic Binding Buffer was added to the tube and mixed thoroughly by vortexing until the mixture was homogenous.

The mixture was transferred to Zymo-Spin$^{TM}$ IIC-XL spin column in a Collection Tube and centrifuged at ≥12,000 ×g for 1 minute after which the collection tube was discarded with the flow through.

Using a new collection tube, 400 µl of DNA pre-Wash was added to the spin column. This was centrifuged at 12,000 ×g for 1 minute and then emptied after which 700 µl of g-DNA wash buffer was added to the spin column. The mix was centrifuged again at 12,000 ×g for 1 minute and the collection tube emptied. Approximately 200 µl of g-DNA wash buffer was directly added onto the matrix in the spin column. This was centrifuged at 12,000 ×g for 1 minute. The collection tube and the flow through were discarded.

The spin column was transferred to a clean microcentrifuge tube and 50µl DNA elution Buffer (at pH >6.0) was added directly on the matrix. DNA elution buffer contained 10mM Tris-HCL, pH 8.5 and 0.1mM EDTA. This was incubated for 5 minutes at room temperature and then centrifuged at 13,000 ×g for 1 minute to elute the DNA. The eluted DNA was stored at ≤-$20^{0}$C awaiting molecular based applications.

The total yield of DNA was optimized by eluting DNA with 60-70$^0$C DNA elution buffer or loading the eluate a second time, incubating for 3 minutes at room temperature, and centrifuging again.

### 3.12.3: SNP Identification

Following DNA extraction, purity was assessed by comparing the $A_{260}$ and $A_{280}$ ratio. DNA was quantified by NanoDrop technology using the NanoDrop Machine (Wellcome Trust Laboratory, Kenya).

Genotyping for the SNPs of *CYP 2C9* , *VKORC1* and *CYP 4F2* was done using MassARRAY Compact mass spectrometer and Agena real-time detection platform at Inqaba Biotechnical Industries (Pty) Ltd, P.O. Box 14356, Hatfield, South Africa.

The SNP Genotyping assay consisted of an initial locus-specific PCR reaction, followed by single base extension using mass modified dideoxynucleotide terminators of an oligonucleotide primer which anneals immediately upstream of the polymorphic site of interest. Using the matrix-assisted laser desorption ionization-time-of-flight (MALDITOF) method, the distinct mass of the extension primer identifies the SNP allele.

Genomic DNA samples were diluted to 25 to 50ng/µL concentration using TE buffer (UV $A_{260}/A_{280}$ ratio was between 1.7 and 2.0). DNA was amplified by PCR prior to performing an extension reaction of the SNP of interest. DNA containing tubes were stored at 4$^o$C.

#### 3.12.3.1: PCR and Extension Primers

Agena Assay Designer software automatically designed PCR and extension primers (probes) for each SNP that was to be investigated. Forward, reverse and extension primers (**Table 3.1**) were ordered and the stocks stored at -20$^o$C.

**Table 3. 1: Primer Sequence for Amplification of *CYP 2C9, VKORC1* and *CYP 4F2* Variant Alleles**

| Allele | Forward Primer Sequence | Reverse Primer Sequence | Extension Primer Sequence |
|---|---|---|---|
| *CYP 2C9* *2 (430C>T) | 5'ACGTTGGATGCTGCGGAATTTTGGGATGG3' | 5'ACGTTGGATGCAGTGATATGGAGTAGGGTC3' | 5'AGAGGAGCATTGAGGAC3' |
| *CYP 2C9* *3 (1075A>C) | 5'ACGTTGGATGATGCAAGACAGGAGCCACAT | 5'ACGTTGGATGTGTCACAGGTCACTGCATGG3' | 5'GCACGAGGTCCAGAGATAC3' |
| *CYP 2C9* *4(1076T>C) | 5'ACGTTGGATGATGCAAGACAGGAGCCACAT | 5'ACGTTGGATGTGTCACAGGTCACTGCATGG3' | 5'CACGAGGTCCAGAGATACA3' |
| *CYP 2C9* *5(1080C>G) | 5'ACGTTGGATGACATGCCCTACACAGATGCT | 5'ACGTTGGATGTGTCACAGGTCACTGCATGG3' | 5'AGGCTGGTGGGGAGAAG3' |
| *CYP 2C9* *6(818delA) | 5'ACGTTGGATGACATGAACAACCCTCAGGAC | 5'ACGTTGGATGCACAAATTCACAAGCAGTCAC3' | 5'GCTTTTGTTTACATTTTACCT3' |
| *CYP 2C9* *8(449G>A) | 5'ACGTTGGATGGGAAGAGGAGCATTGAGGAC | 5'ACGTTGGATGCAGTGATATGGAGTAGGGTC3' | 5'TGTTCAAGAGGAAGCCC3'3' |
| *CYP 2C9* *11(1003C>T) | 5'ACGTTGGATGGTCCAGGAAGAGATTGAACG | 5'ACGTTGGATGCACCACAGCATCTGTGTAGG3' | 5'TTGCATGCAGGGGCTCC3' |
| *CYP 2C9* *13(269T>C) | 5'ACGTTGGATGTGAAGCAGTGAAGGAAGCCC | 5'ACGTTGGATGTGCACACCTACCAAATCCTC3' | 5'CAGAAAACTCCTCTCCA3' |
| *CYP 4F2* (rs2189784; G> A) | 5'ACGTTGGATGACAAGCAAGATGAGATCCTC | 5'ACGTTGGATGTATGTCTGATGCAGTAACTC3' | 5'CTGGTGTTTTTTTTGTTCTC3' |
| *CYP 4F2* *3(1347 C > T;V433M) | 5'ACGTTGGATGCTAGGAGCCTTGGAATGGAC | 5'ACGTTGGATGTGCCTCATCAGTGTTTTCGG3' | 5'ACCTCAGGGTCCGGCCACA3' |
| *VKORC1*(rs9923231) | 5'ACGTTGGATGCCAGGCTTGTCTTAAACTCC | 5'ACGTTGGATGGTCAAGCAAGAGAAGACCTG3' | 5'GGCGTGAGCCACCGCACC3' |

### 3.12.3.2: Amplification of the Target Loci by PCR

The DNA was amplified through PCR in order to make more copies of the DNA. A no template control was included in every PCR reaction in order to detect false positive results.

**PCR Cocktail Mix**

| | **Reagent** | **Concentration in 5µL** | **Volume for 1 reaction** |
|---|---|---|---|
| 1 | Water(HPLC grade) | NA | 1.8µL |
| 2 | 10×PCR Buffer with 20mM $MgCl_2$ | 1×(2mM $MgCl_2$) | 0.5µL |
| 3 | $MgCl_2$(25 mM) | 2mM | 0.4µL |
| 4 | dNTP mix (25mM each) | 500uM | 0.1µL |
| 5 | Primer mix(500nM each) | 100nM | 1.0µL |
| 6 | PCR Enzyme(5 U/µL) | 1.0/reaction | 0.2µL |
| **Total Volume** | | | **4.0µL** |

The 4.0µL of the cocktail mix was transferred to a 96-well microliter plate. Approximately 1.0µL of each genomic DNA was dispensed to each well. The plates were centrifuged at 1000rpm and PCR was performed with the following cycling program:

| Cycle | Temperature | Time |
|---|---|---|
| 1 | 94$^oC$ | 2minutes |
| 2 | 94$^oC$ | 30 Seconds |
| 3 | 56$^oC$ | 30Seconds |
| 4 | 72$^oC$ | 60 Seconds |
| 5 | 94$^oC$ | 30 Seconds* 45 times |
| 6 | 72$^oC$ | 5 minutes |
| 7 | 4$^oC$ | Until SNP identification |

Shrimp alkaline phosphatase (SAP) dephosphorylates unincorporated dNTPs by cleaving the phosphate groups from the 5' termini. Treatment with SAP is thus performed to remove the remaining, non-incorporated dNTPs from amplification products. The SAP mix was prepared thus;

**SAP enzyme solution**

| | Reagent | Volume( 1 reaction) |
|---|---|---|
| 1 | Water(HPLC grade) | 1.53μL |
| 2 | SAP buffer (10×) | 0.17μL |
| 3 | SAP enzyme(1.7U/μL) | 0.30μL |
| Total Volume | | 2.00μL |

This SAP mix was vortexed and centrifuged slightly after which 2μL of the SAP solution was added to each well containing the PCR products and incubated at $37^0$C for 40 minutes, then $85^0$C for 5 minutes and thereafter stored at $4^0$C awaiting SNP identification. This mix formed the iPLEX-SAP mix.

The iPLEX single base extension (SBE) or iPLEX reaction was performed on the products produced after SAP enzyme reaction. The iPLEX reaction is a universal method for detecting single base polymorphisms or small insertion/deletion polymorphisms in amplified DNA. After the PCR clean-up, a primer extension reaction cocktail (containing extension primer, buffer, enzyme, and mass modified ddNTPs) was added to the amplification products. During the iPLEX reaction, the primer was extended by one mass-modified nucleotide depending on the allele and design of the assay.

The iPLEX-SBE master mix was prepared as follows:

| | Reagent | Concentration in 9μL | Volume for 1 reaction |
|---|---|---|---|
| 1 | Water(HPLC grade) | NA | 0.619μL |
| 2 | iPLEX buffer(10×) | ×0.222 | 0.200μL |
| 3 | iPLEX Termination mix | ×1 | 0.200μL |
| 4 | Primer mix(8 μM:10 μM:15 μM) | 0.52 μM:1.04 μM:1.57 μM | 0.940μL |
| 5 | iPLEX enzyme | ×1 | 0.041μL |
| **Total Volume** | | | **2.000μL** |

The 2.0μL iPLEX-SBE master mix was added to each well on SAP treated plate (9.0μL). The mix was vortexed and centrifuged, and incubated at $94^o$C for 30 Seconds then $80^o$C for 3 seconds (for 60 cycles) and later $95^o$C for 5 seconds and $52^o$C for 3 seconds ( for 5 cycles). It was finally incubated at $72^o$C for 3 minutes after which it was held at $15^o$C.

The extension reaction products were subjected to primer extension reaction resin clean-up. This clean-up step was important to optimize mass spectrometry analysis of the products. Slurry of the resin was added directly to the primer extension reaction products to remove $Na^+$, $K^+$ and $Mg^{2+}$ ions. If not removed, these ions could result into high background noise in the mass spectra.

The primer extension reaction resin clean-up involved centrifuging the SBE plate, adding 41µL of nuclease free water to each well in the plate, covering with an adhesive sealing sheet and centrifuging at 3400 rpm for 1 minute. After this, 15mg resin was spread onto the dimple plate and let to dry for one hour. The SBE and the resin plates were held together so that the resin could drop into the wells. The reaction plate was sealed with film, centrifuged at 3400 rpm for 1 minute, and placed on the plate on the rotator for 30 minutes. The reaction plate was centrifuged at 3400 rpm for 5 minutes to pellet the resin. The dimple plate was cleaned with NanoPure water, dried and stored.

**3.12.2.3: Spotting Primer Extension Products on SpectroCHIPs**

The Nanodispenser was conditioned using 1M NaoH and programmed with "Weekly Condition" for 10 minutes. It was then cleaned using "Daily Clean" programme for 30 minutes. Subsequently, the sonicator was drained and the ethanol filled with 50 % ethanol solution. Using the "Maintenance Menu", the Nanodispenser insert was switched to 6-pins. The plate and the new spectroCHIP were then placed on the Nanodispenser ready for the run. "Volume Check" was selected in the method. The following commands were then selected: Aspirate offset=9.5mm, Aspirate time=3sec, Aspirate speed=50mm/sec, Dispense offset=1mm, Dispense speed=140mm/sec and Dispense time=1sec, after which 'Auto-tuning' and 'Analyte and Calibrant' were selected in the method. Approximately 80 µL of 3 pt calibrant was placed in the white reservoir and run. The volumes that were dispensed onto the existing matrix spots on the silica spots were small (~12nl). When this was completed, the SBE plate was sealed and stored at -20$^o$C and the remaining calibrant was pipetted out from the white reservoir, returned to the tube and stored at 4$^o$C.

#### 3.12.2.4: Detection of the Primer Extension Products by MassARRAY Compact Mass Spectrometry and Agena real-time Detection Software

The Compact mass spectrometer was started according to the manufacturer's instructions. The scout plate that holds the chips was opened to remove any detected chip. The spectroCHIPs were then loaded onto the scout plate which was sent into the compact machine. Using the "Assay Editor" application on the workstation, a new customer was created. The assay group was imported and edited for allele calls as well as the sample identities. The sample names and assays were assigned to the wells. The 'Autorun' button was pressed and the instrument begun collecting the data. Once the data was collected, the "Typer Analyzer" application was used to examine the data.

### 3.13: Quality Assurance, Validity and Reliability of the Collected Data

The validity of the questionnaire tool was guaranteed by preparing the tool in line with the proposed study objectives. In addition, the questions were arranged in themes using simple and clear language which could be understood by the participants. Furthermore, the questionnaire was pre-tested prior to the actual research to check whether it met the laid down objectives. The phlebotomists were also trained about the study before the actual process was carried out. Moreover, the phlebotomists were trained and experienced in the medical laboratory field. The study area and site gave a good representation of the Kenyan population since the tertiary hospital attends to patients from all parts of the country. In addition, the sample size and the sampling technique employed in the study were statistically and scientifically applied, respectively.

Genetic testing and INR determination was done in WHO accredited and ISO certified laboratories. GCP and GLP guidelines were adhered to during blood sample collection, INR and genetic analysis. All the laboratory equipment were regularly validated and calibrated to ensure accuracy. Furthermore, close support and supervision of the phlebotomists and laboratory staff were done by the PI to ensure that quality was maintained throughout the study.

### 3.14: Internal and External Validity

Internal validity for this study was maintained by use of a well laid down and comprehensive data collection process in which the instruments were pretested before the actual study. Additionally, standardized, established and official procedures were used in the laboratories

during INR testing, DNA extraction and the genetic analysis. All the laboratory procedures adhered to GLP according to WHO guidelines. All cautionary measures in blood collection, handling, transportation, storage, processing and proper disposal of wastes were adhered to. This included use of personal protection equipment as well as biosafety cabinets. This was enhanced by ensuring that all the laboratory procedures were carried out by trained, qualified, experienced and certified personnel. External validity was maintained by ensuring an adequately prepared study, and carrying out the sample size estimation and the sampling procedures outlined. Additionally, this study was carried out in the largest referral and teaching hospital in Kenya where all categories of anticoagulation patients are most likely to be seen.

## 3.15: Study Variables

The clinical predictor variables included factors which influenced the response to warfarin such as sociodemographic characteristics, clinical indications of anticoagulation, drug/herb interactions, comorbidities and genetic polymorphisms of interest. The other predictor variables were participants' social history including alcohol consumption, as alcohol is likely to interact with warfarin and influence its action.

The main genetic predictor variables in this study were the SNPs of *CYP 2C9 (*2, *3, *4, *5, *6, *8, *11* and *13), VKORC1 rs9923231* and *CYP 4F2 (*3* and *rs2189784)*. On the other hand, the main outcome variable was the response to warfarin which was measured through the phenotypic characteristics of the patient such as the dose of warfarin, INR values and presence or absence of ADRs.

## 3.16: Data Management

### 3.16.1: Data Processing

All the questionnaires were accurately filled at the closure of each day. A database, modeled against the questionnaire, was then created using the IBM SPSS version 23 computer software. Each questionnaire had a unique number which was linked to the created database based on the questionnaire used for the study. Data obtained with the questionnaire were entered directly into the database. Data entries were done on daily basis and routinely checked for accuracy as well as completeness. Inconsistencies between the questionnaires and database were rectified immediately. Data were backed up daily onto a hard drive and kept separately. On completion of the data entry process, the filled questionnaires were preserved in a cabinet under lock and key.

To avoid the breach of confidentiality, the questionnaires were only accessible to the PI. In addition, copies of the signed consent document forms were kept securely and separately from the main data. Once all the data captured with the questionnaires were entered into the database, it was analyzed using IBM SPSS version 23 computer software. Furthermore, the 15 patients did not form part of the main data and, therefore, were excluded from data analysis.

### 3.16.2: Statistical Methods

Three types of analyses were carried out. These included descriptive statistics, bivariate, and multivariate analysis.

#### 3.16.2.1: Univariate Analyses

Exploration of data was carried out to unearth the distribution of the study parameters as well as detect outliers or abnormally entered values. Descriptive indicators were used to survey the overall distribution of the theorized factors and outcomes by calculating the means, median, range for continuous variables and standard deviations. Computations of proportions were done for categorical variables. Frequencies were run to describe the study population in terms of socio-demographic characteristics such as participants' age, gender, occupation, marital status ethnicities, genotype and allele frequencies. These were reported as either proportions or measures of central tendencies. The skewness of the data dictated which measure of central tendency could be used. Descriptive statistics such as mean, standard deviations, median and interquartile ranges were also done for warfarin doses and clinical indications.

The details of the genes of interest as well as their allele frequencies were tabulated. Characterization of genetic variability of warfarin metabolizing enzymes in the study population and across the tribes was done. The results were summarized in form frequency distribution tables, graphs and pie charts. In all the genetic analyses, Hardy-Weinburg equilibrium (HWE), where the genotypes and allele frequencies are constant in a population, was assumed because a homogenous group of black Kenya individuals was involved in the study.

The predictor variables of various SNPs of the warfarin metabolizing enzymes in the black Kenyan population were reported as frequencies. The outcome variable or responses to warfarin were reflected by the warfarin doses prescribed, measured INR values and ADRs for each patient. INR was reported as being within or out-of-therapeutic range of 2-3 as recommended in the international guidelines (Ageno *et al*., 2012).

### 3.16.2.2: Bivariate Analyses

Bivariate analysis was done to determine associations between the predictor and outcome variables. Association between the outcome variables (Warfarin Dose, INRs and Adverse Drug Reactions) and patients characteristics (socio-demographics, patients' clinical characteristics and genetic variations) were conducted by statistical tests for bivariate analyses using ANOVA, student-t-tests, and Pearson's or Spearman's correlation tests to inform the multivariable analyses. Relationships between the genetic variants and warfarin maintenance doses, INRs and ADRs were conducted for the cross-sectional study.

P-values and 95% Confidence Interval (CI) were used to estimate the strength of crude association between predictor and outcome variables. Specifically, the bivariate analysis was done to determine the statistical strength of association between the sociodemographic characteristics versus the initial and maintenance doses of warfarin that the patient was receiving. In addition, tests were done to assess the associations between the level of anticoagulation and ADRs versus participants' sociodemographics, clinical characteristics, and co-prescribed drugs or nutritional and herbal supplements as well. Lastly, statistical associations were computed between the participants' genetic variability versus warfarin doses, the INRs measured and the prevalence of ADRs. The data were presented in charts and tables while setting the threshold for statistical significance at $P \leq 0.05$. Values whose $p \leq 0.05$ were considered statistically significant. Whenever the statistical assumptions for parametric tests were violated their non-parametric options were used instead.

### 3.16.2.3: Multivariable Analyses

The results of the bivariate analyses informed multivariate analysis. The significant variables of the response to warfarin action at bivariate analysis were subjected to multiple regression analysis which was achieved using generalized linear models for a more thorough exploration of outcome variable. For outcomes measured in a continuous scale (Dose and INR) the models used an identity link while for those with binary measures, a logit link was used. This technique was done in order to adjust for potential confounders impacting on the outcome variable and to identify independent determinants of response to warfarin activity. Beta ($\beta$) coefficients and odds ratios (ORs) were computed to determine the strength of the independent predictors of warfarin response. The factors significant at this level predicted the probability of a patient responding

either positively or negatively to the warfarin. All the analyses were conducted using IBM SPSS version 23.

Multiple logistic regression using linear models was done to find out the magnitude of contribution of the predictor variables to the outcome variables studied as well as independent factors, both clinical and genetic, that determined the safe warfarin doses giving therapeutic INRs. R-square (the percentage of the response variable deviation that was explained by a linear model) from the regression model was used to estimate the contribution of individual socio-demographic, clinical and genetic polymorphisms on the variation of warfarin dose. For binary outcomes (ADRs and therapeutic INR ranges), Nagelkerke's $R^2$ from the logistic regression model was used to estimate the amount of variance explained by these predictor variables. In addition, parsimonious modeling was done to adjust for any potential confounders and identify the independent clinical or genetic predictors of warfarin dose that would likely produce therapeutic INRs. This culminated into developing an equation, incorporating the clinical and genetic data, which could be used to determine safe warfarin dose that can provide therapeutic INR in Kenyan participants on anticoagulation.

## 3.17: Ethical Considerations

### 3.17.1: Study Approvals

Approval for the study was sought and acquired from Kenyatta National Hospital/University of Nairobi-Ethics and Research Committee (KNH/UoN-ERC) reference number **KNH-ERC/A/569** (Appendix IV). The study was also registered by KNH Department of Research and Programs reference number **MED/025/14** (Appendix XII) prior to its commencement. All other protocols, including seeking the authority to conduct research from the study sites, were also observed before the study begun. These included approvals from UoN Department of Haematology and Blood Transfusion laboratory, AiBST, heamato-oncology, cardiothoracic and cardiac clinics.

### 3.17.2: Informed Consent

Each of the eligible participants was taken through detailed consenting process. Once they fully understood the consent information and agreed to participate in the study, they were presented with a consent declaration form to sign. Two copies of the consent document were signed by the every participant, where one was given to them to keep and the other was securely stored by the

PI for accountability. The consent documents were stored where there was restricted access to only the PI.

Each participant was informed that participation in the study was voluntary. They were also informed that they were free to withdraw from the study at any point without jeopardizing their treatment. Each participant was encouraged to ask any questions about the study in the course of the interview. Any concerns regarding the use of warfarin were addressed immediately. The participant was also informed that if he/she had any concerns about the ethical rights he/she could contact the Secretary of UoN/KNH-ERC. The participant was also informed that the information he/she gave was confidential and could not be divulged to anyone unless demanded by law or upon institutional instructions.

### 3.17.3: Confidentiality

The information received from participants was treated in strictest confidence without sharing with a third party. Participants' data were kept confidential by concealing with the use of serialized alphanumerical unique identifiers throughout the study. The unique identifiers were used instead of participants' names during the data collection and analysis process to safeguard the identity. All the data collection materials were preserved under lock and key where there was restricted access during the entire study period. The filled data collection tools could only be accessed by the PI or any other authorized agent such as the biostatistician.

### 3.17.4: Benefits from the Study

During participants' interviews, the PI addressed the concerns that they had with regard to the use of warfarin. This was done through providing education on the useful effects of the drug, side effects and the precautions to observe during treatment. Participants were also educated on dietary measures during anticoagulation, especially consumption of supplements or green leafy vegetables containing vitamin K and their effects on the action of the drug. The research findings will be shared with the relevant departments with the hope of improving anticoagulation services in the hospital. Furthermore, some of results have been shared through publications in the peer reviewed journals (Nyamu *et al*., 2017; Nyamu *et al*., 2019; Nyamu *et al*., 2019).

### 3.17.5: Risks from the Study

Obtaining blood from the participant for the INR and genetic testing was carried out using aseptic techniques. GCP and GLP were strictly adhered to ensure that the participants were not harmed. The initial and maintenance doses of warfarin prescribed were determined by the attending clinicians. Furthermore, patients' privacy and confidentiality were maintained at all times and the informed consent declaration forms were signed voluntarily without any coercion whatsoever. There was restricted access to where the signed informed consents were stored. To ensure privacy, the signed consents were detached and stored away from the main data collection tools which had only the alphanumerical codes. Patients who declined to participate due to their own reasons did not lose any benefits from the hospital.

# CHAPTER FOUR: RESULTS

## 4.1: Overall Structure of the Presented Results

This section describes the results obtained after data extraction, entry, cleaning and analysis of the black Kenyan patients on anticoagulation. The section has two main components: The exploratory data report and the inferential analysis. Exploratory data gives the description of the participants' screening process and the reasons for exclusion from the study, followed by description of the sociodemographic characteristics such as the age, gender, education level, marital and obesity status of the participants (**Table 4.1**). The patients' clinical characteristics, which would impact on warfarin anticoagulation, are also described. The clinical characteristics included the number of coexisting diseases, number of other prescription drugs and the use of herbal, nutritional as well as other supplements which were likely to interact with warfarin.

The clinical indications of warfarin anticoagulation, the details of warfarin use such as the doses prescribed, duration of use and the monitoring parameters, which were surrogates for the drug's response are also presented. The principle monitoring parameters of warfarin anticoagulation included the INR measurements and the adverse drug reactions encountered.

The inferential components of data gave the relationships between the predictor and outcome variables of the study. In this part, associations between predictor variables (for example, sociodemographic characteristics, clinical indications of warfarin use and co-prescribed drugs as well as comorbidities) and outcome variables (such as initial and maintenance doses of warfarin as well as the response as measured by INR or adverse drug reactions) and are also included.

Lastly, the section presents the independent predictors of the response to warfarin therapy. This included the independent predictors of; the doses administered and ADRS experienced by the patients.

## 4.2: Pattern and Clinical Determinants of Warfarin Anticoagulation in Black Kenyan Adult Patients

A total of two hundred and seventeen participants were screened to investigate the clinical predictors of warfarin response. However, data were analyzed from 180 participants because thirty seven were not eligible due to various reasons as outlined below (**Figure 4.1**).

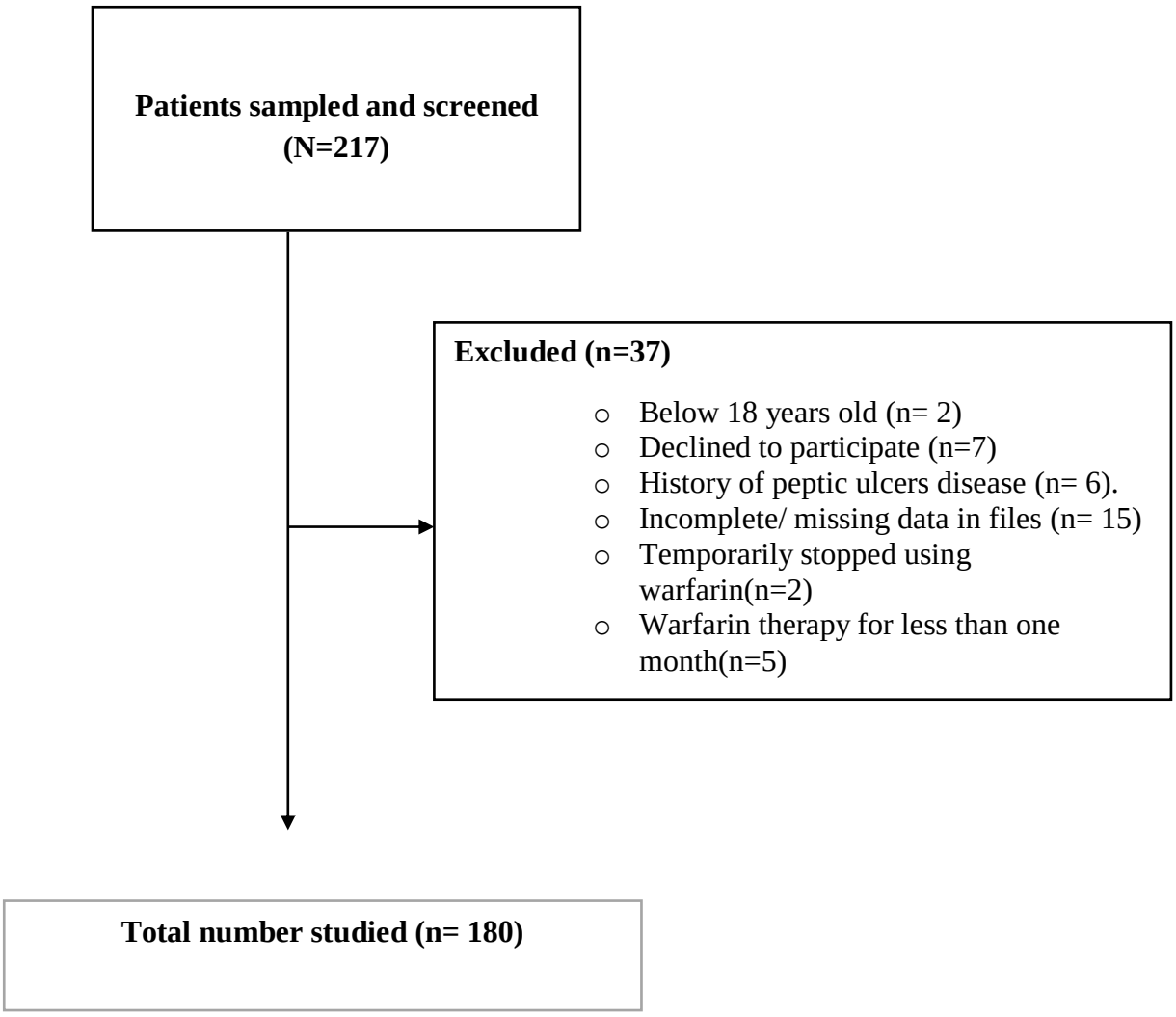



**Figure 4.1: Participants eligibility and reasons for exclusion**

### 4.2.1: Population Characteristics of the Patients on Warfarin Anticoagulation Therapy

The sociodemographic features of the research participants are presented in **Table 4.1**. Approximately 75% of the study participants were females and 67% were married.

**Table 4.1: Sociodemographic Characteristics of Patients on Warfarin Anticoagulation at KNH (N=180)**

| Variable | Category | Frequency (N=180) | Percentage (%) |
|---|---|---|---|
| Sex | Male | 42 | 23.0 |
| | Female | **138** | **77.0** |
| Age categories(years) | 19-30 | 31 | 17.2 |
| | 31-50 | **100** | **55.5** |
| | 51-64 | 37 | 20.6 |
| | 65 and above | 12 | 6.7 |
| Body Mass Index Category | Underweight | 11 | 6.1 |
| | Ideal body weight | 47 | 26.1 |
| | Overweight | **58** | **32.2** |
| | Obese | **32** | **17.8** |
| | Missing data | 32 | 17.8 |
| Marital status | Single | 39 | 22.0 |
| | Married | **118** | **66.0** |
| | Divorced | 11 | 6.0 |
| | Widowed | 12 | 7.0 |
| Employment status | Unemployed | 55 | 31.0 |
| | Salaried | 53 | **29.0** |
| | Self employed | **64** | **36.0** |
| | Student | 8 | 4.0 |
| Highest academic level | College/ University | 35 | **19.0** |
| | Secondary | **81** | **45.0** |
| | Primary | 41 | 23.0 |
| | Non-formal | 23 | 13.0 |
| Denomination | Protestant | 86 | **48.0** |
| | Catholic | 84 | **47.0** |
| | Muslim | 8 | 4.0 |
| | Other | 2 | 1.0 |
| Tobacco use | No | 173 | **96.0** |
| | Yes | 2 | 1.0 |
| | *Missing data* | 5 | 3.0 |
| Alcohol use | No | 162 | **90.0** |
| | Yes | 12 | 7.0 |
| | *Missing data* | 6 | 3.0 |
| *Mean Age(±SD) years; Range* | *43.4(±13.2); 19-87* | | |

Key: SD-Standard Deviation

The mean age of the study participants was 43.4 (±13.2), with a range 19-87 years. More than half (55.5%) were aged between 31-50 years and almost 50.0% had exceeded their ideal body weights. Slightly over 60% of the study participants had attained at least a secondary level of education and had a source of regular income. Over 90% of the participants were Christians and a similar proportion were neither alcohol nor tobacco consumers (**Table 4.1**).

#### 4.1.1.1: Clinical Indications for Warfarin Anticoagulation

**Figure 4.2** shows the clinical indications for warfarin therapy among the participants. Over half (54.4%) of participants were using warfarin due to deep vein thrombosis, while a third (33.3%) had undergone major surgery. The rest were using the drug due to rheumatic heart disease (9.4%), atrial fibrillation (5.6 %) pulmonary embolism (2.2 %) and stroke (1.1%), in that order (**Figure 4.2**).

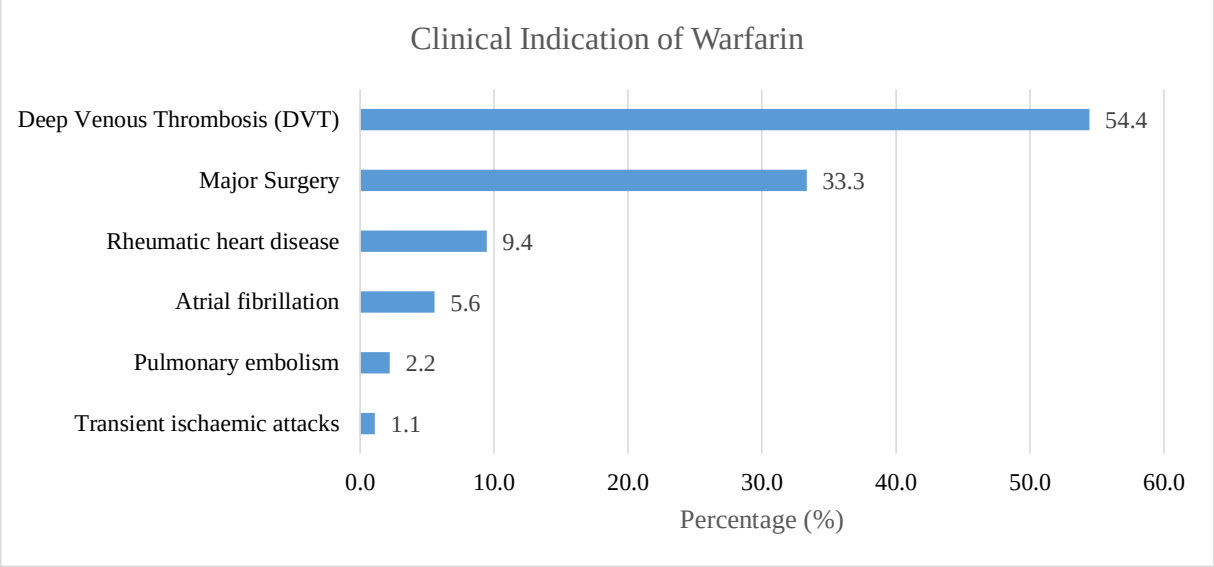



**Figure 4.2: Clinical Indications for Warfarin Therapy among the Study Participants (N=180)**

**Major surgeries included heart surgery such as aortic, mitral or double valve replacement. Atrial fibrillation included the associated conditions such as thrombus in the heart, dilated cardiomyopathy, constrictive pericarditis, ischaemic heart disease and heart failure.*

#### 4.1.1.2: Details of Warfarin Therapy among the Study Participants

The details of warfarin therapy such as the initial, maintenance doses prescribed and duration of use are displayed in **Table 4.2**.

**Table 4.2: Doses and Duration of Warfarin Therapy among the Study Participants**

| Variable | | Proportion of Participants (N=179) | Percentage (%) |
|---|---|---|---|
| Initial dose ranges | 2.5-5mg | 20 | 11.2 |
| | 6-10mg | **133** | **74.3** |
| | >10mg | 26 | 14.5 |
| Mean initial dose (mg) | | 6.03 (SD±4.90) | |
| Median (range) initial dose (mg) | | 5.0 (3-15) | |
| Mean maintenance dose (mg) | | 6.17 (SD±2.75) | |
| Median (Range maintenance) dose (mg) | | 5.0(2-20) | |
| Median (range) duration of warfarin therapy (days) | | 753 (31-11433) | |

SD-Standard Deviation

The mean initial and maintenance daily warfarin doses were 6.03mg (±4.90) and 6.17mg (±2.75), respectively. Almost 75% of the participants were initiated on warfarin at 6-10mg per day for their clinical conditions. The rest received initial daily doses of ≤5mg or >10mg. Furthermore, the study participants had used warfarin for a median duration of 753 days or approximately two years (**Table 4.2**).

#### 4.1.1.3: Number of comorbidities among the Participants

The study explored the clinical characteristics of participants by finding out the prevalence of comorbidities among the study participants. The results are presented in the **Figure 4.3**.

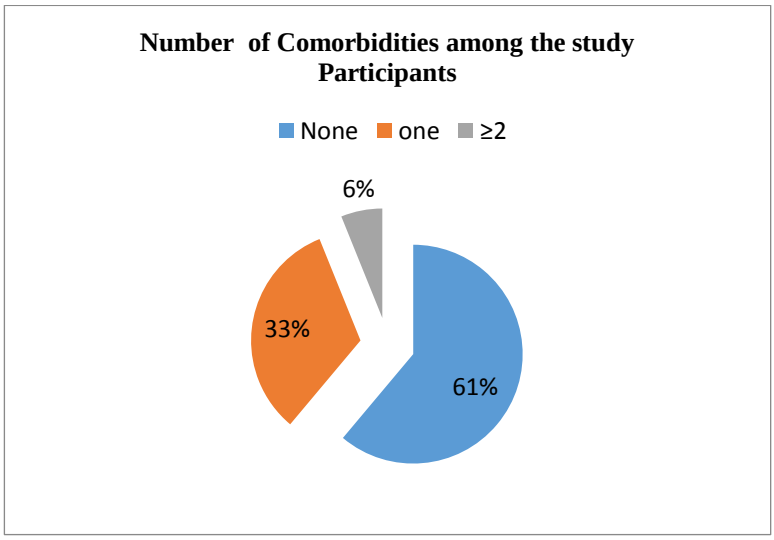



**Figure 4.3: Proportions of participants with comorbidities in the study population (N=180)**

Although the majority of the participants did not have other coexisting diseases, at least 39.0% of them had at least another disease apart from the primary diagnosis of anticoagulation (**Figure 4.3**).

#### 4.1.1.4: Concomitant Medication use by the Study Participants

Concomitant use of other drugs may interfere with warfarin anticoagulation. The study sought to measure the magnitude of utilization of other drugs among the participants as presented in **Figures 4.4** and **4.5.** The study found that 45 % (n=81) of participants were also using other conventional medicines for treatment of comorbidities. The number of the individual drugs prescribed per participant was explored and the findings are shown in **Figure 4.4.**

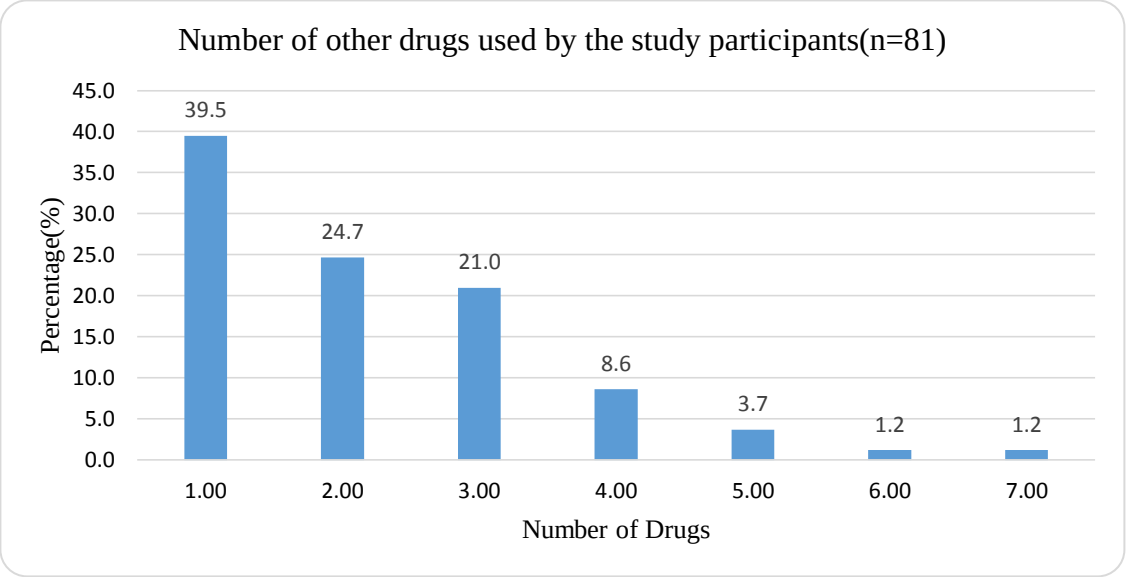



**Figure 4.4: Number of other drugs used by the study participants**

Apart from warfarin, approximately 40 % of participants were on one other drug while about 25% was on ≥2 other drugs. In addition, as the number of drugs per prescription increased, the percentage of participants decreased (**Figure 4.4**).The classes of the other drugs used by the participants are shown in **Figure 4.5**.

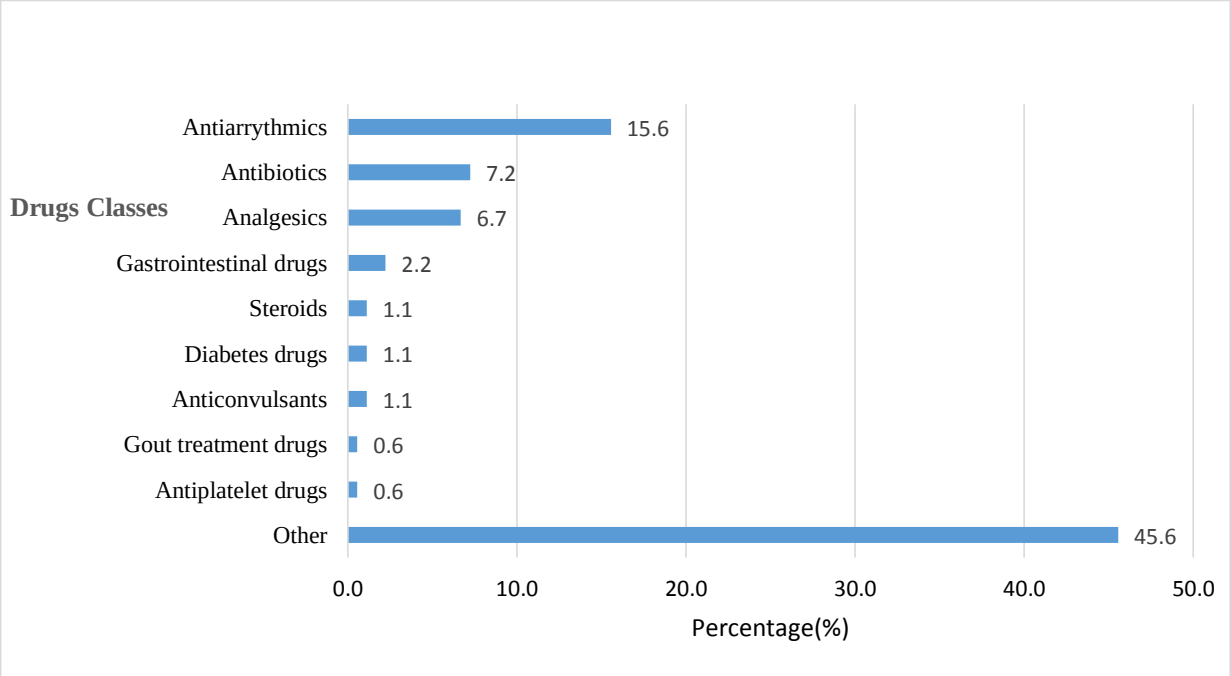



**Figure 4.5: Classes of concomitant drugs used by the participants in the study population**

**Others include antihypertensives, anticancers, antiretrovirals, diuretics, anti-acids*

Antiarrythmics (15.6 %) were the most commonly used concomitant medications followed by antibiotics (7.2%) and analgesics at 6.7% (**Figure 4.5**).

#### 4.1.1.5: Diet, Nutritional and Herbal Supplements use among the Study Participants

The study explored the various dietary foods and their frequency of intake among the participants (**Table 4.3)**.

**Table 4.3: Frequency of Dietary intake by the Study Participants**

| Dietary Type | Frequency of Consumption | n | Percentage |
|---|---|---|---|
| Vegetables (N=135) | <3 Times a Week | 31 | 23.0 |
| | 3-7 Times a Week | **102** | **75.6** |
| | >7 Times a Week | 2 | 1.5 |
| Proteins (N=78) | <3 Times a Week | 21 | 26.9 |
| | 3-7 Times a Week | **56** | **71.8** |
| | >7 Times a Week | 1 | 1.3 |
| Carbohydrates (N=73) | <3 Times a Week | 7 | 9.6 |
| | 3-7 Times a Week | **60** | **82.2** |
| | >7 Times a Week | 6 | 8.2 |
| Fruits (N=124) | <3 Times a Week | 19 | 15.3 |
| | 3-7 Times a Week | **103** | **83.1** |
| | >7 Times a Week | 2 | 1.6 |

Most of the participants were moderating on consumptions of various food types as over 70% were consuming either food type (vegetables, proteins, carbohydrates or fruits) 3-7times per week. Higher frequencies of intake of any food type for >7 times per week were infrequent (**Table 4.3**).

This study explored the use of herbal and nutritional supplements, such as ginger or garlic, which are likely to interact with warfarin. **Table 4.4** displays the frequency of use of the products.

**Table 4.4: Nutritional and Herbal Supplements use among the Study Population (N=180)**

| Nutritional supplements and herbal preparation | Use | Frequency(n) | Percentage |
|---|---|---|---|
| Vitamin E (Greater than 400 IU per day) | No | 179 | 99.4 |
| | Yes | 1 | 0.6 |
| Vitamin C (greater than 500 mg per day) | No | 177 | 98.3 |
| | Yes | 3 | 1.7 |
| Vitamin K supplements | No | 178 | 98.9 |
| | Yes | 2 | 1.1 |
| Garlic herbal | No | 146 | 81.1 |
| | Yes | **34** | **18.9** |
| Ginger herbal | No | 158 | 87.8 |
| | Yes | 22 | **12.2** |
| Green tea herbal | No | 179 | 99.4 |
| | Yes | 1 | 0.6 |

Approximately ninety percent of the study participants were claimed they were not using herbal or nutritional supplements which are likely to interact with warfarin. However, almost a fifth (18.9 %) and 12.2 % of the study participants were using garlic and ginger, respectively (**Table 4.4**). Additionally, it was found that none of the participants was using other supplements such as red clover, sweet clover, St. John's Wort, glucosamine & chondroitin, Coenzyme Q10, alfalfa, aloe vera gel or Ginkgo bilova extracts which were investigated in the present study.

### 4.1.1.6: Warfarin Maintenance Doses for the Clinical Conditions

One of the main outcome variables in the present study was to find out the prescribed warfarin maintenance doses, which may be surrogate markers of anticipated response. As part of the pattern of anticoagulation control as well as measurement of the responses to warfarin therapy, the study therefore, sought to find out the prescribing patterns of warfarin maintenance doses across the clinical indications. The findings are presented in **Figure 4.6**.

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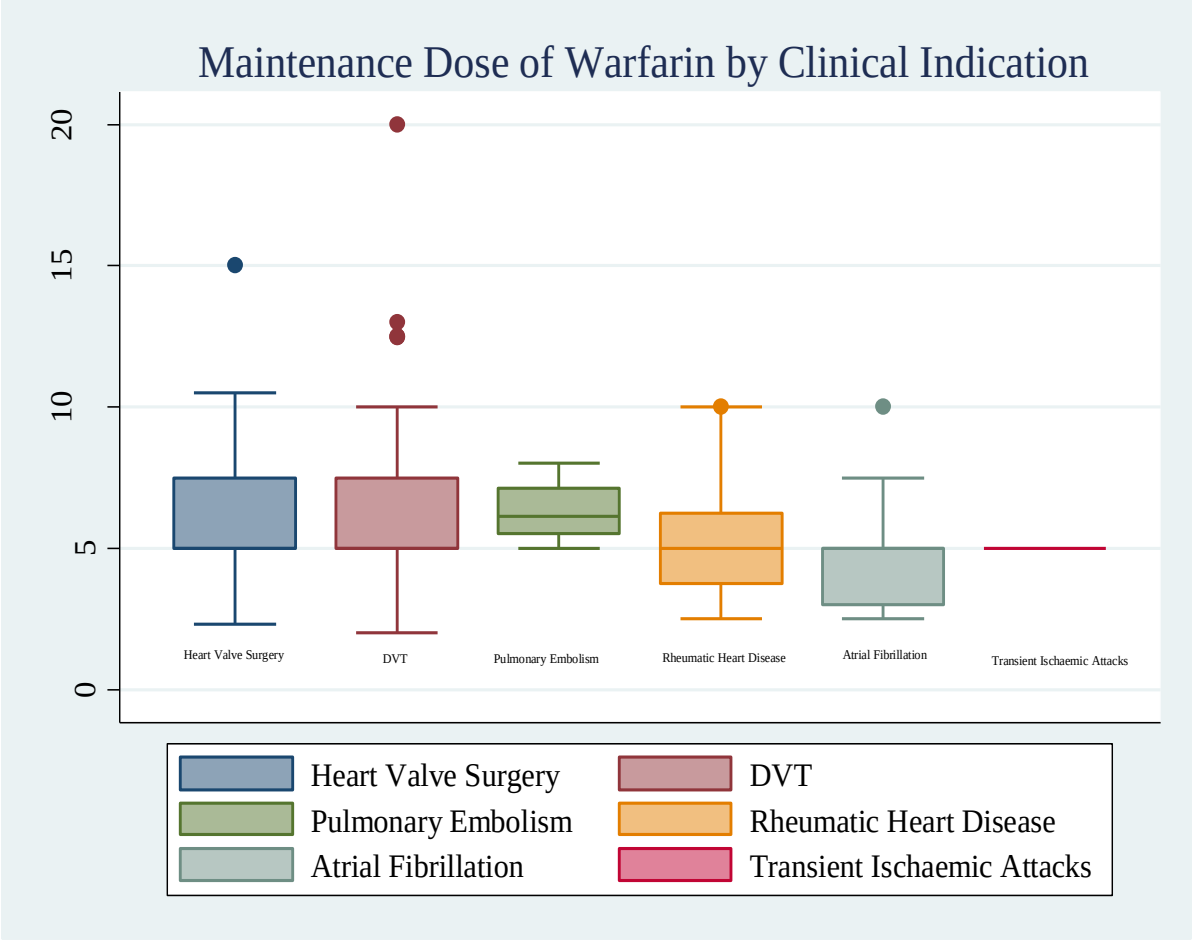


**Figure 4.6: Mean Maintenance and ranges of warfarin doses by the clinical condition**

Key: DVT-Deep Vein Thrombosis.

High warfarin mean daily maintenance doses (>5mg/day) were required in participants who had undergone major surgery (heart valve surgery) or suffering from venous thromboembolic events. Participants with rheumatic heart disease, atrial fibrillation and transient ischaemic attacks required lower warfarin maintenance doses per day (<5.0mg/day). Some participants with DVT and those who had undergone surgery were using higher warfarin maintenance doses (>10mg/day) (**Figure 4.6**). **Table 4.5** shows the ranges of warfarin maintenance doses across the clinical indications.

**Table 4.5: Warfarin Maintenance Doses for the Clinical indications in the Study Population**

| Clinical Indication | Maintenance dose(mg) | Frequency | Percentage |
|---|---|---|---|
| Major Surgery (n=60) | <2.5mg | 1 | 1.7 |
| | 2.5-5mg | 11 | 18.3 |
| | 6-10mg | **44** | **73.3** |
| | >10mg | 4 | 6.7 |
| DVT (n=97) | <2.5mg | 1 | 1.0 |
| | 2.5-5mg | 13 | 13.4 |
| | 6-10mg | **68** | **70.1** |
| | >10mg | 15 | **15.5** |
| Pulmonary embolism (n=4) | 6-10mg | 4 | 100.0 |
| Rheumatic heart disease(n=17) | 2.5-5mg | 5 | 29.4 |
| | 6-10mg | **11** | **64.7** |
| | >10mg | 1 | 5.9 |
| Atrial Fibrillation (n=10) | 2.5-5mg | 4 | 40.0 |
| | 6-10mg | 5 | **50.0** |
| | >10mg | 1 | 10.0 |
| Transient ischemic attacks (n=2) | 6-10mg | 2 | 100.0 |

Key: DVT-Deep Vein Thrombosis

More than half of the participants were receiving warfarin maintenance doses ranging from 6-10mg per day across the clinical indications. Compared to the other clinical indications, a larger proportion (15.5 % vs. ≤10.0%) of participants with DVT received warfarin maintenance doses higher than 10mg per day. Less than 10.0% of participants across other clinical conditions were receiving warfarin doses of 6-10mg per day. In addition, pertaining to atrial fibrillation, the proportion of participants receiving warfarin maintenance doses of 2.5-5mg and higher than 5mg per day was 40% and 60%, respectively. None of the participants with pulmonary embolism received warfarin maintenance doses less than 6mg/day (**Table 4.5**).

For ease of comparing maintenance doses, participants were grouped into two categories according to their clinical indications of warfarin anticoagulation. One group who had diagnosis of rheumatic heart disease, atrial fibrillation or heart valve surgery and repair was collectively categorized as "heart diseases". The other group comprised of any form of venous thromboembolism event such as DVT, pulmonary embolism or thrombosis in the brain (transient ischaemic attacks) which was categorized as "Venous Thromboembolic Events (VTEs)". Warfarin maintenance doses according to these two clusters are displayed in **Table 4.6**.

**Table 4.6: Warfarin daily maintenance doses by clinical indication among the study participants**

| Clinical Indication | Maintenance Dose | | | | Total |
|---|---|---|---|---|---|
| | <2.5mg | 2.5-5mg | 6-10mg | >10mg | |
| Heart Diseases | 0 (0.0%) | **16 (21.3%)** | **54 (72.0%)** | 5 (6.7%) | 75(100.0%) |
| Venous Thromboembolism Events(VTEs) | 1 (1.0%) | 13 (12.9%) | **72 (71.3%)** | **15 (14.9%)** | 101(100.0%) |

Approximately a fifth of the participants (21.3%) with heart diseases were maintained at warfarin doses of 2-5mg daily. Slightly over seventy per cent of participants suffering from VTEs or heart diseases were receiving warfarin maintenance doses of 6-10mg per day. The proportion of participant with VTEs who were receiving warfarin maintenance doses of more than 10mg per day was almost twice that of heart diseases (14.9% vs. 6.7 %) (**Table 4.6**).

### 4.2.2: Measurement of Warfarin Response among the Study Participants

The outcome assessment of participants receiving warfarin therapy involved INRs and ADRs monitoring. Generally, these were the responses to warfarin therapy which were the main outcome variables for the present study.

#### 4.2.2.1: INR Monitoring

In this study, participants were monitored through measurement of INR as a routine for assessing warfarin anticoagulation. The mean INRs obtained across the clinical indications are as shown in **Figure 4.7**.

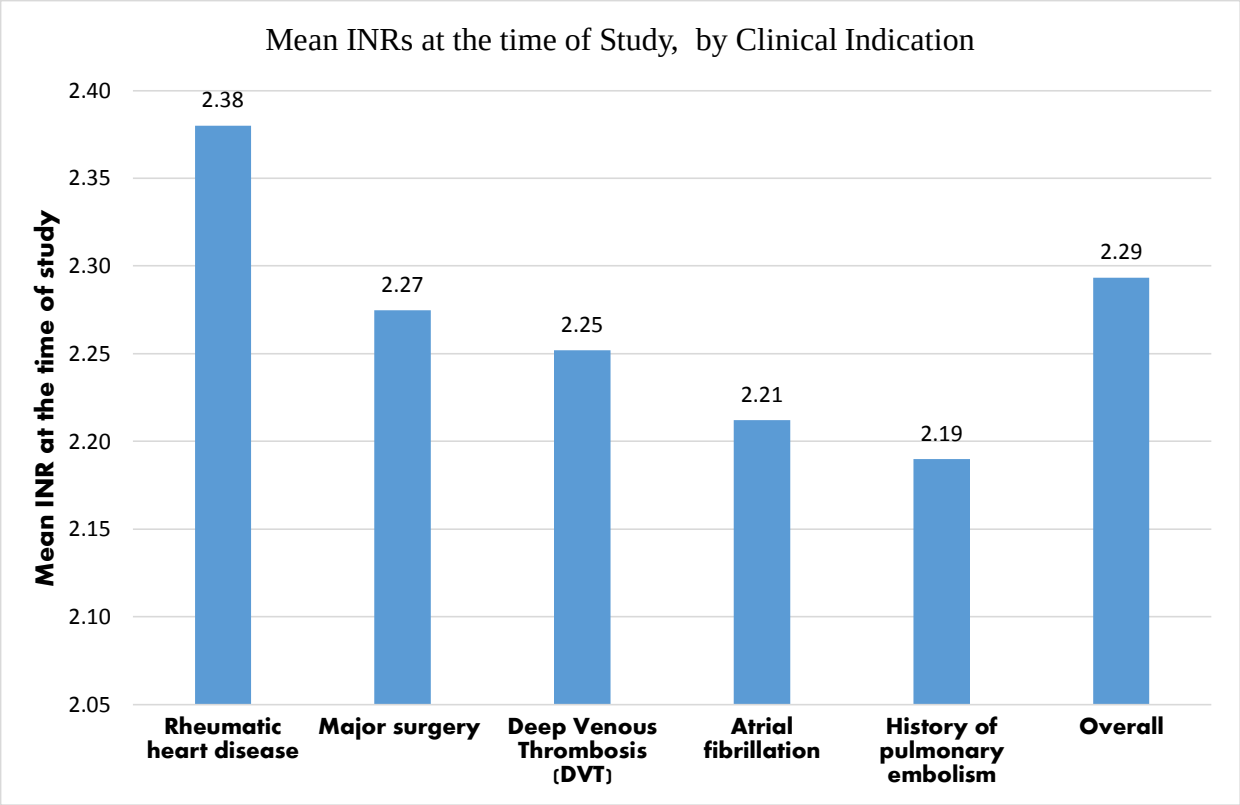


**Figure 4.7: Mean INRs by clinical indication among the study participants**

The overall mean INR for the study participants was 2.29 (normal range 2.0-3.0). Participants with rheumatic heart disease had the highest mean INR (2.28) at the time of the study, followed by those who had undergone major surgery (2.27), DVT (2.25) atrial fibrillation (2.21) and pulmonary embolism (2.19) in that order (**Figure 4.7**).

Participants were followed up for six consecutive clinic visits (as had been determined by the attending clinicians) while assessing the response to warfarin anticoagulation therapy through measurements of INRs. The level of anticoagulation control was assessed by measurement of INRs at various follow up dates. The analysis on the level of anticoagulation is presented in the **Tables 4.7** and **4.8** as well as **Figures 4.8** and **4.9**.

**Table 4.7: Follow up visits and the level of anticoagulation control among the study participants**

| Follow up date | N | Level of Anticoagulation Control | | | | | |
|---|---|---|---|---|---|---|---|
| | | < 2 (Sub-therapeutic) | | 2-3 (Therapeutic) | | >3 (Supra-therapeutic | |
| | | n | % | n | % | n | % |
| Date 1 | 164 | 80 | **48.8** | 58 | **35.4** | 26 | 15.9 |
| Date 2 | 145 | 48 | 33.1 | 66 | **45.5** | 31 | 21.4 |
| Date 3 | 124 | 39 | 31.5 | 60 | **48.4** | 25 | 20.2 |
| Date 4 | 105 | 44 | 41.9 | 37 | **35.2** | 24 | 22.9 |
| Date 5 | 75 | 30 | 40.0 | 29 | **38.7** | 16 | 21.3 |
| Date 6 | 54 | 23 | 42.6 | 26 | **48.1** | 5 | 9.3 |

The proportion of participants who were adequately anticoagulated (INR= 2-3) during follow-ups ranged from 35.2 to 48.4% across the follow-up dates. The ranges were similar for sub therapeutically anticoagulated (INR<2) participants. The highest proportion of adequately anticoagulated participants was found during the third and sixth follow-up (Date 3 and 6, respectively). Almost half of the participants had sub- therapeutic anticoagulation during the first visit (**Table 4.7**).

**Figure 4.8** shows the trend of INRs therapeutic categories at various time points.

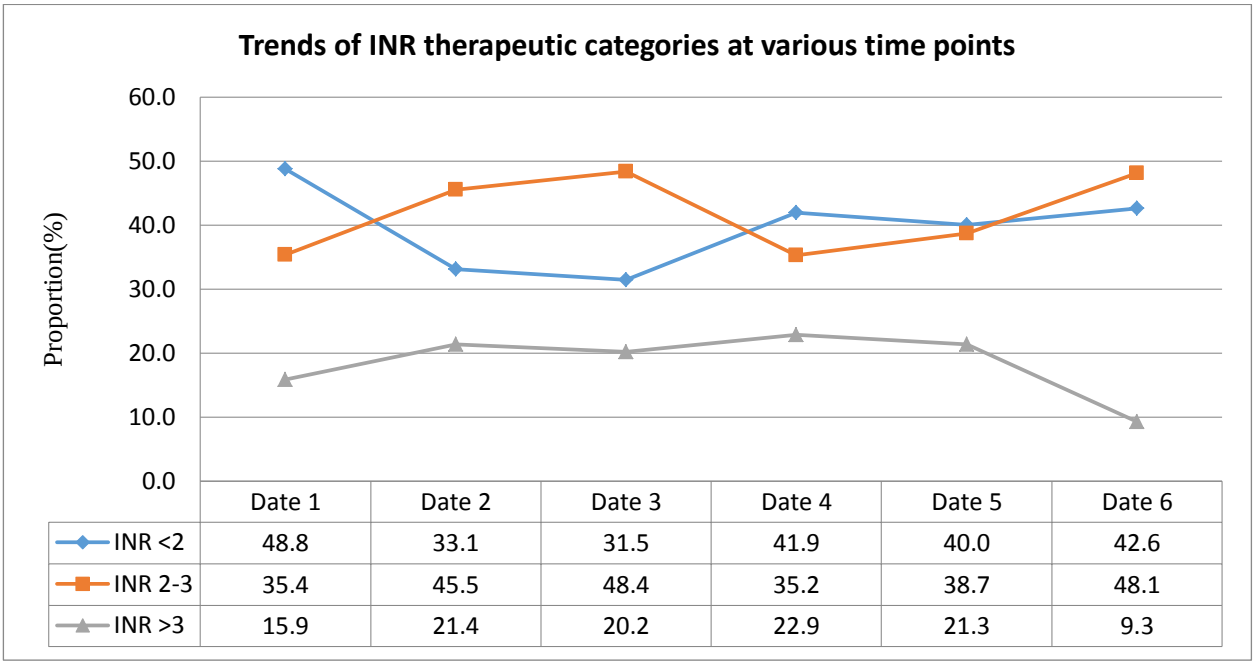


**Figure 4.8: Trend of INR therapeutic categories at various time points**

The proportions of participants with out-of-range INRs were generally more than the therapeutically anticoagulated participants (**Figure 4.8**).

For ease of comparison of the level of anticoagulation control, study participants were classified into two groups according to their clinical indication of heart diseases or VTEs as described above. **Table 4.8** displays the results of analysis.

**Table 4.8: Adequacy of anticoagulation control among the study participants during the follow-up**

| **Indication** | INR | **Date 1** | | **Date 2** | | **Date 3** | | **Date 4** | | **Date 5** | | **Date 6** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Range | n | % | n | % | n | % | n | % | n | % | n | % |
| Heart Diseases (n=75) | <2 | 21 | 44.7 | 17 | 32.7 | 22 | 48.9 | 14 | 35.0 | 9 | 29.0 | 8 | 36.4 |
| | **2-3** | **15** | **31.9** | **24** | **46.2** | **11** | **24.4** | **17** | **42.5** | **14** | **45.2** | **10** | **45.5** |
| | >3 | 11 | 23.4 | 11 | 21.2 | 12 | 26.7 | 9 | 22.5 | 8 | 25.8 | 4 | 18.2 |
| VTEs (n=118) | <2 | 44 | 62.9 | 34 | 26.7 | 28 | 27.8 | 22 | 35.6 | 6 | 56.0 | 4 | 30.8 |
| | **2-3** | **19** | **27.1** | **16** | **16.7** | **15** | **20.4** | **16** | **15.6** | **14** | **20.0** | **4** | **38.5** |
| | >3 | 7 | 10.0 | 10 | 51.9 | 11 | 48.9 | 7 | 24.0 | 5 | 30.8 | 5 | 38.5 |

There were higher proportions of adequate anticoagulated participants (INR 2-3) in the heart disease than in the VTE category across the follow-up dates (**Table 4.8**). This is revealed clearly by the line trend in **Figure 4.9** below. In addition, across the follow up clinics, a larger proportion of participants with INR>3 was seen in VTE group compared to the heart diseases, except on the first follow-up date (**Table 4.8**).

Anticoagulation monitoring during follow up and according to the two broad clinical indications is displayed in **Figure 4.9**.

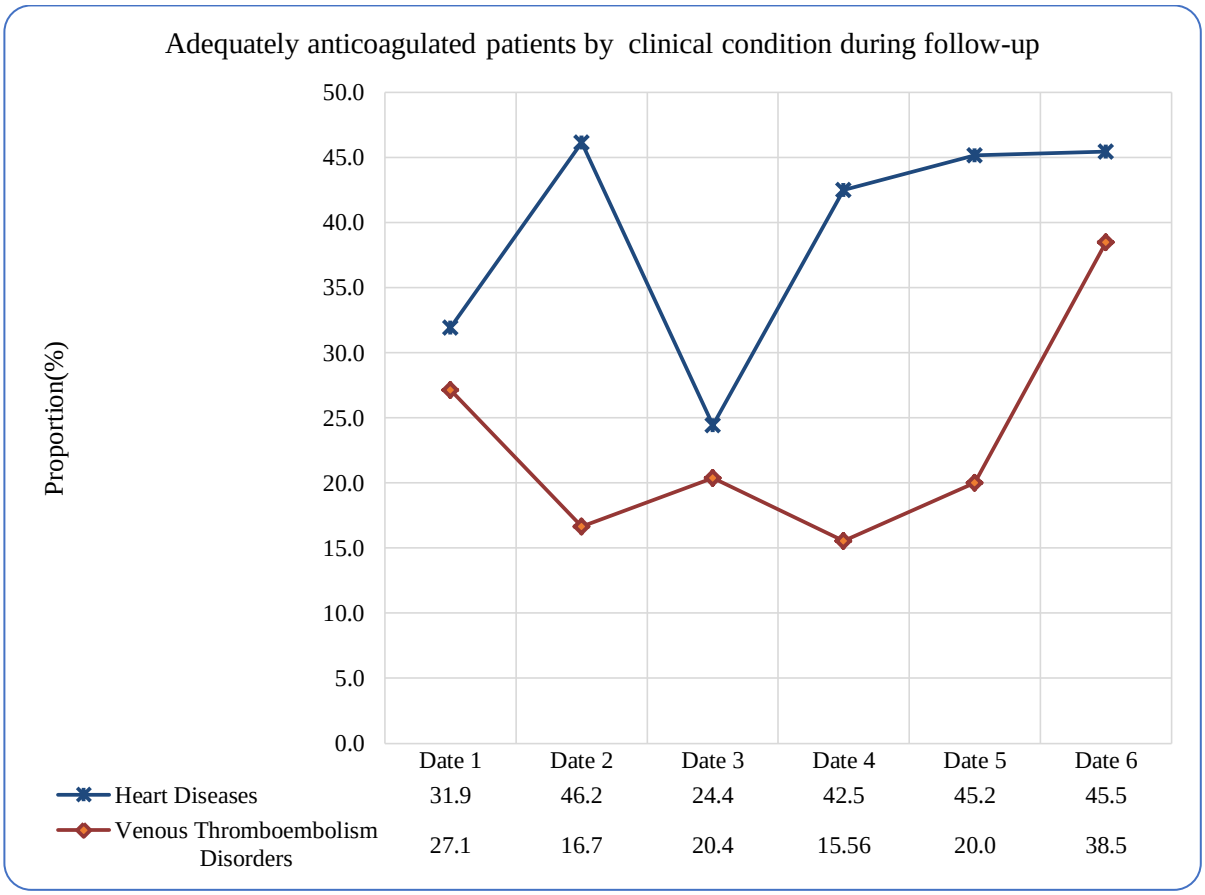


**Figure 4.9: Adequacy of anticoagulation control by clinical condition among the study participants during the participants' follow-up**

Generally, there were higher proportions of adequately anticoagulated participants with heart diseases compared to those with venous thromboembolic events during the participants' follow-up (**Figure 4.9).**

#### 4.2.2.2: Monitoring of Adverse Drug Reactions

Apart from measurement of the INRs, warfarin response was monitored through documentation of ADRs as a result of therapy. Various ADRs and their prevalence as well as the levels of INRs at which they occurred were analyzed. The results are presented in **Figures 4.10** and **4.11**.

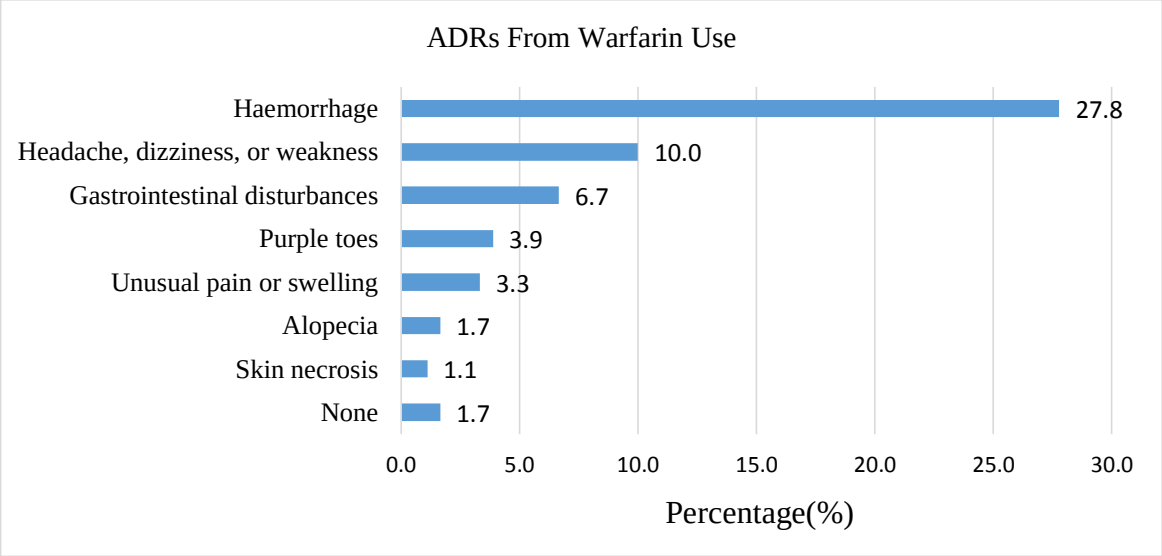


**Figure 4.10: Prevalence of Adverse Drug Reactions of Warfarin as Experienced by the Study Participants**

Key: ADRs-Adverse Drug Reactions

Slightly over half of the participants experienced ADRs with almost 30% presenting with bleeding while 10% had headache, dizziness or weakness. Purple toes, unusual pain or swelling, alopecia and skin necrosis due to warfarin were less common (**Figure 4.10**).

The study also sought to find out the levels of INRs that the ADRs manifested. Only fifty two participants (28.9%) had the INR reported at the time of development of ADRs. **Figure 4.11** shows the INRs ranges at which those ADRs occurred.

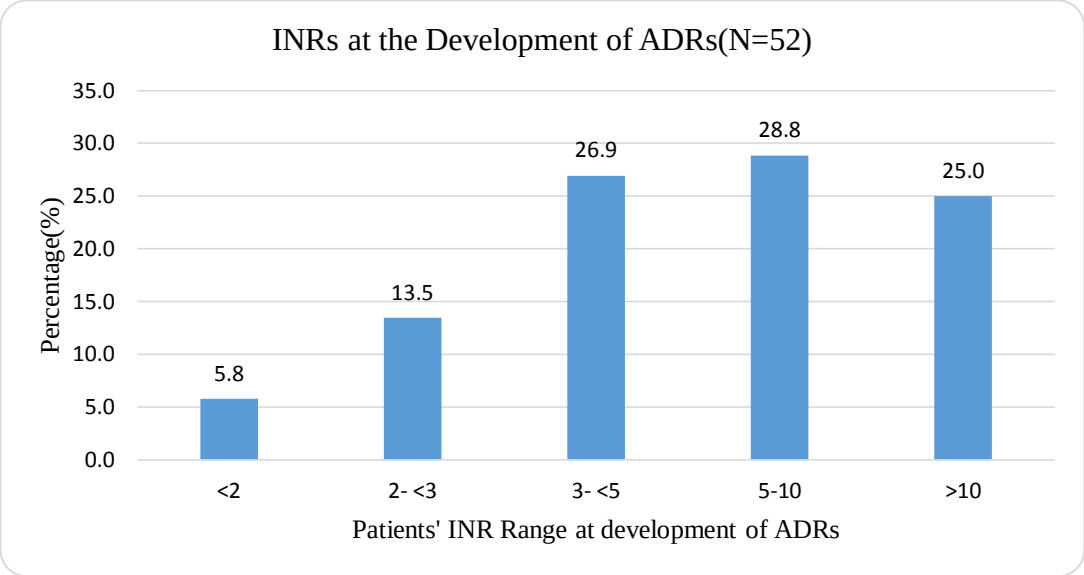


**Figure 4.11: INR ranges at the development of ADRs due to warfarin**

Key: ADRs-Adverse Drug Reactions; INR-International Normalized Ratio

Almost 30% of the ADRs developed at INRs of 5-10. Moreover, over 80% of the ADRs occurred at INRs above 3. However, some ADRs related to warfarin occurred at INR<2(**Figure 4.11**).

### 4.2.3: Inferential Statistical Results for Clinical Predictors of Warfarin Therapy

Inferences were statistically determined between the various predictor and outcome variables of warfarin use as detailed in the conceptual and theoretical framework (**Figure 2.1)** as well as the results from the exploratory analysis described above. For the ease of comparability of outcome variables, participants under the study were classified into two: The ones who were using warfarin because of heart diseases and participants requiring anticoagulation for VTEs.

The sociodemographic characteristics displayed in the descriptive analyses were re-grouped according to the clinical significance in measuring the associations of interest. In this respect, variables with similar characteristics or small numbers were merged in order to make meaningful inferences when using the Pearson's Chi Square or Spearman's correlations. For instance, on the BMI category, participants who were underweight or having normal weight were put together as having BMI≤25. Similarly, participants who had BMI >25 were bundled together. On marital status, participants who were single, divorced or separated or widowed were grouped as 'without

spouses' and vice versa. In addition, small numbers in cells were logically combined. The results on inferential analyses are shown in the succeeding Tables.

The first Table in the bivariate analyses section displays the association between the participants' sociodemographic characteristics and the clinical indication of warfarin anticoagulation.

**Table 4.9: Association between Sociodemographic Characteristics and Clinical Indications of Warfarin Anticoagulation (N=180)**

| Variable | Category | Clinical indication of Warfarin use | | | |
|---|---|---|---|---|---|
| | | Heart Diseases | | VTEs | |
| | | No n (%) | Yes n (%) | No n (%) | Yes n (%) |
| Sex | Male | 18(42.9) | **24(57.1)** | 18(42.9) | 24(57.1) |
| | Female | 85(61.6) | 53(38.4) | 40(29.0) | **98(71.0)** |
| **Group Differences** | | $\chi^2_{(1, 180)}$=4.62; **P=0.032** | | $\chi^2_{(1, 180)}$=2.84; P=0.092 | |
| Age | 50 Years and Below | 69(52.7) | **62(47.3)** | 46(35.1) | **85(64.9)** |
| | Above 50 Years | 34(69.4) | 15(30.6) | 12(24.5) | 37(75.5) |
| **Group differences** | | $\chi^2_{(1, 180)}$=4.07; **P=0.044** | | $\chi^2_{(1, 180)}$=1.84; P=0.175 | |
| Body mass index | Underweight/Normal | 19(32.8) | **39(67.2)** | 27(46.6) | 31(53.4) |
| | Overweight/Obese | 65(72.2) | 25(27.8) | 20(22.2) | **70(77.8)** |
| **Group differences** | | $\chi^2_{(1, 148)}$=22.38; **P<0.001** | | $\chi^2_{(1, 180)}$=9.63; **P=0.002** | |
| Marital status | Without Spouse | 28(45.2) | **34(54.8)** | 27(43.5) | 35(56.5) |
| | With Spouse | 75(63.6) | 43(36.4) | 31(26.3) | **87(73.7)** |
| **Group differences** | | $\chi^2_{(1, 180)}$=5.62; **P=0.018** | | $\chi^2_{(1, 180)}$=5.56**; P=0.018** | |
| Employment status | Non-Regular Income Earners | 72(56.7) | 55(43.3) | 40(31.5) | **87(68.5)** |
| | Regular Income Earners | 31(58.5) | 22(41.5) | 18(34.0) | 35(66.0) |
| **Group differences** | | $\chi^2_{(1, 180)}$=0.05; P=0.824 | | $\chi^2_{(1, 180)}$=0.10; P=0.747 | |
| Highest education level | Below Secondary Education | 36(56.3) | 28(43.8) | 22(34.4) | 42(65.6) |
| | Secondary and Above | 67(57.8) | 49(42.2) | 36(31.0) | 80(69.0) |
| **Group differences** | | $\chi^2_{(1, 180)}$=0.04; P=0.845 | | $\chi^2_{(1, 180)}$=0.21; P=0.646 | |
| Denomination | Christian | 96(56.5) | 74(43.5) | 56(32.9) | 114(67.1) |
| | Muslim and Others | 7(70.0) | 3(30.0) | 2(20.0) | 8(80.0) |
| **Group differences** | | $\chi^2_{(1, 180)}$=0.71; P=0.401 | | $\chi^2_{(1, 180)}$=0.72; P=0.395 | |
| Tobacco use | No | 100(57.8) | 73(42.2) | 55(31.8) | 118(68.2) |
| | Yes | 1(50.0) | 1(50.0) | 1(50.0) | 1(50.0) |
| **Group differences** | | $\chi^2_{(1, 175)}$=0.05; P=0.824 | | $\chi^2_{(1, 175)}$=0.30; P=0.583 | |
| Alcohol Use | No | 93(57.4) | 69(42.6) | 52(32.1) | 110(67.9) |
| | Yes | 7(58.3) | 5(41.7) | 4(33.3) | 8(66.7) |
| **Group differences** | | $\chi^2_{(1, 174)}$=0.00; P=0.950 | | $\chi^2_{(1, 174)}$=0.01; P=0.930 | |

Key: VTEs-Venous Thromboembolic Events

There were statistically significant associations between the gender and use of warfarin for prevention of blood clotting due to heart diseases (p=**0.032**), with a slightly higher proportion of males (57.1%) utilizing the drug. A similar tendency was observed in participants aged ≤ 50 years who showed a statistically significant association with the development of heart diseases (**p=0.0436)** relative to VTEs. The degree of obesity among the participants had statistically significant association with the development of both clinical indications (heart diseases and VTEs) requiring warfarin anticoagulation. Furthermore, there were almost two-thirds, 39 (67.2%) of participants with BMI ≤25 requiring warfarin anticoagulation because of heart diseases (p<0.0001); and 70 (77.8%) participants who had BMI >25 presented with VTEs **(P=0.002)**.

Over half of participants, **34(54.8%)** who were not living with spouses required warfarin anticoagulation because of heart diseases (**p=0.018)** and almost three-quarters, **87 (73.7%)** with spouses were being anticoagulated with vitamin K antagonists for VTEs (**p=0.018**). The status of employment, academic achievement, participants' denomination and recreation activities did not have any statistically significant association with the clinical indication of warfarin anticoagulation (all p>0.05) (**Table 4.9**).

#### 4.2.3.1: Bivariate Analysis on Clinical Determinants of Warfarin Dose Requirements

The various predictor variables studied such as sociodemographic characteristics of the participants as well as the clinical conditions could impact on warfarin doses prescribed. The study explored the relationship between these predictor variables and the warfarin doses prescribed. **Table 4.10** displays the findings.

**Table 4.10: Association between participants' sociodemographic characteristics and the mean initial warfarin doses**

| Variable | Category | Warfarin Mean Initial Dose(mg) | | Group Difference |
|---|---|---|---|---|
| | | n | Mean±SD | |
| Sex | Male | 42 | **6.2±2.5** | $t_{(177)}$=0.72; P=0.474 |
| | Female | 137 | 6.0±2.1 | |
| Age | 50 and Below years | 131 | **6.1±2.2** | $t_{(177)}$=0.41; P=0.682 |
| | Above 50 Years | 48 | 5.9±2.4 | |
| Body mass index | Underweight/Normal | 58 | 6.0±2.3 | $t_{(145)}$=-0.04; P=0.968 |
| | Overweight/Obese | 89 | **6.0±2.2** | |
| Marital status | Without Spouse | 62 | 5.5±2.1 | $t_{(177)}$=-2.18; **P=0.031** |
| | With Spouse | 117 | **6.3±2.3** | |
| Employment status | Non-Regular Income | 127 | 6.0±2.1 | $t_{(177)}$=-0.24; P=0.813 |
| | Regular Income | 52 | 6.1±2.4 | |
| Highest academic level | Below Secondary | 64 | 6.0±2.2 | $t_{(177)}$=-0.31; P=0.760 |
| | Secondary and Above | 115 | 6.1±2.2 | |
| Denomination | Christian | 170 | 6.0±2.2 | $t_{(177)}$=-0.34; P=0.731 |
| | Muslim and Others | 9 | **6.3±1.9** | |
| Tobacco use | No | 172 | 6.0±2.2 | $t_{(172)}$=-0.97; P=0.336 |
| | Yes | 2 | **7.5±0.0** | |
| Alcohol Use | No | 161 | 6.0±2.2 | $t_{(171)}$=0.10; P=0.922 |
| | Yes | 12 | 6.0±1.8 | |

There was a statistically significant association between the mean initial warfarin doses prescribed and the participants' marital status, with participants living with spouses receiving a higher mean initial dose than those without (**6.3±2.3mg vs.** 5.5±2.1mg per day, **p=0.031)**. In contrast, there were no statistically significant associations between mean initial warfarin doses prescribed and; gender, age, body weight, employment status, academic level, denomination and tobacco or alcohol use. However, there were slightly higher prescribed initial warfarin doses among males compared to females; participants aged ≤50 years compared to their older counterparts; Muslims compared to Christians; and those living with spouses compared to the ones without spouses **(Table 4.10).**

The participants' clinical conditions may also determine warfarin dose requirements. The study therefore, sought to find out the relationship between the clinical indications of warfarin

anticoagulation and other clinical conditions with initial doses prescribed by clinicians. The results of analysis are presented in **Table 4.11**.

**Table 4.11: Association between the Participants' Clinical Conditions and the Warfarin doses Prescribed**

| Variable | Category | n | Mean doses | SD | |
|---|---|---|---|---|---|
| | | **Warfarin Initial dose(mg)** | | | |
| Heart Diseases | No | 102 | 6.38 | 2.2 | **$t_{(177)}$=2.49; P=0.014** |
| | Yes | 77 | **5.56** | 2.1 | |
| VTE | No | 58 | 5.69 | 2.1 | t(177)=-1.43; P=0.155 |
| | Yes | 121 | **6.19** | 2.2 | |
| Comorbidities | No | 109 | 5.91 | 2.2 | t(177)=-0.90; P=0.371 |
| | Yes | 70 | **6.21** | 2.3 | |
| | | **Warfarin Maintenance Doses(mg)** | | | |
| Heart Diseases | No | 54 | **5.91** | 2.2 | t(75)=1.71; P=0.092 |
| | Yes | 23 | 5.03 | 1.7 | |
| VTE | No | 58 | 5.95 | 2.6 | t(103)=-0.95; P=0.344 |
| | Yes | 47 | **6.44** | 2.6 | |

Key: SD- Standard Deviation; VTE-Venous Thromboembolic events

Participants who had heart diseases were receiving statistically significant lower initial warfarin doses than those with VTEs or other conditions (5.56±2.2mg vs. 6.38±2.2mg per day, **p=0.014**). Although participants with VTEs and those with comorbidities were receiving higher initial warfarin doses than those who were not suffering from either of the conditions, the findings were not statistically significant (P=0.155 and P=0.371, respectively).

On warfarin maintenance doses, participants being anticoagulated due to heart diseases were receiving slightly lower daily warfarin maintenance doses than those who did not (5.03±1.7mg vs. 5.91±2.2mg). This was, however, not statistically significant. On the other hand, participants diagnosed with VTEs and requiring warfarin maintenance therapy received slightly higher daily doses than those who did not (**6.44±2.6mg vs. 5.95±2.6mg**) and this was also not statistically significant (**Table 4.11**).

Further data analysis was done to explore the relationship between warfarin maintenance doses prescribed and participants' sociodemographic as well as the clinical characteristics. The results of analysis are shown in **Tables 4.12** and **4.13**.

**Table 4.12: Association between sociodemographic Characteristics and the Mean Maintenance dose of warfarin anticoagulation in the study population (N=180)**

| Variable ` | Category | Maintenance Dose (mg) in Heart Diseases | | Maintenance Dose in (mg)VTE | |
|---|---|---|---|---|---|
| | | n | Mean±SD | n | Mean±SD |
| Sex | Male | 24 | **6.3±2.8** | 20 | **6.6±2.5** |
| | Female | 53 | 5.4±1.6 | 85 | 6.1±2.6 |
| **Group Difference** | | $t_{(75)}$=1.80; P=0.076 | | $t_{(103)}$=0.80; P=0.426 | |
| Age | 50 years and Below | 62 | **5.8±2.1** | 70 | 6.2±2.7 |
| | Above 50 Years | 15 | 5.1±1.8 | 35 | 6.2±2.5 |
| **Group Difference** | | $t_{(75)}$=1.12; P=0.265 | | $t_{(103)}$=-0.07; P=0.943 | |
| Body mass index | Underweight/Normal | 39 | 5.7±2.3 | 21 | 5.7±2.5 |
| | Overweight/Obese | 25 | **5.9±2.0** | 64 | **6.5±2.8** |
| **Group Difference** | | $t_{(62)}$=-0.41; P=0.681 | | $t_{(83)}$=-1.11; P=0.271 | |
| Marital status | Without Spouse | 34 | 5.1±1.9 | 28 | 6.0±2.5 |
| | With Spouse | 43 | **6.1±2.2** | 77 | **6.2±2.7** |
| **Group Difference** | | $t_{(75)}$=-1.98; **P=0.051** | | $t_{(103)}$=-0.48; P=0.635 | |
| Employment status | Non-Regular Income | 55 | 5.5±1.8 | 73 | 6.1±2.8 |
| | Regular Income | 22 | **6.0±2.7** | 32 | 6.3±2.2 |
| **Group Difference** | | $t_{(75)}$=-0.84; P=0.402 | | $t_{(103)}$=-0.38; P=0.707 | |
| Highest academic level | Below Secondary | 28 | 5.3±1.8 | 37 | **6.3±2.3** |
| | Secondary and Above | 49 | **5.8±2.2** | 68 | 6.1±2.8 |
| **Group Difference** | | $t_{(75)}$=-0.99; P=0.324 | | $t_{(103)}$=0.28; P=0.781 | |
| Denomination | Christian | 74 | **5.7±2.1** | 99 | **6.2±2.7** |
| | Muslim and Others | 3 | 5.4±1.9 | 6 | 5.6±1.0 |
| **Group Difference** | | $t_{(75)}$=0.19; P=0.850 | | $t_{(103)}$=0.52; P=0.604 | |
| Tobacco use | No | 73 | 5.7±2.1 | 102 | 6.2±2.6 |
| | Yes | 1 | 5.0±UD | 1 | 5.0±UD |
| **Group Difference** | | $t_{(72)}$=0.34; P=0.734 | | $t_{(101)}$=0.45; P=0.652 | |
| Alcohol Use | No | 69 | 5.7±2.1 | 94 | **6.2±2.7** |
| | Yes | 5 | 5.8±2.7 | 8 | 5.6±1.8 |
| **Group Difference** | | $t_{(72)}$=-0.04; P=0.968 | | $t_{(100)}$=0.61; P=0.542 | |

Key: UD-undefined

The mean daily warfarin maintenance dose was higher among the participants with VTEs compared to those suffering from heart diseases. **Table 4.12** above also reveals that male participants required slightly higher daily warfarin maintenance doses than the female counterparts in the management of coagulation due to heart diseases and VTEs although this was not statistically significant. Similarly, compared to participants aged >50 years, those aged ≤50 years required slightly higher warfarin maintenance doses (5.8±2.1mg/d vs. 5.1±1.8mg/day) for the management of heart diseases. This difference was, however, not observed in participants undergoing anticoagulation due to VTEs. Additionally, participants who were overweight or obese required slightly higher warfarin maintenance dose per day than the underweight/ normal weight across the two clinical indications (**Table 4.12**).

There were statistically significant associations between marital status and daily warfarin mean maintenance doses for participants suffering from heart diseases. In this respect, participants living with spouses required slightly higher mean warfarin daily maintenance doses per day compared to the ones without spouses (6.1±2.2mg/d vs. 5.1±1.9mg/d, P=0.05).This finding in the mean maintenance dose was also observed in participants living with spouses in the VTE arm (**6.2±2.7mg Vs. 6.0±2.5mg**), although the findings were not statistically significant (**Table 4.12**).

The regular income earners required higher daily warfarin maintenance doses than the non-earners in the heart disease category, although not statistically significant. This was however, not displayed in the VTE category of participants. In heart disease category, participants with secondary and above level of education required higher daily warfarin maintenance doses than the ones with below secondary level of education. This observation was reversed among the participants presenting with VTEs and requiring warfarin anticoagulation (**Table 4.12**).

Pertaining to the denomination, Christians required higher daily warfarin maintenance dose than non-Christians across the two broad clinical indications. Furthermore, non-alcohol users in the VTE arm had to be maintained at a higher warfarin dose than alcohol users (6.2±2.7mg vs. 5.6±1.8mg).This finding was, however, not statistically significant (**Table 4.12**).

**Table 4.13** shows the associations between the clinical indications, other drugs that the participants were using as well as ADRs observed and the mean maintenance doses of warfarin prescribed. The aim of this exploration was to find out whether the other drugs, coupled with the clinical conditions, could influence the maintenance doses of warfarin prescribed.

**Table 4.13: Association between number of co-prescribed and the Warfarin Mean Maintenance doses across the clinical Conditions of Participants**

| Clinical Condition | Other drugs Co-prescribed | n | Warfarin Mean maintenance Dose (mg) ±SD | Group difference |
|---|---|---|---|---|
| Heart Diseases | None | 40 | 5.80±1.74 | $t_{(75)}$=0.68; P=0.496 |
| | ≥1 | 37 | **5.47±2.43** | |
| VTEs | None | 61 | 6.05±2.74 | $t_{(103)}$=-0.54; P=0.590 |
| | ≥1 | 44 | **6.33±2.43** | |
| ADRs | None | 98 | 6.04±2.00 | $t_{(177)}$=0.09; P=0.927 |
| | ≥1 | 81 | 6.01±2.46 | |

Key: ADRs-Adverse Drug Reactions; VTEs-Venous Thromboembolic events

Participants who were suffering from VTEs and were co-prescribed another drug required slightly higher warfarin maintenance doses than their counterparts with heart diseases **(6.33±2.43mg vs. 5.47±2.43mg).** There were no statistically significant associations between the co-prescribed medications and warfarin maintenance doses across the clinical conditions (**Table 4.13**).

The presence or absence of comorbidities among the participants is likely to influence warfarin maintenance doses. The data was analyzed to find out whether the number of comorbidities among the study participants had any statistically significant associations with warfarin maintenance doses. **Table 4.14** below summarizes the findings.

**Table 4.14: Associations between concomitant diseases and mean maintenance doses of warfarin**

| Clinical Indication | Comorbidities | n | Mean Warfarin maintenance doses(mg) ±SD | Group differences |
|---|---|---|---|---|
| Heart diseases | No | 54 | 5.91±2.2 | $t_{(75)}$=1.71; P=0.092 |
| | Yes | 23 | **5.03±1.7** | |
| VTEs | No | 58 | 5.95±2.6 | $t_{(103)}$=-0.95; P=0.344 |
| | Yes | 47 | **6.44±2.6** | |

Key: SD-Standard Deviation; VTE-Venous thromboembolism disorders

There were no statistically significant associations between the presence of other diseases and warfarin maintenance doses across the clinical conditions. However, participants with comorbidities and undergoing anticoagulation due to VTEs required slightly higher warfarin maintenance doses than their counterparts suffering from heart conditions(6.44±2.6mg vs. 5.03±1.7) (**Table 4.14**).

Relationships between the frequencies of consumption of various food types and the mean daily maintenance doses of warfarin were sought with an aim of identifying their impact on the warfarin dose requirements in anticoagulation. The results are displayed in **Table 4.16.**

**Table 4.15: Associations between frequency of consumptions of food types and Mean maintenance doses of warfarin across the clinical conditions**

| **Clinical Condition** | **Frequency of Consumption**<br><br>**Fruits** | **n** | **Mean Warfarin maintenance dose, mg±SD** | **Group Difference** |
|---|---|---|---|---|
| Heart Diseases | <3 Times a Week | 12 | 5.20±1.9 | $t_{(57)}$=-0.99; P=0.328 |
| | 3 Times and above /Week | 47 | 5.78±1.8 | |
| VTEs | <3 Times a Week | 9 | 5.61±2.2 | $t_{(65)}$=-0.96; P=0.339 |
| | 3 Times and above /Week | 58 | **6.63±3.0** | |
| | **Vegetables** | **n** | **Mean±SD** | **Group Difference** |
| Heart Diseases | <3 Times a Week | 15 | 6.21±1.7 | $t_{(64)}$=1.40; P=0.167 |
| | 3 Times and above /Week | 51 | **5.46±1.8** | |
| VTEs | <3 Times a Week | 17 | 6.28±3.9 | $t_{(69)}$=-0.23; P=0.818 |
| | 3 Times and above /Week | 54 | **6.47±2.5** | |
| | **Proteins** | **n** | **Mean±SD** | **Group Difference** |
| Heart Diseases | <3 Times a Week | 12 | 5.40±1.3 | $t_{(35)}$=-0.07; P=0.946 |
| | 3 Times and above /Week | 25 | 5.44±1.8 | |
| VTEs | <3 Times a Week | 10 | 5.60±2.3 | $t_{(41)=}$.0.83; P=0.412 |
| | 3 Times and above/ Week | 33 | **6.33±2.5** | |
| | **Carbohydrates** | **n** | **Mean±SD** | **Group Difference** |
| Heart Diseases | <3 Times a Week | 3 | 5.42±1.9 | $t_{(31)}$=0.36; P=0.719 |
| | 3 Times and above /Week | 30 | 5.08±1.5 | |
| VTEs | <3 Times a Week | 4 | 4.38±1.3 | $t_{(40)}$=-1.67; P=0.103 |
| | 3 Times and above/ Week | 38 | **6.37±2.3** | |

Key: SD-Standard Deviation; VTEs-Venous thromboembolic events

Largely, participants consuming vegetables at varying frequencies per week required higher warfarin maintenance doses than other category of foods across the clinical conditions. Additionally, participants suffering from VTEs and consuming various food types for 3 or more times per week required slightly higher mean warfarin maintenance doses per day compared to those presenting with heart diseases. The frequency of consumption of the various food types, however, did not statistically significantly impact on warfarin maintenance doses (**Table 4.15**).

Although the number of participants consuming nutritional supplements was low, the relationship between the two main commonly used supplement, garlic and ginger, and warfarin maintenance doses was sought. The findings are presented in **Table 4.16**.

**Table 4.16: Association between warfarin maintenance doses and consumption of herbal supplements**

| Clinical Condition | Garlic Supplements | n | Mean Warfarin maintenance doses(mg) ±SD | Group Differences |
|---|---|---|---|---|
| Heart Diseases | No | 64 | 5.66±2.1 | $t_{(75)=}$0.14; P=0.890 |
| | Yes | 13 | **5.57±2.1** | |
| VTEs | No | 84 | 6.11±2.6 | $t_{(103)}$=-0.44; P=0.660 |
| | Yes | 21 | **6.39±2.8** | |
| | **Ginger supplements** | | | |
| Heart Diseases | No | 68 | 5.69±2.1 | $t_{(75)}$=0.57; P=0.569 |
| | Yes | 9 | **5.27±1.6** | |
| VTEs | No | 92 | 6.17±2.6 | $t_{(103)}$=-0.01; P=0.993 |
| | Yes | 13 | **6.17±2.6** | |

Key: SD-Standard Deviation; VTEs-Venous thromboembolic events

There were no statistically significant associations between consumption of garlic and ginger supplements with warfarin maintenance doses. However, participants with VTEs and using either of the supplements required slightly higher maintenance doses than their counterparts suffering from heart diseases (**Table 4.16**).

#### 4.2.3.2: Bivariate Analysis on Clinical Predictors of Warfarin Response as Measured by INRs

The study explored the relationships between the sociodemographic characteristics (predictor variables) and the response to warfarin anticoagulation among the participants as measured by the levels of INRs (the outcome variable).The results of analysis are displayed in the **Table 4.17**.

**Table 4.17: Association between Participants Sociodemographic Characteristics and Warfarin response as Measured by INRs**

| Variable | Category | INR Level | | | Group Differences |
|---|---|---|---|---|---|
| | | <2 | 2-3 | >3 | |
| | | n (%) | n (%) | n (%) | |
| Sex | Male | 16(41.0) | **20(51.3)** | 3(7.7) | $\chi^2_{(2, 164)}$=6.45; **P=0.0398** |
| | Female | 64(51.2) | 38(30.4) | 23(18.4) | |
| Age | 50 and Below years | 59(50.0) | 41(34.7) | 18(15.3) | $\chi^2_{(2, 164)}$=0.27; P=0.874 |
| | Above 50 Years | 21(45.7) | 17(37.0) | 8(17.4) | |
| Body mass index | Underweight/Normal | 29(58.0) | 16(32.0) | 5(10.0) | $\chi^2_{(2, 134)}$=4.78; P=0.092 |
| | Overweight/Obese | 33(39.3) | 35(41.7) | 16(19.0) | |
| Marital status | Without Spouse | 32(53.3) | 16(26.7) | 12(20.0) | $\chi^2_{(2, 164)}$=3.45; P=0.178 |
| | With Spouse | 48(46.2) | 42(40.4) | 14(13.5) | |
| Employment status | Non-Regular Income | 55(46.2) | 46(38.7) | 18(15.1) | $\chi^2_{(2, 164)}$=2.06; P=0.358 |
| | Regular Income | 25(55.6) | 12(26.7) | 8(17.8) | |
| Highest academic level | Below Secondary | 31(51.7) | 19(31.7) | 10(16.7) | $\chi^2_{(2, 164)}$=0.57; P=0.753 |
| | Secondary and Above | 49(47.1) | **39(37.5)** | 16(15.4) | |
| Denomination | Christian | 74(48.1) | 54(35.1) | 26(16.9) | $\chi^2$ (2, 164)=2.03; P=0.363 |
| | Muslim and Others | 6(60.0) | **4(40.0)** | 0(0.0) | |
| Tobacco use | No | 78(49.7) | 55(35.0) | 24(15.3) | $\chi^2$ (2, 159)=2.21; P=0.331 |
| | Yes | 1(50.0) | 0(0.0) | 1(50.0) | |
| Alcohol Use | No | 73(49.7) | 50(34.0) | 24(16.3) | $\chi^2$ (2, 158)=0.76; P=0.684 |
| | Yes | 5(45.5) | 5(45.5) | 1(9.1) | |

Key: INR-International Normalized Ratio

There was a statistically significant difference between gender and the warfarin response as measured by the INRs ($p$=0.040) where a larger proportion of males were found to be better anticoagulated than the female counterparts. Furthermore, compared to males, there were higher proportions of females with sub-therapeutic and supra-therapeutic levels of INRs. Although there were no statistically significant associations between other sociodemographic variables and level of INRs, the proportions of adequately anticoagulated participants increased with increasing age

and body weight as well as education above secondary level. A higher proportion of income earners had sub-therapeutic INR level compared to non-income earners. In addition, almost half of the few alcohol consumers had sub-therapeutic INR values (**Table 4.17**).

**Table 4.18: Association between Sociodemographic Characteristics and the Response to Warfarin as measured by INR within or out-of-therapeutic range**

| Variable | Category | INR; In-range | INR; Out- of-Range | Group differences |
|---|---|---|---|---|
| | | n (%) | n (%) | |
| Sex | Male | **20(51.3)** | 19(48.7) | $\chi^2_{(1, 164)}$=5.67; **P=0.017** |
| | Female | 38(30.4) | **87(69.6)** | |
| Age | 50 and Below years | 41(34.7) | 77(65.3) | $\chi^2_{(1, 164)}$=0.07; P=0.790 |
| | Above 50 Years | **17(37.0)** | 29(63.0) | |
| Body mass index | Underweight/Normal | 16(32.0) | 34(68.0) | $\chi^2_{(1, 134)}$=1.24; P=0.265 |
| | Overweight/Obese | **35(41.7)** | 49(58.3) | |
| Marital status | Without Spouse | 16(26.7) | 44(73.3) | $\chi^2_{(1, 164)}$=3.13; P=0.077 |
| | With Spouse | **42(40.4)** | 62(59.6) | |
| Employment status | Non-Regular Income | **46(38.7)** | **73(61.3)** | $\chi^2_{(1, 164)}$=2.05; P=0.152 |
| | Regular Income | 12(26.7) | 33(73.3) | |
| Highest academic level | Below Secondary | 19(31.7) | 41(68.3) | $\chi^2_{(1, 164)}$=0.57; P=0.452 |
| | Secondary and Above | **39(37.5)** | 65(62.5) | |
| Denomination | Christian | 54(35.1) | 100(64.9) | $\chi^2_{(1, 164)}$=0.10; P=0.752 |
| | Muslim and Others | 4(40.0) | 6(60.0) | |
| Tobacco use | No | 55(35.0) | 102(65.0) | $\chi^2_{(1, 159)}$=1.07; P=0.301 |
| | Yes | 0(0.0) | 2(100.0) | |
| Alcohol Use | No | 50(34.0) | 97(66.0) | $\chi^2_{(1, 158)}$=0.59; P=0.442 |
| | Yes | 5(45.5) | 6(54.5) | |

Key: INR-International Normalized Ratio

There was a statistically significant association between the participants' gender and the INRs measured. While half of the males had good response to anticoagulation therapy, almost 70% of the females had poor response (out –of-range INRs) to warfarin treatment. Similar proportions of out-of-range INRs were observed in participant aged ≤ 50 years and the older ones.

Approximately two-fifths of participants either living with their spouses or who were obese/overweight had good response although this was not statistically significant. In addition, over 70% of non-regular income earners and those who had attained at least a secondary level of education had poor response to warfarin therapy. This was, however, not statistically significant. More than half of the participants using alcohol had poor response to warfarin (**Table 4.18**).

The data was also analyzed with an aim of determining whether the participants' clinical conditions impacted on warfarin response as measured by the levels of INR therapeutic categories. This is displayed in **Table 4.19**.

**Table 4.19: Relationships between the participants' clinical condition and the warfarin response as measured by the level of INRs**

| Clinical condition | Category | INR | | | Group Difference |
|---|---|---|---|---|---|
| | | <2 n (%) | 2-3 n (%) | >3 n (%) | |
| Comorbidities | No | 49(47.6) | 40(38.8) | 14(13.6) | $\chi^2_{(2, 164)}$=1.92; P=0.383 |
| | Yes | **31(50.8)** | 18(29.5) | 12(19.7) | |
| Heart Diseases | No | 44(49.4) | 28(31.5) | 17(19.1) | $\chi^2_{(2, 164)}$=2.15; P=0.341 |
| | Yes | 36(48.0) | **30(40.0)** | 9(12.0) | |
| Venous Thromboembolism Disorders | No | 28(49.1) | 21(36.8) | 8(14.0) | $\chi^2_{(2, 164)}$=0.24; P=0.888 |
| | Yes | 52(48.6) | **37(34.6)** | 18(16.8) | |

Key: INR-International Normalized Ratio

There was no statistically significant association between the participants' clinical conditions and the warfarin response as measured by levels of INR. However, there was a larger proportion of participants with heart diseases who were therapeutically anticoagulated compared to VTEs (40.0%vs. 34.6%) (**Table 4.19**).

The relationship between the numbers of co-prescribed medications warfarin response as measured by the INR was also investigated. The relationship is displayed in **Table 4.20**.

**Table 4.20: Association between the number of co-prescribed medicines and Warfarin response as Measured by INRs**

| Number of other drugs prescribed | INR Range | | | Group Difference |
|---|---|---|---|---|
| | <2 n (%) | 2-3 n (%) | >3 n (%) | |
| None | 46(51.1) | 30(33.3) | 14(15.6) | $\chi^2_{(14, 164)}$=14.94; P=0.382 |
| One | 16(55.2) | 10(34.5) | 3(10.3) | |
| Two | 4(20.0) | 11(55.0) | 5(25.0) | |
| Three | 8(53.3) | 4(26.7) | 3(20.0) | |
| Four | 4(66.7) | 1(16.7) | 1(16.7) | |
| Five | 0(0.0) | 2(100.0) | 0(0.0) | |
| Six | 1(100.0) | 0(0.0) | 0(0.0) | |
| Seven | 1(100.0) | 0(0.0) | 0(0.0) | |
| **None** | 46(51.1) | 30(33.3) | 14(15.6) | $\chi^2_{(2, 164)}$=2.94; P=0.792 |
| **One and above** | 34(45.9) | 28(37.8) | 12(16.2) | |

Key: INR-International Normalized Ratio

There were no statistically significant associations between the number of co-prescribed medications and the level of INR measurements. The proportions of under-anticoagulated participants mostly remained above 50.0% across the number of co-prescribed medicines. Additionally, an average of 10-20 % participants remained over-anticoagulated across the number of co-prescribed medications. Additionally, participants maintaining therapeutic INRs decreased as the number of concomitant medications increased (**Table 4.20**).

Analysis was also done to establish the existence of associations between the number of other co-prescribed medications and out-of-range or within range INRs. The findings are as presented in **Table 4.21**.

**Table 4.21: Association between the number of co-prescribed medicines and warfarin response as measured by INR within or out-of-therapeutic range**

| Other Drugs prescribed | INR Range | | Group Difference |
|---|---|---|---|
| | In-range n (%) | Out- of- Range n (%) | |
| None | 30(33.3) | 60(66.7) | $\chi^2_{(7, 164)}$=7.94; P=0.206 |
| One | 10(34.5) | 19(65.5) | |
| Two | 11(55.0) | 9(45.0) | |
| Three | 4(26.7) | 11(73.3) | |
| Four | 1(16.7) | 5(83.3) | |
| Five | 2(100.0) | 0(0.0) | |
| Six | 0(0.0) | 1(100.0) | |
| Seven | 0(0.0) | 1(100.0) | |
| **None** | 30(33.3) | 60(66.7) | $\chi^2_{(1, 164)}$=1.94; P=0.548 |
| **One and above** | 28(37.8) | 46(62.2) | |

Key: INR-International Normalized Ratio

Regardless of the number of co-prescribed medications, the proportions of participants maintaining INR within range remained slightly above 30.0%. There was inverse relationship on the proportions of participants having in-range INRs and the number of concomitant medications. However, there were no statistically significant associations between the numbers of co-prescribed medications and warfarin response as measured by INRs when classified as within or out-of-range (**Table 4.21**).

Some foods may also interact with warfarin and thereby affect its response, in terms of the measured INRs. Associations between frequency of consumption of foods and the level of INRs were determined. Results are shown in **Tables 4.22** and **4.23**.

**Table 4.22: Association between the frequency of consumption of food types and Warfarin response as measured by INRs**

| Type of Food | Frequency of Consumption | INR Range | | | Group Difference |
|---|---|---|---|---|---|
| | | <2 n (%) | 2-3 n (%) | >3 n (%) | |
| **Fruits** | <3 Times a Week | 9(52.9) | 4(23.5) | 4(23.5) | $\chi^2_{(2, 116)}$=1.63; P=0.442 |
| | 3 Times and above /Week | 46(46.5) | 38(38.4) | 15(15.2) | |
| **Vegetables** | <3 Times a Week | 15(55.6) | 7(25.9) | 5(18.5) | $\chi^2_{(2, 123)}$=1.27; P=0.531 |
| | 3 Times and above /Week | 46(47.9) | **36(37.5)** | 14(14.6) | |
| **Proteins** | <3 Times a Week | 9(47.4) | 6(31.6) | 4(21.1) | $\chi^2_{(2, 72)}$=1.63; P=0.442 |
| | 3 Times and above /Week | 23(43.4) | 24(45.3) | 6(11.3) | |
| **Carbohydrates** | <3 Times a Week | 3(50.0) | 2(33.3) | 1(16.7) | $\chi^2_{(2, 65)}$=0.38; P=0.827 |
| | 3 Times and above /Week | 27(45.8) | 26(44.1) | 6(10.2) | |

Key: INR-International Normalized Ratio

**Table 4.23: Association between frequencies of consumption of food types and the response to warfarin as measured by INRs within or out-of-therapeutic range**

| Type of Food | Frequency of Taking | INR Range | | Group Difference |
|---|---|---|---|---|
| | | Within Range n (%) | Out- of -Range n (%) | |
| **Fruits** | <3 Times a Week | 4(23.5) | 13(76.5) | $\chi^2_{(1, 116)}$=1.39; P=0.239 |
| | 3 Times and above /Week | 38(38.4) | 61(61.6) | |
| **Vegetables** | <3 Times a Week | 7(25.9) | 20(74.1) | $\chi^2_{(1, 123)}$=1.24; P=0.265 |
| | 3 Times and above /Week | 36(37.5) | 60(62.5) | |
| **Proteins** | <3 Times a Week | 6(31.6) | 13(68.4) | $\chi^2_{(1, 72)}$=1.08; P=0.299 |
| | 3 Times and above /Week | 24(45.3) | 29(54.7) | |
| **Carbohydrates** | <3 Times a Week | 2(33.3) | 4(66.7) | $\chi^2_{(1, 65)}$=0.26; P=0.613 |
| | 3 Times and above /Week | 26(44.1) | 33(55.9) | |

Key: INR-International Normalized Ratio

There were no statistically significant associations between the frequency of consumption of various food types and the participants' level of INRs. The proportions participants with therapeutic response to warfarin therapy remained below 40% regardless of the frequency of consumption of any particular food type (**Tables 4.22** and **4.23**).

The present study explored the relationship between use of the nutritional and herbal preparations versus the warfarin response as measured by the level of INRs. The findings are presented below.

**Table 4.24: Association between use of nutritional supplements and level of INR among the study population**

| Supplement | Use | INR Range | | | Group Difference |
|---|---|---|---|---|---|
| | | <2 | 2-3 | >3 | |
| Garlic herbal preparations | No | 65(48.1%) | **47(34.8%)** | 23(17.0%) | $\chi^2_{(2, 164)}$=0.80; P=0.670 |
| | Yes | 15(51.7%) | **11(37.9%)** | 3(10.3%) | |
| Ginger herbal preparations | No | 70(48.3%) | **52(35.9%)** | 23(15.9%) | $\chi^2_{(2, 164)}$=0.15; P=0.927 |
| | Yes | 10(52.6%) | **6(31.6%)** | 3(15.8%) | |

Key: INR-International Normalized Ratio

Approximately a third of the participants had INR 2-3 across the use of the nutritional supplements. Additionally, less than 20% were over-anticoagulated (INR>3) while almost half were under-anticoagulated (INR<2). However, there were no statistically significant associations between the use of various nutritional supplements and level of INRs (**Table 4.24**).

The study also sought to find out whether use of garlic and ginger had any statistical significance with within range and out-of-range INRs. The findings are presented below.

**Table 4.25: Association between use of nutritional supplements and the Response to Warfarin as measured by INR within or out-of-therapeutic range**

| Supplement | Use | INR Range | | Group Difference |
|---|---|---|---|---|
| | | Within Range n (%) | Out -of –Range n (%) | |
| Garlic herbal preparations | No | 47(34.8) | 88(65.2) | $\chi^2_{(1, 164)}$=0.10; P=0.750 |
| | Yes | 11(37.9) | 18(62.1) | |
| Ginger herbal preparations | No | 52(35.9) | 93(64.1) | $\chi^2_{(1, 164)}$=0.14; P=0.713 |
| | Yes | 6(31.6) | 13(68.4) | |

Key: INR-International Normalized Ratio

There were no statistically significant associations between use of the nutritional supplements and warfarin response as measurement by within or out-of-range INRs. However, almost two-thirds of the participants, either using garlic or ginger supplements had out-of-range INR (**Table 4.25**).

### 4.2.3.3: Bivariate Analysis on Clinical Determinants of ADRs to Warfarin Therapy

The response to warfarin was measured by occurrence of adverse reaction to the drug. To find out the factors associated with this response, associations were determined between the sociodemographic characteristics and ADRs. There was a statistically significant association between marital status and development of ADRs to warfarin therapy, where 59.7 % of participants without spouses experienced some ADRs to warfarin therapy (**P=0.008**).

**Table 4.26: Association between participants' sociodemographic and warfarin response as measured by presence or absence of ADRs**

| Variable | Category | ADRs | | Group differences |
|---|---|---|---|---|
| | | No | Yes | |
| | | n (%) | n (%) | |
| Sex | Male | 23(54.8) | **19(45.2)** | $\chi^2_{(1, 180)}$=0.02; P=0.897 |
| | Female | 74(53.6) | **64(46.4)** | |
| Age | 50 and Below years | 66(50.4) | **65(49.6)** | $\chi^2_{(1, 180)}$=2.38; P=0.123 |
| | Above 50 Years | 31(63.3) | 18(36.7) | |
| Body mass index | Underweight/Normal | 30(51.7) | 28(48.3) | $\chi^2_{(1, 148)}$=0.11; P=0.746 |
| | Overweight/Obese | 49(54.4) | 41(45.6) | |
| Marital status | Without Spouse | 25(40.3) | **37(59.7)** | $\chi^2_{(1, 180)}$=7.01; **P=0.008** |
| | With Spouse | 72(61.0) | 46(39.0) | |
| Employment status | Non-Regular Income | 71(55.9) | 56(44.1) | $\chi^2_{(1, 180)}$=0.71; P=0.401 |
| | Regular Income | 26(49.1) | **27(50.9)** | |
| Highest academic level | Below Secondary | 37(57.8) | 27(42.2) | $\chi^2_{(1, 180)}$=0.62; P=0.433 |
| | Secondary and Above | 60(51.7) | **56(48.3)** | |
| Denomination | Christian | 90(52.9) | **80(47.1)** | $\chi^2_{(1, 180)}$=1.11; P=0.293 |
| | Muslim and Others | 7(70.0) | 3(30.0) | |
| Tobacco use | No | 94(54.3) | 79(45.7) | $\chi^2_{(1, 175)}$=2.35; P=0.126 |
| | Yes | 0(0.0) | 2(100.0) | |
| Alcohol use | No | 88(54.3) | 74(45.7) | $\chi^2_{(1, 174)}$=0.72; P=0.397 |
| | Yes | 5(41.7) | **7(58.3)** | |

Key: ADRs-Adverse Drug Reactions

The proportions of males and females developing ADRs to warfarin were almost the same. However, there was a larger proportion of participants aged ≤50 years who developed ADRs to warfarin compared to those aged >50 years (49.6 % vs. 36.7 %), although not statistically significant. Almost half (48.3%) of the participants who were underweight/normal weight developed some form of ADRs whereas slightly more than half (54.4%) of the obese/overweight participants did not. Half of the regular income earners and 44.1% of non-regular income earners had ADRs. Similar proportions were noted for highest academic levels of participants. More than half of the alcohol users and all the smokers experienced the ADRs to warfarin therapy. All these observations were however, not statistically significantly associated with development of ADRs (**Table 4.26**).

The study further sought to measure whether the participants' clinical characteristics impacted on the ADRs to warfarin therapy. The Table below summarizes the revelations.

**Table 4.27: Relationships between participants' clinical conditions and warfarin response as measured by presence or absence of ADRs**

| Clinical Characteristics | Category | Adverse Drug Reaction | | Group Difference |
|---|---|---|---|---|
| | | No, n (%) | Yes, n (%) | |
| Comorbidities | No | 64(58.2) | 46(41.8) | $\chi^2_{(1, 180)}$=2.10; P=0.148 |
| | Yes | 33(47.1) | 37(52.9) | |
| Number of other drugs | None | 55(55.6) | 44(44.4) | $\chi^2_{(1, 180)}$=0.25; P=0.620 |
| | One and above | 42(51.9) | 39(48.1) | |
| Heart Diseases | No | 62(60.2) | 41(39.8) | $\chi^2_{(1, 180)}$=3.85; **P=0.049** |
| | Yes | 35(45.5) | 42(54.5) | |
| Venous Thromboembolism Disorders | No | 26(44.8) | 32(55.2) | $\chi^2_{(1, 180)}$=2.83; P=0.093 |
| | Yes | 71(58.2) | 51(41.8) | |

There was a statistically significant association between participants using warfarin due to heart diseases and the development of ADRs (**p=0.049**). Slightly more than half of the participants (54.5%) with heart diseases developed ADRs to warfarin therapy. Other clinical characteristics of the participants such as presence of comorbidities, polypharmacy and VTEs did not have any statistically significant associations with the development of ADRs (**Table 4.27**).

Dietary intake may impact on the development of ADRs to warfarin therapy. The association between the frequency of consumption of various food types and the response to warfarin as

measured by the adverse drug reaction that the participants experienced was explored. These findings are presented in **Table 4.28**.

**Table 4.28: Association between Frequency of Consumption of the various food types and the presence or absence of ADRs to Warfarin among the Study Participants**

| Food Type | Frequency of Consumption | Warfarin Adverse Drug Reaction | | Group Differences |
|---|---|---|---|---|
| | | No | Yes | |
| | | n (%) | n (%) | |
| Fruits | <3 Times a Week | 9(47.4) | 10(52.6) | $\chi^2_{(1, 124)}$=0.16; P=0.687 |
| | ≥3 Times a Week | 55(52.4) | 50(47.6) | |
| Vegetables | <3 Times a Week | 18(58.1) | 13(41.9) | $\chi^2_{(1, 135)}$=0.62; P=0.430 |
| | ≥3 Times a Week | 52(50.0) | 52(50.0) | |
| Proteins | <3 Times a Week | 9(42.9) | 12(57.1) | $\chi^2_{(1, 78)}$=0.59; P=0.444 |
| | ≥3 Times a Week | 30(52.6) | 27(47.4) | |
| Carbohydrates | <3 Times a Week | 2(28.6) | 5(71.4) | $\chi^2_{(1, 73)}$=1.92; P=0.166 |
| | ≥3 Times a Week | 37(56.1) | 29(43.9) | |

There were no statistically significant associations between the weekly consumption of various food types and the occurrence of ADRs to warfarin. However, more than 40% of the participants consuming various food types had experienced the ADRs to warfarin. Furthermore, half of the participants consuming vegetables for three or more times per week experienced some ADRs to warfarin. In addition, slightly more than half (52.6%) of the participants taking fruits for less than 3 three times per week experienced some ADRs to warfarin therapy(**Table 4.28**).

The study also explored whether consumption of various food supplements may influence on the development of ADRs to warfarin among the study population. The Table below gives the findings.

**Table 4.29: Relationship between consumption of various food supplements and warfarin response as measured by presence or absence of ADRs**

| Supplement | Use | Adverse Drug Reaction | | Group Difference |
|---|---|---|---|---|
| | | No | Yes | |
| Garlic herbal preparations | No | 83(56.8%) | 63(43.2%) | $\chi^2_{(1, 180)}$=2.73; P=0.099 |
| | Yes | 14(41.2%) | **20(58.8%)** | |
| Ginger herbal preparations | No | 86(54.4%) | 72(45.6%) | $\chi^2_{(1, 180)}$=0.15; P=0.696 |
| | Yes | 11(50.0%) | **11(50.0%)** | |

The proportion of participants who developed ADRs to warfarin and were consuming garlic was higher than the ones taking ginger (**58.8 % vs. 50.0%**). However, there were no statistically significant difference between consumption of herbal supplements and development of ADRs to warfarin therapy (**Table 4.29**).

### 4.2.4: Generalized Linear Regression Model for Independent Predictors of Warfarin Dose and Response

#### 4.2.4.1: Multivariate Analysis on Factors Impacting on Warfarin Dose Requirements

At bivariate analysis, marital status (p=0.031) and use of warfarin due to diagnosis of heart disease (p=0.014) were found to be statistically significantly associated with initial warfarin dose requirements. Additionally, marital status was statistically significantly associated with warfarin maintenance dose requirements at bivariate analysis (p=0.05). Therefore, marital status and diagnosis of heart diseases were subjected to multivariate generalized linear model using identity link with the participants without spouses and clinical indication of heart disease as references. The analysis revealed that other diagnosis of anticoagulation apart from heart disease were the independent predictor of higher initial doses (6.24mg vs. 5.52mg) of warfarin **(β=0.72, CI: 0.07, 1.36; p=0.030) (Table 4.30).**

**Table 4.30: Multivariate Analysis of Factors associated with Warfarin Dose**

| Parameter | Category significant at bivariate analysis | Estimated marginal means of warfarin doses(mg) | β | S.E | 95% C.I. | | P-Value |
|---|---|---|---|---|---|---|---|
| | | | | | Lower | Upper | |
| Marital status | Without Spouse | 5.57 | -0.62 | 0.34 | -1.29 | 0.05 | 0.071 |
| | With Spouse | 6.19 | Ref. | | | | |
| Indication | Other indications | **6.24** | **0.72** | 0.33 | 0.07 | 1.36 | **0.030** |
| | Heart diseases | 5.52 | Ref. | | | | |
| Intercept | | | 5.83 | 0.29 | 5.27 | 6.40 | <0.001 |

Key: β-Regression coefficient; C.I-Confidence Interval; S.E-Standard error

**4.2.4.2: Multivariate Analysis on Factors Impacting on Warfarin Response as Measured by ADRs**

Bivariate analysis revealed that only participants' gender was statistically significant on the levels of therapeutic INRs in anticoagulation using warfarin. Additionally, marital status ($p$=0.008) and a clinical indication of heart disease ($p$=0.05) were statistically significant factors for the development of ADRs to warfarin therapy. Therefore, marital status and diagnosis of heart diseases were subjected to multivariate analysis using generalized linear model with a logit link to identify independent predictors of warfarin response as measured by ADRs. The multilevel analysis showed that only the marital status was the independent predictor of ADRs to warfarin, with those living without spouses being two times more likely to have adverse drug reaction to warfarin therapy **(β=0.76; 95% CI: 1.13, 4.06; A.O.R=2.14, $p$=0.019) (Table 4.31).**

**Table 4.31: Multivariate Analysis of Factors associated with Adverse Drug Reactions**

| Parameter | Category significant at bivariate analysis | β | S.E | A.O.R | 95% C.I. | | *p*-value |
|---|---|---|---|---|---|---|---|
| | | | | | Lower | Upper | |
| Marital status | Without Spouse | **0.76** | 0.33 | **2.14** | 1.13 | 4.06 | **0.019** |
| | With Spouse | Ref. | | 1 | | | |
| Indication | Other indications | -0.48 | 0.31 | 0.62 | 0.33 | 1.14 | 0.122 |
| | Heart Diseases | Ref. | | 1 | | | |

Key: A.O.R-Adjusted Odds Ratio; β-Regression coefficient; C.I-Confidence Interval; S.E-Standard error

## 4.3: Genetic Determinants of Warfarin Response in the Study Population

### 4.3.1. Socio-demographics and Clinical Characteristics of Participants Genotyped

A total of one hundred and ten participants provided the blood samples for the genetic testing. However, DNA was extracted from 105 specimens because five were excluded due to various reasons (**Figure 4.12)**. In addition, genetic testing was done among 40 participants owing to the exorbitant costs of genotyping. **Appendix IX** shows the DNA yields in ng/ml in the blood samples whose DNA was extracted.

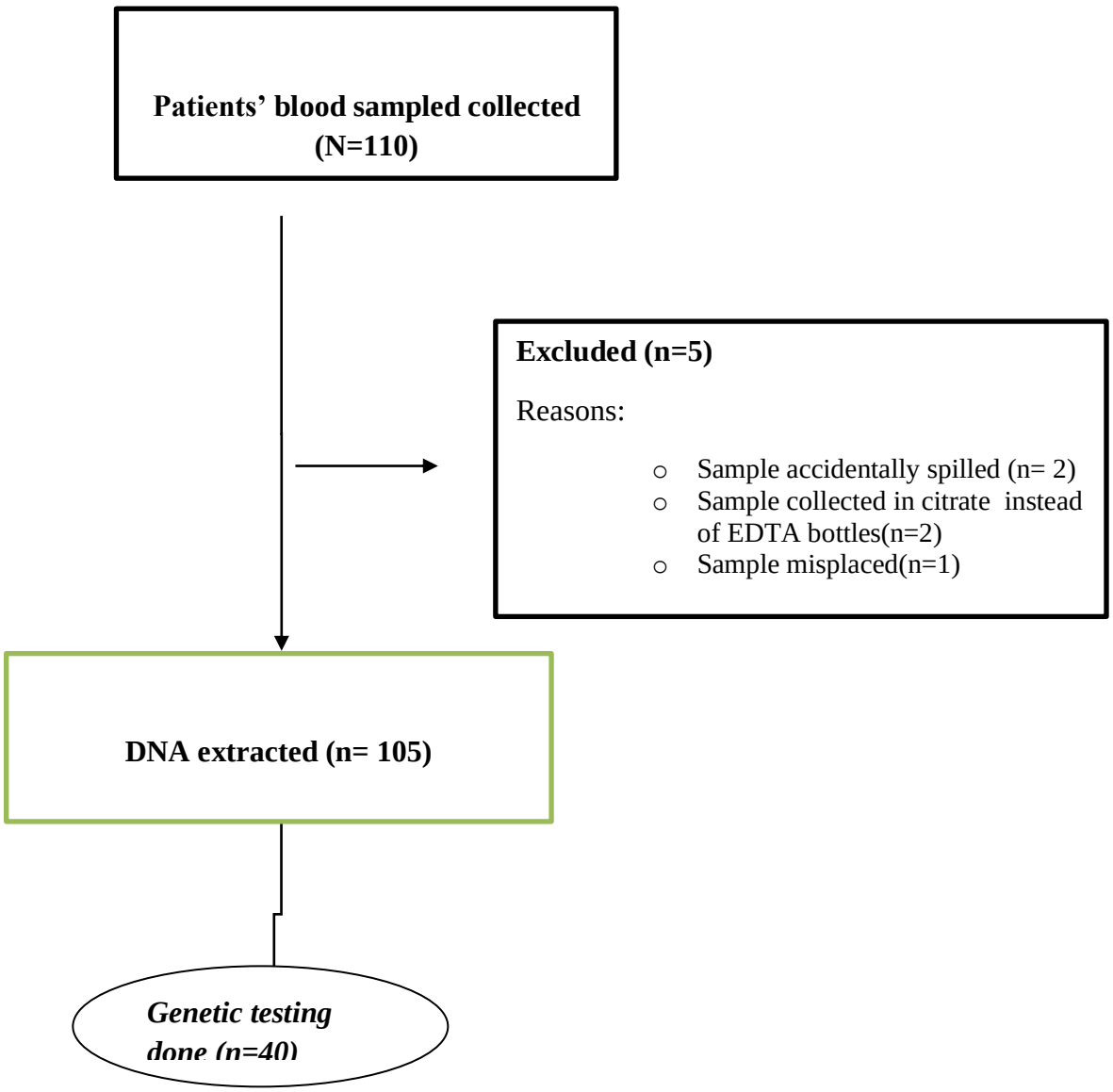


**Figure 4.12: Consort diagram showing participant blood samples collected and reasons for exclusion in genetic testing**

The sociodemographic characteristics of the study participants are shown in Table 4.32.

**Table 4.32: Sociodemographic Characteristics of the Study Participants (N=40)**

| Variable | Category | Frequency(n) | Percentage |
|---|---|---|---|
| Gender | Male | 8 | 20.0 |
| | Female | **32** | **80.0** |
| Age Categories | 18-40 Years | 21 | 52.5 |
| | 41-65 Years | 16 | 40.0 |
| | >65 Years | 3 | 7.5 |
| Mean/ Median Age(years) | | 44.3±14.2; Median 40 (range 18-87) | |
| Mean/Median Weight(Kg) | | 70.6±11.6; Median 71.5(52.0-107.4) | |
| BMI | Underweight | 1 | 2.7 |
| | Normal Weight | 11 | 29.7 |
| | Overweight/Obese | 25 | 67.6 |
| | *Missing* | *3* | |
| Mean/Median BMI | | 28.0±7.5; Median 27.0(18.4-55.8) | |
| Height(Meters);Median/Range | | 1.6±0.1; Median 1.6; Range (1.2-1.8) | |
| Mean/Median BSA($M^2$) | | 1.8±0.2; Median 1.8(1.5-2.1) | |
| Marital status | Without Spouse | 12 | 30.0 |
| | With Spouse | 28 | 70.0 |
| Income Sources | Non-regular Income | 27 | 67.5 |
| | Regular Income | 13 | 32.5 |
| Education Level | Below Secondary | 18 | 45.0 |
| | Secondary and Above | 22 | 55.0 |
| Religion | Christian | 34 | 85.0 |
| | Muslim | 6 | 15.0 |
| Alcohol Use | No | 36 | 90.0 |
| | Yes | 4 | 10.0 |

Key: BMI-Body Mass Index; BSA-Body Surface Area; Underweight (BMI <20) Normal weight (BMI 20-24.99); overweight/ Obese (BMI≥25)

There was female preponderance (80.0%) and the mean age of the study participants was 44.3±14.2 years with an age range of 18-87 years. Approximately half of the participants were aged 40 years and below. In addition, most of the participants had exceeded the ideal body weight (67.6%), were married (70.0%), did not have a regular source of income (67.5%) but had attained at least a secondary level of education (55.0%). The study comprised of Christians (90.0%) who were non-alcohol consumers (90.0%) (**Table 4.32**).

**Table 4.33: Clinical characteristics of the Study Population and Details of warfarin Therapy**

| Variable | Category | Frequency | Percentage |
|---|---|---|---|
| Comorbidities Present | No | 27 | 67.5 |
| | Yes | 13 | 32.5 |
| Number of Concomitant Drugs used | None | 19 | 47.5 |
| | 1.00 | 9 | 22.5 |
| | 2.00 | 6 | 15.0 |
| | 3.00 | 3 | 7.5 |
| | >3 | 3 | 7.5 |
| Adverse Drug Reaction Developed | No | 22 | 55.0 |
| | Yes | 18 | 45.0 |
| INR levels at the time of study | <2 | 14 | 35.0 |
| | 2-3 | 21 | 52.5 |
| | >3 | 5 | 12.5 |
| Mean INR at the time of study | 2.3±0.8; Median 2.2, range (1.0-4.8) | | |
| Warfarin mean initial doses prescribed(mg) | 5.9±1.9; Median 5.0, range (2.5-10) | | |
| Warfarin maintenance Dose categories | 2.5-5mg | 5 | 12.8 |
| | 6-10mg | 31 | 79.5 |
| | >10mg | 3 | 7.7 |
| Warfarin mean maintenance doses prescribed(mg) | 6.0±1.9; Median 5.0, range (2.5-10.3) | | |
| Mean Duration of Therapy (years) | 5.9±6.4; Median 4.2, range (0.1-31.3) | | |

Key: INR-International Normalized Ratio

The median duration of warfarin therapy was 4.2years (range 0.1-31.3). The mean daily initial and maintenance warfarin prescribed dose were 5.9±1.9mg and 6.0±1.9mg, respectively. Majority of the participants (79.5%) were stabilized on 6-10mg of warfarin. A third of the study participants had some form of comorbidities apart from the primary indication of warfarin anticoagulation and slightly over half had one other drug that was prescribed besides the anticoagulant. Forty-five percent developed ADRs to warfarin therapy although adequacy of anticoagulation was at 52.5% (**Table 4.33**). The clinical indications of warfarin anticoagulation are shown in **Figure 4.13**.

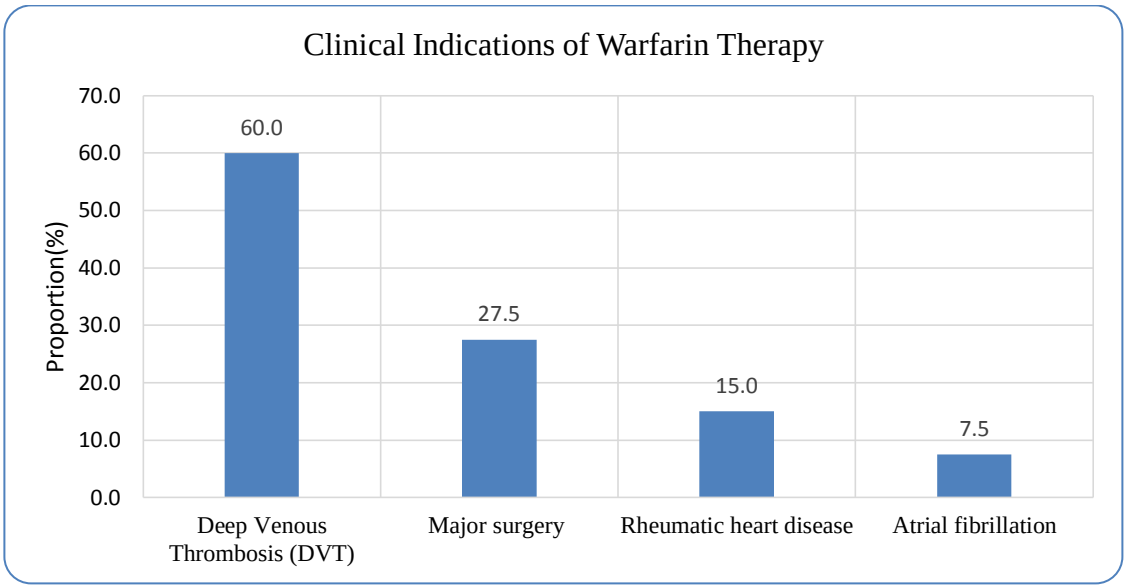


**Figure 4.13: Clinical indications for Warfarin Anticoagulation in the Study Population (N=40)**

The most common indication of warfarin anticoagulation was DVT (60%) followed by major surgery (27.5%), rheumatic heart disease (15.0%) and atrial fibrillation (7.5%). Major surgeries included heart valve surgery such as single or double valve replacement or repair (**Figure 4.13**).

### 4.3.2: Allele Frequencies of the Study Sample

A total of 11 SNP targets (**Table 4.34**) were investigated in each of the 40 participants from ten Kenyan tribes as shown in **Table 4.35**.

**Table 4.34: Genes and the SNPs of Interest in the Study Population**

| SNPS | *CYP 2C9* | Base Change | Amino Acid Change |
|---|---|---|---|
| rs1799853 | *2 | 430C>T | R144C |
| rs1057910 | *3 | 1075A>C | I359L |
| rs56165452 | *4 | 1076T>C | I359T |
| rs28371686 | *5 | 1080C>G | D360E |
| rs9332131 | *6 | 818delA | 273frameshift |
| rs7900194 | *8 | 449G>A | R150H |
| rs28371685 | *11 | 1003C>T | R335W |
| rs72558187 | *13 | 269T>C | L90P |
| | ***VKORC1*** | | |
| rs9923231 | *VKORC1**2 | -1639G>A | |
| | ***CYP 4F2*** | | |
| rs2108622 | *3 | 1347 C > T | V433M |
| rs2189784 | | G > A | |

**Table 4.35: Kenyan Tribes Genotyped for Variants of Warfarin Metabolizing Enzymes (N=40)**

| Kenyan Tribes | n | % |
|---|---|---|
| Duruma | 1 | 2.5 |
| Embu | 2 | 5.0 |
| Kalenjin | 1 | 2.5 |
| Kikuyu | 15 | 37.5 |
| Kamba | 5 | 12.5 |
| Kisii | 4 | 10.0 |
| Luhya | 5 | 12.5 |
| Luo | 4 | 10.0 |
| Meru | 2 | 5.0 |
| Taita | 1 | 2.5 |
| Total | 40 | 100.0 |

These were further divided into two main ethnolinguistic groups of Africa: Bantus and Nilotes. Bantus comprised of Duruma, Embu, Kikuyu, Kamba, Kisii, Luhya, Meru and Taita while the Nilotes comprised of Kalenjin and Luo (**Table 4.35**).

The prevalence of the SNPs in the study population was estimated by gene counting-method. **Table 4.36** shows the allele frequencies of the various SNPs in the black Kenyan population.

**Table 4.36: Genotype and Allele frequencies for Variants of *CYP 2C9, VKORC1* and *CYP 4F2* in Patients on Warfarin Therapy at Kenyatta National Hospital**

| | Genotype (N=40) | | | |
|---|---|---|---|---|
| **Gene** | **Wild type n (%)** | **Homozygous n (%)** | **Heterozygous n (%)** | **Allele Frequency** |
| ***CYP 2C9* *2 (430C>T)** | *CC* 39 (97.5%)$^{\beta}$ | *TT* 0(0) | *CT* 0(0) | *C*=0.975 |
| ***CYP 2C9* *3 (1075A>C)** | *AA* 39(97.5 %)$^{\beta}$ | *CC* 0(0) | *AC* 0(0) | *A*=0.975 |
| ***CYP 2C9* *4(1076T>C)** | *TT* 40(100.0%) | *CC* 0(0) | *TC* 0(0) | *T*=1.000 |
| ***CYP 2C9* *5(1080C>G)** | *CC* 40(100.0%) | *GG* 0(0) | *CG* 0(0) | *C*=1.000 |
| ***CYP 2C9* *6(818delA)** | AA40 (100.0%) | - | - | *A*=1.000 |
| ***CYP 2C9* *8(449G>A)** | *GG* 33(82.5%) | *AA* 0(0) | *GA* 7(17.5%) | *A*=0.0875;*G*=0.9125 |
| ***CYP 2C9* *11(1003C>T)** | *CC* 38(95.0%)$^{\beta}$ | *TT* 0(0) | *CT* 1(2.5%) | *C*=0.975; *T*=0.025 |
| ***CYP 2C9* *13(269T>C)** | *TT* 39(95.5%)$^{\beta}$ | *CC* 0(0) | CC 0 (0) | *T*=0.975 |
| ***CYP 4F2* (rs2189784; G> A)** | *GG* 16(40.0%) | *AA* 5(12.5%) | *GA* 19(47.5%) | *A*=0.3625;*G*=0.6375 |
| ***CYP 4F2* *3(1347 C > T; V433M)** | *CC* 33(82.5%) | *TT* 2(5.0%) | *CT* 5(12.5%) | *T*=0.8775;*C*=0.1125 |
| ***VKORC1*(rs9923231)** | *CC* 32 (80.0%)$^{\delta}$ | *TT* 0 (0) | *CT* 4(10.0%) | *C*=0.85; *T*=0.100 |

β=No call on one sample; δ=No call on four samples

*No call=Number of samples with no genotype call (usually if the ask did not work for that specific sample)

There were no variants of *CYP 2C9* *2, *3, *4, *5, *6 and *13 in this sample of ten tribes. The variant allele frequency of *CYP 2C9* *11 was lowest (*T*=0.025) compared to the other *CYP 2C9* variants. Among the *CYP 4F2* variants, *CYP 4F2* *3 variant allele frequency (*C*=0.1125) was lowest compared to *CYP 4F2* (rs2189784) (*A*=0.3625) (**Table 4.36**).

### 4.3.3: Prevalence of SNPs in the Study Sample

The prevalence of the genetic variants in the study sample is displayed in the subsequent Tables.

**Table 4.37: Prevalence of CYP *2C9, VKORC1* and *CYP 4F2* Variants Patients on Warfarin Therapy at Kenyatta National Hospital**

| Genetic Variant | Characteristic | n | Percentage |
|---|---|---|---|
| *CYP 2C9* *2(430C>T) variants | Wild type/Homozygous(*CC*) | 39 | 97.5 |
| | *No call | 1 | 2.5 |
| *CYP 2C9* *3(1075A>C) variants | Wild type/Homozygous(*AA*) | 40 | 100.0 |
| *CYP 2C9* *4 (1076T>C) Variants | Wild type/Homozygous(*TT*) | 40 | 100.0 |
| *CYP 2C9* *5 (1080C>G) variants | Wild type/Homozygous(*CC*) | 40 | 100.0 |
| *CYP 2C9* *6(818delA) Variants | Wild type/Homozygous(*AA*) | 40 | 100.0 |
| *CYP 2C9* *8(449G>A) variants | Wild type/Homozygous(*GG*) | 33 | 82.5 |
| | Heterozygous(*GA*) | 7 | 17.5 |
| *CYP* 2C*9* *11(1003C>T) Variants | Wild type/Homozygous(*CC*) | 38 | 95.0 |
| | Heterozygous(*TC*) | 1 | 2.5 |
| | *No call | 1 | 2.5 |
| *CYP 2C9* *13(269T>C) variants | Wild type/Homozygous(*TT*) | 39 | 97.5 |
| | *No call | 1 | 2.5 |
| *CYP 4F2* *3(1347 C > T; V433M) variants | Wild type/Homozygous(*CC*) | 33 | 82.5 |
| | Homozygous(*TT*) | 2 | 5.0 |
| | Heterozygous(*CT*) | 5 | 12.5 |
| *CYP 4F2*(rs2189784; G> A) variants | Wild type/Homozygous(*GG*) | 17 | 42.5 |
| | Homozygous(*AA*) | 4 | 10.0 |
| | Heterozygous(*AG*) | 19 | 47.5 |
| *VKORC1* (rs9923231)variants | Wild type/Homozygous(*CC*) | 32 | 80.0 |
| | Heterozygous(*CT*) | 4 | 10.0 |
| | *No call | 4 | 10.0 |

*No call=Number of samples with no allele call

The genetic variants of *CYP 2C9* *2, *3, *4, *5, *6, *13 in the study population were entirely the wild types of homozygous traits *CC*, *AA*, *TT*, *CC*, *AA* and *TT*, respectively. *CYP 2C9 *11(1003C>T)* had heterozygous (*CT*) at a prevalence of 2.5% (n=1). *CYP 4F2 *3(1347 C > T; V433M)* had 82.5 % (n=33) wild type (*CC*), 5.0% (n=2) homozygous (*TT*) and 12.5 % (n=5) heterozygous (*TC*). Most genetic variations were observed in *CYP 4F2 (rs2189784; G> A)* where 42.5% (n=17) were the wild type (*GG*), 10.0% (n=4) were homozygous (*AA*) and 47.5% (n=19) were heterozygous (*AG*). The variations in *VKORC1* were observed among the 10.0 % (n=4) of the participants who displayed heterozygousity (*CT*) traits (**Table 4.37**).

The study sought to find out the prevalence of various SNPs across the ten Kenyan ethnicities studied. This was done for the polymorphic *CYP 2C9* *8, *11, *CYP 4F2* and *VKORC1*. The findings are presented below.

**Table 4.38: Prevalence of *CYP 2C9, CYP 4F2* and *VKORC1* SNPs across the ethnolinguistic groups of Africa studied (N=2)**

| Genetic Variant | Characteristic | Ethnolinguistic group (N=2) | | | |
|---|---|---|---|---|---|
| | | Bantu | | Nilotes | |
| | | n | (%) | n | (%) |
| *CYP 2C9*8(449G>A)* | Wild type/Homozygous(*GG*) | 29 | 82.9 | 3 | 75.0 |
| | Heterozygous(*GA*) | 6 | **17.1** | 1 | **25.0** |
| *CYP 2C9*11(1003C>T)* | Wild type/Homozygous(*CC*) | 33 | 97.1 | 4 | 100.0 |
| | Heterozygous(*TC*) | 1 | **2.9** | 0 | 0.0 |
| *CYP 4F2*3(1347 C > T; V433M)* | Wild type/Homozygous(*CC*) | 29 | 82.9 | 3 | 75.0 |
| | Homozygous(*TT*) | 2 | **5.7** | 0 | 0.0 |
| | Heterozygous(CT) | 4 | 11.4 | 1 | 25.0 |
| *CYP 4F2(rs2189784; G> A)* | Wild type/Homozygous(*GG*) | 15 | 42.9 | 2 | **50.0** |
| | Homozygous(*AA*) | 4 | 11.4 | 0 | 0.0 |
| | Heterozygous(*AG*) | 16 | 45.7 | 2 | **50.0** |
| *VKORC1 (rs9923231)* | Wild type/Homozygous(*CC*) | 28 | 87.5 | 4 | 100.0 |
| | Heterozygous(*CT*) | 4 | **12.5** | 0 | 0.0 |

*CYP 2C9 *8* polymorphism was displayed across the two ethnolinguistic groups of Africa but was highest among the Nilotes at 25.0% (n=1). *CYP 2C9 *11* and *VKORC1* polymorphisms were displayed by the Bantus though the prevalence was low at 2.9% (n=1) and 12.5% (n=4), respectively. *CYP 4F2* was the most polymorphic across the two groups with Bantus revealing *CYP 4F2*3* heterozygousity *CT* at 11.4 % (n=4) vs. Nilotes, 25% (n=1); and *CYP 4F2 (rs2189784)* showing heterozygousity *AG* at 45.7 %( n=16) among the Bantus and 50.0% (n=2) among the Nilotes (Table **4.38**).

### 4.3.4: Warfarin Maintenance Doses during Genetic Testing

**Table 4.39: Mean Warfarin Maintenance Doses across the Ethnicities Studied**

| Tribe | n | Mean Warfarin Maintenance dose(mg) | Std. Deviation |
|---|---|---|---|
| Duruma | 1 | 5.0 | |
| Embu | 2 | 5.6 | 0.9 |
| Kalenjin | 1 | 7.5 | |
| Kamba | 5 | 7.4 | 2.9 |
| Kikuyu | 15 | 5.9 | 1.6 |
| Kisii | 4 | 5.4 | 3.4 |
| Luhya | 5 | 5.7 | 1.1 |
| Luo | 4 | 5.6 | 2.4 |
| Meru | 2 | 5.0 | 0.0 |
| Taita | 1 | 7.5 | |
| | Overall | 6.0 | 1.9 |

The overall mean warfarin maintenance dose was 6.0 ±1.9mg per day across the tribes. In addition Kalenjins, Taita and Kambas required higher warfarin maintenance doses (>7.0mg) than the average for the study population. On the other hand, Meru, Duruma and Kisii communities required lower warfarin maintenance doses (approximately 5.0mg per day) (**Table 4.39**). **Figure 4.14** shows the trends in warfarin maintenance dose requirements among the tribes.

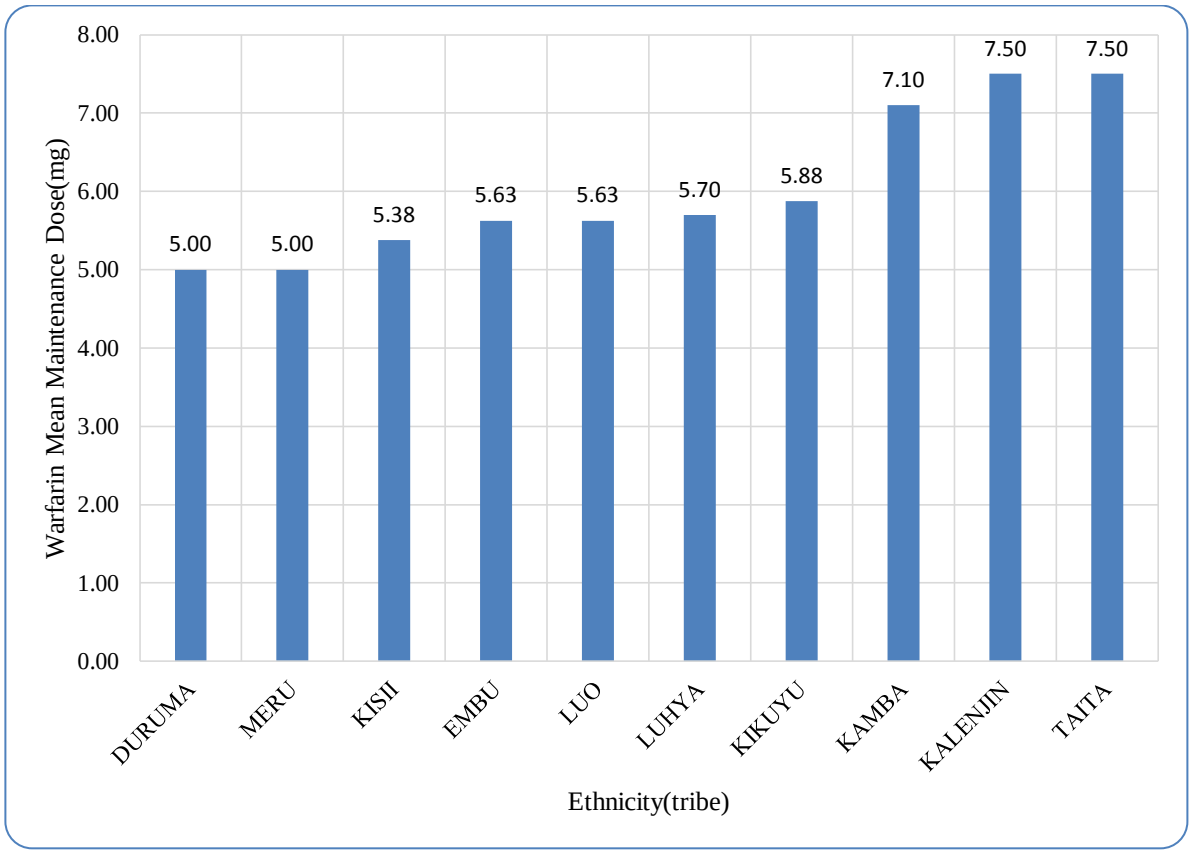


**Figure 4.14: Trends of Mean Warfarin Maintenance Doses Requirements across the tribes**

The highest warfarin maintenance dose was required by the Kalenjins and Taita followed by Kambas, while the lowest was prescribed for Duruma and Meru participants (**Figure 4.14**).

The study also sought to find out the warfarin maintenance dose requirements by the participants' place of residence as they came for clinic appointments. The findings of the results are summarized in **Figure 4.15**.

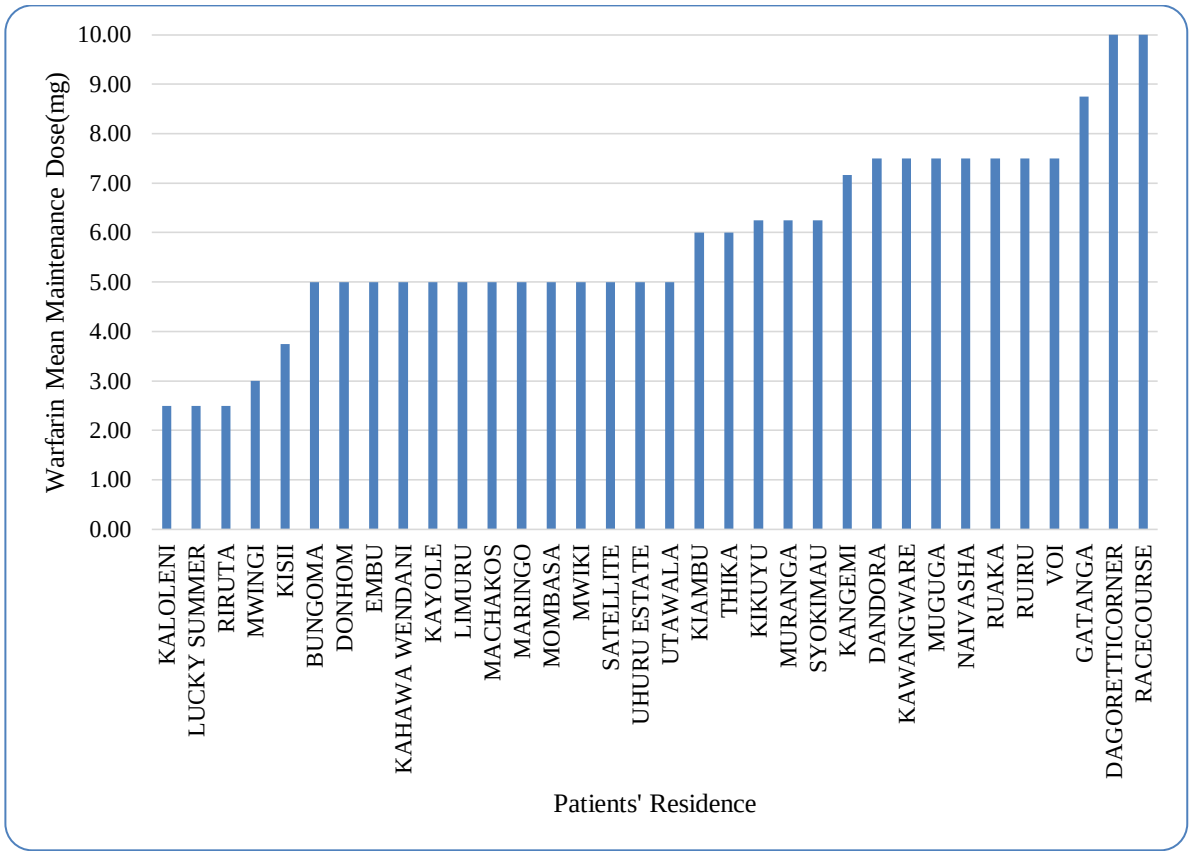


**Figure 4.15: Warfarin Maintenance dose requirements across participants' places of residence**

Participants living in Nairobi suburbs of Dagoretti Corner and Racecourse as well as Gatanga in Murang'a required higher warfarin maintenance doses compared to the other residence. In contrast, participants living in Nairobi Suburbs of Kaloleni, Lucky Summer and Riruta required the lowest warfarin maintenance doses (**Figure 4.15**).

### 4.3.5: Bivariate Analyses on Genetic Testing Data

Bivariate analysis was done to identify the associations between the predictor and outcome variables of the study. The main predictor variables for this study were the sociodemographic and clinical characteristics of the study population as well as the genetic variations portrayed by the Kenyan tribes. On the other hand, the main outcome variables were the mean maintenance doses of warfarin prescribed as well as its response as measured by the levels anticoagulation (INRs) and adverse drug reactions experienced by participants. There was no statistically significant differences between the mean warfarin maintenance doses between the two ethnolinguistic groups (Bantu 5.93±1.94 mg vs. Nilotes 6.00mg±2.24 mg, $p$=0.939).

#### 4.3.5.1: Trends of Warfarin Response as Measured by INRs and ADRs for Genotyped Participants

The trends of the level of anticoagulation and presence or absence of ADRs among the patients who underwent genetic testing are described in the subsequent Tables and bar graphs.

**Table 4.40: Relationship between Sociodemographics and Warfarin Response as Measured by INRs**

| Variable | Category | n | INRs | | | p-Value |
|---|---|---|---|---|---|---|
| | | | Mean | SD | Mean Difference | |
| Gender | Male | 8 | 2.10 | 0.60 | -0.26 | 0.42 |
| | Female | 32 | 2.35 | 0.84 | | |
| Age(Years) | 50 and Below years | 27 | 2.29 | 0.70 | -0.02 | 0.93 |
| | Above 50 Years | 13 | 2.32 | 0.99 | | |
| BMI | Underweight/Normal | 12 | 2.22 | 0.59 | -0.24 | 0.40 |
| | Overweight/Obese | 25 | 2.45 | 0.86 | | |
| Marital status | Without Spouse | 12 | 2.48 | 0.95 | 0.26 | 0.360 |
| | With Spouse | 28 | 2.22 | 0.72 | | |
| Income | Non-Regular Income | 27 | 2.34 | 0.87 | 0.12 | 0.653 |
| | Regular Income | 13 | 2.22 | 0.63 | | |
| Education Level | Below Secondary | 18 | 2.26 | 0.75 | -0.08 | 0.744 |
| | Secondary and Above | 22 | 2.34 | 0.84 | | |
| Religion | Christian | 34 | 2.36 | 0.82 | 0.37 | 0.304 |
| | Muslims | 6 | 1.99 | 0.55 | | |
| Alcohol Use | No | 35 | 2.33 | 0.82 | 0.11 | 0.798 |
| | Yes | 4 | 2.22 | 0.60 | | |

Key: BMI-Body Mass Index; INR-International Normalized Ratio; SD-Standard deviation

Generally, the mean INR for the study sample was in the therapeutic range of 2-3. Females had a slightly higher mean INR than males (2.35±0.84 vs. 2.10±0.60). A similar trend was observed in participants who were overweight /obese compared to underweight/normal weight, and the ones without spouses compared to with spouses. The striking difference in INRs was greatest in the denomination whereby Christians showed slightly higher mean INR than Muslims (mean difference=0.37), with the latter being in the sub-therapeutic levels (INR<2). These findings were however, not statistically significant (**Table 4.40**).

**Table 4.41: Association between Sociodemographics and INR therapeutic Categories**

| Variable | Category | INR therapeutic Categories | | | P-Value |
|---|---|---|---|---|---|
| | | <2 n (%) | 2-3 n (%) | >3 n (%) | |
| Gender | Male | 2(25.0) | 6(75.0) | 0(0.0) | 0.287 |
| | Female | 12(37.5) | 15(46.9) | 5(15.6) | |
| Age (Years) | 50 and Below years | 9(33.3) | 14(51.9) | 4(14.8) | 0.807 |
| | Above 50 Years | 5(38.5) | 7(53.8) | 1(7.7) | |
| BMI | Underweight/Normal | 5(41.7) | 6(50.0) | 1(8.3) | 0.510 |
| | Overweight/Obese | 6(24.0) | 15(60.0) | 4(16.0) | |
| Marital status | Without Spouse | 3(25.0) | 7(58.3) | 2(16.7) | 0.657 |
| | With Spouse | 11(39.3) | 14(50.0) | 3(10.7) | |
| Income | Non-Regular Income | 9(33.3) | 14(51.9) | 4(14.8) | 0.807 |
| | Regular Income | 5(38.5) | 7(53.8) | 1(7.7) | |
| Education Level | Below Secondary | 7(38.9) | 9(50.0) | 2(11.1) | 0.891 |
| | Secondary and Above | 7(31.8) | 12(54.5) | 3(13.6) | |
| Religion | Christian | 11(32.4) | 18(52.9) | 5(14.7) | 0.511 |
| | Muslim and Others | 3(50.0) | 3(50.0) | 0(0.0) | |
| Alcohol Use | No | 12(34.3) | 18(51.4) | 5(14.3) | 0.596 |
| | Yes | 1(25.0) | 3(75.0) | 0(0.0) | |

Key: BMI-Body Mass Index

There were no statistically significant associations between participants' sociodemographic characteristics and the INR therapeutic categories. Over half of the participants in all sociodemographic categories had achieved therapeutic levels of INR 2-3. Besides, the proportion of males with adequate anticoagulation response was more than the females (75.0 % vs. 46.9%). The proportion of participants who had reached at least a secondary level of education and had therapeutic response was slightly more than the ones with below secondary level of education.

Additionally, a quarter to third of the participants in all sociodemographic categories had sub-therapeutic response to warfarin therapy (**Table 4.41**).

**Table 4.42: Relationship between Tribes and Warfarin Response as Measured by INRs**

| Tribe | n | INR | | |
|---|---|---|---|---|
| | | Totals INRs | Mean | SD |
| Duruma | 1 | 2.70 | 2.70 | |
| Embu | 2 | 4.00 | 2.00 | 0.71 |
| Kalenjin | 1 | 1.90 | 1.90 | |
| Kamba | 5 | 11.95 | 2.39 | 0.96 |
| Kikuyu | 15 | 34.95 | 2.33 | 0.71 |
| Kisii | 4 | 7.24 | 1.81 | 0.55 |
| Luhya | 5 | 11.65 | 2.33 | 0.59 |
| Luo | 4 | 7.76 | 1.94 | 0.74 |
| Meru | 2 | 8.20 | 4.10 | 0.99 |
| Taita | 1 | 1.70 | 1.70 | |

Key: INR-International Normalized Ratio; SD-Standard deviation

The mean INR recorded was within therapeutic range (2-3) for Duruma, Embu, Kamba, Kikuyu and Luhya ethnicities. The mean INR was sub-therapeutic (<2) for Kalenjin, Kisii, Luo and Taita communities. Participants from Meru community were over-anticoagulated with a mean INR=4.10±0.99 (**Table 4.42**).

The trend in the mean INR recorded for the study participants is shown in the **Figure 4.16**.

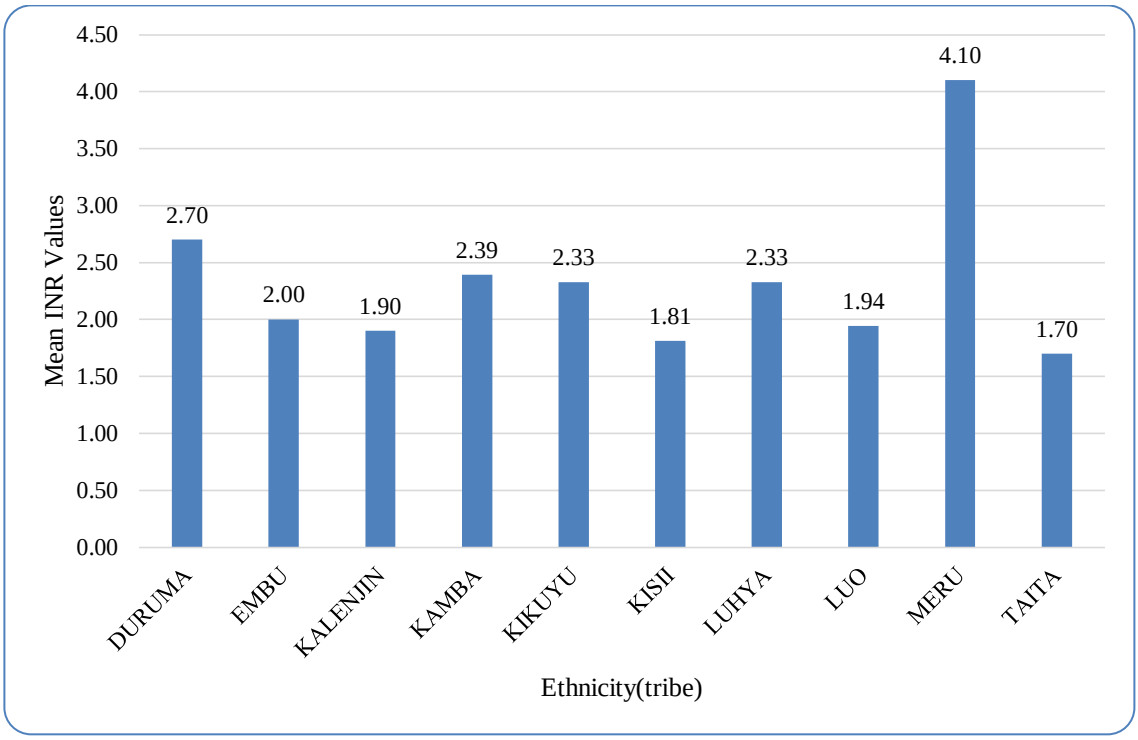


**Figure 4.16: Trends in the mean INRs obtained across the Kenyan tribes studied**

There was no statistically significant differences between the mean INRs observed between the two ethnolinguistic groups (Bantu 2.35±0.81 vs. Nilotes 1.93±0.64, p=0.275).

The study further explored the relationship between the participants' residence and the mean INRs obtained. The aim was to find out whether the participants' residence had a bearing on the warfarin response. The Figure below reveals the findings.

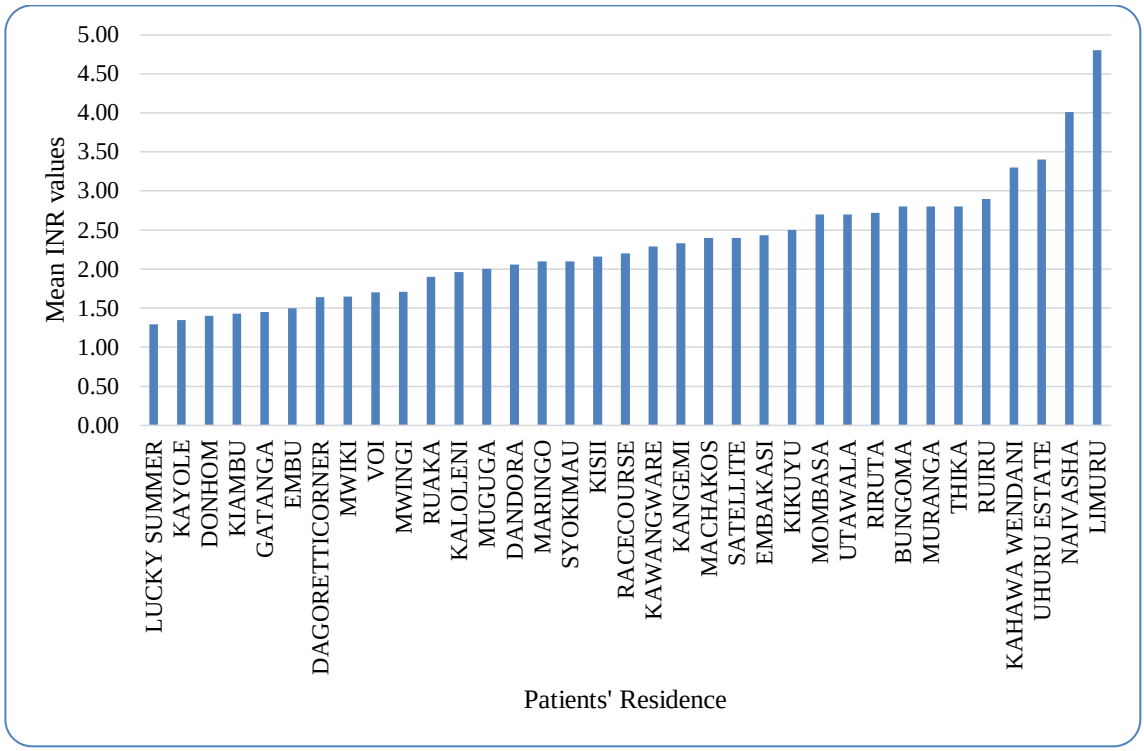


**Figure 4.17: Mean INRs obtained versus the participants' residence**

The highest mean INRs were obtained in participants living in Kahawa Wendani, Limuru, Naivasha and Thika. A number of participants had sub-therapeutic INR levels, of which the lowest were found in people living in Donhom, Gatanga, and Lucky summer (**Figure 4.17**).

Warfarin response was also measured by finding out the prevalence of ADRs to the drug. The study explored the relationship between sociodemographic characteristics and presence or absence of ADRs and the findings are presented below.

**Table 4.43: Relationship between Sociodemographic characteristics of the participants and warfarin response as measured by presence or absence of ADRs**

| Variable | Category | Total | ADRs | | *p*-value |
|---|---|---|---|---|---|
| | | | No n (%) | Yes n (%) | |
| Gender | Male | 8 | 6(75.0) | 2(25.0) | 0.204 |
| | Female | 32 | 16(50.0) | **16(50.0)** | |
| Age | 50 years and below | 27 | 13(48.1) | **14(51.9)** | 0.209 |
| | Above 50 years | 13 | 9(69.2) | 4(30.8) | |
| BMI | Underweight/Normal | 12 | 6(50.0) | **6(50.0)** | 0.732 |
| | Overweight/Obese | 25 | 14(56.0) | 11(44.0) | |
| Marital status | Without Spouse | 12 | 6(50.0) | 6(50.0) | 0.677 |
| | With Spouse | 28 | 16(57.1) | 12(42.9) | |
| Income | Non-Regular Income | 27 | 13(48.1) | 14(51.9) | 0.209 |
| | Regular Income | 13 | 9(69.2) | 4(30.8) | |
| Education Level | Below Secondary | 18 | 8(44.4) | **10(55.6)** | 0.225 |
| | Secondary and Above | 22 | 14(63.6) | 8(36.4) | |
| Religion | Christian | 34 | 18(52.9) | 16(47.1) | 0.533 |
| | Muslim and Others | 6 | 4(66.7) | 2(33.3) | |
| Alcohol Use | No | 35 | 20(57.1) | 15(42.9) | 0.785 |
| | Yes | 4 | 2(50.0) | **2(50.0)** | |

Key: ADRS-Adverse Drug reactions; BMI-Body Mass Index

There was no statistically significant association between the participants' sociodemographic characteristics and the warfarin response as measured by presence or absence of ADRs. However, half of the females, underweight/normal weight participants, those below secondary level of education and alcohol consumers suffered from ADRs to warfarin therapy (**Table 4.43**).

### 4.3.5.2: Role of Genetic Variations in Warfarin Response and Dose Requirements

The role of genetic variation and warfarin maintenance doses as well as the response was also explored in this study. The findings are presented below.

**Table 4.44: Association between Genetic variants and the warfarin Maintenance Doses**

| Genetic Variant | Type | n | Warfarin Maintenance Dose(mg) | | Mean Rank | *p*-value |
|---|---|---|---|---|---|---|
| | | | Mean | SD | | |
| *CYP 2C9* *8(449G>A) variants | Wild type/Homozygous (*GG*) | 32 | **6.1** | **1.7** | 21.1 | 0.166 |
| | Heterozygous (*GA*) | 7 | 5.2 | 2.7 | 14.8 | |
| *CYP* 2C9 *11(1003C>T) Variants | Wild type/Homozygous (*CC*) | 37 | **6.0** | **1.9** | 19.9 | 0.142 |
| | Heterozygous (*TC*) | 1 | 3.0 | | 4.0 | |
| *VKORC1* (rs9923231)variants | Wild type/Homozygous (*CC*) | 31 | 6.1 | 2.0 | 17.8 | 0.789 |
| | Heterozygous (*CT*) | 4 | **6.3** | **1.4** | 19.3 | |
| *CYP 4F2*(rs2189784; G> A) variants | Wild type/Homozygous (*GG*) | 17 | **6.3** | **2.1** | 20.9 | 0.719 |
| | Homozygous (*AA*) | 3 | 5.0 | 2.5 | 15.3 | |
| | Heterozygous (*AG*) | 19 | 5.9 | 1.7 | 19.9 | |
| *CYP 4F2* *3(1347 C > T; V433M) variants | Wild type/Homozygous (*CC*) | 33 | 6.0 | 2.0 | 20.2 | 0.185 |
| | Homozygous (*TT*) | 2 | 3.8 | 1.8 | 7.5 | |
| | Heterozygous (*CT*) | 5 | **6.6** | **1.2** | 24.9 | |

Key: SD-Standard Deviation

Participants with *CYP 2C9* *8(449G>A) wild type (*GG*) required slightly higher warfarin mean maintenance doses than those with heterozygous (*GA*) alleles (6.1±1.7mg vs. 5.2±2.7mg per day). Participants with *CYP 2C9* *11(1003C>T) wild type required a higher mean warfarin maintenance dose than the heterozygous (*GA*) types (**6.0 mg vs. 3.0mg per day**). On the other hand, the heterozygous (*CT*) of *VKORC1* (rs9923231) and *CYP 4F2* *3(1347 C > T; V433M) required a higher warfarin maintenance doses than their wild type. Participants with the wild type (*GG*) of *CYP 4F2* (rs2189784; G> A) required higher warfarin maintenance doses (6.3±2.1mg) than the homozygous (*AA*) genotype (5.0±2.5mg) and heterozygous (*AG*) variants (5.9±1.7mg). Individuals with heterozygous (*CT*) of *CYP 4F2* *3 required the highest warfarin maintenance doses of 6.6±1.2mg per day (**Table 4.44**).

**Table 4.45: Association between Genetic variants and the warfarin responses as measured by mean INRs**

| Genetic Variant | Type | n | INR | | Mean Rank | P-Value |
|---|---|---|---|---|---|---|
| | | | Mean | SD | | |
| *CYP 2C9* *8(449G>A) variants | Wild type/Homozygous (*GG*) | 33 | 2.3 | 0.8 | 20.1 | 0.656 |
| | Heterozygous (*GA*) | 7 | 2.3 | 0.5 | 22.3 | |
| CYP 2C9 *11(1003C>T) Variants | Wild type/Homozygous (*CC*) | 38 | 2.3 | 0.8 | 20.2 | 0.477 |
| | Heterozygous (*TC*) | 1 | **1.7** | | 12.0 | |
| *VKORC1* (rs9923231)variants | Wild type/Homozygous (*CC*) | 32 | 2.2 | 0.7 | 17.9 | 0.365 |
| | Heterozygous (*CT*) | 4 | 2.7 | 0.9 | 23.0 | |
| *CYP 4F2*(rs2189784; G> A) variants | Wild type/Homozygous (*GG*) | 17 | 2.5 | 0.8 | 23.9 | 0.157 |
| | Homozygous (*AA*) | 4 | 2.4 | 0.5 | 23.8 | |
| | Heterozygous (*AG*) | 19 | 2.1 | 0.8 | 16.8 | |
| *CYP 4F2* *3(1347 C > T; V433M) variants | Wild type/Homozygous (*CC*) | 33 | 2.3 | 0.8 | 20.9 | 0.712 |
| | Homozygous (*TT*) | 2 | 2.4 | 0.4 | 23.3 | |
| | Heterozygous (*CT*) | 5 | **2.0** | 0.6 | 16.7 | |

The mean INRs were within therapeutic range (2-3) across the genetic variants except for heterozygous (*TC*) of *CYP 2C9* *11(1003C>T) variants (INR=1.7). In addition, the heterozygous (*CT*) of *CYP 4F2* *3(1347 C > T; V433M) variants recorded a lower mean INR (2.0±0.6) compared to other genetic variants studied. The findings were, however, not statistically significant (**Table 4.45**).

**Table 4.46: Association between genetic polymorphisms and the warfarin responses as measured by INR therapeutic Categories**

| Variant | Type | INR Therapeutic Categories | | | P-Value |
|---|---|---|---|---|---|
| | | <2 n (%) | 2-3 n(%) | >3 n(%) | |
| *CYP 2C9* *8(449G>A) variants | Wild type/Homozygous (*GG*) | 13(39.4) | 15(45.5) | 5(15.2) | 0.153 |
| | Heterozygous (*GA*) | 1(14.3) | 6(85.7) | 0(0.0) | |
| *CYP 2C9* *11(1003C>T) Variants | Wild type/Homozygous (*CC*) | 13(34.2) | 20(52.6) | 5(13.2) | 0.487 |
| | Heterozygous (*TC*) | **1(100.0)** | 0(0.0) | 0(0.0) | |
| *CYP 4F2* *3(1347 C > T; V433M) variants | Wild type/Homozygous (*CC*) | 12(36.4) | 16(48.5) | 5(15.2) | 0.656 |
| | Homozygous (*TT*) | 0(0.0) | 2(100.0) | 0(0.0) | |
| | Heterozygous (*CT*) | 2(40.0) | 3(60.0) | 0(0.0) | |
| *CYP 4F2*(rs2189784; G> A) variants | Wild type/Homozygous (*GG*) | 4(23.5) | 10(58.8) | 3(17.6) | 0.526 |
| | Homozygous (*AA*) | 1(25.0) | 3(75.0) | 0(0.0) | |
| | Heterozygous (*AG*) | **9(47.4)** | 8(42.1) | 2(10.5) | |
| *VKORC1* (rs9923231)variants | Wild type/Homozygous (*CC*) | 12(37.5) | 17(53.1) | 3(9.4) | 0.310 |
| | Heterozygous (*CT*) | 0(0.0) | 3(75.0) | 1(25.0) | |

Although there were no statistically significant associations between the genetic variants studied and the INR therapeutic categories, almost half (47.4%) of heterozygous (*AG*) of *CYP 4F2* (rs2189784; G> A) and heterozygous (*TC*) of *CYP 2C9* * 11 variants were under-anticoagulated (INR<2). The findings were, however, not statistically significant (**Table 4.46**).

**Table 4.47: Association between Genetic polymorphisms and the warfarin responses as measured by presence or absence of ADRs to warfarin therapy**

| Genetic Variant | Type | Adverse Drug Reaction | | P-Value |
|---|---|---|---|---|
| | | No n (%) | Yes n (%) | |
| *CYP 2C9* *8(449G>A) variants | Wild type/Homozygous (*GG*) | 20(60.6) | 13(39.4) | 0.211 |
| | Heterozygous (*GA*) | 2(28.6) | **5(71.4)** | |
| CYP 2C9 *11(1003C>T) Variants | Wild type/Homozygous (*CC*) | 22(57.9) | 16(42.1) | 0.436 |
| | Heterozygous (*TC*) | 0(0.0) | **1(100.0)** | |
| *CYP 4F2* *3(1347 C > T; V433M) variants | Wild type/Homozygous (*CC*) | 18(54.5) | 15(45.5) | 1.000 |
| | Homozygous (*TT*) | 1(50.0) | **1(50.0)** | |
| | Heterozygous (*CT*) | 3(60.0) | **2(40.0)** | |
| *CYP 4F2*(rs2189784; G> A) variants | Wild type/Homozygous (*GG*) | 8(47.1) | 9(52.9) | 0.219 |
| | Homozygous (*AA*) | 1(25.0) | **3(75.0)** | |
| | Heterozygous (AG) | 13(68.4) | **6(31.6)** | |
| *VKORC1* (rs9923231)variants | Wild type/Homozygous (*CC*) | 17(53.1) | **15(46.9)** | 0.613 |
| | Heterozygous (*CT*) | 3(75.0) | **1(25.0)** | |

There were no statistically significant associations between the development of ADRs and the genetic variants studied. However, compared to other genotypes, there were higher proportions of participants with heterozygous (*GA*) of *CYP 2C9* *8 (71.4%) and homozygous (*AA*) of *CYP 4F2* (rs2189784; G> A) (75.0%) as well as the wild type of *VKORC1* (46.6%) who experienced ADRs to warfarin therapy (**Table 4.47**).

### 4.4: Relative Contribution of the Predictor Variables to Variability in Warfarin Response

Each of the predictor variables, as described in the conceptual framework, was assessed through multiple linear regression models and ranked to find out the degree of contribution to the outcome variables, including the warfarin maintenance doses, INRs measured and ADRs. R-Square ($R^2$) from the regression model was used to estimate the contribution of individual socio-demographics, clinical characteristics and genetic polymorphisms on the variation of warfarin maintenance doses. The subsequent Tables illustrate the findings of analysis.

**Table 4.48: Relative contribution of participants' sociodemographic, food and clinical characteristics to warfarin maintenance doses**

| Predictor Variable | Description | R | R Square | % Variance |
|---|---|---|---|---|
| Body Surface Area($M^2$) | Body Surface Area($M^2$) | 0.263 | 0.0692 | 6.92 * |
| Marital status | With spouse vs. without | 0.138 | 0.0190 | 1.90* |
| Sex | Female vs. male | 0.119 | 0.0142 | 1.42 * |
| Heart Diseases | Yes vs. No | 0.114 | 0.0130 | 1.30^ |
| Body Mass Index | Body Mass Index (BMI) | 0.103 | 0.0106 | 1.06* |
| Employment Status | Regular income vs. non-regular income | 0.067 | 0.0045 | 0.45* |
| Venous Thromboembolism Disorders | Yes vs. No | 0.057 | 0.0032 | 0.32^ |
| Diet(Vegetables) | ≥3 times/ week vs. <3 times/ week | 0.049 | 0.0024 | 0.24# |
| Age in years | Age in years | 0.042 | 0.0018 | 0.18* |
| Denomination | Muslim and others vs. Christians | 0.038 | 0.0015 | 0.15* |
| Highest Academic Level | Secondary and above vs. primary and below | 0.022 | 0.0005 | 0.05* |
| Comorbidities | With vs. without | 0.004 | 0.0000 | 0.00 |
| Concomitant use of other drugs | Yes vs. No | 0.004 | 0.0000 | 0.00 |
| Herbal Supplements (Ginger and garlic) | Yes vs. No | 0.002 | 0.0000 | 0.00# |
| **Total** | | | | **13.99** |

Key: * Sociodemographic factors; ^ Clinical indications of warfarin anticoagulation; # Dietary intake and herbal supplements; R-square is the percentage of the response variable variation that is explained by a linear model

The sociodemographic and clinical characteristics of the participants contributed to a 13.99% of warfarin maintenance dose variation between participants. The sociodemographic factors alone contributed to 12.14% of dose variation while the clinical indication for anticoagulation provided a 1.62 % warfarin dose variation between participants. Among the sociodemographics, participants' BSA contributed the greatest variability (6.92 %) in warfarin dose requirements. Dietary intake alone contributed to 0.24% of warfarin dose variation. Comorbidities, concomitant use of other drugs and herbal supplements did not contribute any variation in warfarin dose requirements (**Table 4.48**).

**Table 4.49: Relative contribution of participants' sociodemographic, food and clinical characteristics to INRs observed**

| | | INR | |
|---|---|---|---|
| **Predictor variable** | **Description** | **Nagelkerke's R Square** | **% Variance** |
| Body Surface Area($M^2$) | Body Surface Area($M^2$) | 0.0454 | 4.54* |
| Body Mass Index (BMI) | Body Mass Index (BMI) | 0.0381 | 3.81* |
| Marital status | With spouse vs. without | 0.0266 | 2.66* |
| Sex | Female vs. male | 0.0223 | 2.23* |
| Education | Secondary and above vs. primary and below | 0.0176 | 1.76* |
| Vegetables/Diet | ≥3 times a week vs. less than 3 times a week | 0.0143 | 1.43# |
| Comorbidities | With vs. without | 0.0123 | 1.23 |
| Heart Diseases | Yes vs. No | 0.0108 | 1.08^ |
| Employment | Regular income vs. non- regular income | 0.0048 | 0.48* |
| Concomitant use of other drugs | Yes vs. No | 0.0030 | 0.30 |
| Herbal Supplements (Ginger and garlic) | Yes vs. No | 0.0014 | 0.14# |
| Denomination | Muslim and others vs. Christians | 0.0008 | 0.08* |
| Venous Thromboembolism Disorders | Yes vs. No | 0.0007 | 0.07^ |
| Age in years | Age in years | 0.0003 | 0.03* |
| Total | | | 19.84 |

Key: * Sociodemographic factors; ^ Clinical indications of warfarin anticoagulation; # Dietary intake and herbal supplements; For binary outcomes (ADR and therapeutic INR range) Nagelkerke's $R^2$ from the logistic regression model was used to estimate the amount of variance explained by these factors

The sociodemographic, clinical characteristics and food contributed to 19.84% variability in the observed INR responses. Sociodemographic factors alone contributed to 15.59% of the variability in INR responses, with the greatest contributor being the BSA at 4.54% followed by BMI at 3.81%. Dietary habits and herbal supplementation contributed to 1.57% of the variability of INR responses while clinical diagnosis, comorbidities and concomitant drugs had a contribution of 1.15%, 1.23% and 0.3%, respectively (**Table 4.49**).

**Table 4.50: Relative contribution of participants' sociodemographic, food and clinical characteristics to ADRs**

| | | Adverse Drug Reaction | |
|---|---|---|---|
| | | Nagelkerke's R Square | % Variance |
| Predictor | Description | | |
| Marital status | With spouse vs.. without | 0.051 | 5.11* |
| Heart Diseases | Yes vs. No | 0.028 | 2.83^ |
| Venous Thromboembolism Disorders | Yes vs. No | 0.021 | 2.08^ |
| Body Surface Area($M^2$) | Body Surface Area($M^2$) | 0.019 | 1.89 * |
| Herbal Supplements (Ginger and garlic) | Yes vs. No | 0.016 | 1.56# |
| Comorbidities | With vs. without | 0.015 | 1.55^ |
| Body Mass Index (BMI) | Body Mass Index (BMI) | 0.012 | 1.17* |
| Denomination | Muslims vs. Christians | 0.008 | 0.85* |
| Vegetables/Diet | 3 times a week vs. less than 3 times a week | 0.006 | 0.62# |
| Education | Secondary and above vs. primary and below | 0.005 | 0.52 * |
| Employment | Regular income vs. non-regular income | 0.005 | 0.46* |
| Concomitant use of other drugs | Yes vs. No | 0.002 | 0.18^ |
| Age in years | Age in years | 0.001 | 0.06* |
| Sex | Female vs. male | 0.000 | 0.01* |
| Total | | | 18.89 |

Key: * Sociodemographic factors; ^ Clinical indications of warfarin anticoagulation; # Dietary intake and herbal supplements For binary outcomes (ADR and therapeutic INR range) Nagelkerke's $R^2$ from the logistic regression model was used to estimate the amount of variance explained by these factors

The sociodemographic, clinical characteristics and food contributed to 18.89% of the observed ADRs to warfarin therapy. The sociodemographic characteristics alone contributed to 10.07% of the observed ADRs to warfarin therapy, with the greatest contributor being the marital status at 5.11% followed by BSA (1.89%). The clinical conditions such as the indications for anticoagulation, comorbidities and concomitant use of other drugs contributed 6.64%, with a diagnosis of heart diseases having the greatest contribution to ADRs at 2.83%. Dietary contributions to ADRs were minimal at 2.18 %( **Table 4.50**).

**Table 4.51: Relative contribution of genetic polymorphisms to warfarin maintenance doses and responses**

| Predictor/Variable | | Warfarin Maintenance Dose | | | INR | | Adverse Drug Reaction | |
|---|---|---|---|---|---|---|---|---|
| | | R | R Square | % Variance | Nagelkerke's R Square | % Variance | Nagelkerke's R Square | %Variance |
| *CYP 4F2* *3 (1347 C > T; V433M) variants | Homozygous (*TT*) | 0.268 | 0.0721 | **7.21*** | 0.086246 | 8.62 | 0.000708 | 0.07 |
| CYP 2C9 *11 (1003C>T) variants | Heterozygous (*TC*) | 0.249 | 0.0622 | 6.22 | 0.049199 | 4.92 | 0.057018 | 5.70 |
| *CYP 2C9* *8 (449G>A) variants | Heterozygous (*GA*) | 0.192 | 0.0369 | 3.69 | 0.131073 | **13.11*** | 0.078658 | 7.87 |
| *CYP 4F2* (rs2189784; G> A) variants | Homozygous (*AA*) | 0.145 | 0.0212 | 2.12 | 0.031204 | 3.12 | 0.054079 | 5.41 |
| *CYP 4F2* *3 (1347 C > T; V433M) variants | Heterozygous (*CT*) | 0.105 | 0.0109 | 1.09 | 0.004326 | 0.43 | 0.001943 | 0.19 |
| *CYP 4F2* (rs2189784; G> A) variants | Heterozygous (*AG*) | 0.057 | 0.0033 | 0.33 | 0.051607 | 5.16 | 0.086387 | **8.64*** |
| *VKORC1* (rs9923231)variants | Heterozygous (*CT*) | 0.032 | 0.0010 | 0.10 | 0.026738 | 2.67 | 0.026738 | 2.67 |
| **Total** | | | | **20.77** | | **38.03** | | **30.55** |

Key: * Greatest contributors to the assessed warfarin response; For binary outcomes (ADR and therapeutic INR range) Nagelkerke's $R^2$ from the logistic regression model was used to estimate the amount of variance explained by these factors

Genetic polymorphisms of *CYP 2C9*, *VKORC1* and *CYP 4F2* contributed to 20.77% of the variability in warfarin maintenance doses between participants. The variability of INRs between participants that could be explained by genetic variability was 38.03%. A third of the variability in ADRs between participants could be accounted by genetic polymorphisms.

Polymorphisms in *CYP 4F2* *3(1347 C > T; V433M) *TT* variants contributed the greatest variability in warfarin maintenance doses at 7.21%, followed by *CYP 2C9* *11(1003C>T) heterozygous *TC* at 6.22%. *CYP 4F2* polymorphisms in general contributed 10.75% variability in dose variation. The individual *CYP 4F2*, *CYP 2C9* and *VKORC1* polymorphisms accounted for 10.75%, 9.91% and 0.10%, respectively of the observed differences in warfarin maintenance doses.

The greatest variability in the INRs observed between participants was contributed by *CYP 2C9* *8(449G>A) polymorphisms at 13.11% followed by *CYP 4F2* *3(1347 C > T; V433M) variants at 8.62%. When Combined, *CYP 2C9*, *CYP 4F2* and *VKORC1* contributed to 18.03%, 17.33%

and 2.67% of the variability in the INRs observed. *CYP 2C9* and *VKORC1* polymorphisms contributed to 13.57% and 2.67% of the ADRs, respectively.

*CYP 4F2* (rs2189784; G> A) heterozygous *AG* contributed the greatest in the variability observed in the development of ADRs at 8.64%, followed by *CYP 2C9* *8(449G>A) heterozygous *GA* at 7.87%. When put together, *CYP 4F2* contributed to 14.31% variability in ADRs experienced (**Table 4.51**).

### 4.5: Independent genetic and non-genetic factors for warfarin maintenance doses which produce therapeutic INR values

In order to estimate factors that were associated with dose that produce an INR of 2-3, multivariate generalized linear regression model was conducted using the dose as the outcome variable and participants' socio-demographics and genetic markers as predictors. Maximum likelihood estimations were used to estimate the parameters of individual factors associated with warfarin dose. Participants' sociodemographics (such as age, gender and BMI) and clinical characteristics (such as comorbidities and other drugs prescribed) were regressed against the warfarin maintenance doses producing INR of 2-3. In addition the various genetic variations were logistically regressed against the warfarin maintenance doses producing therapeutic INRs (2-3). The results of logistic regression are presented (**Table 4.52**).

**Table 4.52: Multivariate Generalized Linear Regression on Factors associated with Warfarin Maintenance dose giving INR reading of 2-3 among the study participants**

| Parameter | Category | β | S.E. | 95% C.I. | | Sig. |
|---|---|---|---|---|---|---|
| | | | | Lower | Upper | |
| Intercept | - | 6.28 | 6.0 | -5.44 | 18.00 | 0.294 |
| Sex | Male | -3.38 | 1.0 | -5.38 | -1.38 | **0.001*** |
| | Female | Ref. | | | | |
| Adverse Reaction | No | -0.09 | 0.6 | -1.26 | 1.08 | 0.880 |
| | Yes | Ref. | | | | |
| Comorbidities | No | 1.26 | 1.3 | -1.26 | 3.78 | 0.327 |
| | Yes | Ref. | | | | |
| Age (Years) | ≤50 vs. >50 | -0.06 | 0.0 | -0.13 | 0.00 | **0.054*** |
| Body Surface Area (BSA) $M^2$ | - | 0.13 | 2.1 | -4.07 | 4.33 | 0.952 |
| Number of other Drugs | None vs. ≥1 | -0.14 | 0.5 | -1.12 | 0.84 | 0.782 |
| *CYP 2C9* *8(449G>A) | Wild type/Homozygous (*GG*) | 1.33 | 0.8 | -0.28 | 2.93 | 0.105 |
| | Heterozygous (*GA*) | Ref. | | | | |
| *CYP 4F2* *3(1347 C > T; V433M) | Wild type/Homozygous (*CC*) | -3.07 | 2.0 | -7.01 | 0.88 | 0.127 |
| | Homozygous (*TT*) | 1.11 | 1.3 | -1.48 | 3.71 | 0.400 |
| | Heterozygous (*CT*) | Ref. | | | | |
| *CYP 4F2*(rs2189784; G> A) | Wild type/Homozygous (*GG*) | 3.01 | 0.8 | 1.36 | 4.65 | **<0.001*** |
| | Homozygous (*AA*) | -1.69 | 1.7 | -4.96 | 1.58 | 0.312 |
| | Heterozygous (*AG*) | Ref. | | | | |
| *VKORC1* (rs9923231) | Wild type/Homozygous (*CC*) | 3.77 | 1.2 | 1.51 | 6.03 | **0.001*** |
| | Heterozygous (*CT*) | Ref. | | | | |

Key: **β** –Beta Coefficient; C.I-Confidence Interval; SE-Standard Error; Sig-Significance. *Statistically significant parameters

Among the sociodemographic characteristics, only participants' gender (β=-3.38, 95%CI: -5.38 to -1.38, p=0.001) and age (β=-0.06, 95%CI: -50.13 to -0.00, p=0.054) predicted the warfarin maintenance dose that would give INR of 2-3, with males requiring almost three times lower doses than females. None of the clinical characteristics of participants, including primary diagnosis, comorbidities, concomitant drugs and ADRs predicted the warfarin maintenance dose producing therapeutic INRs. Furthermore, among the genetic variations, only participants with *CYP 4F2*(rs2189784; G> A) wild type/ homozygousity(*GG*) [β =3.01, 95%CI:1.36-4.65, p<0.001] and *VKORC1* (rs9923231) wild type/homozygousity(*CC*)[ β =3.77, 95%CI:1.51-6.03, p=0.001) required higher warfarin maintenance doses to achieve therapeutic response of INR(2-3) (**Table 4.52**).

To identify whether there is a single clinical or genetic factor impacting on warfarin maintenance dose that is likely to produce therapeutic INR, parsimonious modeling revealed that none of the variables was the independent predictor of the safest initial warfarin dose for the patients.

Parsimonious model accomplishes a desired level of explanation or prediction with as few predictor variables as possible. In this regard, we first conducted bivariate regression analysis and all the variables that did not meet the threshold of statistical significance were removed. The variables which remained significant at that level were fitted into the model. None of the variables entered had a variance of more than 20% and therefore, there was no need of adding the removed variables at bivariate level. The stepwise regression by including all the variables arrived at the same conclusion. The $R^2$ regression model which was used had the highest good-ness- of-fit.

Using the above clinical and genetic information, we developed equation 3 below that could yield a safe warfarin dose producing therapeutic INR (**Equation 3**).

**Equation 3: Nyamu's Equation for Predicting Safe Warfarin Dose that produces therapeutic level of INR**

$$\text{Safe Warfarin Dose}_{(\text{Mg})} = -3.38(\text{Male}) + 3.01\ \textit{CYP 4F2}(\text{rs2189784; G> A})_{(\text{Wild type/Homozygous } (\textit{GG}))} + 3.77\ \textit{VKORC1}\ (\text{rs9923231})_{(\text{Wild type/Homozygous } (\textit{CC}))}.$$

# CHAPTER FIVE: DISCUSSIONS, CONCLUSIONS AND RECOMMENDATIONS

## 5.1: Outline of Discussions

This chapter interrogates the study findings and compares or contrasts them with published literature. Plausible explanations for the deviations or similarities are also highlighted. In addition, scientific explanations on some findings, which may not corroborate with other results, have been proposed.

The chapter begins by examining the clinical determinants of oral anticoagulation use among participants attending Kenyatta National Hospital. It then proceeds to look into the population characteristics of participants, the types of thrombotic disorders encountered and the various warfarin doses prescribed. It also explores the pattern of anticoagulation control among the participants and endeavors to explain the findings with other similar studies. The associations between the sociodemographics and clinical characteristics versus the warfarin dosing patterns, INRs and ADRs have been discussed.

The second part of the discussion looks into the genetic variations among Kenyan patients with respect to oral anticoagulation therapy. Specifically, the discussion dwells on the major genes that impact on the metabolism of warfarin. The allele frequencies and prevalence of SNPs of the genes as well as their polymorphisms in Kenyan tribes have been discussed along with other related findings reported elsewhere. The impact of the polymorphisms on the dosing patterns of warfarin and responses to the drug as measured by INRs and ADRs has been explained. Lastly, the section looks into the level of contribution of each of the predictor variables to the measured response to warfarin as well as doses prescribed. The study has also characterized the independent genetic and non-genetic factors impacting on warfarin dose and level of INRs. This characterization led to derivation of the equation that would produce safe initial warfarin doses.

## 5.2: DISCUSSIONS

### 5.2.1: Clinical Variables Impacting on Warfarin Dose and Response

#### 5.2.1.1: Clinical Indications of Warfarin Anticoagulation

The major clinical indications of warfarin anticoagulation among the study participants were venous and cardiac thromboembolic disorders. Related studies done in the same settings (Karuri, 2016; Kibiru, 2012; Mariita *et al*., 2016) found varying proportions of the clinical indications of warfarin anticoagulation. Specifically, Mariita *et al* (2016) found the prevalence of VTEs and heart diseases at 48.3% and 17.7 %, respectively (Mariita *et al*., 2016). Karuri *et al* (2016) found higher prevalence of VTEs at 82.5% but a lower proportion of heart diseases at 7.6% (Karuri, 2016). Although the reported figures were slightly different, probably due to differences in study methodologies employed all of them highlighted VTEs as the most common clinical indications of warfarin anticoagulation among Kenyans patients.

The proportions of VTEs were higher than cardiac diseases in all studies suggesting that warfarin is majorly used for the management of DVT and pulmonary embolism in the Kenyan healthcare settings. Anticoagulation studies done in other countries had been more specific to one disease condition. For instance, atrial fibrillation among some Western countries (Le Heuzey *et al*., 2014) and mechanical heart valves in Tanzania (Makubi *et al*., 2008) have been studied. As such, there were no proportions of VTEs or cardiac diseases for comparison with the present study.

The current study also found out that the proportion of males with heart diseases was significantly higher than females. Probably majority of male participants were patients with valvular abnormalities, which were the major heart-related diseases in the present study. Furthermore, similar studies have reported higher proportions of men with such cardiovascular diseases (Appelman *et al*., 2015). In contrast, there were more females with VTEs compared to males although this was not statistically significant despite other studies revealing significance of female gender as a risk for VTEs (Rogers Jr *et al*., 2007).

There was a slightly larger proportion of participants aged 50 years and below with a diagnosis of heart disease compared to those aged >50 years ($p=0.044$). It was observed that most of the patients suffered from heart diseases comprising of valvular abnormalities secondary to rheumatic fever. Studies have revealed that valvular abnormalities occur in young and middle

aged persons (Iung and Vahanian, 2011) probably due to rheumatic heart disease which commonly afflicts people of such age in the developing countries. Conversely, in the developed countries, valvular heart diseases occur more commonly among the elderly patients due to degenerative diseases of the heart valves.

Obesity status of the participant had a statistical significance with the clinical diagnosis of anticoagulation. Participants with heart diseases appeared to be underweight or normal weight ($p<0.001$) whereas those with VTEs such as DVT were overweight/obese ($p=0.002$). Although the present study was not intended to find out the risk factors for VTEs, the degree of obesity has been linearly linked to the development of thrombotic events (Anderson Jr and Spencer, 2003; Hansson *et al*., 1999; Stein *et al*., 2005). Besides, the development of valvular heart disease was not correlated to obesity status but participants were generally normal or underweight suggesting that these participants may have lost weight over time due to the chronicity of their disease conditions.

Statistically significant proportion of participants with heart diseases was comprised of young persons and without spouses ($p=0.002$). Probably they were not married at the time of study. Furthermore, there was a statistically significant proportion (73.7 %) of participants with spouses suffering from VTEs ($p=0.018$). The study found out that participants with VTEs were generally of older age and perhaps married. Other sociodemographic factors such as employment status, education level, denomination and tobacco or alcohol use did not yield any statistically significant associations with the clinical indications of warfarin anticoagulation among the study participants.

#### 5.2.1.2: Food, Nutritional and Herbal Medicine use among the Participants

Studies have reported that some diets interact with warfarin (Delaney *et al*., 2007; Fugh-Berman, 2000; Nutescu *et al*., 2011). Frequency and amounts consumed among patients using warfarin is, therefore, important for patients' counseling. In particular, frequent consumption of vegetables and fruits with high levels of vitamin K may interact with warfarin necessitating higher warfarin maintenance doses. Majority of participants (70 %) in this study were consuming vegetables and fruits for 3-7 times in a week but the frequency of consumption of various food types did not have significant associations with warfarin maintenance doses.

Although the present study did not individually characterize the green leafy vegetables consumed by the participants, previous local studies have reported that the commonest used herbs include *Sukuma wiki (kales), Amaranthus hybridus L., Sesamum calycimum Welw. var. angustifolium (Oliv., Crotalaria ochroleuca (Kotschy) Polhill, Senna occidentalis L., Sida acuta Burm. F, Portulaca quadrifida L.and Cucurbita maxima Duchesne ex Lam.* (Orech *et al*., 2005). These green leafy vegetables consumed locally contain mainly vitamin A (Oiye *et al*., 2009) vitamin C, beta carotene, iron, oxalate as well as calcium (Chweya, 1983; Mwajumwa *et al*., 1991) and protein (Chweya, 1983) . Other related studies have found that most of the vegetables consumed, especially by the Nyanza and western communities in Kenya contain polyphenols and flavonoids (Orech *et al*., 2005). All these constituents have not been reported to impact on warfarin anticoagulation therapy. However, the present data revealed that participants with VTEs and taking various food types for more than three times per week required higher mean warfarin maintenance doses, which was a general observation in this group of patients.

Almost a fifth and 12.2 % of the study population was using garlic and ginger supplements, respectively. Several herbal supplements have been implicated in influencing the action and response to warfarin therapy (Jiang *et al*., 2006, 2005; Krüth *et al*., 2004; Vaes and Chyka, 2000). For instance, use of ginger (Krüth *et al*., 2004) and garlic (Vaes and Chyka, 2000) have been associated with over-anticoagulation in patients using vitamin K antagonists. In the present study, participants suffering from VTEs and consuming garlic or ginger supplements required clinically higher warfarin maintenance doses than their counterparts suffering from heart diseases (>6.2 vs. <5.6 mg per day). Certainly, these were the general observations for participants with VTEs. However, use of ginger and garlic did not show any statistically significant associations with warfarin maintenance doses.

Consumption of the two herbal products did not reveal any significant association with the levels of INRs or development of ADRs. Possibly the proportions of participants using the herbal supplements were relatively small to elicit statistically significant associations with warfarin response. Furthermore, the frequencies of their consumption and the amounts consumed thereof were not measured to assess the degree of any possible clinical significance with the level of anticoagulation as well as ADRs. It is likely that the quantities consumed as well as the frequencies of their consumption were not significantly impacting on the warfarin response.

Besides, participants may have forgotten to give adequate information because the data collected relied on them recalling use of herbal remedies.

Other herbal products such as gingko biloba (Jiang *et al*., 2005; Vaes and Chyka, 2000) and ginseng (Vaes and Chyka, 2000) as well as red clover, St John's Wort , alfalfa, aloe vera, vitamin C, E and K have been shown to interact with warfarin thereby producing varying responses but the use of these supplements among study participants was rare. However, it is worth to note that use of herbal remedies is increasingly becoming common in developing countries among patients on chronic diseases (Bodeker and Ong, 2005) and therefore, this finding should not be underestimated, but instead interrogated through specific structured studies.

**5.2.1.2: Doses of Warfarin Prescribed**

Three quarters of study participants had been initiated on warfarin at 6-10mg per day. Few participants were receiving higher (>10mg) or lower doses (<6mg). The revelations deviated from the recommendations from internationally recognized guidelines which suggests that all participants requiring warfarin anticoagulation should be initiated at 10mg/day unless there are special precautions (Ageno *et al*., 2012).

Participants aged 50 years and below received clinically higher mean initial daily doses of the drug than their older counterparts. This tallies with other studies that found comparable higher average daily doses among younger patients (Shahin *et al*., 2011) but lower daily doses of up to 3.5 mg among the individuals with advanced years (Garcia *et al*., 2005). Moreover, it was apparent that the initial daily doses of warfarin was inversely related to the age and strongly associated with gender where females were receiving clinically lower doses than men.

The present study revealed that the average warfarin maintenance dose was 6.17±2.75mg per day (range 2-20mg) which is supported by a previously related study done in the same setting that found 6.81±2.67mg (Ogendo, 2000). Since the median duration of warfarin therapy was approximately 2 years, this revelation suggests that majority of the participants were already on the stabilized warfarin maintenance amounts equivalent to the average doses found. In contrast to these findings, the mean daily maintenance warfarin dose among the Chinese was lower at

approximately 3mg per day (Jia *et al*., 2017) . The differences observed could be attributable to genetic and environmental variations between the populations studied.

A previous study relating to factors affecting warfarin maintenance doses in clinical practice found that the dose was inversely proportional to age and was associated with participants' sex. In this study, the median daily dose ranged from 6.4 mg/day for males who were aged < 50 years to 3.1 mg/d for females ≥ 80 years (Garcia *et al*., 2005). In the current study, this observation was also maintained even after aggregating the clinical indications and trying to find out the association between warfarin maintenance doses across the gender. Furthermore, men were found to receive clinically significant higher warfarin maintenance doses than women across the two main aggregated clinical indications: VTEs (6.6 ±2.5mg vs. 6.1±2.6mg/day) and heart diseases (6.3±2.8mg vs. 5.4±1.6mg/day). Aggregation of clinical indications of warfarin anticoagulation also revealed that participants with VTEs received a higher warfarin maintenance dose than those with heart diseases. Additionally, participants aged 50 years and below were found to receive higher daily warfarin maintenance doses than their older ones, especially in the heart disease category. Related studies have also indicated that as the patient's age advances, sensitivity to warfarin increases (Saleh, 2016).

There was statistically significant relationship between the status of marriage and initial warfarin doses prescribed (p=0.031), with participants living with spouses receiving higher warfarin doses compared to those without spouses (6.3 ±2.3mg vs. 5.5 ± 2.1mg). It was observed that majority (~75%) of participants with spouses had diagnoses of VTEs. Participants suffering from VTEs required higher doses than other patients. Similarly, in other related studies diagnosis of VTEs was associated with higher warfarin dose requirements (Kahlon *et al*., 2016). Additionally, participants suffering from VTEs received clinically higher initial and maintenance daily doses of warfarin compared to those suffering from other clotting disorders (6.19±2.2mg vs. 5.69±2.1mg and 6.44±2.6 vs. 5.95±2.6mg, respectively).

Exploration of the data on warfarin dosing patterns revealed that participants with heart diseases received a statistically lower initial warfarin doses (5.56 ±2.1mg vs. 6.38±2.2 mg, p=0.014) and clinically lower maintenance doses than other participants requiring anticoagulation for other clinical indications such as VTEs. This finding is supported by other related studies (Shahin *et*

*al*., 2011). For instance, among the Egyptians, patients with VTEs such as pulmonary embolism were found to receive statistically significant higher warfarin maintenance doses (Shahin *et al*., 2011).

Several comorbidities have been shown to impact on the development of venous thromboembolic events. For instance, atrial fibrillation has been linked to development of thrombosis or aggravate the existing thromboembolic conditions (Watson *et al*., 2009) thereby necessitating higher warfarin maintenance doses. The present study found that nearly two-fifths of the participants had at least one other disease apart from the clinical indication of anticoagulation. These other diseases included the hypercoagulable states as well as non-coagulable conditions such as seizure disorders. However, the number of comorbidities present in a participant did not significantly impact on the maintenance doses of warfarin prescribed. Though, participants with VTEs and comorbidities seemed to require slightly higher warfarin maintenance doses than those without concomitant illnesses (6.44±2.6 vs. 5.03±1.7mg per day).

In an attempt to find out the magnitude of other diagnoses apart from the primary VTEs, it was observed that some of the participants with DVT had secondary hypercoagulable conditions such as heart failure, HIV and malignancies which have also been highlighted in similar studies (Chan *et al*., 2008). These are probably the explanations behind the higher warfarin maintenance doses observed in patients suffering from VTEs.

Commonly co-prescribed medicines have been reported to interact with warfarin (Rikala *et al*., 2015) and necessitate maintenance dose titrations. However, less than half (45.0%) of the participants were using at least one other drug apart from warfarin but out of these, 40.0% had a single additional agent. The principal agents were antiarrythmics (15.6%), probably for the management of atrial fibrillations, followed by antibiotics (7.2 %) and analgesics (6.7 %) for pain which is common in thrombotic episodes. However, addition of these drugs to the regimen did not have any statistically significant associations with the warfarin maintenance doses in the present study. In the present study, participants with heart diseases receiving one or more additional drugs required clinically lower maintenance doses of warfarin compared to those who had none. In contrast, participants with VTEs and also receiving at least one extra drug required higher daily warfarin maintenance doses than those without. It is possible that there were no substantial drug-drug interactions to elicit statistically significant adjustment of the warfarin

maintenance doses. Nevertheless, the potential for drug-drug interaction and its clinical relevance should not be underestimated because studies have shown that hemorrhagic episodes are common in patients using warfarin and other drugs (Delaney *et al*., 2007; Linkins *et al*., 2003; Ogendo, 2001).

**5.2.1.3: Factors Associated with Warfarin Maintenance Doses**

Few participants (<10.0%) were receiving warfarin maintenance doses of less than 5mg or 10mg and above across the clinical indications, which is comparable with findings from other similar studies that indicated a lower proportion (Kahlon *et al.*, 2016) However, over 50% of participants were receiving warfarin maintenance doses of 6-10mg per day across the clinical indications. Furthermore, a significant proportion of participants (15.5 %) with DVT were receiving slightly more than10mg warfarin maintenance dose per day. Interestingly, unlike other clinical indications, none of the participants with pulmonary embolism was receiving warfarin maintenance doses of less than 6mg suggesting that for adequate anticoagulation in this population with DVT and PE, higher maintenance doses are required. Furthermore, it was observed that a larger proportion of participants with VTEs required more than 5mg of warfarin maintenance doses compared to participants with heart diseases (86.2% vs. 78.7%).

Participants with chronic rheumatic heart disease and atrial fibrillation generally required lower mean warfarin maintenance doses than those with heart valve surgery (≤5mg per day). Among the Chinese population, studies have also indicated that patients with rheumatic heart disease required statistically significantly smaller maintenance doses than those with heart valve replacements (2.9 ± 1.2 vs. 3.7 ± 1.4 mg) (Yu *et al*., 1996).

Participants who were obese tended to receive clinically significant higher warfarin maintenance doses than the non-obese counterparts across the clinical indications. In addition, obese participants with diagnosis of VTEs seemed to receive higher daily warfarin maintenance doses than their counterparts with heart diseases (6.5±2.8mg vs. 5.9±2.0mg). Several studies have established such linear relationship of obesity and higher warfarin dose requirements (Mueller *et al*., 2014; Patel *et al*., 2011; Wallace *et al*., 2013). Wallace *et al*, for instance, on their study on comparison of warfarin dose requirements in obese and non-obese participants found that compared to normal weight, morbidly obese patients required higher average daily maintenance doses of warfarin, 7.6 ± 0.5 vs. 5.0 ± 0.3 mg (Wallace *et al*., 2013). Furthermore, another study

demonstrated that for every single increase in body mass index, the weekly maintenance dose of warfarin had to be positively adjusted by approximately 0.7mg (Mueller *et al*., 2014). Available literature suggests that the clearance of vitamin K antagonists increases with patients' obesity status (Patel *et al*., 2011) and probably explains the higher dose requirements in this participant category.

The present study found out that there was a significant relationship between marital status and warfarin maintenance dose requirements in participants receiving anticoagulation due to heart diseases. Participants who were living with spouses and the same time suffering from heart diseases required statistically significant higher daily warfarin maintenance doses than the unmarried ones (6.1 ± 2.2 vs. 5.1 ± 1.9 mg, $p$=0.05). Probably the married participants suffering from heart diseases tended to be obese and hence the higher warfarin maintenance dose requirements as has been found in related studies (Mueller *et al*., 2014). On the other hand, married participants with diagnoses of VTEs received clinically significant higher doses than their unmarried counterparts (6.2 ± 2.7mg vs. 6.0 ± 2.5 mg per day) although this was not statistically significant. There were slightly varying warfarin maintenance doses across the other sociodemographic categories and clinical indications of anticoagulation but their associations were not statistically or clinically significant (P>0.05).

On multilevel analysis, marital status and diagnosis of heart diseases were subjected to multivariate generalized linear model using identity link with the participants with spouses and clinical indication of heart disease as references. Analysis revealed that diagnosis of VTEs was independent predictors of higher warfarin dose requirements compared to other requirements for anticoagulation.

**5.2.1.4: Methods of the Assessment of Outcome of Anticoagulation in the Study Population**

INR values and spectrum of ADRs to warfarin therapy were the target outcomes of the present study. The mean INR was 2.29, ranging from 2.19-2.38 across the clinical indications of warfarin. Similar mean INR values of 2.5±1.18 have been reported (Ogendo, 2000) although this was among patients with valvular abnormalities. Participants with rheumatic heart disease showed the highest mean INR at 2.38 which could have been attributed to higher doses as the clinicians tried to achieve the recommended INR target of 2.5-3.5 (Ageno *et al*., 2012) among this patient population. The finding is comparable with a previously obtained INR value of 2.32

±1.04 for aortic valve replacement patients (Ogendo, 2000). All participants with pulmonary embolism were receiving warfarin doses of greater than 6mg per day although they had the lowest mean INR at 2.19 suggesting other factors may have influenced the level of anticoagulation in this group.

INR determinations were conducted for six clinic visits and the results showed that therapeutic anticoagulation was maintained by 35.2-48.4% participants. This reflects poor anticoagulation control as most of the participants had out-of-range INRs. These findings agree with those from previous local studies reporting 40.2% (Ogendo, 2000) among the patients with valvular abnormalities and 43.5% among those assessed for adherence (Mariita *et al*., 2015). Additionally, Karuri *et al* indicated that only 20% of the patients maintained adequate anticoagulation for 50% or more of their follow up time, and the overall time that patients spent in therapeutic INR was approximately 30% (Karuri, 2016). A similar regional study in Tanzania showed that only 35.5 % achieved adequate anticoagulation control among patients with mechanical heart valves (Makubi *et al*., 2008). The present findings, however, contrast the revelations from a British population where patients suffering from non-valvular atrial fibrillation were within therapeutic INR for almost 70% of the follow up time (Jones *et al*., 2005).

There were more under-anticoagulated (31.5-48.8%) than over anticoagulated participants (9.3-22.9%) across the six clinic follow ups. The proportions were high compared to the participants from the Western countries, 16.7 % for INR<2 and 15.4% for INR > 3 (Jones *et al*., 2005). These conflicting reports could be attributable to a number of factors including different patients' population, study settings and the target ranges of therapeutic INR that were considered. For instance while in the present study we considered INR 2-3 to be therapeutic, Jones *et al* took theirs to be 2-3.5(Jones *et al*., 2005).

Based on the clinical indications, there were higher proportions of participants with heart diseases who achieved therapeutic INR than those with VTEs. This compares with reports from a group of patients followed up at cardiothoracic clinics and attributed it to the use of predesigned anticoagulation clinic re-appointment cards which had details of INR monitoring (Mariita *et al*., 2016; Nyamu *et al*., 2017). Perhaps previous related publications from the local studies, which

were entirely on valvular heart patients (Ogendo, 2000; Ogendo, 2001), may also have improved the anticoagulation among these patients.

The proportion of males who were optimally anticoagulated was statistically significantly more than that of females (51.3% vs. 30.4%; p=0.040). Furthermore, more females than males were likely to be over-anticoagulated (p=0.017). Related studies have shown that female gender is a risk factor for over-anticoagulation using vitamin K antagonists (Apostolakis *et al*., 2013; Cadiou *et al*., 2008). Other regional studies have also indicated that there are statistically significant proportions of females who are either over-anticoagulated (Mariita *et al*., 2016) or with out-of-therapeutic ranges of INRs (Karuri, 2016; Mariita *et al*., 2015; Ogendo, 2001). Moreover, previous related studies on the outcome of anticoagulation management among the Western countries with cardiovascular disorders have indicated that males have better outcome than females (Dagres *et al*., 2007).

The proportion of participants maintaining therapeutic INRs increased with advancing age as has been established in related studies (Mariita *et al*., 2016; Nyamu *et al*., 2017). There is a possibility that as the patient's age advanced, they get accustomed to the treatment of the disease and thus better manage it as compared to the young individuals.

There was a larger proportion of participants (BMI>25) who were therapeutically anticoagulated compared to those with BMI≤25 (41.7% vs. 32.0 %). In contrast, related studies indicate that higher BMI values are associated with under-anticoagulation and therefore, need for higher warfarin doses (Mueller *et al*., 2014). The present finding supports that other factors such as knowledge and adherence as proposed in related studies (Mariita *et al*., 2016; Mariita *et al*., 2015) are associated with INR within the therapeutic range. In addition, there were more under-anticoagulated non-income earners than regular earners (55.6% vs. 46.2%) probably due to differences in affordability of the comprehensive anticoagulation care. Similarly, there were greater proportions of participants with secondary education level and above who were adequately anticoagulated compared to the ones with lower education level probably due to differences in understanding and handling of the clinical indication of anticoagulation. Other sociodemographic variables such as marital status and alcohol use had no statistically significant relationship with the level of anticoagulation.

It was also observed that there was larger proportion of patients with heart diseases who had therapeutic INRs compared with VTEs (40.0% vs. 43.6 %). This difference may be attributed to the differences in the clinics that the two groups of participants were managed. Whereas the heart diseased participants were attended at cardiac and cardiothoracic clinics, the participants with VTEs were treated at medical outpatient hemato-oncology clinic. The attending clinicians in the clinics differed in their specializations. It was observed that most of the participants with cardiac diseases were attended by cardiothoracic surgeons while significant proportions of patients suffering from VTEs were treated by physicians.

Although the present study did not reveal any statistically significant associations between the number of co-prescribed medicines and INRs of 2-3, the proportions of participants who were adequately anticoagulated was inversely proportional to the number of other drugs co-prescribed. Several factors are likely to contribute to this observation. For instance, as the pill burden increased, non-adherence to warfarin therapy, which has been attributed to poor anticoagulation (Mariita *et al*., 2015), is likely. Secondly, the many drugs could have partially interacted with warfarin and thus impacting negatively on anticoagulation although this was not shown to be statistically significant probably due to the small number of participants with one or more other co-prescribed drugs. Furthermore, an average of 10-20% participants remained over-anticoagulated across the number of co-prescribed medications. Studies had previously indicated that some of the concomitant medications were predictors of excessive anticoagulation (Rikala *et al*., 2015).

A significant proportion of participants with one or more comorbidities was either over anticoagulated (19.7% vs. 13.6 %) or under anticoagulated (50.8% vs. 47.6%) compared to those with no other diseases. Perhaps some of the comorbidities could have intensified or decreased warfarin's action and hence the observations made. These findings corroborate literature (Apostolakis *et al*., 2013). Furthermore, the proportion of participants with therapeutic INRs was significantly lower for participants with comorbidities compared to those without (29.5% vs. 38.8%).

The response to warfarin therapy was also monitored through documentation of ADRs. Almost half of the patients experienced ADRs due to warfarin therapy including bleeding, gastrointestinal disturbances, purple toes, alopecia and skin necrosis, among others.

Haemorrhage accounted for 27.8% and was the most common ADRs among patients receiving warfarin therapy. Reports from UK indicate that the incidence of idiopathic warfarin-related bleeding was 15.2 per 100 patient-years (Hollowell *et al*., 2003). In Denmark, the incidence rate of gastrointestinal bleeding in patients using vitamin K antagonists was 2.8% (Johnsen *et al*., 2001). Other studies have reported the incidence of gastrointestinal bleeding at almost 6% (Gallagher *et al*., 2014). Local literature reported bleeding episodes at 20.7% among patients with open heart surgery (Ogendo, 2001). Neurological ADRs such headache, dizziness and weakness as well as GIT disturbances were also reported in the present study. However, these could not be purely attributed to warfarin because they could be caused by a several other diseases whose assessments were beyond the scope of the present research.

The ADRs to warfarin therapy occurred at therapeutic as well as out-of range INRs, including sub-therapeutic levels. This corroborates other studies which have revealed that ADRs to warfarin, such as bleeding, can occur at therapeutic doses and any INRs (Wysowski *et al*., 2007). However, in the current study, more than three-quarters of the ADRs occurred at INRs above 3.These were probably bleeding events associated with warfarin, which are more likely to feature at higher INRs as has been revealed in related studies (Kucher *et al*., 2004).

There was no gender and age preference in the development of ADRs to warfarin therapy ($p>0.05$) in contrast to some studies that have highlighted that males and older age were independent factors of major adverse effects of the drug treatment (Lindh *et al*., 2008). Similarly, the participants' BMI, employment status, the level of education and tobacco or smoking habits were not statistically significantly associated with development of ADRs to warfarin.

There was a significant relationship between marital status and adverse drug reaction to warfarin, with significant proportion of participants without spouses experiencing ADRs. Although there may be no plausible scientific explanation for this revelation, exploration of the data revealed that majority of the participants without spouses were suffering from heart diseases and had lower BMI $\leq 25$. Related studies have revealed that the major adverse effects to warfarin such as bleeding are common in patients on anticoagulation due to heart valve diseases (Ogendo, 2001). In addition, some studies have also highlighted that the independent factors for the major adverse effects of warfarin are heart diseases (Hughes and Lip, 2007). Furthermore, a significant

proportion of patients (54.5%) with heart diseases developed ADRs. These patients were found to have lower BMI which has been linked to higher INRs and bleeding. Probably cardiac patients (with lower BMIs) were using similar warfarin doses to other participants and as such were likely to experience more bleeding episodes than their counterparts with VTEs.

On multilevel analysis only the marital status was the independent predictor of ADRs to warfarin, with those living without spouses being two times more likely to have adverse drug reaction than those with spouses. The present study revealed that majority of participants without spouses had BMI≤25 and were suffering from heart diseases which have been associated with increased ADRs to warfarin therapy (Mueller *et al*., 2014; Ogendo, 2001). Furthermore, there was a significant proportion (54.5%) of participants with heart diseases who experienced ADRs to warfarin. The present findings suggest that there could have been other factors associated with ADRs apart from the clinical diagnosis, concomitant drugs, food types and comorbidities among the participants.

### 5.2.2: Genetic Variables Impacting on Warfarin Dose and Response

This section compares and contrasts the genetic findings with related studies done elsewhere. Where the contrasts are found, scientific explanations have been sought. Moreover, a detailed discussion of the genetic analysis of results such as allele frequencies as well as prevalence of SNPs in the study population and across the Kenyan ethnicities has been attempted. In addition, the contributions of the genetic variants to the warfarin maintenance dose and responses (INR and ADRs) have been illustrated. The magnitude of contribution of each genetic variable to warfarin dose and response has been highlighted. Lastly, the participants' clinical and genetic information was used to find out the independent determinants of prescribed warfarin doses that are likely to produce therapeutic INR of 2-3.

#### 5.2.2.1: Characteristics of the Study Participants Who Underwent Genetic Testing

The population was comprised of relatively middle-aged individuals, married and with female majority who were receiving long-term warfarin anticoagulation treatment. Related local studies on anticoagulation using warfarin have generated similar findings of middle-aged persons and female majority (Iqbal, 2017; Karuri, 2016; Kibiru, 2012; Mariita *et al*., 2016; Mariita *et al*., 2015; Nyamu *et al*., 2017). Most of the studies done in the Western countries have revealed female preponderance as well but elderly population (Apostolakis *et al*., 2013; Dagres *et al*.,

2007) owing to differences in the clinical indications of anticoagulation. Western studies were principally for patients with atrial fibrillations, a condition which is more likely to occur among the elderly due to changes that occur in the heart as a result of aging processes.

Most of the participants (62.5%) were obese or overweight with a median BMI of 27.0 (range 18.4-55.8). Although obesity has been linked to the development of thromboembolic events in related studies (Stein *et al*., 2005), it is not clear whether participants were obese before the primary diagnosis or the obesity developed in the course of management of disease because this information was not captured in the present study. This notwithstanding , healthcare workers should encourage anticoagulation patients to maintain healthy BMI because obesity is associated with increased warfarin dose requirements (Patel *et al*., 2011).

Approximately 70% of the participants did not have any comorbidity and half had no concomitant drugs prescribed. This means that anticoagulation control achieved by 52.5% of the participants while taking 6-10mg (mean 6.0±1.9mg) warfarin daily maintenance doses (79.5 %), was minimally influenced by other diseases or drugs. In addition, the mean INR of 2.3±0.8 was therapeutic perhaps due to the same observation. The finding that participants had used warfarin for a median duration of 51.5 months (range 1.3-381.1 months) suggests that stable warfarin maintenance doses had been established. Therefore, the genetic information obtained could confidently be correlated with the stable warfarin doses. However, the initial warfarin doses prescribed (5.9±1.9mg per day) were below the recommended international guidelines (Ageno *et al*., 2012) although this could have been attributed to unavailability of local anticoagulation protocols as has been found in related studies (Nyamu and Guantai, 2018).

The main indication of warfarin anticoagulation was DVT (60.0%) which corroborates other locally related studies (Iqbal, 2017; Mariita *et al*., 2016; Nyamu *et al*., 2017). Other indications included major surgeries (27.5 %), rheumatic heart disease (15.0%) and atrial fibrillation (7.5 %). It was observed that the major surgeries were either heart valve replacements or repairs. These findings suggested that there were two main types of participants undergoing anticoagulation therapy: VTEs (which was principally DVT), comprising 60.0% and heart disease (40.0%). Although most of the local studies have found similar high proportions of VTEs (Mariita *et al*., 2016; Pastakia *et al*., 2010), studies done on the high income countries have

mainly dealt with anticoagulation on patients with heart diseases, especially atrial fibrillation (Apostolakis *et al*., 2013; Gladstone *et al*., 2009).

#### 5.2.2.2: Ethnic, Residence and Denominational Differences in Warfarin Dose Requirements and INRs among Participants

The highest warfarin maintenance dose requirement was exhibited by the Kalenjins and Taita (approximately 7.5mg daily) while the lowest warfarin doses were found among the Duruma and Meru participants (5.0mg per day). However, it is not feasible to make significant inferences on this revelation because the number of participants involved was relatively small (n=1 for Kalenjin, Duruma, Taita and n=2 for Meru) but this calls for larger studies to verify the findings.

There are no published studies that report established relationship between patients' place of residence and warfarin dose requirements as well as the response as measured by INRs. Therefore, as part of exploring the contribution of environmental factors affecting warfarin response, it was revealed that participants living in some suburbs of Nairobi required higher warfarin maintenance dose than participants living elsewhere. In addition, participants living far from the hospital required lower maintenance doses of warfarin compared to the others. There was, however, no predictable pattern in regard to the participants' place of residence and the requirements of warfarin doses and INR values. For example, some participants living near the hospital required higher warfarin doses while others were prescribed lower maintenance doses. It is important to report that timing of the sampling of blood, in relation to the last warfarin dose, for INR determination was not uniform across the participants. As such, the distance that the participant travelled to hospital, the time that the last dose of warfarin was taken and the mode of transport may have impacted on the INRs measured.

The mean INRs for the study participants who underwent genetic testing were within therapeutic levels across the sociodemographic variables, though 25.0 to 33.0% of participants had sub-therapeutic INRs. Furthermore, there were no significant relationships between sociodemographic characteristics and INR therapeutic categories. INR responses also varied across the participants' places of residence. A similar observation was made for participants living far from Nairobi. For instance, participants living in Voi, which is almost 330Km from Nairobi required mean warfarin maintenance doses of approximately 8.0 mg per day but the mean INR was <2. Participants living in Bungoma (300km from Nairobi City) required 5.0mg

daily but the mean INR was approximately 3.0. These findings suggest that participants' residence, though impacting on the lifestyle activities, may not be a predictor of warfarin dose requirements and response as measured by INR. Perhaps the recorded permanent residence was not where participants regularly lived. Additionally, the place of residence could affect the cultural (dietary habits) and consequently warfarin dose requirements and measured INRs.

There is scanty published literature on the role of ethnicity and warfarin response in the Kenyan population. In the present study, the tribes which revealed therapeutic INRs during follow-up were Duruma, Embu, Kamba, Kikuyu and Luhya. Participants from Kalenjin, Kisii, Luo, Taita and Meru tribes had out-of-range INRs. Studies have shown that the differences in cultural activities and environmental factors may play some roles in the response to warfarin therapy. Furthermore, the role of ethnic differences in drug response has been established in related studies (Yasuda *et al*., 2008) and has been thought to be due to differences in pharmacodynamics and pharmacogenetics as well as genetic, dietary habits or both (Yasuda *et al*., 2008).

#### 5.2.2.3: *CYP 2C9, VKORC1* and *CYP 4F2* Polymorphisms and their Impact on Warfarin Dose Requirements among Kenyan Patients

Genetic study showed did not detect allelic variants of *CYP 2C9* *2, *3, *4 *5 *6 and *13. However, the *CYP 2C9* variants were exhibited by *8 and *11. *CYP 2C9* *8 showed heterozygousity (*GA*) variants at 17.5% while the rest were wild type (*GG*) alleles. This heterozygousity was observed among the two ethnolinguistic groups. In addition, there was small proportion of variants of *CYP 2C9* * 11 with heterozygousity (CT), which was only exhibited by the Bantus.

The *CYP 2C9* frequencies observed have similarities with some African studies. For instance, in some African studies, the variants of *CYP 2C9* were found to be rare (Matimba *et al*., 2009; Ndadza *et al*., 2019b). Among the south Africans, unlike *CYP 2C9* *8 whose variants were prevalent (Mitchell *et al*., 2011), *CYP C9 *2* was not found to be polymorphic (Dandara *et al*., 2014; Mitchell *et al*., 2011). However, *CYP2C9*2* and *3* alleles were highly frequent among Egyptians (Ndadza *et al*., 2019b) but rare in other African populations, including Kenya (Dandara *et al*., 2014; Ndadza *et al*., 2019b). Other related findings have indicated the polymorphisms in *CYP 2C9* in African populations accounted for <1 % unlike in European populations where they were prevalent (Suarez-Kurtz and Botton, 2013). Furthermore, African

populations have also been shown to display the largest genetic diversity unlike the Caucasians where *CYP 2C9* *2 and *3 are prevalent and contribute significantly to warfarin dose requirements (Dandara *et al*., 2014; Li *et al*., 2009; Ozgon *et al*., 2008). Among the Indian population high allelic frequencies of *CYP 2C9* *2 and *3 were observed but polymorphisms were low and *CYP 2C9* *2 wild type(*CC*) a*cc*ounted for 90.7% , where C=0.95 and T=0.046 (Shalia *et al*., 2012). In the present study, *CYP 2C9* *2, the wild type (*CC*) accounted for 97.5%.

*CYP 2C9* polymorphisms which included variants in *CYP 2C9* *8 (449G>A) and *CYP 2C9* *11(1003C>T) were associated with clinically significant lower warfarin maintenance doses. For instance, participants with *CYP 2C9* *8 heterozygous (*GA*) were shown to require lower warfarin maintenance doses compared to the wild type/homozygous (*GG*) (5.2±2.7mg vs. 6.1±1.7mg per day). Additionally, participants with *CYP 2C9* *11 *TC* heterozygousity alleles were prescribed lower mean maintenance dose of warfarin than the wild type (*CC*) (3.0mg vs. 6.0±1.9 mg per day).

There is scant published literature on the impact of *CYP 2C9* *11 allelic variants on warfarin dose requirements. However, polymorphisms in *CYP 2C9* gene, which is responsible for termination of warfarin anticoagulation (Sanderson *et al*., 2005), are associated with the decreased activity of the enzyme and hence lower warfarin dose requirements (Ndadza *et al*., 2019b; Yin and Miyata, 2007).*CYP 2C9* encodes for the enzyme which metabolizes S-warfarin and the allelic variants contribute to lower warfarin dose requirements due to impaired hydroxylation of warfarin (Aithal *et al*., 1999; Scordo *et al*., 2002; Voora *et al*., 2005; Yin and Miyata, 2007). In addition, the mean warfarin maintenance doses have been found to be highest in participants with the *CYP 2C9* wild type alleles (*GG*) compared to those with variants (Ndadza *et al*., 2019a; Sanderson *et al*., 2005; Sconce *et al*., 2005). Specifically, among the African-Americans, *CYP 2C9* *8 polymorphisms were associated with lower warfarin dose requirements (Cavallari *et al*., 2010). In a South African black patients' study on the impact of *CYP 2C9* polymorphisms on warfarin dose variability among 213 participants , it was demonstrated that *CYP 2C9* *8 variants were associated with a reduction in the doses of warfarin required (Mitchell *et al*., 2011),while among the Han-Chinese, the variant CYP2 C*9* *3 required lower warfarin dose than the wild type (Liang *et al*., 2012).

The single *VKORC1* (rs9923231) SNP investigated in the present study was found to be polymorphic. It revealed heterozygousity variants (*CT*) at 12.5 % whereas the wild type, homozygous (*CC*), accounted for 87.5%. The heterozygousity was found among the Bantus. *VKORC1*(-1639G>A) variant alleles, though less than the wild type, have been found among the Indian population where the wild type and heterozygousity were 22.4% and 75.4%, respectively (Shalia *et al*., 2012). In addition, the variants of *VKORC1*(rs9923231) have been revealed among the south African blacks and shown to influence warfarin response (Mitchell *et al*., 2011). Moreover, among the Ethiopians, studies have shown polymorphisms in *VKORC1* contributing to warfarin resistance (Aklillu *et al*., 2008). Studies have also shown a 6% prevalence of *VKORC1* Asp36Tyr SNP among the Kenyans and Sudanese (Shahin *et al*., 2013) while the prevalence of *VKORC1* (rs9923231) polymorphisms among the South Indians was revealed at 12.0% (Kumar *et al*., 2013) which corroborated the present findings.

The present study revealed that participants with *VKORC1* (rs9923231) heterozygous (*CT*) alleles variants required clinically higher warfarin maintenance doses than the wild type/homozygous (*CC*) counterparts. This finding corroborates other related studies which indicated that the mean daily warfarin's maintenance dose among patients carrying the *CC* genotype was approximately (D'Andrea *et al*., 2005). In contrast, other studies from Indonesia showed that *VKORC1*(rs9923231) *AA* were associated with low warfarin dose requirements of 2.05 ± 0.77 mg/day (Suriapranata *et al*., 2011). The observed differences could be attributed to the difference in functionality status of the enzyme with the amino acid substitution involved.

The polymorphisms of *VKORC1* have been widely characterized and found to be associated with warfarin dose variability among patients (Aklillu *et al*., 2008; Ozgon *et al*., 2008; Scott *et al*., 2010; Shahin *et al*., 2013). Among the Egyptians, for instance, patients with *VKORC1* allelic variants required significantly higher daily warfarin maintenance doses than those with wild type alleles (Shahin *et al*., 2013). In the Han-Chinese population, *VKORC1* polymorphisms have been found to have the strongest effects on the requirements of warfarin doses (Lee *et al*., 2009) and studies have suggested that genotyping of *VKORC1* −1639 G>A SNP could be clinically significant for estimating the safest doses for warfarin therapy (Obayashi *et al*., 2006). Among the Ethiopians, polymorphisms in *VKORC1* have been linked to warfarin resistance and higher dose requirements (Aklillu *et al*., 2008) while in South Africa, *VKORC1* variants necessitated

increase in warfarin doses among black patients (Mitchell *et al*., 2011). Furthermore, patients requiring very high warfarin maintenance doses (>10mg per day) have been shown to exhibit *VKORC1* p.Asp36Tyr mutations (Bodin *et al*., 2008).

*CYP 4F2* gene had the most common variants across all the genes investigated in the present study, with *CYP 4F2* (rs2189784; G>A) revealing 60.0 % polymorphisms (heterozygous, 47.5% and homozygous 12.5%). The homozygous variant (*AA*) was found among the Bantu while the heterozygous (*AG*) allele was detected in the majority of the two ethnolinguistic groups studied. In addition, *CYP 4F2* *3 (1347C>T) homozygous (*TT*) variants were detected among the Bantus whereas the heterozygous (*CT*) alleles were found across the two ethnolinguistic groups. The present study revealed higher frequencies of *CYP 4F2* SNPs among Kenyans compared to other populations. For instance, though lower frequencies have been reported , related studies indicated *CYP 4F2* *3 variants to be significantly prevalent among Ashkenazi Jewish (AJ), Caucasian, Asian and Hispanics than African–Americans (0.233–0.342 vs. 0.117) (Scott *et al*., 2010).

The polymorphic nature of *CYP 4F2* is well documented (Borgiani *et al*., 2009; Cen *et al*., 2010; Kim *et al*., 2018; Liang *et al*., 2012; Scott *et al*., 2010). For example, among the Italians, *CYP 4F2* (rs2189784;G>A) variants have been shown to be prevalent and contributing to warfarin dose variations in patients (Borgiani *et al*., 2009). Furthermore, *CYP 4F2* *3(1347C>T) has been shown to be polymorphic in systematic reviews involving Africans, Caucasian and Asian populations (Liang *et al*., 2012).

This research revealed that allelic variants of *CYP 4F2* *3 *(rs2108622)* and *rs2189784* were clinically associated with inter-patient variability in the doses of warfarin required. Participants with *CYP 4F2 V433M (rs2108622)* heterozygous (*CT*) required higher warfarin doses than those with the wild/homozygous (*CC*) type (6.6±1.2mg vs. 6.0±2.0mg) and the homozygous (*TT*) variants (6.6±1.2mg vs. 3.8±1.8mg). The clinical relevance of *CYP 4F2* polymorphisms on warfarin dose variability has been described in several studies (Borgiani *et al*., 2009; Cen *et al*., 2010; Fohner *et al*., 2015; Liang *et al*., 2012; McDonald *et al*., 2009; Nakamura *et al*., 2012). In USA, for instance, *CYP 4F2* polymorphisms revealed clinically differences in the requirements of warfarin doses (Fohner *et al*., 2015), accounting for a variation of 1mg per day between the patients exhibiting *CC* and *TT* (Caldwell *et al*., 2008). Among the Han-Chinese, patients carrying

*CYP 4F2* *3(rs2108622) *TT* or *CT* allele required statistically significantly higher doses of warfarin than those having *CC* (3.40 ± 0.14 mg/day vs. 2.80 ± 0.14 mg/day) (Liang *et al*., 2012).

In Asia, the mean dose of warfarin was statistically significantly lower in participants having *CYP 4F2* *3 (rs2108622) wild-type/homozygous *CC* than with individuals having the variant alleles of *CT* and *TT* (wild type vs. *TT*+ *CT*= 3.0 $mgd^{-1}$ vs. 3.8 $mgd^{-1}$) (Singh *et al*., 2011). A similar observation was found among the Japanese whereby the carriers of *CC* and *CT* required warfarin maintenance doses of 2·88 ± 1·00 mg/day and 3·59 ±1·80mg/day, respectively (Nakamura *et al*., 2012), yet these doses are lower than found in the present study. In a meta-analyses of 22 studies, patients with *CT* or *TT* alleles of *CYP 4F2* *3 required higher warfarin doses compared to carriers of *CC* (Sun *et al*., 2016). Reports indicated that participants with the *CYP 4F2 V433M (rs2108622)* polymorphisms are expected to have raised blood levels of Vitamin K1, demanding an escalation in the dose of warfarin to produce similar anticoagulant outcome (McDonald *et al*., 2009).

In the current study, participants with *CYP 4F2 (rs2189784)* wild type/homozygous *GG* required clinically higher warfarin doses than the variant homozygous *AA* and heterozygous *GA* (6.3±2.1mg vs. 5.0±2.5mg and 5.9±1.7mg, respectively). Related studies have yielded conflicting reports. For instance, in a study on multiethnic population, warfarin doses did not vary significantly according to *CYP 4F2 (rs2189784)* variants (Lubitz *et al*., 2010). In another related study, Burmester *et al* (2011) indicated that *CYP 4F2* polymorphisms were associated with higher warfarin dose requirements although the findings were not statistically significant (Burmester *et al*., 2011) while among the northern Chinese patients, polymorphisms in *CYP 4F2* rs2189784 were associated with lower warfarin dose requirements (Liu *et al*., 2017).

There are limited published studies on warfarin pharmacogenetics among African populations. In one study African-specific variants of *CYP 2C9* were confirmed, but no significant differences were found in the frequencies of the SNPs between ethnolinguistic groups (Matimba *et al*., 2009). Related African studies on two ethnolinguistic groups revealed that unlike Nilotes, there was similarity between the South African black people pharmacogenetics and Bantus in Kenya. The study suggested that the *CYP2C9* SNP *(rs1799853)* was not polymorphic among the Bantus and some *VKORC1* SNPs had low variant allele frequencies, restricting their significance to warfarin dose in this population (Dandara *et al*., 2011). Another study on worldwide distribution

of the important genes impacting on warfarin's action showed that *CYP 2C9, VKORC1* and *CYP 4F2* are polymorphic among the Bantus in African content (Ross et al., 2010).

#### 5.2.2.4: Genetic Variations in Warfarin Response as Measured by INRs

The present results did not reveal any statistically significant relationship between the genetic variations studied and level of anticoagulation achieved. The mean INRs were within therapeutic anticoagulation ranges (2-3) across the genetic variants studied. Furthermore, none of the participants with *CYP 2C9* *8 and *11 variants was over-anticoagulated despite related studies revealing that *CYP 2C9* polymorphisms, due to their effect of reduced activity on warfarin metabolizing enzyme, are linked to higher risks of over-anticoagulation and bleeding among participants (Higashi *et al*., 2002).

The influence of genetic polymorphisms on the warfarin's activity as measured by the level of INR has been widely studied but has mixed findings (Higashi *et al*., 2002; Limdi *et al*., 2008; Spreafico *et al*., 2008; Taube *et al*., 2000). One study confirmed the association between *CYP 2C9* variant allele and increased warfarin sensitivity but no risk of over-anticoagulation (Taube *et al*., 2000). Another study found no variation between the genotype and INR (Haug *et al*., 2008). However, these observations were made among the Americans genotyped for *CYP 2C9* *2 and *3. There is a possibility that the variations observed were attributed to differences in study methodologies, the genotypes explored and the sample sizes. Some of the genotypes in the present study had small numbers and the data needed some caution in interpretation. For instance, only one participant with *CYP 2C9*11* had heterozygous *CT* and was under-anticoagulated.

With respect to the effect of *VKORC1* polymorphisms on warfarin response as measured by INR, over-anticoagulation was found in one individual who had *VKORC1 (rs9923231)*, but the mean INR for this subgroup was within range (2.7±0.9). Polymorphisms of *VKORC1* have been reported to necessitate raised doses of warfarin and under or over-anticoagulation (Spreafico *et al*., 2008). Other studies have found no association between *VKORC1* and INR in warfarin anticoagulation (Haug *et al*., 2008) while some have associated the polymorphisms with over-anticoagulation (Pautas *et al*., 2010; Schalekamp *et al*., 2006) in association with other multiple polymorphisms (Pautas *et al*., 2010). The observed differences may be attributed to the *VKORC1*

genotypes studied and the sample size as well as the pharmacokinetics of warfarin. For instance, the present research studied the effect of *VKORC1* *2 on INR but other studies have explored the roles of *VKORC1* *1, *3 and *4 on anticoagulation level (Haug *et al*., 2008; Schalekamp *et al*., 2006; Spreafico *et al*., 2008). Also, some studies have identified these differences as well and implicated the pharmacokinetics(half-life) of warfarin (Meckley *et al*., 2008).

The present study found that *CYP 4F2 *3(rs2108622)* genotypes were not statistically significantly associated with over-anticoagulation but there were 2 participants with *CT* genotype who were under-anticoagulated. Previous studies which followed up patients over time revealed that INR greater than 3 was more than four times lower in the patients having *CYP 4F2* *3 (rs2108622) *TT* genotype than in those having *CC* (wild type) or *CT*, which suggested that participants with the *TT* allele were resistant to warfarin therapy and were less likely to be over-anticoagulated (Bejarano-Achache *et al*., 2012). On the other hand, *CYP 4F2(rs2189784)* genotypes in the present study were generally associated with out-of-range INRs, with majority of the patients with *GA* genotype being under-anticoagulated and 10.5% of heterozygous AG genotype having supra-therapeutic INR. In related studies, a SNP of rs2189784, which was in strong linkage disequilibrium with rs2108622, revealed an inverse relationship with time-to-therapeutic INR (Zhang *et al*., 2009) while another study found no association between the genotype variations of *CYP 4F2* and warfarin response as measured by INR (Rusdiana *et al*., 2013).These conflicting findings may be partly attributed to dissimilarities in populations studied and the *CYP 4F2* SNPs that were investigated.

**5.2.2.5: Genetic Variations and Occurrence of ADRs to Warfarin Therapy**

The association between genetic variants of warfarin metabolism and adverse drug reactions have been highlighted in several studies among the white populations (Aithal *et al*., 1999; Visser *et al*., 2004). Some researchers revealed that polymorphisms of *CYP 2C9* were associated with increased risk of haemorrhage in participants on warfarin anticoagulation therapy (Aithal *et al*., 1999; Kawai *et al*., 2014). Although there was no statistically significant association between genetic variants and ADRs (which was mainly bleeding) in the current study, a number of participants with various genotypes developed adverse reactions to warfarin. For instance, 71.4% participants with *CYP 2C9* *8 *GA* genotype and the single participant with *CYP 2C9* *11 *TC* genotype experienced some ADRs to warfarin therapy. Associated studies have indicated that

participants with *CYP 2C9* *2 and *3 polymorphisms had a significantly higher risk of major haemorrhagic episodes while on acenocoumarol therapy (HR 1.83, 95% CI: 1.01-3.32) (Visser *et al*., 2004). However, there is limited data on the influence of *CYP 2C9* *8 and *11 on the ADRs of Vitamin K antagonists among the black population.

There was a larger proportion of participants with wild type *CC* genotype of *VKORC1 (rs9923231)* who experienced ADRs compared to the *CT* genotype (46.9% vs. 25.0%) in the present study. In contrast, literature revealed that polymorphisms in *VKORC1*, especially carriers of *T* allele, were associated with increased bleeding risk among phenprocoumon but not acenocoumarol users (Reitsma *et al*., 2005) although this was a different genetic variant of C1173T in *VKORC1* which was investigated among patients. Increased bleeding tendency to warfarin therapy has been found in patients with *VKORC1*(-1639G>A) polymorphisms in related case control studies (Sridharan *et al*., 2016). However, some studies did not reveal statistically significant relationship between increased risk of bleeding and this genotype variant (Montes *et al*., 2008).

Polymorphisms of *CYP 4F2* were associated with fewer adverse drug reactions compared to the wild type variants. However, *CYP 4F2* (rs2189784) *AA* genotype was associated with more ADRs (75.0%) than the heterozygous *AG* (31.6%) and the wild type *GG* (52.9 %) genotype. Previous genetic studies on the influence of *CYP 4F2* polymorphisms on ADRs to warfarin therapy have not yielded any statistical or clinical associations (Kawai *et al*., 2014). However, among the European-Americans, possession of *CYP 4F2* *3 variant influenced ADRs to warfarin therapy following a recessive pattern whereby *CYP 4F2* *3/*3 genotype demonstrated a protective effect of lower risk of bleeding (Shendre *et al*., 2016). The different observations made in these studies may be related to the different loci of *CYP 4F2* and the sample sizes studied.

### 5.2.3: Relative Contribution of the Predictor Variables to Variability in Warfarin Dose and Response

In the present study, both clinical predictors and genetic determinants accounted for 35.0%, 58.0% and 50.0% of inter-individual warfarin dose variation, INR responses and ADRs, respectively.

The clinical predictors which included sociodemographic and clinical characteristics as well as dietary habits of the participants contributed to 14.0% of the interindividual variation in the dose of warfarin in the current study. A study among Chinese patients revealed that two clinical predictors (age and weight) contributed to 14.7% dose variation between patients (Miao *et al*., 2007), which was similar to the present findings. Other studies have revealed conflicting reports and higher proportions. For instance, one study revealed that the clinical determinants contributed to 27.5% of interindividual warfarin dose variation (Kamali *et al*., 2004) while other related studies indicated proportions of 17-22% (Gage *et al*., 2008). These conflicting findings could be related to the different sociodemographic and clinical predictors evaluated.

In the current study, among the sociodemographic characteristics, BSA yielded the greatest inter-individual dose variations at 6.9%. Participants' age had a small impact of 0.2% which contrasts other studies that have indicated higher figures of 14.6% (Hillman *et al*., 2004). In related studies, age and weight provided 62.8% of warfarin dose variation (Miao *et al*., 2007), while in other studies, age, BSA and weight could explain 16.8%, 4.4% and 6.3%, respectively of dose difference between patients (Kamali *et al*., 2004). Furthermore, previous findings had revealed that BSA had an impact on the requirements of warfarin doses (Kirking *et al*., 1985). In one study, BSA contributed 7.5% of the interindividual warfarin dose variation (Hillman *et al*., 2004) which tallies with the present finding. Of note is that dietary habits among the study participants contributed minimally to the inter-individual warfarin dose variation at 0.2%.

The combined effect of the sociodemographic, clinical characteristics and food contributed to 19.8% variability in the observed INR responses, with BSA and BMI accounting for 4.5% and 3.8%, respectively. In addition, the sociodemographic, clinical characteristics and food contributed to 18.9% of the observed ADRs to warfarin therapy. There is limited published literature on the contribution of clinical predictors to INRs and ADRs. However, since the greatest contributor to the differences observed in INR was BSA at 4.5%, it is possible that this is a signal to incorporate BSA in warfarin dosing among Kenyans. The observation that marital status had the highest impact in the variability of the observed ADRs at 5.1% in the study requires further investigation which was beyond the scope of the present investigation.

Genetic polymorphisms in CYP *2C9*, *VKORC1* and *CYP 4F2* together contributed to 20.8% of inter-participant variability in the dose of warfarin required in the present study. These findings

were similar to those of a study which indicated that the genetic variability alone accounted for approximately 20.0% of inter-patient variability in warfarin doses (Hillman *et al*., 2004). Studies have indicated that among patients on warfarin therapy, *CYP 2C9* and *VKORC1* combined accounted for 23.5% of dose variation between patients (Michaud *et al*., 2008). Other studies have revealed higher rates of 53-54% (Gage *et al*., 2008) and 51.4% (Aquilante *et al*., 2006) probably due to differences in study methodologies and the SNPs or genes investigated.

Collectively, genetic and non-genetic determinants of response to warfarin in the present study accounted for 35.0% variability of the dose requirements which corroborates another study which revealed 36.0% (Cavallari *et al*., 2010). Besides, among the African-Americans, *CYP 2C9* genetic variability, participants' age and BSA explained a third of the variability in the doses of warfarin prescribed (Momary *et al*., 2007). Others have indicated higher proportions at 62.8% among the Chinese (Miao *et al*., 2007), 55.0% among the Americans (Sconce *et al*., 2005) , 54.8% in the Japanese (Ohno *et al*., 2009), 60.5% among the Italians (Borgiani *et al*., 2009), and 62.0 % among the Han-Chinese (Wen *et al*., 2008) as well as 31.0% among the Egyptians (Shahin *et al*., 2011). The differences observed could be related to variations in number of the clinical indicators and genes investigated. For example the Japanese study investigated other factors impacting on warfarin metabolism such as gamma glutamyl carboxylase enzyme (*GGCX)* and Factor VII(FVII) (Ohno *et al*., 2009) unlike the present study.

The individual contribution of *CYP 4F2*, *CYP 2C9* and *VKORC1* polymorphisms accounted for 10.8%, 9.9% and 0.1%, respectively of the observed differences in warfarin maintenance doses. Other related studies have indicated that the contribution to inter-patient variability in the dose of warfarin was approximately 5.0% for *CYP 2C9* and 6.0% for *VKORC1* − 1639G>A (Kimura *et al*., 2007) as well as 49.4% for *VKORC1* in some previous work (Miao *et al*., 2007). Contrary to the present findings, one Italian study indicated that *CYP 4F2* polymorphisms alone contributed 7.0% of the inter-patient variability in warfarin doses (Borgiani *et al*., 2009). In addition, previous studies had suggested that *CYP 4F2* contributed 1.0-2.0% of dose variation among patients (Takeuchi *et al*., 2009), and in some Egyptian studies, this gene was not significant (Shahin *et al*., 2011) unlike the present study.

The impact of genetic polymorphisms on INRs and ADRs to warfarin has not been widely explored in previous nticoagulation studies. The present study found that *CYP 2C9, CYP 4F2*

and *VKORC1* polymorphisms contributed to 18.0%, 17.3% and 2.7% of the variability in the INRs observed. The greatest variability in the INRs observed between patients was contributed by *CYP 2C9* *8(449G>A) polymorphisms at 13.1% followed by *CYP 4F2* *3(1347 C > T; V433M) variants at 8.6%. Furthermore, *CYP 4F2* (rs2189784; G> A) heterozygous *AG* genotype contributed the greatest in the variability observed in development of ADRs at 8.6%, followed by *CYP 2C9* *8 (449G>A) heterozygous *GA* genotype at 7.8%. *CYP 4F2* polymorphisms contributed to 14.3% variability in ADRs experienced.

### 5.2.4: Clinical and Genetic Determinants of Warfarin Maintenance Doses that and Therapeutic INR values

In order to determine which of the clinical or genetic factors independently predicted warfarin doses that produced INR of 2-3, multiple linear regression models were done. These models revealed that only participants' gender (β=-3.38, 95% CI: -5.38 to -1.38, p=0.001) and age (β=-0.06, 95% CI: -50.13 to -0.00, p=0.054) predicted the warfarin maintenance dose that would give INR of 2-3, with males requiring almost three times lower doses than females. None of the clinical characteristics of patients, including primary diagnosis, comorbidities, concomitant drugs and ADRs predicted the warfarin maintenance dose producing therapeutic INRs. Likewise, among the genotypes, only participants with *CYP 4F2 (rs2189784; G> A*) wild type (*GG*) [β =3.01, 95%CI: 1.36-4.65, p<0.001] and *VKORC1* (rs9923231) wild type (*CC*)[ β =3.77, 95%CI:1.51-6.03, p=0.001) required higher warfarin maintenance doses to achieve therapeutic response of INR (2-3).

This information led to derivation of the present study equation (**Equation 3**) that could be used to estimate a safe warfarin dose which can produce therapeutic INR:

**Equation 3:**

**Warfarin Dose $_{(Mg)}$ = -3.38(Male) +3.01 *CYP 4F2* (rs2189784; wild type *GG*) + 3.77 *VKORC1* (rs9923231; wild type *CC*)**

This equation implies that the gender and genotyping for polymorphic *CYP 4F2* and *VKORC1* as revealed in the Kenyan population studied are important parameters which can guide optimization of warfarin anticoagulation.

### 5.2.5: Strengths, Weaknesses and Limitations of the Present Study

Genotyping was carried out on a limited number of genetic markers of impacting on warfarin pharmacokinetics and pharmacodynamics. There are other several genes which are involved in warfarin's action. However, this is the first Kenyan genetic study that has established a foundation for other future related local genetic studies on warfarin anticoagulation.

Kenya has more than 40 tribes but the present study captured only ten Kenyan communities. Additionally, there were tribes with very low representation. It was not practicable to do genetic studies to all the Kenyan tribes owing to the study eligibility criteria, study setting as well as exorbitant costs. Furthermore, the clinical indications of warfarin anticoagulation are not extensively prevalent in all the Kenyan tribes so as to be able to capture all of them. Nevertheless, the ten tribes represented the two main ethnolinguistic groups which account for approximately 90% of the Kenyan population.

### 5.2.6: New Knowledge Acquired from this Study

This is the first Kenyan study to characterize the non-genetic and genetic determinants of warfarin response. Furthermore, 3 important genes comprising 11 SNPs supposedly impacting on warfarin dose and response among the Kenyans were characterized.

This present research has led to the characterization of prominent genetic influencing factors in three genes: *CYP 2C9*, *VKORC1* and *CYP 4F2*, accounting for a significant part of the inter-patient variation in the required dose of warfarin, INR responses and ADRs among Kenyans patients on anticoagulation therapy. It has also proposed an equation, using the clinical and genetic data, which may be used to estimate safe and effective warfarin dose that can produce therapeutic INR. The study has found that the non-genetic determinants account for approximately 14.0%, 19.0% and 20.0% in determining warfarin response as measured by maintenance dose, INRs and ADRs, respectively .Additionally, it has been demonstrated that the genetic determinants accounted for approximately 21.0%, 38.0% and 31.0% of dose variability, INR responses and ADRs, respectively among the Kenyan patients.

### 5.2.7: Study Limitations

Determination of some clinical predictors of warfarin response such as frequency of food and nutrional consumption, use of herbal products and recreation activities were purely subjective. Therefore, participants may have overstated or understated their experiences. In addition, some participants declined to be interviewed while others failed to provide blood samples for genetic testing even after consenting to participate. Secondly, genetic testing was done on a limited number of participants owing to the high costs of genotyping.

## 5.3: CONCLUSIONS

### 5.3.1: Clinical Predictors of Warfarin Doses Requirements and Response

Patients were undergoing anticoagulation with warfarin due to VTEs and cardioembolic disorders. VTEs were observed among women and obese participants while cardiac related thromboembolic disorders were seen among men and lean individuals. There were limited comorbidities among the participants and therefore, the concomitant medications were also few except for the antiarrythmics.

Participants with VTEs generally required higher warfarin maintenance doses than those with heart diseases. A large proportion of participants with spouses was found to have VTEs and therefore, required significantly higher maintenance doses of warfarin. Males and younger participants ($\leq$ 50 years) required higher warfarin maintenance doses. Higher warfarin maintenance doses were also required with advancing BMI. Multivariable analysis revealed that compared to other clinical diagnoses, VTEs were independent predictors of higher warfarin dose requirements. There were no significant associations between warfarin maintenance doses and consumption of herbal products in the present study owing to the small proportion of consumers of these products.

The clinical variables of the participants contributed to a 14.0% of warfarin maintenance dose variation between patients, with BSA contributing the greatest variability at 6.9 %.

There was poor anticoagulation control with less than 50.0% of the participants being therapeutically anticoagulated across the follow-up clinics. However, participants with heart

diseases were better anticoagulated than their counterparts suffering from VTEs. In addition a significant proportion of males were adequately anticoagulated compared to females.

ADRs due to warfarin were observed with the commonest being bleeding, which was reported by almost a third of the participants. However, over two-thirds of all the participants did not have INR indicated when they developed ADRs. Frequency of consumption of various food categories and use of herbal medicine did not significantly impact on the ADRs to warfarin therapy. ADRs were significantly associated heart diseases and living without spouses. Participants without spouses were twice more likely to suffer from ADRs than those with spouses

### 5.3.2: Genetic Determinants of Response to Warfarin Therapy

There were no variant alleles for *CYP 2C9* *2, *3, *4, *5, *6 and *13 among the ethnolinguistic groups studied. Variant alleles among *CYP 2C9* were expressed by *8 and *11 with the latter having the lowest frequencies (T=0.025). *CYP 4F2* exhibited the greatest allelic variants with *CYP 4F2 (rs2189784)* revealing higher prevalence than *CYP 4F2* *3*(rs2108622)*. The prevalence of *VKORC1* (rs9923231) polymorphisms were at 12.5%, all heterozygous *CT*.

*CYP 2C9* *8 variant alleles were detected across the two ethnolinguistic groups while *11 variants were only demonstrated by the Nilotes. *VKORC1* (rs9923231) variants were exhibited by the Bantus while *CYP 4F2*3 (rs2108622)* and *CYP 4F2* (rs2189784) were detected in the two ethnolinguistic groups studied.

Polymorphisms in *CYP 2C9* were clinically associated with lower doses of warfarin among the participants and generally better therapeutic INRs but more ADRs. In contrast, polymorphisms in *VKORC1* and *CYP 4F2* were generally associated with a general increase in the dose of warfarin required; lower therapeutic INRs but fewer ADRs. The individual contribution of *CYP 4F2*, *CYP 2C9* and *VKORC1* polymorphisms explained 10.8%, 9.9% and 0.1%, respectively, of the warfarin dose variability between patients.

*CYP 2C9*, *CYP 4F2* and *VKORC1* polymorphisms accounted for 18.0%, 17.3% and 2.7% of the variability in the INRs observed, respectively. *CYP 4F2* polymorphisms contributed the greatest in the variability observed in the development of ADRs at 14.3 %,

## 5.4: RECOMMENDATIONS

### 5.4.1: Recommendations for Practice and Policy Change

This study has demonstrated the clinical predictors and genetic determinants of warfarin dose requirements and response among the black Kenyan population on long-term warfarin anticoagulation therapy. It shows that clinicians should be aware that certain patients' characteristics including male gender, younger age, advanced BMI and a diagnosis of VTE necessitate higher warfarin dose requirements. BSA contributed the greatest variability in inter-patient warfarin dose and, therefore, should be incorporated when optimizing long-term warfarin anticoagulation. Data from this study recommends development of screening and follow-up protocol for patients on warfarin therapy where all monitoring parameters such as patient's body weight, height, ADRs, warfarin dose and corresponding INRs can be captured. Patients should be advised to keep the chart to be used during the next clinic attendance.

Intensification of monitoring of warfarin therapy is recommended among female patients who were poorly anticoagulated. Despite participants with heart diseases revealing better anticoagulation through measurements of INRs, this patient population suffered from more ADRs. Healthcare workers should therefore be encouraged to monitor the ADRs in patients on warfarin anticoagulation due to heart-related conditions.

The present study has revealed that *CYP 2C9* *2, *3, *4, *5, *6 and *13 variants are rare among the ethnolinguistic groups on anticoagulation. Clinicians should consider genotyping for the polymorphic *CYP 2C9* *8 and *11, *VKORC1* and *CYP 4F2* when optimizing warfarin anticoagulation among Kenyans of the two ethnolinguistics origin. Furthermore, genetic polymorphisms of *CYP 4F2* variants accounted for 10.0% inter-individual variability in warfarin dose and may be specifically factored in warfarin dose determination.

### 5.4.2: Recommendations for Further Research Work

Prospective studies involving large sample sizes should be carried out to characterize the types, content and the magnitude of contribution of herbal products to warfarin dose requirements, the level of anticoagulation and the possible drug-food interactions and ADRs among patients on long-term warfarin therapy.

There is also need to characterize the types and constituents of the vegetables consumed, and their impact on warfarin dose and the level of anticoagulation in patients on long-term warfarin therapy.

Extensive genetic studies involving many study sites are necessary in order to widen the population catchment for the allelic frequencies for all the tribes and to further build on the present findings. In addition, large Kenyan pharmacogenetic studies exploring the role of each gene involved in warfarin pharmacokinetics and pharmacodynamics are recommended in order to improve long term warfarin anticoagulation.

When combined both clinical predictors and genetic determinants accounted for 35.0%, 58.0% and 50.0% of inter-individual warfarin dose variation, INR responses and ADRs, respectively. This implies that there are other clinical, pharmacodynamics and pharmacokinetic factors affecting warfarin response. Therefore, other determinants such as health facility, pharmacoeconomics and prescriber-related factors require to be investigated for optimal warfarin therapy. Furthermore, future studies should review the available coagulation management protocols and correlate them to the practice.

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# APPENDICES

**Appendix IA: Informed Consent Document**

**CONSENT EXPLANATION**

**Dear Respondent,**

My name is Dr. David Nyamu, a Clinical Pharmacist by profession. I am currently undertaking a study on the warfarin, which is a blood thinning drug.

I am kindly asking you to take part in this research study to find out the effect of certain genetic make-up on the amount of blood thinning medicine (called warfarin) which you are receiving. Genetic make-up is the unit of heredity. The basic unit of heredity in everyone is called a gene. Everyone has genes. Genes hold the information to build and maintain one's cells and pass genetic traits to children. We hope to learn whether certain genes influence the initial amount of warfarin required by patients in order to avoid side effects such as bleeding. You were selected as a possible participant in this study because you are on warfarin. In order to decide whether or not you want to be a part of this research study, you should understand what is involved and if there are any potential risks or benefits. This form gives information about the study which will be discussed with you. Once you understand the study, you will be asked to sign this form if you wish to participate.

**THE STUDY**

This study is being done to find out if certain basic units of heredity in the body necessitate an increase or decrease in the amount of warfarin for optimal management of your condition. Knowing this will enable health care providers give initial quantities that are just sufficient while avoiding side effects. In order for this research to be carried out you will be asked a few

questions and be requested to give a small amount of your blood (roughly 5 milliliters). Our researchers will withdraw this blood from a visible vein on your hand into a small container. You may experience slight pain for a short moment. The sample will be studied in our laboratory. However, should we find that our laboratory is not having all the required reagents or equipment for the analysis, then some blood samples may be shipped out for that particular analysis.

**PARTICIPATION**

Your participation in the research is purely voluntary and one CAN withdraw anytime at his or her own will. Your withdrawal will not affect the treatment or services you get in any way.You are encouraged to ask any question(s) regarding the study. You are also encouraged to ask any question(s) for clarity, in case the questions are unclear to you or unsatisfactory. Please take your time to make your decision. Feel free to discuss it with your friends and family.

**RISKS**

There is no known potential health harm or benefit associated with giving this blood. You may not receive any direct benefit from being in the research study. However, the research study has been designed to obtain knowledge that may help other people in future.

**CONFIDENTIALITY**

All the information recorded about you will be kept with strictest confidence. The information will be kept under lock and key and only the researcher will have an access to it. If you have any questions, please do not hesitate to ask us. In case you have any additional questions later, please contact the person(s) given below who will be happy to answer them.

**Dr. David Nyamu (B.Pharm, M.Pharm.),** Lead Investigator,

Department of Pharmaceutics and Pharmacy Practice

School of Pharmacy, University of Nairobi

PO Box 19676-00202 KNH, NAIROBI,

Tel. No. 0722 403671

**Prof. A. N. Guantai (PhD)**

Department of Pharmacology and Pharmacognosy

School of pharmacy, University of Nairobi

Tel 2726300 Ext 43673

**Prof G. O. Osanjo( PhD)**

Department of Pharmacology and Pharmacognosy

School of pharmacy, University of Nairobi

Tel 2726300 Ext 43673

**RESEARCHER'S STATEMENT**

I, **DR. DAVID GITONGA NYAMU, NATIONAL ID NO., 11608865 OF P.O. BOX 1003-00200 CITY SQUARE NAIROBI, TELEPHONE NUMBER 0722 40 36 71,** have clearly explained the purpose and the benefits of the interview to the participant.

I have also explained that this is purely voluntary and the research will not jeopardize the patient treatment in any way.

**Contact Name: Dr. David Gitonga Nyamu.**

**National Id No: 11608865**

**Address: Department of Pharmaceutics & Pharmacy Practice**

**School of Pharmacy, P.O. Box 19676, Nairobi**.

**Telephone Number: 0722- 40 36 71**

SIGNATURE---------- DATE----------

**PARTCIPANT'S STATEMENT**

STUDY SERIAL NO …….

I,…….. (Name of the participant) being 18 years or more and having fully the capacity to consent have been informed about the study entitled:

**"Clinical Predictors and Genetic Determinants of Warfarin Response among Kenyans"**

The implications, duration, purpose, voluntary nature and conveniences or hazards that may reasonably be expected have been explained to me by _____

I have been given opportunity to ask questions concerning the study and these have been answered to my satisfaction. If I have further questions I may contact Dr. D. Nyamu, Tel No. 0722403671 or the Secretary KNH/UoN-ERC, P.O. Box 20723, Telephone number 2726300 Ext 44102, 44355 Nairobi

I understand that I may at any time during the study revoke my consent and withdraw without any penalty. However, my refusal to participate will involve no loss of benefit to which I am otherwise entitled.

**YES** I wish to participate in this study

☐☐ ☐☐ ☐☐

Participant's name …….. Signature …….. …… Date D ☐☐☐☐☐☐

| | |
|---|---|
| | **Inclusion criteria** |
| 1 | Is the participant 18 years of age or older? Yes ☐ No ☐ |
| 2 | Is the participant been consistently on warfarin for one month? Yes ☐ No ☐ |
| 3 | Is the participant undergoing follow-up for anticoagulation at KNH clinics<br>Yes ☐ No ☐ |
| 4 | Has the participant given voluntary informed consent and signed consent declaration form? Yes ☐ No ☐ |
| **Exclusion criteria** *questions 6-11 need to be answered in the negative for eligibility* | |
| 5 | Is the participant not on warfarin therapy either due to non-adherence or treatment alteration based on clinical judgment of attending physician?<br>Yes ☐ No ☐ |
| 6 | For ladies: is she pregnant? This may make participant unsafe, complicate interpretation of the study outcome and may interfere with achieving the set objectives.<br>Yes ☐ No ☐ |
| 7 | Does the participant suffer from any one of the following medical conditions?<br>a. Uncontrolled hypertension ☐ Yes ☐ No<br>b. Peptic Ulcer Disease ☐ Yes ☐ No<br>c. Inherited coagulation disorders ☐ Yes ☐ No |
| 8 | Is the participant mentally challenged such that verbal communication would be problematic in getting the clinical data? Yes ☐ No ☐ |
| 9 | Has participant been advised not to take warfarin by physician? Yes No |
| 10 | Is the patient of black Kenya descent? Yes ☐ No ☐ |
| 11 | Based on the information from item 1-10, is the participant eligible or not?<br>Eligible ☐ Not eligible ☐ |
| 12 | If not eligible, reasons for exclusion…… |
| **Completed by:_____ (***initials/date***) Researcher Personnel** | |

**Appendix IIIA: Data Extraction Tool**

Study Title: **"Clinical Predictors and Genetic Determinants of Warfarin Response among Kenyans"**

**Section A: Participant's demographic data**:-

***Date*** ☐☐ ☐☐ ☐☐ **Participant ID** D - ☐☐☐☐

| **Screening for Demographics** *This is an interviewer administered form. Read each item aloud to the patient* | |
|---|---|
| 1. | Gender of the participant Male ☐ Female ☐ |
| 2. | When were you born? dd ☐☐ mm ☐☐ yy ☐☐ OR What is your age? ☐☐ *years* |
| 3. | What is your marital status?<br>1. Single ☐<br>2. Married ☐<br>3. Separated ☐<br>4. Divorced ☐<br>5. Widowed ☐ |
| 4. | What is your occupation?<br>1. Unemployed ☐<br>2. Salaried ☐<br>3. Self-Employed ☐<br>4. Student ☐ |
| 5. | What is your ethnic group or tribe? |
| 6 | Information on participant's parents:-<br>1. Parents' place of current residence----------<br>2. Parents' place of permanent residence [if different from (a) ]above ----------<br>3. Mother's place of birth (ethnic origin)----------<br>4. Father's place of birth (ethnic origin)---------- |
| 6. | What is your highest academic/Education level?<br>1. Informal ☐<br>2. Primary ☐<br>3. Secondary ☐<br>4. College/University ☐ |
| 7. | What is your denomination?<br>a. Protestant ☐<br>b. Catholic ☐<br>c. Muslim ☐<br>d. Other ☐ Specify_____ |

| | |
|---|---|
| 8 | What is your current place of residence? |
| 9 | Place of residence over the last five years (if different from 7 above) ---------- |
| 10 | Body weight(Kg)----------Height (Metres) ---------- |
| 11 | Body Mass Index (BMI) ---------- |
| 12 | Body Surface Area($M^2$) |
| 13 | Do you smoke cigarettes? Yes ☐ No ☐ |
| 14 | Do you consume alcohol? Yes ☐ No ☐ |
| | |
| | **Completed by:** _____in*itials/date)* **Research Personnel** |

**Section B: Details of warfarin Therapy**

1. Date started on warfarin therapy----------Dose----------mg
2. Duration of warfarin use (days) ----------
3. Patient reminders: What reminds the patient of the next clinic appointment?

   a) Caregiver [ ] b) Relative[ ] b) Calendar[ ] d) Mobile alerts[ ]

   e) Other (specify)

4. Have there been any adverse reactions of warfarin? (Tick):

   a)Haemorrhage (visible or internal) [ ] b) Purple toes…..[ ] c) Skin necrosis……[ ] d) jaundice……[ ] e) alopecia…..[ ] f) Hypersensitivity rash……..[ ] g) Gastrointestinal disturbances; nausea, vomiting, diarrhoea…[ ] h)Headache, dizziness, or weakness [ ] i)Unusual pain or swelling [ ]

5. What was the INR at the development of ADRs?---------- a) <2[ ] b 2-<3[ ] c)3-<5[ ] d) 5-10[ ] e)>10[ ]

6. Actions taken after development of adverse reactions :

    a) Medication change---------- [ ] Name------ Dose----------

    b) Admission---------- [ ]

    c) Stopping of medication---- [ ]

    d) Given antidote [ ] Name--------- Dose--------

    e) None---------- [ ]

7. Resolution of ADRs

    a) Did the ADR resolve? [Yes] [No]

    b) If Yes, time taken to resolve (days) ----------.

    c) If No, fate of the ADR: ----------

8. Contra-indications to warfarin therapy: (Tick whichever is applicable): a)Peptic ulcer disease-----[ ] b)Severe and uncontrolled hypertension------[ ] c)Renal disease….[ ] d) Pregnancy---------[ ]

9. Does the patient have any inherited coagulation disorders? 1Yes----------[ ]

    2No----------[ ]

10. If yes, to number 9 above, please specify----------

**Section C: Details of concomitant medication use**

| | Type of medication (class) | Specific type | Duration of use (days) |
|---|---|---|---|
| 1. | Antibiotics | | |
| 2. | Analgesics | | |
| 3. | Anticonvulsants | | |
| 4. | Antidepressants | | |
| 5. | Antiplatelet drugs | | |
| 6. | Diabetes drugs | | |
| 7. | Gastrointestinal drugs | | |
| 8. | Gout treatment drugs | | |
| 9. | Lipid-lowering drugs | | |
| 10. | Steroids | | |
| 11. | Thyroid drugs | | |
| 12. | Antiarrythmics | | |
| 13. | Antifungal drugs | | |
| 14. | Others, Please specify | | |

**Section D: What are the indications/predisposing factors of warfarin therapy in the patient? (Tick)**

| | **Indications** | **Maintenance Daily dose (mg)** | **Range of therapeutic INR** | |
|---|---|---|---|---|
| | | | **Min** | **Max** |
| 1 | Major surgery ( please give details/ type of the surgery) e.g. Total Hip Replacement | | | |
| 2 | Deep Venous Thrombosis (DVT) | | | |
| 3 | History of pulmonary embolism | | | |
| 4 | Rheumatic heart disease | | | |
| 5 | Atrial fibrillation | | | |
| 6 | Transient ischaemic attacks | | | |
| 7 | Others (specify) | | | |

**Section E) Details of co-morbid illness (es) if any**

| | **Type of illness** | **Duration of illness (months)** |
|---|---|---|
| 1. | | |
| 2. | | |
| 3. | | |
| 4. | | |
| 5. | | |

**Section F: Information on patient's current nutritional status**

1) Diet

| | **Type of diet** | **Frequency (Average number of intakes per week)** |
|---|---|---|
| 1. | | |
| 2. | | |
| 3. | | |
| 4. | | |
| 5. | | |

2.) Does the patient use **any** of the following nutritional supplements? (Tick)

a) Vitamin E (Greater than 400 IU per day)

b) Vitamin C (greater than 500 mg per day)

c) Multivitamins containing Vitamin K

d) Glucosamine & Chondroitin

e) Coenzyme Q10

f) Others, Specify----------

3) Does the patient use any of the following herbal preparations?

| | | | | |
|---|---|---|---|---|
| a) Alfalfa | b) Green tea | c) Garlic | d) St. John's Wort | e) Ginkgo Biloba |
| f) Aloe Gel | g) Red Clover | h) Ginger | i) Sweet Clover | j) Others, please specify |

**Section G: Information on source of warfarin used by patient**

1) Where does the patient normally warfarin from….1[ ] KNH 2[ ] Other Channels

2) Last supply of warfarin was obtained from…..1[ ] KNH 2[ ] Other Channels

**Section H: History of alcohol intake**

1. Does the patient take alcohol? a)Yes---------- b)NO----------
2. If yes, for how long (months) has the patient been taking alcohol?----------
3. Which is the preferred alcoholic beverage/brand----------a)Beer b)Wine c) Spirits d) Local brew
4. Number of units consumed per day----------

**Section I: History of tobacco use**

1. Does the patient use tobacco? **a)** Yes---------- b) NO----------

2. If yes, for how long (months) has the patient been using tobacco?……

**Section J: Warfarin dosing and INR values**

1) What was the INR at the development of ADRs, if any?----------

2) Document at least the **latest four** INR readings and the corresponding warfarin doses (Starting with the current dose and corresponding INR)

| | **Date** | **Dose of warfarin (mg)** | **INR Readings (Produced by the Dose given)** |
|---|---|---|---|
| **1st (Current)** | | | |
| **2nd** | | | |
| **3rd** | | | |
| **4th** | | | |
| **5th** | | | |
| **6th** | | | |

3) Document at least the **first four** INR readings and the corresponding warfarin doses (Starting with the initial dose & corresponding INR)

| | **Date** | **Dose of warfarin (mg)** | **INR Readings** |
|---|---|---|---|
| **1st (Initial )** | | | |
| **2nd** | | | |
| **3rd** | | | |
| **4th** | | | |
| **5th** | | | |
| | | | |

**4) Documentation for genotyping**

1. Time for sampling……..
2. Date and time drug taken……
3. Hours elapsed after the dose…….
4. Dose given……mg

**Section K: Genetic Testing Results**

**KA: *CYP 2C9* Variants:**

1. *CYP 2C9* *2 (430*C>T*) variants present(tick)
    1. Wild type/Homozygous(*CC*)
    2. Heterozygous(*CT*)
    3. Other(Specify)

2. *CYP 2C9* *3 (1075A>C) variants Present(tick)
    1. Wild type/Homozygous(*AA*)
    2. Heterozygous(*AG*)
    3. Other(Specify)

3. *CYP 2C9* *4 (1076T>C) Variants present(Tick)
    1. Wild type/Homozygous(*TT*)
    2. Heterozygous(TA)
    3. Other(Specify)

4. *CYP 2C9* *5 (1080C>G) variants Present(Tick)
    1. Wild type/Homozygous(*CC*)
    2. Heterozygous(CA)
    3. Other(Specify)

5. *CYP 2C9* *6(818delA) Variants Present(Tick)
    1. Wild type/Homozygous(*AA*)
    2. Heterozygous(*AG*)
    3. Other(Specify)

6. *CYP 2C9* *8(449G>A) variants Present(Tick)
    1. Wild type/Homozygous(*GG*)
    2. Heterozygous(*GA*)
    3. Other(Specify)

7. CYP 2C*9* *11(1003C>T) Variants Present(Tick)
    1. Wild type/Homozygous(*CC*)
    2. Heterozygous(*TC*)
    3. Other(Specify)
8. *CYP 2C9* *13(269T>C) variants Present(Tick)
    1. Wild type/Homozygous(*TT*)
    2. Heterozygous(*TC*)
    3. Other(Specify)

**KB: *CYP 4F2* Variants:**

9. *CYP 4F2* *3(1347 C > T; V433M) variants Present(Tick)
    1. Wild type/Homozygous(*CC*)
    2. Homozygous(*TT*)
    3. Heterozygous(*CT*)
    4. Other(specify)
10. *CYP 4F2*(rs2189784; G> A) variants Present(tick)
    1. Wild type/Homozygous(*GG*)
    2. Homozygous(*AA*)
    3. Heterozygous(*AG*)
    4. Other(specify)

**KC: *VKORC1* variants:**

11. *VKORC1* (rs9923231)variants present (Tick)
    1. Wild type/Homozygous(*CC*)
    2. Heterozygous(*CT*)
    3. Other(specify)

*Thank you for your assistance*

**Kiambatisho IB: Waraka Wa Idhini**

**UFAFANUZI**

**Mpendwa Mshiriki,**

Jina langu ni  Dr. David Nyamu, taaluma yangu ni mfamasia tabibu. Ninafanya utafiti wa dawa aina ya warfarin ambayo hulainisha damu.

Ninakuomba  kwa uhuru, ukubali kushiriki katika utafiti huu wa kuchunguza madhara ya baadhi ya vinasaba vya maumbile kwenye kiwango cha dawa ya kulainisha damu (aina ya warfarin) unayotumia. Vinasaba vya maumbile ni chembechembe za kurithi.chembechembe hizi za kurithi zinaitwa kinasaba.kila mmoja wetu ana vinasaba. Vinasaba hubeba taharifa za kujenga na kuimarisha afya za seli zetu na usafirisha tabia kwenda kwa watoto (wazawa) wetu.Tunatumai kujifunza kwamba baadhi ya vinasaba vinaathiri kiwango cha mwanzo kinachohitajiwa na mgonjwa ili kuhepuka madhara ambatanishi ya dawa kama kutokwa damu.Umechaguliwa kama mshiriki katika utafiti huu kwasababu unatumia warfarin.ili kuamua kuendelea au kutoendelea kuwa mshiriki katika utafiti huu inakupasa kuelewa nini kinahitajika na kama kuna athari au faida zozote.waraka huu unakupa taharifa kuhusiana na utafit huu ambazo tutajadiliana na wewe. Ukielewa kuhusu utafiti huu, utatakiwa kutia sahini kwenye waraka huu kama umeridhika kuwa mshiriki.

**UTAFITI**

Utafiti huu unafanyika ili kujua kama baadhi ya vinasaba vya kurithi mwilini vinasababisha kuongezeka au kupungua kwa kiwango cha warfarin kwenye damu katika kutibu tatizo lako.Kutambua hili kutamsaidia mtoa huduma ya afya kukupatia kiwango cha dawa cha mwanzo kinachokutoshereza huku ukihepuka madhara ambatanishi ya dawa.Ili utafiti huu ufanyike,

utaulizwa maswali machache na kuombwa kutoa kiwango kidogo cha damu (kama mililita 5).Watafiti wetu watakutoa damu kidogo kutoka katika mishipa yako ya mkono na kuweka katika kichupa kidogo. Unaweza kuhisi maumivu kidogo kwa muda mfupi sana. Sampuli ya damu itasomwa katika maabara yetu.Aidha, kama tukikuta maabara yetu haina vitendanishi au vifaa vya kutosha , baadhi ya sampuli itapelekwa n'gambo kwa uchunguzi maalumu.

**USHIRIKI**

Ushiriki wako katika utafiti huu in hiari na unaweza kujitoa muda wowote kwa maamuzi yako. Kujitoa kwako hakutaathiri matibabu yako na huduma unazopata kwa njia yoyote ile. Unashauriwa kuuliza swali/maswali kuhusu utafiti huu. Unashauriwa pia kuuliza maswali kwa ufafanuzi zaidi kama maswali yako hayajaeleweka au haujaridhika na majibu uliyopewa.Tafadhali tumia muda wako kufanya uamuzi.Pia jisikie huru kujadiliana na marafiki pamoja na familia yako.

**ATHARI**

Hakuna athari zozote kwa afya yako au faida utakayopata kwa kutoa damu.Unaweza usipate faida ya moja kwa moja kutokana na utafiti huu.Lakini, utafiti hutayarishwa kupata maarifa yatakayosaidia watu siku za usoni (zijazo).

**USIRI**

Taharifa zote zitakazorekodiwa zitatunzwa kwa usiri mkubwa. Taharifa zote zitafungiwa kabatini kwa kufuri na ni mtafiti mkuu pekee ndie atakaeweza kuziona.Kama una swali lolote

tafadhali usisite kutuuliza.Ikiwa una maswali ya ziada baadae, tafadhari wasiliana nami/nasi kwa anuani ifuatayo hapo chini, tutafurai kuyajibu.

**Dr. David Nyamu (B.Pharm.,M.Pharm.),** Mtafiti kiongozi,

Idara ya Pharmaceutics na Pharmacy Practice

Shule ya famasia, Chuo kikuu cha Nairobi

S.L.P 19676-00202 KNH, NAIROBI,

Simu. No. 0722 403671

**Prof. A. N. Guantai (PhD)**

Idara ya Pharmacology na Pharmacognosy

Shule ya famasi, Chuo kikuu cha Nairobi

Simu, No 2726300 Ext 43673

**Prof G. O Osanjo( PhD)**

Idara ya Pharmacology na Pharmacognosy

Shule ya famasi, Chuo kikuu cha Nairobi

Simu, No 2726300 Ext 43673

**KAULI YA MTAFITI**

Mimi, **Daktari. DAVID GITONGA NYAMU, kitambulisho cha taifa NO., 11608865 Wa S.L.P 1003-00200 CITY SQUARENAIROBI, NAMBA YA SIMU 0722 40 36 71,** Nimeelezea kwa ufasaha madhumuni na faida za kumhoji mshiriki.

Pia nimeelezea kwamba kushiriki utafiti huu ni hiari na haitaathiri kwa vyovyote matibabu anayopata mshiriki.

WASILIANA NAMI: DR. DAVID GITONGA NYAMU.

KITAMBULISHO CHA TAIFA NO: 11608865

ANUANI: IDARA YA PHARMACEUTICS & PHARMACY PRACTICE

SHULE YA FAMASI, S.L.P 19676, NAIROBI.

SIMU NAMBA: 0722- 40 36 71

SAHIHI---------- TAREHE----------

**KAULI YA MSHIRIKI**

NAMBA YA USHIRIKI …….

Mini,…….. (Jina la mshiriki) nikiwa na miaka 18 au zaidi nikiwa na uwezo wote, na nikiwa nimehabarishwa kuhusu utafiti wenye kichwa:

adhumuni, muda, malengo, uhiari na urahisi au athari zinazoweza kutarajiwa zimeelezwa kwangu na

…….

Nimepewa nafasi ya kuuliza maswali kuhusu utafiti huu na nimeridhishwa na majibu nliyopewa. Kama nina swali la ziada naweza kuwasiliana na Dr. D. Nyamu, Simu No. 0722403671 au katibu KNH/UoN-ERC, P.O. Box 20723, Simu namba 2726300 Ext 44102, 44355 Nairobi

Ninanfahamu kwama ninaweza kufuta uamuzi wangu wa kushiriki na kujitoa bila tatizo lolote. Aidha, kukataa kwangu kushiriki hakutaathiri huduma au faida ninazotakiwa kupata.

**Ndio** Nimekubali kushiriki katika utafiti huu

Jina la mshiriki …… Sahihi …….. …… Tarahe ……

| | | | | | |
|---|---|---|---|---|---|
| D | | | | | |

**Utambulisho wa mshiriki**

| | |
|---|---|
| | **Vigezo vya kushiriki** |
| 1 | Je mshiriki ana miaka 18 au zaidi? Ndio ☐ Hapana ☐ |
| 2 | Je mashiriki amekua akitumia warfarin mfululizo kwa mwezi mmoja?<br>Ndio ☐ Hapana ☐ |
| 3 | Je mshiriki amekuwa akifuatilia na kupata matibabu kwa kutumia vilainisha damu katika kliniki za KNH?<br>Ndio ☐ Hapana ☐ |
| 4 | Je mshiriki amekubali kwa ridhaa yake na amesaini fomu ya tamko la kukubali?<br>Ndio ☐ Hapana ☐ |
| **Vigezo vya kutoshiriki** *swali la 6 mpaka la11 lijibiwe kupinga ushiriki* | |
| 5 | Je mgonjwa hatumii warfarin kwa sababu hafuati taratibu za matumizi au matibabu kwa mujibu wa daktari anemtibu?<br>Ndio ☐ Hapana ☐ |
| 6 | Kwa wanawake:Je ni mjamzito? Hi inaweza kufanya isiwe salama kwake kushiriki, ikaaribu tafsiri ya matokeo ya utafiti na inaweza kuharibu kupatikana kwa malengo yaliyopangwa<br>Ndio ☐ Hapana ☐ |
| 7 | Je mshiriki anaugua lolote kati ya magonjwa yafuatayo?<br>a. Shinikizo la damu Ndio ☐ Hapana ☐<br>b. vidonda vya tumbo Ndio ☐ Hapana ☐<br>c. magonjwa ya kurithi ya damu Ndio ☐ Hapana ☐ |
| 8 | Je mshiriki ana matatizo ya akili kama kushindwa kuzungumza ambayo italeta tatizo la kupata taharifa zake za kitabibu?<br>Ndio ☐ Hapana ☐ |
| 9 | Je mshiriki ameshauriwa na daktari kutotumia warfarini?<br>Ndio ☐ Hapana ☐ |
| 10 | Je, wewe ni Mkenya kwa kuzaliwa na wazazi wa kabila za Kenya?<br>Ndio ☐ Hapana ☐ |

| | |
|---|---|
| 11 | Kwa mujibu wa taharifa kutoka swali namba 1 mpaka 9 je mshiriki anastahili au hastahili?<br><br>Anastahili kushiriki ☐ Hastahili kushiriki☐ |
| 12 | Kama hastahili, taja sababu za kutomshirikisha<br>…… |
| **Imejazwa na:_____ (***herufi za mwanzo/tarehe***) Mtafiti** | |

**Kiambatisho IIIB: Fomu ya kukusanyia taharifa za mshiriki**

**Kichwa cha utafiti:"Mchango wa vinasaba juu ya matokeo ya utibuji wa warfarini kwa Wakenya"**

**Sehemu A: Taharifa za kijamii za mshiriki**:-

***Tarehe*** ☐☐ ☐☐ ☐☐

**Kitambulisho cha mshiriki ID**

| D | | | | |
|---|---|---|---|---|

| **Kuchunguza taharifa za mshiriki.** *Hi ni fomu ya mahojiano. Soma kila swali kwa sauti mbele ya mshiriki* | |
|---|---|
| 1. | Jinsia ya mshiriki Me ☐ Ke ☐ |
| 2. | Tarehe ya kuzaliwa? dd ☐☐ mm ☐☐ yy ☐☐ AU Una miaka mingapi? ☐☐ |
| 3. | Hali yako ya ndoa ni nini?<br>1. Sijaoa/olewa ☐<br>2. Nimeoa/olewa ☐<br>3. Tumeachana ☐<br>4. Tmetalakiana ☐<br>5. Mjana/mgane ☐ |
| 4. | Kazi yako ni nini?<br>1. Sijaajiriwa ☐<br>2. nimeajiriwa ☐<br>3. Nimejiajiri ☐<br>4. Mwanafunzi ☐ |
| 5. | Wewe ni jamii gani au kabila gani? |
| 6 | Taharifa za wazazi wa mshiriki:-<br>1. Mahali wanapoishi wazazi kwa sasa----------<br>2. Makazi ya kudumu ya wazazi [kama ni tofauti na iliyotajwa hapo juu (1) ]----------<br>3. Mahali alipozaliwa mama (asili ya jamii)----------<br>4. Mahali alipozaliwa baba (asili ya jamii)---------- |
| 6. | Ni nini kiwango chako cha juu cha elimu?<br>1. Sijasoma ☐<br>2. Shule ya msingi ☐<br>3. Shule ya sekondari ☐<br>4. Chuo/chuo kikuu ☐ |
| 7. | Wewe ni dhehebu gani la dini?<br>a. Mprotestanti ☐<br>☐<br>☐<br>☐ |

| | |
|---|---|
| | b. Katoliki<br>c. Muisilamu<br>d. Nyingine Elezea_____ |
| 8 | Taja mahali unapoishi kwa sasa |
| 9 | Mahali ulipoishi kwa miaka mitano iliyopita (kama ni tofauti na iliyotajwa hapo juu) ---------- |
| 10 | Uzito(Kg)----------Urefu (Mita) ---------- |
| 11 | Kipimo cha uzito kwa kulinganisha na urefu wake (BMI) ---------- |
| 12 | Kipimo cha ujazo($M^2$) |
| 13 | Je, unavuta sigara? Ndio ☐ Hapana ☐ |
| 14 | je unakunywa pombe? Ndio ☐ Hapana ☐ |
| | **Imejaza na:** _____(herufi za mwanzo/tarehe)**Mtafiti** |

**Sehemu B: Taharifa za matumizi ya warfarin**

1. Tarehe uliyoanza kutumia warfarin----------Dozi----------mg
2. Muda ambao umekuwa ukitumia warfarin (siku) ----------
3. Ukumbusho kwa mgonjwa: Ni nini humkumbusha mgojwa kwenda kliniki wakati ukifika?
    a) Mtoa huduma [ ] b) Ndugu wa mgojwa[ ] b) kalenda[ ] d) tahadrari ya simu[ ]
    f) Nyingine (elezea)
4. Umewahi kupata madhara ambatano baada ya kutumia warfarin kama yafuatayo? (Tiki):

    a)kutokwa damu (inayoonekana au ndani kwa ndani) [ ] b) vidole vya miguu kuwa vya kahawia…..[ ] c) ngozi kupata vidonda……[ ] d) kuwa na rangi ya njano kwenye macho au ngozi……[ ] e) kupata upara…..[ ] f) vipere miwasho vya

aleji……..[ ] g) Matatizo ya tumbo; kichefuchefu, kutapika, kuharisha…[ ]h)kuumwa kichwa, kizunguzungu, au kukosa nguvu [ ] i)Maumivu yasiyo ya kawaida au uvimbe [ ]

5. INR yako ilikua ngapi wakati unapata madhara ambatani (ADRs)?---------- a) <2[ ] b 2-<3[ ] c)3-<5[ ] d) 5-10[ ] e)>10[ ]
6. Ulichukua hatua gani baada ya kupata madhara ambatano ya dawa:
    a) Nilibadili dawa---------- [ ] Jina------ Dozi----------
    b) Nilipelekwa hospitali---------- [ ]
    c) Niliacha kutumia dawa---- [ ]
    d) Nilitumia dawa pingamizi [ ] Jina-------- Dozi--------
    e) Sikufanya chochote---------- [ ]
7. Kupona ADRs
    a) Je ulipona ADR ? [Ndio] [Hapana]
    b) Kama jibu ni ndio, Ulitumia muda gani kupona? (siku) ----------.
    c) Kama jibu ni hapana, Nini ilikua mwisho wa ADR?: ----------
8. Vizuizi vya matumizi ya warfarin: (Tiki kinachostahili): a)Vidonda vya tumbo-----[ ] b)Shinikizo kali la damu------[ ] c)Magonjwa ya figo….[ ] d) Ujauzito------[ ]
9. Je mgonjwa anayo magonjwa ya kurithi yanayoathiri mfumo wa damu kuganda? 1Ndio---------[ ] 2Hapana----------[ ]
10. Kama jibu ni ndio, kwa swali namba 9 hapo juu, fafanua----------

**Sehemu C: Taharifa za matumizi mseto ya dawa**

| | **Aina ya dawa (daraja)** | **Aina mahususi** | **Muda wa matumizi (siku)** |
|---|---|---|---|
| 1. | viuavijasumu | | |
| 2. | Dawa za maumivu | | |
| 3. | Dawa za degedege | | |
| 4. | Dawa za sonona | | |
| 5. | Dawa za kuzuia kuganda damu | | |
| 6. | Dawa za kisukari | | |
| 7. | Dawa za magonjwa ya tumbo | | |
| 8. | Dawa za kutibu gauti | | |
| 9. | Dawa za kupunguza mafuta | | |
| 10. | Dawa aina steroidi | | |
| 11. | Dawa za tezi shingo | | |
| 12. | Dawa za mapiga ya moyo | | |
| 13. | Dawa za fangasi | | |
| 14. | Nyingine, Elezea | | |

**Sehemu D: Ni nini magonjwa na hari zinazosababisha matumizi ya warfarin kwa mgonjwa? (Tiki inayostahili)**

| | **Sababu za kutumia warfarin** | **Dozi halisi ya kutumia kila siku (mg)** | **Kiwango cha utibuji INR** | |
|---|---|---|---|---|
| | | | **Cha chini** | **Cha juu** |
| 1 | Upasuaji mkubwa ( Tafadhari fafanua/ aina ya upasuaji) e.g. kubadilisha nyonga | | | |
| 2 | Mgando wa damu kwenye mishipa mikuu ya miguu (DVT) | | | |
| 3 | Historia ya kuganda damu kwenye mapafu | | | |
| 4 | Ugonjwa wa homa ya moyo | | | |
| 5 | Kuongezeka mapigo ya moyo | | | |
| 6 | Dalili za kiharusi | | | |
| 7 | Nyingine (elezea) | | | |

**Sehemu E: Taharifa za magonjwa mengine unayougua, kama yapo**

| | **Aina ya ugonjwa** | **Muda wa ugonjwa (miezi)** |
|---|---|---|
| 1. | | |
| 2. | | |
| 3. | | |

| | | |
|---|---|---|
| 4. | | |
| 5. | | |

**Sehemu F: Taharifa kuhusu hari ya afya ya ulaji wa virutubisho kwa mgonjwa**

1) Lishe

| | **Aina ya lishe** | **Unakula mara ngapi (namba ya wastani unaokula kwa wiki)** |
|---|---|---|
| 1. | | |
| 2. | | |
| 3. | | |
| 4. | | |
| 5. | | |

2.) Je mgonjwa anatumia **chochote** kati ya virutubisho vifuatavyo? (Tiki)

a) Vitamini E (Zaidi ya 400 IU kwa siku)

b) Vitamini C (Zaidi ya 500 mg kwa siku)

c) Vitamin nyingi zenye Vitamini K

d) Glucosamine & Chondroitin

e) Coenzyme Q10

f) Nyingine, Elezea----------

3) Je, mshiriki anatumia mitishamba kama yoyote kati ya zifuatazo?

| | | | | |
|---|---|---|---|---|
| a) Alfalfa | b) Green tea | c) Kitunguu saumu | d) St. John's Wort | e) Ginkgo Biloba |
| f) Aloe Gel | g) Red Clover | h) Ginger | i) Sweet Clover | j) Zingine, eleza |

**Sehemu G: Taharifa kuhusu mahali anapopata warfarin mshiriki**

1) Ni wapi unapata warfarin?…..1[ ] KNH 2[ ] sehemu nyingine

2) Kiasi cha mwisho cha warfarin ulipata wapi?…..1[ ] KNH 2[ ] sehemu nyinige

**Sehemu H: Historia ya matumivi ya vileo**

1. Je mgonjwa anatumia pombe? a)Ndio---------- b)Hapana----------
2. Kama jibu ni ndio,Ni kwa muda gani umekua ukitumia pombe (pombe)?----------
3. Unakunywa pombe aina gani----------a)Bia b)mvinyo c) pombe kali d) pombe za kienyeji
4. Kiasi unachokunywa kwa siku----------

**Sehemu I: Historia ya matumizi ya tumbaku**

1.Je mgonjwa anatumia tumbaku?**a)** Ndio---------- b) Hapana----------

2. Kama jibu ni ndio,Ni kwa muda gani mgonjwa amekuwa akitumia tumbaku(miezi)?……

**Sehemu J: Matumizi ya wafarin kwa kuangalia viwango vya INR**

1) Ulikua na kiwano gani cha INR wakati unapata madhara ambatano(ADRs) kwa mara ya kwanza, kama imetokea?----------

2) Orodhesha walau viwango vinne vya hivi karibuni vya INR kwa kuhusianisha na dozi zake za warfarin (Ukianzia na dozi ya sasa na INR yake)

| | **Tarehe** | **Dozi ya warfarin (mg)** | **INR iliyorekodiwa(imesababishwa na dozi iliyotolewa)** |
|---|---|---|---|
| **1st (Ya sasa)** | | | |
| **2nd** | | | |
| **3rd** | | | |
| **4th** | | | |
| **5th** | | | |
| **6th** | | | |

3) Orodhesha walau INR **za kwanza nne** kwa kuhusianisha na dozi za warfarin alizotumia mgonjwa (Ukianzia na dozi ya kwanza na INR yake)

| | **Tarehe** | **Dozi ya warfarin (mg)** | **INR Iliyorekodiwa(imesababishwa na dozi ya warfarin aliyotumia mgonjwa)** |
|---|---|---|---|
| **1st (Ya mwanzo )** | | | |
| **2nd** | | | |
| **3rd** | | | |
| **4th** | | | |
| **5th** | | | |
| **6th** | | | |

4) Taharifa za vinasaba

1. Muda sampuli ilipochukuliwa……..
2. Tarehe na muda dawa ilipotumiwa……
3. Masaa yaliyopita baada ya kupata dozi…….
4. Dozi iliyotolewa……mg

*Ahsante kwa ushirikiano wako!*

## Appendix IV: UoN/KNH-ERC Approval to carry out the study

UNIVERSITY OF NAIROBI
COLLEGE OF HEALTH SCIENCES
P O BOX 19676 Code 00202
Telegrams: varsity
Tel:(254-020) 2726300 Ext 44355

KNH-UON ERC
Email: uonknh_erc@uonbi.ac.ke
Website: http://www.erc.uonbi.ac.ke
Facebook: https://www.facebook.com/uonknh.erc
Twitter: @UONKNH_ERC https://twitter.com/UONKNH_ERC

KENYATTA NATIONAL HOSPITAL
P O BOX 20723 Code 00202
Tel: 726300-9
Fax: 725272
Telegrams: MEDSUP, Nairobi

Ref. No.KNH/ERC/R/173

August 20, 2018

Dr. David Gitonga Nyamu
Dept. of Pharmaceutics and Pharmacy Practice
School of Pharmacy
College of Health Sciences
University of Nairobi

Dear Dr. Nyamu

**Re: Approval of Annual Renewal Genetic Determinants of Warfarin Response in Kenyan Patients (P206/06/2010)**

Refer to your communication dated August 15, 2018.

This is to acknowledge receipt of your study progress report and hereby grant you annual extension approval for ethics research protocol P206/06/2010.

The approval dates are 25th August 2018 – 24th August 2019.

This approval is subject to compliance with the following requirements:

a) Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b) All changes (amendments, deviations, violations etc) are submitted for review and approval by KNH/UoN ERC before implementation.
c) Death and life threatening problems and severe adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH/UoN- ERC within 72 hours of notification.
d) Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH/UoN ERC within 72 hours.
e) Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (*Attach a comprehensive progress report to support the renewal*).
f) Clearance for export of biological specimens must be obtained from KNH/UoN-Ethics & Research Committee for each batch of shipment.
g) Submission of an *executive summary* report within 90 days upon completion of the study
This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/or plagiarism.

Protect to discover

For more details consult the KNH- UoN ERC website **http://www.erc.uonbi.ac.ke**

Yours sincerely,

**PROF. M.L. CHINDIA**
**SECRETARY, KNH-UON ERC**

c.c. The Principal, College of Health Sciences, UoN
The Director CS, KNH
The Chairperson, KNH-UoN ERC

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**Appendix V: Study Registration Certificate by Department of Research and Programs**

KNH/R&P/FORM/01

KENYATTA NATIONAL HOSPITAL
P.O. Box 20723-00202 Nairobi

Tel.: 2726300/2726450/2726565
Research & Programs: Ext. 44705
Fax: 2725272
Email: knhresearch@gmail.com

## Study Registration Certificate

1. Name of the Principle Investigator/Researcher
   DR. DAVID NYAMU
2. Email address: dgnyamu@yahoo.com Tel No. 0722403671
3. Contact person (if different from PI).....
4. Email address: ..... Tel No. .....
5. Study Title
   GENETIC DETERMINANTS OF WARFARIN RESPONSE IN KENYAN PATIENTS
6. Department where the study will be conducted Haemato Medicine & Surgery
   *(Please attach copy of Abstract)*
7. Endorsed by Research Coordinator of the Department where the study will be conducted.
   Name: ..... Signature ..... Date.....
8. Endorsed by Head of Department where study will be conducted.
   Name: Dr William K-Sigilai Signature ..... Date 3.12.2014
9. KNH UoN Ethics Research Committee approval number _____
   *(Please attach copy of ERC approval)*
10. I DR. DAVID NYAMU commit to submit a report of my study findings to the Department where the study will be conducted and to the Department of Research and Programs.
    Signature ..... Date 2/12/2014
11. Study Registration number (Dept/Number/Year) MED /025/2014
    (To be completed by Research and Programs Department)
12. Research and Program Stamp _____

KENYATTA NATIONAL HOSPITAL H.O.D. RESEARCH & PROGRAMS 04 DEC 2014 P.O. Box 20723 NAIROBI

All studies conducted at Kenyatta National Hospital **must** be registered with the Department of Research and Programs and investigators **must commit** to share results with the hospital.

Version 2: August. 2014

**Appendix VI: Approval to carry out the Study in the Department of Medicine KNH**

KENYATTA NATIONAL HOSPITAL
P. O. Box 20723, 00202 Nairobi

Tel: 2726300/2726450/2726550
Fax: 2725272
Email: knhadmin@knh.or.ke

Ref: KNH/SAD-MED/42B/VOL.I

Date: 4th December, 2014

Dr. David Nyamu
Department of Pharmaceuticals & Pharmacy Practice
School of Pharmacy
**UNIVERSITY OF NAIROBI.**

**RE:APPROVAL TO CONDUCT A STUDY AT THE KNH MEDICINE DEPARTMENT**

Following approval of your study by the KNH/UoN ERC and completion of the KNH study registration form, permission is hereby granted for you to collect data from the KNH Medical Department to enable you complete your study on "Genetic ***determinants of warfarin response in Kenyan patients"*** at *Kenyatta National Hospital, Nairobi County, Kenya.*

Kindly liaise with the Assistant Chief Nurse of Medicine Department for facilitation.

DR. W.K.SIGILAI
AD - MEDICINE

Copy to: ACN , Medicine
KNH

*Vision: A world class patient-centered specialized care hospital*

*ISO 9001: 2008 CERTIFIED*

**Appendix VII: Approval to carry out the Study in the Department of Surgery of KNH**

KENYATTA NATIONAL HOSPITAL
P.O. BOX 20723, 00202 Nairobi

Tel.: 2726300/2726450/2726550
Fax: 2725272
Email: knhadmin@knh.or.ke

**FILE NO.KNH/SURG/** **DATE: 10th December,2014**

To

**ACN- Surgery**

**RE: PERMISSION TO CARRY OUT THE STUDY**

Dr. David Nyamu has approval from KNH-Ethics Committee to undertake Determinants of Warfarin Response.

The patients will be drawn from the surgical haematology and Cardiothoracic surgical clinics

Please give him all the necessary support.

Thank you.

Dr. B. GITHAE
SENIOR ASSISTANT DIRECTOR
**SURGICAL SERVICES**

**CC**: SNO – Surgical Clinic

## Appendix VIII: Number of Participants Enrolled for Anticoagulation at KNH

**OUT-PATIENT ATTENDANCE - CARDIOTHORACIC AND HAEMATOLOGY CLINICS - 2012**

**CARDIOTHORACIC**

| Month | New | Revisit | Total |
|---|---|---|---|
| Jan | 65 | 142 | **207** |
| Feb | 68 | 159 | **227** |
| Mar | 48 | 172 | **220** |
| Apr | 47 | 119 | **166** |
| May | 71 | 157 | **228** |
| Jun | 63 | 169 | **232** |
| Jul | 55 | 130 | **185** |
| Aug | 53 | 231 | **284** |
| Sep | 26 | 71 | **97** |
| Oct | 73 | 125 | **198** |
| Nov | 96 | 154 | **250** |
| Dec | 58 | 115 | **173** |
| **Total** | **723** | **1744** | **2467** |

**HAEMATOLOGY**

| Month | New | Revisit | Total |
|---|---|---|---|
| Jan | 73 | 753 | **826** |
| Feb | 76 | 603 | **679** |
| Mar | 41 | 712 | **753** |
| Apr | 78 | 793 | **871** |
| May | 50 | 628 | **678** |
| Jun | 54 | 656 | **710** |
| Jul | 58 | 844 | **902** |
| Aug | 30 | 406 | **436** |
| Sep | 22 | 238 | **260** |
| Oct | 54 | 637 | **691** |
| Nov | 60 | 647 | **707** |
| Dec | 58 | 631 | **689** |
| **Total** | **654** | **7548** | **8202** |

*Source: Health Information Department*

*21/08/2013*

## Appendix IX: Extracted DNA Yields by Sample

| Sample ID | Date and Time | Nucleic Acid Conc. | Unit | A260 | A280 | 260/280 | 260/230 | Type | Factor |
|---|---|---|---|---|---|---|---|---|---|
| D1001 | 5/18/2018 12:32:04 PM | 27.4 | ng/µl | 0.548 | 0.316 | 1.74 | 0.2 | DNA | 50 |
| D1002 | 5/18/2018 12:33:39 PM | 40 | ng/µl | 0.8 | 0.525 | 1.52 | 0.25 | DNA | 50 |
| D1005 | 5/18/2018 2:25:35 PM | 7.9 | ng/µl | 0.159 | 0.088 | 1.8 | 0.23 | DNA | 50 |
| D1006 | 5/18/2018 11:45:08 AM | 31.8 | ng/µl | 0.636 | 0.333 | 1.91 | 1.5 | DNA | 50 |
| D1007 | 5/18/2018 1:10:26 PM | 132.6 | ng/µl | 2.652 | 1.453 | 1.83 | 1.42 | DNA | 50 |
| D1012 | 5/18/2018 2:22:43 PM | 21.4 | ng/µl | 0.428 | 0.229 | 1.87 | 1.54 | DNA | 50 |
| D1014 | 5/18/2018 12:09:46 PM | 12.7 | ng/µl | 0.254 | 0.146 | 1.74 | 0.64 | DNA | 50 |
| D1015 | 5/18/2018 1:56:10 PM | 64.9 | ng/µl | 1.297 | 0.702 | 1.85 | 1.63 | DNA | 50 |
| D1018 | 5/18/2018 12:06:47 PM | 2.4 | ng/µl | 0.049 | 0.01 | 4.76 | 1.57 | DNA | 50 |
| D1019 | 5/18/2018 12:01:09 PM | 4.7 | ng/µl | 0.095 | 0.05 | 1.9 | 0.19 | DNA | 50 |
| D1022 | 5/18/2018 2:10:51 PM | 10.1 | ng/µl | 0.202 | 0.11 | 1.83 | 0.31 | DNA | 50 |
| D1042 | 5/18/2018 12:07:57 PM | 67 | ng/µl | 1.34 | 0.819 | 1.64 | 0.57 | DNA | 50 |
| D1043 | 5/18/2018 12:48:27 PM | 38.1 | ng/µl | 0.763 | 0.412 | 1.85 | 0.2 | DNA | 50 |
| D1044 | 5/18/2018 11:53:31 AM | 3.9 | ng/µl | 0.077 | 0.035 | 2.23 | 0.22 | DNA | 50 |
| D1045 | 5/18/2018 12:17:36 PM | 22.4 | ng/µl | 0.448 | 0.235 | 1.9 | 0.96 | DNA | 50 |
| D1052 | 5/18/2018 12:25:54 PM | 15.7 | ng/µl | 0.315 | 0.23 | 1.37 | 0.35 | DNA | 50 |
| D1054 | 5/18/2018 12:39:46 PM | 14.3 | ng/µl | 0.285 | 0.169 | 1.68 | 0.76 | DNA | 50 |
| D1056 | 5/18/2018 12:46:38 PM | 45.2 | ng/µl | 0.904 | 0.551 | 1.64 | 0.49 | DNA | 50 |
| D1057 | 5/18/2018 12:03:27 PM | 25.4 | ng/µl | 0.507 | 0.272 | 1.87 | 0.93 | DNA | 50 |
| D1058 | 5/18/2018 12:49:44 PM | 28.3 | ng/µl | 0.565 | 0.3 | 1.88 | 1.04 | DNA | 50 |
| D1059 | 5/18/2018 2:27:04 PM | 58.9 | ng/µl | 1.177 | 0.904 | 1.3 | 0.93 | DNA | 50 |
| D1060 | 5/18/2018 11:54:41 AM | 27.2 | ng/µl | 0.545 | 0.332 | 1.64 | 0.9 | DNA | 50 |
| D1064 | 5/18/2018 12:28:54 PM | 69.2 | ng/µl | 1.383 | 0.793 | 1.74 | 0.67 | DNA | 50 |
| D1065 | 5/18/2018 2:14:41 PM | 5.1 | ng/µl | 0.102 | 0.057 | 1.78 | 0.04 | DNA | 50 |
| D1066 | 5/18/2018 2:19:19 PM | 0.6 | ng/µl | 0.012 | 0.006 | 2.19 | 0.03 | DNA | 50 |
| D1067 | 5/18/2018 12:52:14 PM | 46.1 | ng/µl | 0.921 | 0.507 | 1.82 | 1.64 | DNA | 50 |
| D1080 | 5/18/2018 12:27:29 PM | 4.9 | ng/µl | 0.098 | 0.061 | 1.61 | 0.1 | DNA | 50 |
| D1083 | 5/18/2018 12:14:18 PM | 53.4 | ng/µl | 1.068 | 0.58 | 1.84 | 1.59 | DNA | 50 |
| D1084 | 5/18/2018 12:20:05 PM | 9.7 | ng/µl | 0.194 | 0.111 | 1.75 | 0.9 | DNA | 50 |
| D1090 | 5/18/2018 2:18:22 PM | 4 | ng/µl | 0.08 | 0.044 | 1.81 | 0.09 | DNA | 50 |
| D1093 | 5/18/2018 12:21:36 PM | 25.6 | ng/µl | 0.512 | 0.337 | 1.52 | 0.15 | DNA | 50 |
| D1100 | 5/18/2018 12:53:38 PM | 11.6 | ng/µl | 0.232 | 0.143 | 1.62 | 0.11 | DNA | 50 |
| D1101 | 5/18/2018 1:50:07 PM | 36 | ng/µl | 0.72 | 0.437 | 1.65 | 0.07 | DNA | 50 |
| D1102 | 5/18/2018 11:59:50 AM | 10.2 | ng/µl | 0.204 | 0.115 | 1.79 | 0.46 | DNA | 50 |
| D1103 | 5/18/2018 11:47:44 AM | 8.2 | ng/µl | 0.163 | 0.09 | 1.81 | 0.97 | DNA | 50 |
| D1104 | 5/18/2018 2:16:31 PM | 45.7 | ng/µl | 0.913 | 0.491 | 1.86 | 1.35 | DNA | 50 |
| D1106 | 5/18/2018 12:42:31 PM | 9.9 | ng/µl | 0.199 | 0.113 | 1.77 | 0.5 | DNA | 50 |
| D1107 | 5/18/2018 2:24:38 PM | 113.4 | ng/µl | 2.268 | 1.232 | 1.84 | 2.15 | DNA | 50 |
| D2001 | 5/18/2018 2:02:55 PM | 42.3 | ng/µl | 0.846 | 0.461 | 1.83 | 1.37 | DNA | 50 |
| D2002 | 5/18/2018 12:57:31 PM | 131.5 | ng/µl | 2.631 | 1.436 | 1.83 | 0.52 | DNA | 50 |
| D2003 | 5/18/2018 1:15:44 PM | 168.7 | ng/µl | 3.374 | 2.058 | 1.64 | 0.52 | DNA | 50 |
| D2004 | 5/18/2018 1:44:39 PM | 44.6 | ng/µl | 0.891 | 0.522 | 1.71 | 0.61 | DNA | 50 |
| D2005 | 5/18/2018 2:04:02 PM | 17.3 | ng/µl | 0.346 | 0.193 | 1.79 | 0.93 | DNA | 50 |
| D2006 | 5/18/2018 1:09:06 PM | 45.6 | ng/µl | 0.912 | 0.491 | 1.86 | 0.85 | DNA | 50 |
| D2009 | 5/18/2018 11:58:34 AM | 39.4 | ng/µl | 0.788 | 0.424 | 1.86 | 1.97 | DNA | 50 |
| D2010 | 5/18/2018 1:36:22 PM | 63.8 | ng/µl | 1.275 | 0.783 | 1.63 | 0.64 | DNA | 50 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| D2011 | 5/18/2018 11:50:32 AM | 22 | ng/µl | 0.44 | 0.23 | 1.92 | 1.72 | DNA | 50 |
| D2013 | 5/18/2018 2:00:54 PM | 113.6 | ng/µl | 2.272 | 1.26 | 1.8 | 1.7 | DNA | 50 |
| D2014 | 5/18/2018 1:30:28 PM | 98.2 | ng/µl | 1.964 | 1.095 | 1.79 | 1.78 | DNA | 50 |
| D2015 | 5/18/2018 11:56:06 AM | 33.9 | ng/µl | 0.678 | 0.37 | 1.83 | 1.74 | DNA | 50 |
| D2016 | 5/18/2018 12:12:00 PM | 11.1 | ng/µl | 0.223 | 0.112 | 1.98 | 0.38 | DNA | 50 |
| D2017 | 5/18/2018 12:02:10 PM | 26.4 | ng/µl | 0.527 | 0.285 | 1.85 | 0.7 | DNA | 50 |
| D2018 | 5/18/2018 12:05:27 PM | 12.2 | ng/µl | 0.244 | 0.123 | 1.98 | 1.65 | DNA | 50 |
| D2019 | 5/18/2018 1:23:09 PM | 90.7 | ng/µl | 1.814 | 1.008 | 1.8 | 0.81 | DNA | 50 |
| D2020 | 5/18/2018 2:04:54 PM | 48.5 | ng/µl | 0.971 | 0.527 | 1.84 | 1.78 | DNA | 50 |
| D2024 | 5/18/2018 12:23:19 PM | 40.5 | ng/µl | 0.81 | 0.437 | 1.85 | 0.6 | DNA | 50 |
| D2025 | 5/18/2018 2:17:25 PM | 55.1 | ng/µl | 1.102 | 0.599 | 1.84 | 0.77 | DNA | 50 |
| D2026 | 5/18/2018 11:57:23 AM | 14.1 | ng/µl | 0.282 | 0.152 | 1.85 | 1.8 | DNA | 50 |
| D2028 | 5/18/2018 11:51:54 AM | 1.9 | ng/µl | 0.038 | 0.011 | 3.38 | 0.06 | DNA | 50 |
| D2029 | 5/18/2018 12:24:42 PM | 67.5 | ng/µl | 1.35 | 0.744 | 1.81 | 0.81 | DNA | 50 |
| D2033 | 5/18/2018 12:41:07 PM | 70.8 | ng/µl | 1.415 | 0.765 | 1.85 | 0.96 | DNA | 50 |
| D2034 | 5/18/2018 11:49:09 AM | 6.8 | ng/µl | 0.136 | 0.069 | 1.96 | 1.28 | DNA | 50 |
| D2035 | 5/18/2018 1:29:27 PM | 50.3 | ng/µl | 1.007 | 0.569 | 1.77 | 1.41 | DNA | 50 |
| D2036 | 5/18/2018 1:53:45 PM | 17.7 | ng/µl | 0.355 | 0.192 | 1.85 | 0.1 | DNA | 50 |
| D2037 | 5/18/2018 1:13:20 PM | 15 | ng/µl | 0.3 | 0.174 | 1.72 | 0.32 | DNA | 50 |
| D2038 | 5/18/2018 1:19:27 PM | 26.9 | ng/µl | 0.538 | 0.29 | 1.86 | 1.73 | DNA | 50 |
| D2040 | 5/18/2018 1:27:57 PM | 103.5 | ng/µl | 2.07 | 1.167 | 1.77 | 1.38 | DNA | 50 |
| D2041 | 5/18/2018 1:11:54 PM | 47.5 | ng/µl | 0.95 | 0.521 | 1.82 | 0.93 | DNA | 50 |
| D2042 | 5/18/2018 1:21:59 PM | 34.7 | ng/µl | 0.694 | 0.393 | 1.76 | 1.06 | DNA | 50 |
| D2043 | 5/18/2018 1:25:38 PM | 112 | ng/µl | 2.24 | 1.278 | 1.75 | 1.14 | DNA | 50 |
| D2044 | 5/18/2018 1:47:07 PM | 39.8 | ng/µl | 0.795 | 0.454 | 1.75 | 1.39 | DNA | 50 |
| D2045 | 5/18/2018 2:21:47 PM | 27.7 | ng/µl | 0.553 | 0.302 | 1.83 | 0.76 | DNA | 50 |
| D2048 | 5/18/2018 1:35:09 PM | 43 | ng/µl | 0.86 | 0.465 | 1.85 | 1.4 | DNA | 50 |
| D2049 | 5/18/2018 1:14:31 PM | 8 | ng/µl | 0.16 | 0.078 | 2.05 | 0.87 | DNA | 50 |
| D2050 | 5/18/2018 1:08:02 PM | 24.3 | ng/µl | 0.486 | 0.289 | 1.68 | 0.1 | DNA | 50 |
| D2051 | 5/18/2018 1:06:46 PM | 26.7 | ng/µl | 0.534 | 0.297 | 1.8 | 0.21 | DNA | 50 |
| D2052 | 5/18/2018 1:20:59 PM | 42.6 | ng/µl | 0.853 | 0.483 | 1.76 | 0.78 | DNA | 50 |
| D2053 | 5/18/2018 1:24:20 PM | 18.6 | ng/µl | 0.372 | 0.197 | 1.89 | 0.96 | DNA | 50 |
| D2054 | 5/18/2018 1:38:51 PM | 22.7 | ng/µl | 0.454 | 0.245 | 1.85 | 2 | DNA | 50 |
| D2055 | 5/18/2018 1:59:52 PM | 22.5 | ng/µl | 0.449 | 0.248 | 1.81 | 1.08 | DNA | 50 |
| D2056 | 5/18/2018 1:33:52 PM | 13.9 | ng/µl | 0.277 | 0.145 | 1.91 | 0.04 | DNA | 50 |
| D2057 | 5/18/2018 1:40:01 PM | 107 | ng/µl | 2.139 | 1.212 | 1.76 | 0.42 | DNA | 50 |
| D2058 | 5/18/2018 1:31:44 PM | 19.8 | ng/µl | 0.396 | 0.218 | 1.82 | 0.91 | DNA | 50 |
| D2059 | 5/18/2018 1:37:47 PM | 33.2 | ng/µl | 0.665 | 0.359 | 1.85 | 0.24 | DNA | 50 |
| D2060 | 5/18/2018 1:18:15 PM | 9.9 | ng/µl | 0.199 | 0.1 | 2 | 1.99 | DNA | 50 |
| D2061 | 5/18/2018 1:32:41 PM | 22.4 | ng/µl | 0.448 | 0.236 | 1.9 | 1.4 | DNA | 50 |
| D2062 | 5/18/2018 1:01:02 PM | 46.2 | ng/µl | 0.925 | 0.502 | 1.84 | 0.36 | DNA | 50 |
| D2063 | 5/18/2018 1:54:54 PM | 13.1 | ng/µl | 0.261 | 0.146 | 1.78 | 0.11 | DNA | 50 |
| D2064 | 5/18/2018 1:52:37 PM | 21.4 | ng/µl | 0.428 | 0.229 | 1.86 | 0.72 | DNA | 50 |
| D2065 | 5/18/2018 1:57:56 PM | 13.1 | ng/µl | 0.262 | 0.133 | 1.97 | 0.27 | DNA | 50 |
| D2066 | 5/18/2018 2:06:49 PM | 17 | ng/µl | 0.34 | 0.189 | 1.8 | 0.28 | DNA | 50 |
| D2067 | 5/18/2018 1:48:11 PM | 25.4 | ng/µl | 0.508 | 0.278 | 1.82 | 1.53 | DNA | 50 |
| D2068 | 5/18/2018 1:51:27 PM | 16.8 | ng/µl | 0.335 | 0.164 | 2.04 | 1.52 | DNA | 50 |
| D2069 | 5/18/2018 2:05:44 PM | 27 | ng/µl | 0.54 | 0.315 | 1.71 | 0.16 | DNA | 50 |
| D2070 | 5/18/2018 1:58:55 PM | 7.2 | ng/µl | 0.143 | 0.077 | 1.85 | 0.07 | DNA | 50 |
| D2071 | 5/18/2018 1:46:02 PM | 8.7 | ng/µl | 0.173 | 0.086 | 2.02 | 0.92 | DNA | 50 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| D2072 | 5/18/2018 12:13:10 PM | 38 | ng/µl | 0.76 | 0.417 | 1.82 | 1.35 | DNA | 50 |
| D2073 | 5/18/2018 12:35:10 PM | 26.8 | ng/µl | 0.535 | 0.486 | 1.1 | 0.39 | DNA | 50 |
| D2074 | 5/18/2018 12:45:11 PM | 52.4 | ng/µl | 1.048 | 0.661 | 1.58 | 0.75 | DNA | 50 |
| D2076 | 5/18/2018 2:15:42 PM | 50.7 | ng/µl | 1.015 | 0.558 | 1.82 | 0.12 | DNA | 50 |
| D2077 | 5/18/2018 12:38:23 PM | 15.3 | ng/µl | 0.305 | 0.166 | 1.84 | 1.02 | DNA | 50 |
| D2078 | 5/18/2018 12:43:51 PM | 64.7 | ng/µl | 1.294 | 0.72 | 1.8 | 0.93 | DNA | 50 |
| D2079 | 5/18/2018 12:36:37 PM | 77.1 | ng/µl | 1.542 | 0.862 | 1.79 | 0.33 | DNA | 50 |
| D2080 | 5/18/2018 2:13:44 PM | 20 | ng/µl | 0.4 | 0.219 | 1.83 | 0.2 | DNA | 50 |
| D2081 | 5/18/2018 2:12:45 PM | 23.4 | ng/µl | 0.468 | 0.255 | 1.83 | 0.65 | DNA | 50 |
| D2083 | 5/18/2018 2:11:54 PM | 27.1 | ng/µl | 0.543 | 0.291 | 1.86 | 1.17 | DNA | 50 |
| Negative Control | 5/18/2018 2:07:45 PM | 2.2 | ng/µl | 0.043 | 0.019 | 2.26 | 0.03 | DNA | 50 |
| Negative Control | 5/18/2018 2:08:47 PM | 1.7 | ng/µl | 0.035 | 0.01 | 3.46 | 0.03 | DNA | 50 |

## Appendix X: Material Transfer Approval Document by UoN/KNH-ERC

UNIVERSITY OF NAIROBI
COLLEGE OF HEALTH SCIENCES
P O BOX 19676 Code 00202
Telegrams: varsity
(254-020) 2726300 Ext 44355

KNH-UoN ERC
Email: uonknh_erc@uonbi.ac.ke
Website: http://www.erc.uonbi.ac.ke
Facebook: https://www.facebook.com/uonknh.erc
Twitter: @UONKNH_ERC https://twitter.com/UONKNH_ERC

KENYATTA NATIONAL HOSPITAL
P O BOX 20723 Code 00202
Tel: 726300-9
Fax: 725272
Telegrams: MEDSUP, Nairobi

Ref: KNH-ERC/Shipment/24

17th August, 2018

Dr.David Gitonga Nyamu
Principal Investigator
Dept.of Pharmaceutics and Pharmacy Practice
School of Pharmacy
College of Health Sciences
University of Nairobi

Dear Dr. Nyamu

**Re: Approval for shipment of study samples – study titled Genetic Determinants of Warfarin Response in Kenyan Patients (P206/06/2010)**

Refer to your communication of August 13, 2018.

The KNH-UON ERC has reviewed and granted permission to ship 40 samples each containing twenty microliters(20µl) of DNA for limited genetic testing of CYP2C9,VORC1 and CYP 4F2 variants in Inqaba Biotechnical Industries(Pty) Ltd, South Africa.

The samples will be under the custody of the following contact:

Dr. Aron Abera
Technical Support Manager
Inqaba Biotechnical Industries (Pty) Ltd
P O Box 14356, Hatfield 0028, South Africa
Phone +27 12 343 5829.

Yours sincerely,

**PROF. M. L. CHINDIA**
**SECRETARY, KNH-UON ERC**

c.c. The Principal, College of Health Sciences, UoN
The Director, CS, KNH
The Chair, KNH-UoN ERC

Protect to discover

**Appendix XI: Acceptance to Analyze Samples at Inqaba Biotechnical (Pty) Industries Ltd –South Africa**

inqaba biotec

**Dr Aron Berhanie Abera**
Technical Support Manager
Inqaba Biotechnical Industries (Pty) Ltd
Phone: +27 12 343 5829
Fax: +27 86 677 8409
Email: aron.abear@inqababiotec.co.za

14 August 2018

To Kenyatta National Hospital/University of Nairobi Ethics and Research Committee
Kenyatta National Hospital
Hospital Rd. along, Ngong Road
P.O Box 20723, Nairobi

**Re: DNA Samples for MassARRAY Analysis**

This letter confirms that DNA samples provided for MassARRAY SNP genotyping analysis will be destroyed following satisfactory results being derived from the samples. Inqaba Biotec does not have a gene bank facility and we do not store DNA samples after analysis is completed.

If you require additional information, please contact me via email or telephone listed above.

Sincerely

Dr Aron Abera

**Appendix XII: Approval to carry out the Study in Cardiothoracic Clinic**

KNH/R&P/FORM/01

**KENYATTA NATIONAL HOSPITAL**
P.O. Box 20723-00202 Nairobi

Tel.: 2726300/2726450/2726565
Research & Programs: Ext. 44705
Fax: 2725272
Email: knhresearch@gmail.com

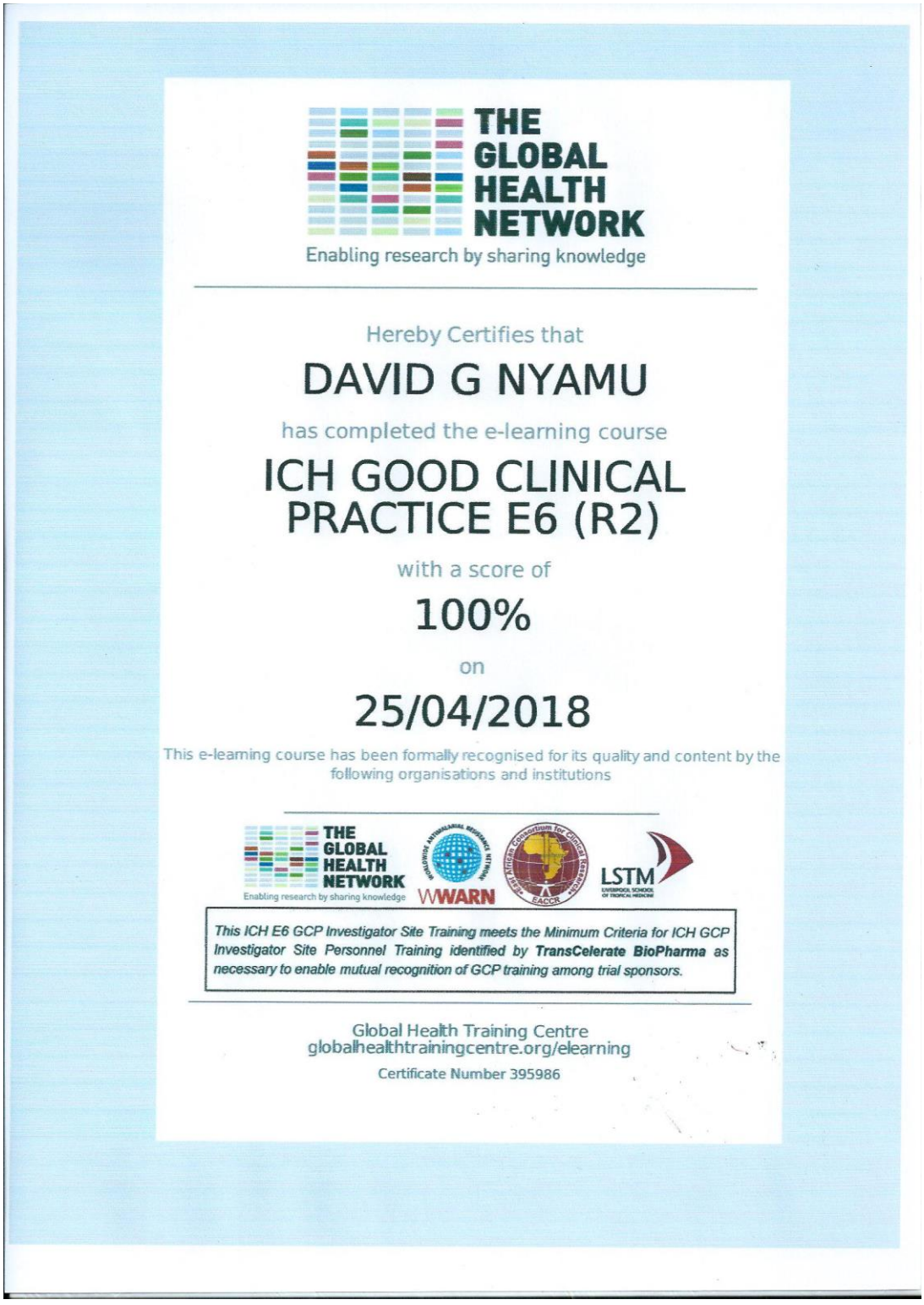
## Study Registration Certificate

1. Name of the Principle Investigator/Researcher
   DR. D.G. NYAMU
2. Email address: dgnyamu@yahoo.com Tel No. 0722 403671
3. Contact person (if different from PI) –
4. Email address: – Tel No. –
5. Study Title
   GENETIC DETERMINANTS OF WARFARIN RESPONSE IN KENYAN PATIENTS
6. Department where the study will be conducted SURGERY
   *(Please attach copy of Abstract)*
7. Endorsed by Research Coordinator of the Department where the study will be conducted.
   Name: ..... Signature ..... Date.....
8. Endorsed by Head of Department where study will be conducted
   Name: ..... Signature ..... Date 9/12/2014
9. KNH UoN Ethics Research Committee approval number P206/06/2010
   *(Please attach copy of ERC approval)*
10. I DR D.G. NYAMU commit to submit a report of my study findings to the Department where the study will be conducted and to the Department of Research and Programs.
    Signature ..... Date 8/12/2014
11. Study Registration number (Dept/Number/Year) MED / 25 / 14
    (To be completed by Research and Programs Department)
12. Research and Program Stamp_____

All studies conducted at Kenyatta National Hospital **must** be registered with the Department of Research and Programs and investigators **must commit** to share results with the hospital.

Version 2: August, 2014

**Appendix XIII: Researcher's Certificate of ICH**



THE GLOBAL HEALTH NETWORK

Enabling research by sharing knowledge

Hereby Certifies that

DAVID G NYAMU

has completed the e-learning course

ICH GOOD CLINICAL PRACTICE E6 (R2)

with a score of

100%

on

25/04/2018

This e-learning course has been formally recognised for its quality and content by the following organisations and institutions

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Enabling research by sharing knowledge
WWARN
EACCR
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*This ICH E6 GCP Investigator Site Training meets the Minimum Criteria for ICH GCP Investigator Site Personnel Training identified by* ***TransCelerate BioPharma*** *as necessary to enable mutual recognition of GCP training among trial sponsors.*

Global Health Training Centre
globalhealthtrainingcentre.org/elearning

Certificate Number 395986