Association of plasma Warfarin level with monitoring parameters among patients treated at Hematology Referral Clinic, Tikur Anbessa Specialized Hospital

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This is to certify that the thesis prepared by Gediwon Negash entitled: *Association of plasma warfarin level with monitoring parameters among patients treated at Hematology Referral Clinic, Tikur Anbessa Specialized Hospital* and submitted in partial fulfilment of the requirements for the Degree of Master of Science (Medical Pharmacology) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

Signed by the Examining Committee:

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ABSTRACT

Association of plasma warfarin level with monitoring parameters among patients treated at hematology referral clinic

Gediwon Negash

Warfarin monitoring poses challenge to the treatment of thromboembolic disorders because out-of-range INRs which is encountered as a result of changes in numerous factors. The fluctuation of INR in some patients might be dangerous because of the narrow therapeutic index of warfarin. The objective of this study is to evaluate the association of plasma warfarin level with warfarin the clinical monitoring parameter, INR and related parameters. Institution based cross-sectional prospective study was conducted recruiting 57 eligible patients on warfarin treatment at hematology referral clinic of Tikur Anbessa Specialized Hospital. Clinical data was retrieved from patient cards and plasma warfarin level were determined using High Pressure Liquid Chromatography with Ultra Violet – Visible detector. The associations of plasma concentration with INR and weekly dose were low (rho = 0.375 at p = 0.006 and r = 0.283 at p = 0.04) respectively. There was also statistically significant difference in the mean plasma warfarin concentration of patients who took effect enhancing co-medication and those who took non-effect changing co-medication ($p < 0.001$) as well between the mean plasma concentration of patients who took effect diminishing co-medication and those who took non-effect changing co-medications was also statistically significant (p = 0.005). In conclusion clinical monitoring parameters of warfarin have low association with plasma warfarin level. The low correlation indicates the importance of plasma warfarin concentration monitoring in some situations, especially when the ideal INR is difficult to achieve the target where by patient safety can be affected.

Key Words: Warfarin, INR, Dose, Association, co-medications

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List of acronyms

AAU	Addis Ababa University
AKI	Acute Kidney Injury
ANOVA	Analysis of Variance
CHS	College of Health Sciences
CKD	Chronic Kidney Disease
CYP2C9	Cytochrome P450 2C9
GGCX	γ-glutamyl carboxylase
HPLC	High Performance Liquid Chromatography
INR	International Normalized Ratio
KH_2	Vitamin K quinol
LOD	Limit of Detection
LOQ	Limit of Quantitation
PT	Prothrombin time
SNP	Single Nucleotide Polymorphism
SPE	Solid Phase Extraction
TASH	TikurAnbessa Specialized Hospital
VKA	Vitamin K antagonists
VKO	Vitamin K-epoxide
VKOR	Vitamin K epoxide reductase
VKORC1	Vitamin K epoxide reductase complex subunit 1
VKR	Vitamin K Reductase

Introduction

Overview of oral anti-coagulation

Thrombosis and its associated complications are a common cause of death throughout the world and many patients suffer long term physical consequences from the thrombotic event, resulting in an adverse quality of life and millions of dollars spent for healthcare (Battinelli et al. 2012). Venous thromboembolism and atherothrombosis share common risk factors and the common pathophysiological characteristics of inflammation, hypercoagulability, and endothelial injury (Goldhaber & Bounameaux 2012). Oral anticoagulants are thus used when an accurate control of coagulation is required, in conditions of thromboembolic disorders (Ghimenti et al. 2011).

Warfarin and other Coumarin derivatives, collectively termed as Coumarins or Vitamin K antagonists (VKA) are useful for the prophylaxis as well as secondary prevention of arterial and venous thromboembolism, as a preventive measure against systemic embolism in patients with prosthetic heart valves or atrial fibrillation, for the primary prevention of acute myocardial infarction, for the prevention of stroke and recurrent infarction in patients with acute myocardial infarction (Ansell et al. 2006). Since the introduction in the 1950s, warfarin has become the commonly used oral anticoagulant (Michael Lee M.T., *et al,* 2014). Patients with Antiphospholipid antibody syndrome, a thrombophilic condition manifested by vascular thrombosis or recurrent pregnancy loss with the presence of antibodies against anionic phospholipid protein complexes benefit from the use of moderate intensity anticoagulation (Freire et al. 2014). Oral anticoagulants are also effective in the prevention of venous thrombosis after hip surgery (Mannucci & Poller 2001).

The Vitamin K Cycle and coagulation

Warfarin exerts its anticoagulant effect by interfering with the hepatic synthesis of vitamin K-dependent clotting factors. Therefore understanding the vitamin K cycle and the coagulation mechanism is vital.

Vitamin K is a family of structurally related fat-soluble compounds including phylloquinone (K1), menaquinone (K2) and menadione (K3). All vitamin K molecules contain 2-methyl-1,4-naphthoquinone and are characterized by a naphthalene ring containing two carbonyl moieties at positions 1 and 4. The best known of them is vitamin K1 (phylloquinone), which is found mainly in green vegetables. In chloroplasts, phylloquinone is an important molecule for energy transfer in photosynthesis. Vitamin K2 (menaquinone) is synthesized by bacteria but is also found in liver, milk, cheese and fermented soy products. Menadione (K3) is chemically synthesized as pro-vitamin because vertebrate intestinal bacteria can convert it to K2 by adding a 4-prenyl side-chain at the 3-position (Stafford 2005).

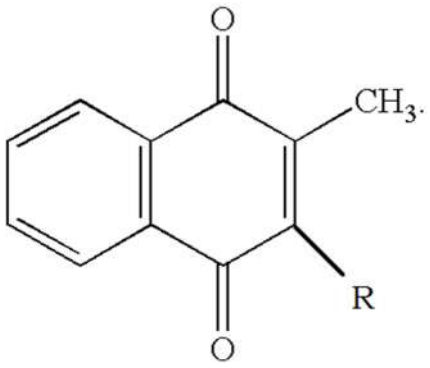


Figure 1: Vitamin K (Perez-Soler & Ling 2014)

In hemostasis, procoagulant factors II, VII, IX, X as well as anticoagulant proteins C and S need vitamin K for their physiological function. γ-Carboxyglutamic acid contained in the different Vitamin K dependent proteins confers metal binding properties. In the

presence of calcium ions, these proteins undergo a conformational change that leads to the expression of membrane binding properties (Borowskis et al. 1986; Stry & Nelsestuen 1976). In the presence of membrane bound cofactors, blood clotting enzymes, some of which are vitamin K-dependent (eg, factors IXa, Xa, and VIIa), assemble on membrane surfaces and act on membrane bound substrates (eg, factor IX, factor X, and prothrombin). Thus, the γ-carboxyglutamic acid-rich domain of the vitamin K-dependent proteins allows for calcium-dependent, reversible protein complex formation on cell surfaces (Furie 1990).

Vitamin K quinone, received from dietary sources, is reduced to vitamin K quinol (KH_2) by vitamin K reductase (VKR). Formed KH_2 is a cofactor for γ-glutamyl carboxylase (GGCX). This enzyme uses CO_2 and O_2 to convert Glu amino acids on the N-terminal of vitamin K-dependent proteins to Gla residues. In the same reaction and by the same enzyme, KH_2 is oxidised to vitamin K-epoxide (VKO). Thus, GGCX is also an epoxidase. VKO is reduced back to the quinone form by vitamin K epoxide reductase (VKOR). VKOR is the enzyme inhibited by VKA drugs such as warfarin, whereas VKR is less sensitive to these drugs. All of the enzymes involved in the vitamin K cycle are embedded in the membrane of the endoplasmic reticulum (Wallin & Hutson 2004).

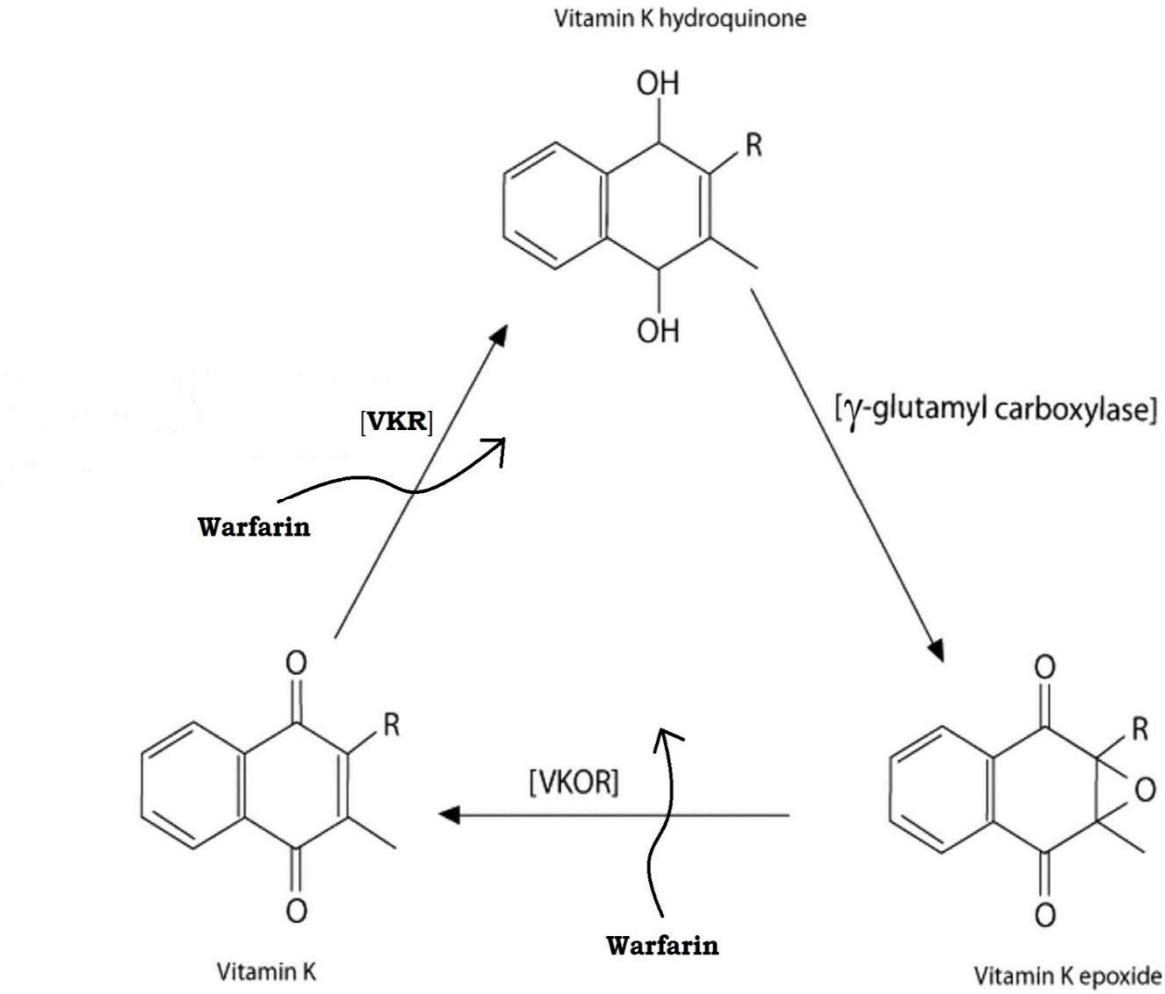


Figure 2: Vitamin K Cycle (Teichert et al. 2008)

Discovery of warfarin

Warfarin discovery have its origins in an odd bleeding disorder in cattle that broke out in the United States and Canada in the 1920s. In the early 1920s, farmers reported a new cattle disease that was characterized by fatal bleeding associated with feeding on spoiled Sweet Clover. The disease was manifested as hemorrhagic type, with the presence of subcutaneous swellings and the anemic type which could be seen following animal dehorning. In the anemic type, examination of the blood shows greatly reduced hemoglobin and markedly delayed clotting time which to the naked eye also reveals thin

and watery blood seeping from the base of the horn and fails to clot or clots very slowly. It was Frank Schofield, a veterinary pathologist in Canada, who eventually recognized that the disease originated from the consumption of mouldy hay made from sweet clover (Schofield 1984).

Then, in 1929 Roderick, a veterinarian in the USA, demonstrated from the pathophysiology of the disease that spoiled sweet clover contained an unknown anticoagulant agent involved a reduction of prothrombin and consequently a prolongation of coagulation time (Roderick 1931). Schofield and Roderick recognized that the disease was reversible and it could be controlled in cattle by the withdrawal of the spoiled hay from the diet and by transfusion of blood freshly drawn from normal cattle, provided the hemorrhagic extravasation had not proceeded too far (Link 1959).

In February 1933, a farmer named Ed Carlson took a milk can containing blood completely destitute of clotting capacity, and about 100 pounds of spoiled sweet clover- the only hay he had to feed his cattle to the Biochemistry Building of University of Wisconsin-Madison. Between February 1933 and June 1939 a long and arduous trial was followed by Co-workers of Link KP: Smith, Roberts, and especially Campbell, to lay the anticoagulant out on the bench. Finally on June 28, 1939 Campbell could extract and collect 6 mg the crystalline Dicumarol (Link 1959).

Later in 1940, C. F. Huebner determined structure of Dicumarol, as 3, 3'-methylenebis (4-hydroxycoumarin). He also set the sights for the synthesis. The biological synthesis of Dicumarol during spoilage could be rationalized as an oxidation of coumarin to 4-hydroxycoumarin which upon coupling with formaldehyde leads to Dicumarol. Link and his co-workers then conducted dose-response trials using pure Dicumarol on rabbits and they also suggested that the observed hypoprothrombinaemia caused by Dicumarol

could be induced by some of its analogues and derivatives. Then after warfarin and many other molecules were synthesized (Crops 1942).

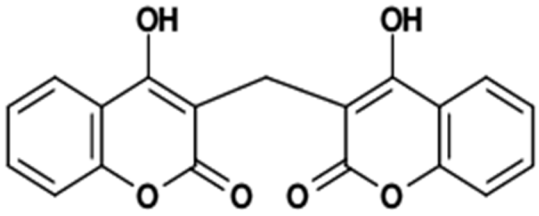


Figure 3: Dicumarol (PubChem n.d.)

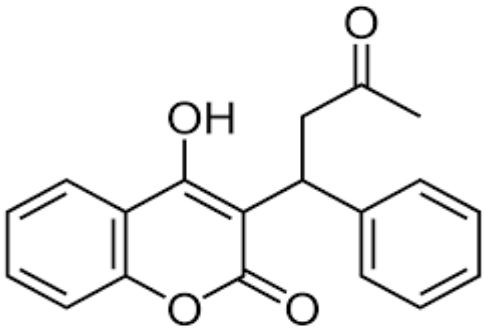


Figure 4: Warfarin (Petitpas et al. 2001)

The reversibility of the prolongation of the prothrombin time (PT) and coagulation time induced by Dicumarol was demonstrated in Sweden by Jörgen Lehmann, who emphasised on the structural similarities between coumarin and the naphthoquinone part of vitamin K Synthesis. He was also effective in determining the reversal of bleeding using vitamin K in patients (Lehmann 1959).

Pharmacokinetics of warfarin

Warfarin is rapidly and extensively absorbed from the stomach and small intestine following oral administration suggesting almost 100% bioavailability. No differences in absorption rates or bioavailability have been reported for R and S-warfarin. Warfarin is extensively bound to plasma proteins, primarily albumin. Only the remaining free fraction is pharmacologically active. The volumes of distribution for the two enantiomers also appear to be similar, with an average of 0.15 l/kg for each enantiomer and 0.11 to 0.18 l/kg for racemic warfarin, similar to that of albumin (Zhou & Chan 2009; Stirling et al. 1982; Wittkowsky 2003).

Warfarin undergoes stereo-selective metabolism in the endoplasmic reticulum of the liver parenchyma by cytochrome p450 hepatic microsomal enzymes. Once formed, the hepatic metabolites are eliminated via urine. Differences in clearance are reflected in differences in elimination half-lives, estimated as an average of 29 hours (range 18 to 52 hours) for S-warfarin compared with an average of 45 hours (range 20 to 70 hours) for R-warfarin (Lewis et al. 1974).

Warfarin Monitoring

Initiation of warfarin therapy is challenging, since the pharmacodynamic response is delayed and difficult to predict. The clinically important antithrombotic effect of warfarin is reflected by the prevention of clots and is believed to largely depend on the clearance of prothrombin, which has a relatively long half-life of up to 5 days (Hirsh et al. 2001). It is therefore common to give 5 – 10-day courses of heparin before the long-term warfarin therapy.

Monitoring of warfarin in patients is usually done by the pharmacodynamic effects (Such as PT). Confirmation of the presence of coumarin-type anticoagulants in biologic tissues

is required in cases of increased PT, when there is no apparent cause for this condition, to confirm an accidental or intentional ingestion as coumarins are in some situations used as rodenticides (Kollroser & Schober 2002). While warfarin is effective, regulating warfarin dosing is challenging because of the drug's narrow therapeutic range. Patients must undergo frequent coagulation testing (i.e, PT) to monitor their response to the drug. Because PT assays are subject to significant variation across laboratories and testing systems due to differences in reagent sensitivity, the international normalized ratio (INR) was developed and is now the standard method for monitoring warfarin response (Hirsh et al. 2003). The pharmacokinetic and pharmacodynamic properties of warfarin as well as its narrow therapeutic index make it particularly vulnerable to increased PT monitoring and warfarin dosing adjustments to maintain safe and effective anticoagulation (Wittkowsky 2003). A supra-therapeutic INR may put patients at risk of bleeding, whereas a sub-therapeutic INR may not protect against thromboembolic complications (Merli & Tzanis 2009).

Statement of the problem

Warfarin monitoring poses challenge to the treatment of thromboembolic disorders because out-of-range INRs which is encountered as a result of changes in numerous factors (Wittkowsky & Devine 2004). Out of the ranges, a supra-therapeutic INR puts patients at risk of bleeding, and a sub-therapeutic INR may not protect against thromboembolic complications (Merli & Tzanis 2009).

Genetic Polymorphism of Cytochrome P450 2C9 (CYP2C9) and Vitamin K epoxide reductase complex subunit 1 (VKORC1) accompanies significantly higher risk of major bleeding events with a warfarin dose-dependent effect (Tomek et al. 2013). Despite the major issue of plasma testing of warfarin, that is a significant proportion of serious adverse events occur in the first six weeks of warfarin therapy (Flockhart et al. 2008), mutations in the vitamin K–dependent protein genes are associated with a lower ratio of INR to Plasma concentration (INR/Cp), suggesting reduced warfarin sensitivity (Shikata et al. 2004).

Even though different studies (Cho et al. 2007; Shikata et al. 2004; Li et al. 2015) have demonstrated the effect of genetic variations on warfarin response and variation of dose requirements, no clear studies are conducted in Ethiopia so far on the relationship between warfarin concentration and its response. This study aims at constructing measuring the plasma warfarin level of Ethiopian patients at Tikur Anbessa Specialized Hospital (TASH) taking variable doses, to scientifically predict the need for genotype study. The study anticipates to determine the association of plasma warfarin level with dose and INR, and discuss the possible factors on the anticoagulant response.

Objective

General objective

The objective of this study was to evaluate the association of plasma warfarin level with warfarin clinical monitoring parameters.

Specific objective

- To evaluate the association between patients' plasma warfarin level and the demographic variables such as age, sex, & weight.
- To evaluate the association between patients' Plasma warfarin level and dose
- To evaluate the association between patients' Plasma warfarin level and INR
- To evaluate the association between patients' plasma warfarin level and Co-medications

Literature Review

Plasma drug analysis provides appropriate information if the patient is at steady-state, 5–7 half-lives of drug elimination. Warfarin has a half-life of one week which means a minimum of five weeks is required to achieve steady state. The major issue with this testing is that a significant proportion of serious adverse events occur in the first six weeks of warfarin therapy. Nevertheless, warfarin steady-state concentration determined by plasma drug analysis can be used to assess patient compliance and adjust warfarin dosing (Flockhart et al. 2008). While INR is a standardized method of warfarin monitoring, it still has limitations in detecting the factors affecting the anticoagulants such as patient compliance, resistance to anticoagulant drug interaction and food variety. Besides the fluctuation of INR in some patients might be dangerous because of the narrow therapeutic index of warfarin. Thus other monitoring approach is helpful in some situations and plasma warfarin measurement can be helpful especially in the management of complicated patients Confirmation of the plasma concentration of warfarin can also facilitate diagnosis and allows for the effective treatment of severe intoxication (Sun et al. 2006).

Even though the anti-coagulant effect or clinical value of warfarin is due to the free fraction of the drug in blood, most studies indicate that patients' INR and/or dose does not correlate to its plasma concentration. A study has reported in the application of plasma concentration development method that the coefficients of correlation between free concentration of warfarin and the INR or the weekly dosage for 108 treated patients were low, 0.207 and 0.378 respectively (Huang et al. 2008). Despite the inevitable study differences, similarly low correlations of plasma concentration to INR (r=0.23) was also noted in other study (Osman et al. 2005). Another study (Kulkarni et al. 2008) however studied the correlation between ratio of INR : plasma total warfarin concentration with

the weekly dose of warfarin and found good correlation (r=0.81) yet having extremely poor correlation of INR with the 7-hydroxywarfarin ratio and the weekly dose of warfarin (r=0.14). This could possibly be explained by the fact that it was a pilot study with smaller sample size of twenty five and the applicability of their findings to predict the therapeutic dose of warfarin in subjects in the initial dose titration phase of therapy than the maintenance phase as explained by the authors.

Krishna and colleagues (2013) have studied the plasma concentration of 7-hydroxywarfarin, major metabolite of warfarin and used it in studying the correlation of metabolic ratio (plasma concentrations of warfarin/7-hydroxy warfarin) to stratified dose groups of low, standard and high dose. They found that when higher doses are given, the clearance is decreased and the concentration of the drug is increased which resulted in apparent reduced metabolism of warfarin at higher doses (with higher metabolic ratio). Thus they recommended determination of plasma warfarin to be considered in managing patients with fluctuating INR as the higher dosage leads to warfarin related toxicities.

Several factors, including age, body size, diet, and drugs that interfere with warfarin metabolism, are well known to influence warfarin dose requirements (Ansell et al. 2008). A case report (Aomori et al. 2014) of patient with therapy on carbamazepine and who had to start warfarin, INR was 1·40 despite taking a dose three times higher than the average yet S-warfarin concentration was 0·15 μg/mL and R-warfarin was 0·52 μg/ml. The VKORC1 genotype and induction of CYP2C9 by carbamazepine indicated that the patient require an even higher dose. Besides the role of drug interaction in terms of enzyme induction as factor affecting warfarin monitoring, much emphasis is given to the inter-individual difference of enzyme expression.

Studies have indicated that the contribution of variable warfarin metabolism by CYP2C9 and VKORC1 genotypes are 10% and 25% respectively, whereas clinical factors, such as

age, sex, diet, drugs, and body mass index, contribute another 20% (Ma & Lu 2011). The variable metabolism of S-warfarin, the more effective isomer in anticoagulation, is believed responsible for most of the variability in warfarin dosing. The major pharmacokinetic change associated with deficient CYP2C9 is that the half-life of bioactive S-warfarin is increased (Linder et al. 2002). This will increase the time to reaching steady-state concentrations of warfarin and the INR determined at a set time in a protocol may not reflect the final steady-state INR on the warfarin dose used for the patient who is heterozygous or homozygous for CYP2C9 polymorphisms (Flockhart et al. 2008).

Another genetic polymorphism of VKORC1 is associated with the variation of inter-individual warfarin dose requirement in different ethnic populations (Yang et al. 2010). VKORC1 polymorphism, for example in Korean patients, has a dominant genetic influence on inter-individual variability for warfarin dose. It explains approximately 32% of the overall variability in warfarin dose requirements given all of the variables studied (Cho et al. 2007). As indicated in studies, mutations in the vitamin K–dependent protein genes are associated with a lower ratio of INR/Cp value, suggesting reduced warfarin sensitivity (Shikata et al. 2004). (Li et al. 2015) evaluated the relationship between plasma concentration and INR to investigate the effect of single nucleotide polymorphisms (SNP) in VKORC1, CYP2C9, CYP4F2, and GGCX on the pharmacodynamics of warfarin in Chinese population. They found significant association in the requirement for lower plasma concentration with SNP of VKORC1 to achieve a target INR and analogously, patients with SNP CYP2C9 already had a lower plasma concentration under similar maintenance dose of warfarin, though not statistically significant.

CYP2C9*2 and CYP2C9*3 polymorphisms are associated with an increased risk of over anti-coagulation and of a serious or life-threatening bleeding event in a warfarin anticoagulation clinic setting. Patients with at least one variant allele have increased risk of above-range INRs and require more time to achieve stable dosing with a median difference of 95 days. (Higashi et al. 2002) In addition, despite the insignificant association of the VKORC1 genotype with bleeding, a warfarin dose-dependent effect with significantly higher risk of major bleeding events during the initiation and maintenance therapy is noted in patients who are carriers of 3 variant alleles of the genes CYP2C9 and VKORC1 (Tomek et al. 2013). Further more acute kidney injury (AKI) may occur shortly in warfarin-treated chronic kidney disease (CKD) patients after an acute increase in the International Normalization Ratio (INR) > 3.0 with formation of occlusive red blood casts; and recovery from this warfarin-associated AKI is poor. Over-anticoagulation is associated with faster progression of CKD in a high percentage of patients (Brodsky et al. 2010). The International warfarin pharmacogenetics consortium have thus recommended that pharmacogenetic algorithm accurately identifies larger proportions of patients on different doses of warfarin to achieve the target INR than the clinical algorithm. (Consortium* 2009)

Study Methodology

Study setting, Design & population

This study was conducted using cross-sectional prospective study design at the Hematology Referral Clinic of the Internal Medicine department, TASH. The study population was patients taking warfarin treatment for at least six weeks and on follow up at the referral clinic.

Sample size calculation for correlation

In order to detect a simple correlation *r* (r=0.4) using a two sided test of 5% significance level test (α=0.05) with power 80% power (β=0.2), the required minimum sample size is approximately 47. The formula used to calculate the sample size is depicted as follows,

$$N = \left(\frac{z_{\alpha} + z_{\beta}}{C(r)} \right)^2 + 3$$

Where;

C(r) = Fisher's arctanh transformation
r = a sample correlation r
N = Sample size

To control for confounding factor role in sample size calculated, 20% more contingency sample was included. This made the total sample required for this study to be 57. The minimum number of participants were then recruited based on the eligibility criteria from the date of approval of the proposal. Patients charts were also reviewed for identification of patients' demographics and History, types of medications given, and the doctor's evaluations using a data abstraction form (Annex-I)

Eligibility criteria

Inclusion Criteria

All patients on warfarin therapy at the Hematology Referral Clinic of TASH, AAU were included in screening for inclusion in the study. Fifty seven patients who fulfilled the follow criteria were chosen for inclusion: Adult patients (>18 years old), who took warfarin for at least six weeks, assuming steady state concentration of warfarin is achieved in five weeks, and willing to give their informed consent of participation.

Exclusion criteria

Pregnant women, Children of ≤ 18 years of age, patients with serious complication such as those on blood transfusion, and those who did not give an informed consent were excluded from the study.

Ethical Considerations

Review and Permission

Approval from the Department of Pharmacology and Department of Internal Medicine research and ethical committees, at the School of Medicine, College of Health Sciences (CHS), Addis Ababa University (AAU), was sought and permission was obtained from the study site prior to beginning of the study.

Confidentiality and Data protection

Confidentiality of participants' information and results of analysis was assured by giving non-traceable codes to participants and their blood samples. The data collected is protected and only the researcher and advisor will have access to the data.

Informed Consent

Verbal Consent: The study's benefit and risk were read clearly from the participant information sheet to the research participants and the participant were be asked for his/her willingness to participate.

Written Consent: The participants were then asked to sign a written consent form which states what he/she has verbally agreed using the local language (Annex-III).

Statistical Analysis

The collected data were cleared and entered into SPSS® version 16 for statistical analysis at 95% confidence interval. Associations of patient demographics: Age, weight and sex with the plasma concentration and the association between plasma concentration and weekly warfarin dose were evaluated by using Pearson's Correlation. The association between plasma concentration and INR was evaluated by using non-parametric spearman's correlation. The association between plasma concentration and the different co-medications were evaluated by comparing means using one way analysis of variance (ANOVA).

Experimental Section

Chemicals and reagents

All organic solvents and water used in this work were HPLC-grade. Methanol and acetonitrile were purchased from Carlo Erba Reagents through local distributors. Racemic warfarin (Pestanal® analytical standard 97.2%) and Discovery® DSC – 18 Supelco Solid Phase Extraction (SPE) cartridges were purchased from Sigma–Aldrich

(Germany). HPLC grade glacial acetic acid was kindly donated from Pharmacognosy Laboratory, School of Pharmacy, CHS, AAU.

All procedures were adopted from similar studies (Krishna et al. 2013; Huang et al. 2008) and modified as to best suit the working conditions.

Blood samples and plasma preparation

Five milliliter whole blood was collected, into test tubes containing Ethylene-Diamine-Tetra-Acetic acid (EDTA), from patients on warfarin treatment. The samples were centrifuged using Eppendorf® 5804R centrifuge at 2000×g for 10 min to get cell free plasma. The tubes were then kept at–20◦C. Plasma for the Quality Controls was prepared in the same way from a healthy consented blood donor.

Standard preparation

Stock solution of pure warfarin was prepared at 0.5 mg/mL in methanol. Working solutions at the levels 0.5, 1, 2, 5, 10, 15 µg/mL were then prepared from the stock solution. The solutions for the standard curve were freshly prepared before the analysis.

Sample preparation and extraction

The SPE Cartridge was conditioned with 2% Methanol. One ml of plasma was then rigorously vortexed and transferred to the SPE cartridge. Extraction was performed using positive pressure method. The warfarin retained in the column were finally eluted with 2 ml of acetonitrile and 20 µl was injected into the High Performance Liquid Chromatography (HPLC).

Chromatography

HPLC was performed at the Center for Food Science and Nutrition, Faculty of Science, AAU. The chromatographic system consisted of Shimadzu LC–20AB pump, SPD-20AV Ultra Violet-Visible (UV-VIS) detector, SIL-20A auto sampler, VP-ODS Shim Pack reverse phase column (250 mm x 4.6 mm) (Shimadzu Corporation, Kyoto, Japan), at Column oven temperature of 25 ^{0}C.

An isocratic mobile phase of acetonitrile/water (60/40, v/v) with 1% glacial acetic acid was developed by changing ratio of the solvent composition to achieve economical ratio that give reasonable pick. The flow rate was maintained at 1.2 mL/min and the column was equilibrated with the mobile phase before analysis.

Linear calibration curves were generated by setting the concentration of the standards on the x-axis and the response of standards on they-axis. To fit the curve to the calibration data, least-squares fit was used (y=A+Bx).The absorbance at 280 nm was measured with a total run-time of 13 min.

Method validation

The linearity of the method was investigated by establishing an initial standard curve containing six points over the range 0.5–15μg/ml. The limit of detection (LOD) was defined as the concentration corresponding to a signal to noise ratio of 3:1. The limit of quantitation (LOQ) was defined as 10×LOD. Plasma from a healthy volunteer was equally divided in two volumes and spiked with known amounts of racemic warfarin in duplicates (2 μg/mL and 5.0 μg/ml) and the recovery was calculated by comparing with unextracted (directly injected) warfarin of the same levels. Repeatability on the same day and reproducibility for five days were calculated from sets of five pairs of standards (2 and 5.0μg/ml).

Results

Patient demographics

The demographic parameters and plasma levels of warfarin of study participants are given in Table 1. Among 57 patient samples, 4 were excluded from analysis due to loss of samples during extraction process.

Table 1: Patient demographic data

Parameters	All Patients (n = 53)
Age (Years) (Mean ± SD)	41.55 ± 14.30
Gender	
Male (%)	41.5
Female (%)	58.5
Weight (Kgs)	64.40
Average weekly dose of warfarin (mg ± SD)	38.30 ± 16.47
Mean Plasma warfarin conc. (µg/ml ± SD)	8.075 ± 6.15
INR (Mean ± SD)	2.37 ± 0.95
Indications for warfarin	
Deep Venous Thrombosis (%)	73.6
Pulmonary Embolism (%)	13.2
Others (%)	13.2
Co-Medications	
Effect enhancing[a] (n, %)	16 (30.2)
Effect diminishing[b] (n, %)	9 (17.0)
No co-Medication/No effect[c](n, %)	28 (52.8)

[a] Co-medications which have warfarin effect enhancing potential

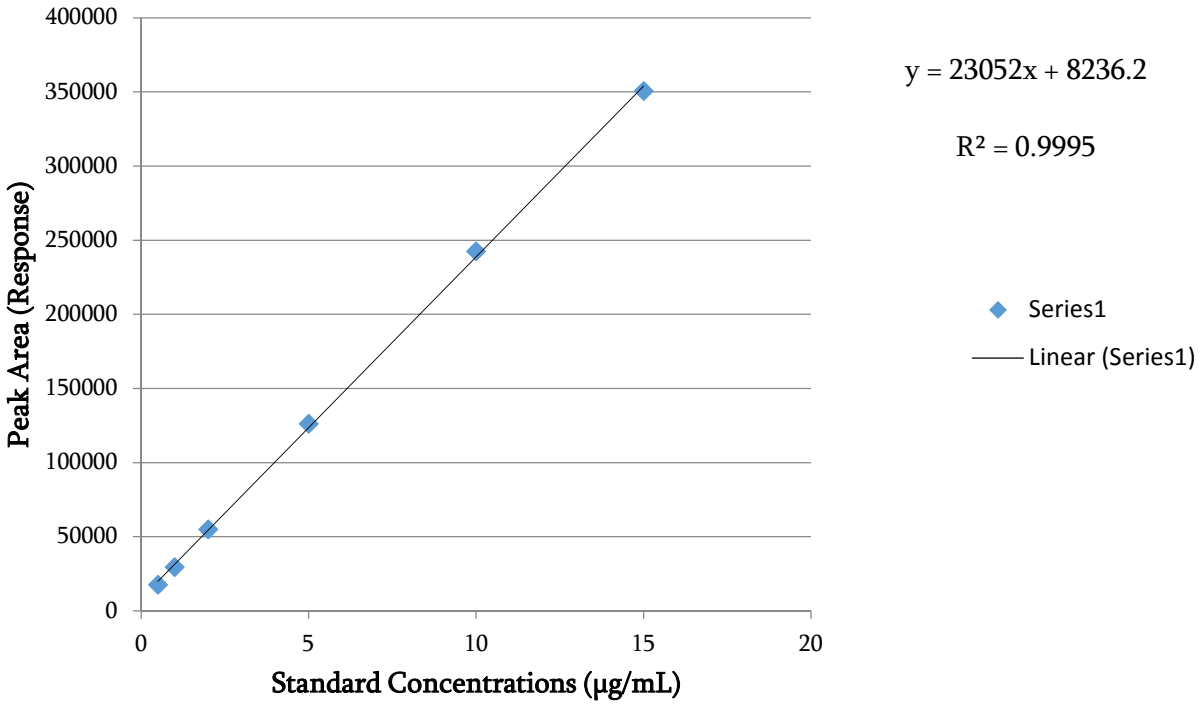
[b] Co-medications which have warfarin effect enhancing potential

[c] No co-medication or co-medications which have no warfarin effect enhancing or diminishing potential

Analysis outputs

Warfarin determination was linear over the range 0.5 – 15 µg/ml. Six points were used and gave linearity with $r^2 = 0.9995$; and linearity equation was Y = 23052x + 8236.2. The LOD determined was 40 ng/mL and LOQ defined as 10×LOD was calculated to be 400 ng/ml. The entire population analyzed had values well above the calculated LOQ. Total recovery was checked using spiked healthy volunteer plasma spiked with (2 µg/mL and 5.0µg/mL) and found approximately 87.4% ± 3.84.

Retention time of warfarin, time for elution from the stationary phase or also defined as peak time, was between 5.54 and 5.64 minutes as obtained by comparing with the reference standard peak response. Representative chromatogram of standard (15 µg/ml) and anonymized patient sample (6.824 µg/mL detected) is depicted in Figures 5 and 6.



Graph 1: Calibration Curve of Standard reference warfarin

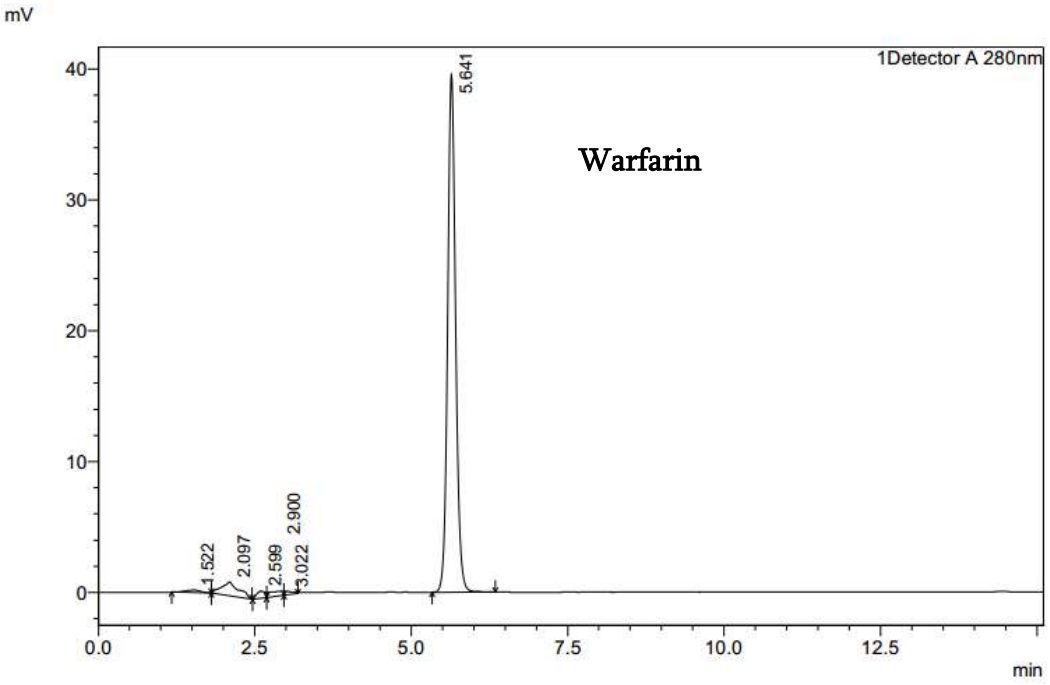


Figure 5: Chromatogram of pure racemic warfarin (15µg/ml)

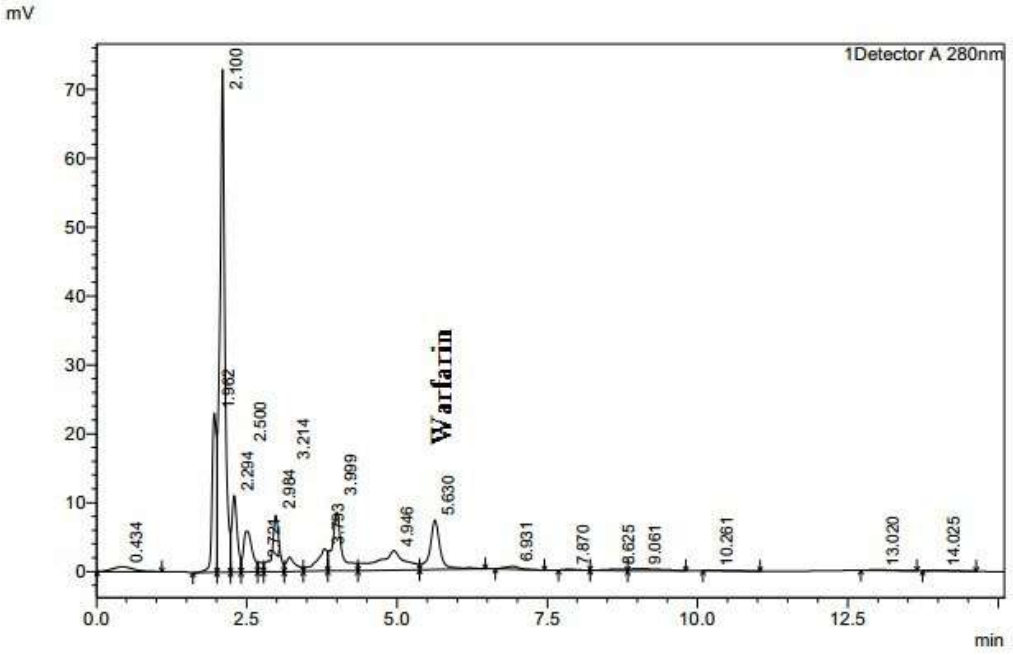
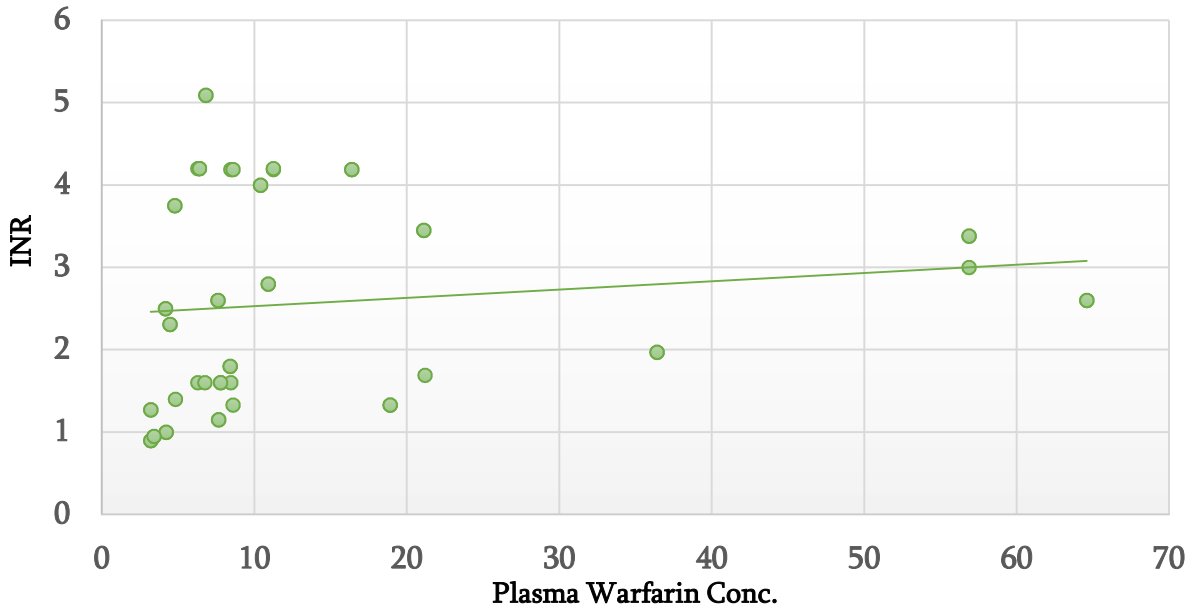


Figure 6: A typical patient chromatogram

Association of plasma warfarin concentration with INR and weekly dose

There were no statistically significant correlation of age, weight or sex with the plasma warfarin concentration ($p>0.1$). The plasma concentration and weekly dose data were fit for Pearson's correlation analysis and have low correlation ($r = 0.283$; $p = 0.04$). The association between plasma warfarin concentration and INR was evaluated using non-parametric Spearman's correlation and had also low correlation (Spearman's rho = 0.375; $p = 0.006$).



Graph 2 Scatter plot of plasma warfarin concentration against INR

Association of mean plasma warfarin concentrations of ranges of INR and weekly dose

Patients were also stratified in to three groups of INR classes; those having INR < 2, those having INR between 2 and 3, and those having INR > 3. Then mean plasma concentration of patients among the three INR classes were compared.

Table 2: Descriptive statistics of mean plasma concentration of range of patients' INR

Range of INR	N	Mean Plasma Conc.	Std. Deviation	Std. Error	95% CI for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
<2	24	10.100	9.552	1.949	6.066	14.134	3.201	36.408
2 – 3	10	18.346	24.508	7.750	.814	35.878	4.176	64.590
> 3	19	15.335	15.467	3.548	7.880	22.789	4.769	56.882
Total	53	13.532	15.434	2.120	9.278	17.787	3.201	64.590

Similarly, patients were also stratified into three groups of weekly doses: those receiving weekly doses of warfarin < 35 mg, those receiving weekly doses between 35 and 52.5 mg, and those receiving weekly doses > 52.5 mg.

Table 3: Association of mean plasma concentration of range of patients' weekly dose

Range of weekly dose	N	Mean Plasma Conc.	Std. Deviation	Std. Error	95% CI for Mean		Min.	Max.
					Lower Bound	Upper Bound		
<35 mg	24	10.121	7.646	1.560	6.892	13.349	3.201	36.408
35 - 52.5mg	16	17.607	20.003	5.000	6.948	28.266	4.460	64.590
> 52.5 mg	13	14.816	19.307	5.355	3.148	26.484	3.201	56.882
Total	53	13.532	15.434	2.120	9.278	17.787	3.201	64.590

Then means of plasma concentrations of patients among the three different weekly dose groups were then compared. In both cases, INR classed and Weekly dose grouped patients, there were no statistically significant differences in their mean plasma concentration ($p = 0.304$, $p = 0.310$ respectively)

Association of mean plasma warfarin concentrations with Co-medications

The mean plasma concentration of patients taking co-medications were compared using one way ANOVA analysis. Patients were first stratified according to the effect of their co-medications on warfarin efficacy; those taking effect enhancing, effect diminishing and non-effect changing co-medication.

Table 4: Association of mean plasma concentration over effect enhancing and no drug effect co-medication taken

Plasma Conc.

	N	Mean	Std. Deviation	Std. Error	95% CI for Mean		Min.	Max.
					Lower Bound	Upper Bound		
No drug effect	27	7.959	3.563	.685	6.549	9.369	3.201	21.185
Effect enhancing	16	28.195	21.640	5.410	16.663	39.726	4.205	64.590
Total	43	15.489	16.524	2.520	10.403	20.574	3.201	64.590

Table 5: Association of mean plasma concentration over effect diminishing and no drug effect co-medications taken

Plasma Conc.

	N	Mean	Std. Deviation	Std. Error	95% CI for Mean		Min.	Max.
					Lower Bound	Upper Bound		
No drug effect	28	8.064	3.540	.669	6.691	9.437	3.201	21.185
Effect Diminishing	9	4.478	1.053	.351	3.669	5.288	3.201	6.748
Total	37	7.192	3.476	.571	6.033	8.351	3.201	21.185

The analysis gave a statistically significant difference in the mean plasma warfarin concentration of patients who took effect enhancing co-medication and those who took non-effect changing co-medication ($p<0.001$). The difference in the mean plasma concentration of patients who took effect diminishing co-medication and those who took non-effect changing co-medications was also statistically significant ($p = 0.005$).

Discussion

Great strides have been made in monitoring the anticoagulant effect of warfarin with the development of INR to standardize PT. But warfarin monitoring is still challenge in treating thromboembolic disorders because out-of-range INRs may be encountered frequently as a result of changes in numerous factors (Wittkowsky & Devine 2004). Such fluctuation might be dangerous because of the narrow therapeutic index of warfarin. Thus other monitoring approach is helpful in some situations and Plasma warfarin measurement can be helpful especially in the management of complicated patients. The most used methods for plasma warfarin concentration determination is based on extraction of warfarin from plasma followed by chromatography. The extraction is the crucial step in such methods, which requires careful conditions to ensure good recovery of warfarin before sample injection. In this study, extraction of the drug from plasma was performed using C18 SPE cartridges and elution was done using acetonitrile. Total recovery was approximately 87.4% ± 3.84.

The study aimed at determining plasma warfarin level and evaluating the association of patients' plasma warfarin level with the major demographic variables and non-demographic variables. The non-demographic variables to be compared with were dose of warfarin, indication for warfarin, INR and co-medications. Not surprisingly, the correlation between concentration with weekly dosage was low ($r = 0.283$; $p = 0.04$). And, the association between plasma warfarin concentration and INR was also low (Spearman's rho = 0.375; $p = 0.006$). This is in line with the low correlations of most other findings as in (rho = 0.207 with INR, $p=0.03$ and rho=0.378 with weekly dosage, $p < 0.001$; $r=0.23$ for S-warfarin concentration with INR and $r=0.30$ with INR, $p < 0.05$ respectively) (Osman et al. 2005; Sun et al. 2006; Huang et al. 2008). This can be explained by numerous factors and inter-individual differences such as interaction with

other drugs, resistance, diet, differing drug metabolisms, Vitamin K status, and other factors which make the INR poorly dependent on warfarin concentration and thus affect the subjective judgment to dose adjustment (Osman et al. 2005).

To further evaluate the influence of subjective judgment towards dose adjustment based on the INR, which may not be necessarily effective, patients' weekly doses were classified in to three presumptive groups: those taking <35mg/wk., those taking between 35mg/wk. and 52.5 mg/wk. and those taking above 52.5 mg/wk. Then the one way ANOVA revealed there was no statistically significant differences in mean plasma concentrations among the different weekly dose group of patients. Similarly patients INR were classified in to three: those who achieve INR less than 2, those who achieved INR between 2 and 3, and those whose INR is above 3. Again, there was no statistically significant differences in mean plasma concentrations among the different INR class patients. There are no specific studies which use dose stratification unless the Krishna *et al* that used dose stratification to study metabolic ratio. (Krishna et al. 2013) and INR stratification methods. Nevertheless, the poor significance from one way ANOVA study tell us dose increment can be merely subjective judgment based on the INR for an individual patient and did not consider other possible factors which affect the patient INR. This gives a glimpse that INR measurement alone would be only of limited value for dose-adjustment in patients with complicated situations. Thus, there can be danger of bleeding for the patient if we do not make sure the warfarin concentration is low when the INR is still not falling in the targeted range. So warfarin plasma concentration measurement may be helpful in managing patients, especially to the patients with difficulty to manage fluctuating INRs.

However there was one finding, (Kulkarni et al. 2008) - a pilot study, which found there was a good correlation between INR : plasma total warfarin concentration and the

weekly dose of warfarin (r=0.81) and yet extremely poor correlation of INR with the 7-hydroxywarfarin ratio and the weekly dose of warfarin (r=0.14). The difference of finding in this study may probably be attributed to the nature of study that it is a pilot study involving small patient sample size (25) and limitation of the findings that its applicability is limited to predict the therapeutic dose of warfarin in the initial dose titration phase of therapy than the maintenance phase, as explained by the authors.

No recent studies on direct plasma warfarin and co-medication association were found. Yet it is established for long that warfarin effect is enhanced as well diminished by drugs for different reasons. For instance a literature review (Wells et al. 1994) had reported warfarin's anticoagulant effect was potentiated by drugs such as cotrimoxazole, erythromycin, propranolol, cimetidine and omeprazole. Three patients were reported to have a hemorrhage at the time of a potentiating interaction. Diminished warfarin's anticoagulant effect was also observed by drugs such as rifampin, barbiturates and carbamazepine.

In the current study, the effect of co-medications on warfarin was studied by comparing the mean plasma concentrations of patients taking effect enhancing and effect diminishing co-medication with those taking none effect inducing co-medications. Patients were thus stratified by their co-medications accordingly. The result of one way ANOVA gave a highly statistically significant difference in the mean plasma warfarin concentration of patients who took effect enhancing co-medication and those who took non-effect changing co-medication ($p < 0.001$). Similarly, the difference in the mean plasma concentration of patients who took effect diminishing co-medication and those who took non-effect changing co-medications was also statistically significant ($p = 0.005$).

The present study has not come across direct association studies but different case reports have shown association on the effect of co-medication on plasma warfarin level with INR and/or warfarin dose. A case report (Aomori et al. 2014) of patient with therapy on carbamazepine, an effect diminishing co-medication, and warfarin, the INR was low (1·40) despite taking a dose three times higher than the average. Yet S-warfarin concentration (0·15 μg/mL) and R-warfarin (0·52 μg/ml) were very low. Another case report, (Almog et al. 1988) have shown the diminishing effect of warfarin by Rifampin co-medication. They reported warfarin fractional clearance decreased from 15.2 to 4.2 ml/min after cessation of rifampin, and thus warfarin concentrations had risen back. And hence, PT was maintained by a 50% reduction of warfarin doses.

Among the factors that contribute to the difference in response to warfarin treatment and poor dependence of INR on warfarin concentration is inter-individual difference. This study could not address this factor due to financial and time limitations. A study by (Krishna et al. 2013) have already determined plasma concentration of the total warfarin and its predominant metabolite 7-hydroxy warfarin and used their metabolic ratio for comparison with stratified dose groups; Sensitive, standard and resistant. In their finding, the metabolic ratio of the sensitive group was significantly different from the standard dose and high dose groups. 7-hydroxy warfarin is the predominant metabolite of warfarin by CYP2C9.

While the correlation study between metabolic ratio and dose group indirectly indicates role of inter-individual difference, pharmacogenomics study is the direct method of research. Variants of the major genes involved in warfarin pharmacokinetics and pharmacodynamic, CYP2C9 and VKORC1, account for 30–50% of the variability in dosing of warfarin; thus, testing of these genes will aid in warfarin dosing recommendations (Flockhart et al. 2008). While some form of polymorphism in these

genes necessitate lower dose requirement, the other may necessitate increase dose requirement to achieve target INR. For example some polymorph in VKORC1 elevates hepatic level of Vitamin K, a warfarin antagonist, and some polymorphism in CYP2C9 increases warfarin metabolism. Despite their similar effect in lowering INR value, the effect on plasma warfarin concentration is different. Higher plasma concentration is expected in the first, and dose increment in the later is important to compensate for the loss through metabolism. (McDonald et al. 2009) (Li et al. 2015) evaluated the relationship between plasma concentration and INR to investigate the effect of SNP in VKORC1, CYP2C9, CYP4F2, and GGCX on the pharmacodynamics of warfarin in Chinese population. They found significant association in the requirement for lower plasma concentration with SNP of VKORC1 to achieve a target INR and analogously, patients with SNP CYP2C9 already had a lower plasma concentration under similar maintenance dose of warfarin, though not statistically significant. Other studies (Shikata et al. 2004; Maddison et al. 2013; Lane et al. 2012) also show similar finding on the role of genotypes important in warfarin anticoagulation in relation to plasma concentration and pharmacokinetic parameter.

Limitations of the study

The findings of this study should be interpreted with some limitations. Because it was conducted at a single site, the findings may not be generalizable to other clinical settings. We were unable to relate the obtained association between monitoring parameters with plasma concentration to various factors that could affect the monitoring parameters. We also were not able to study better predictors such as metabolic ratio and genotype that could affect the association due to financial limitation due to financial barriers. Moreover, patient's personal factor such as smoking and alcohol drinking habits, diet types and adherence were not included because the study was designed in such a way that patients are met only once.

Conclusion

There was significantly low correlation between the dosage, concentration of warfarin and INR. It is revealed that the anticoagulant effect of warfarin could be affected by many factors. Moreover, analysis has showed insignificant difference in mean plasma concentration among differing groups of patients in terms INR and Dose. This is however in contrast to one's pharmacological expectation. So although the INR is widely accepted as a golden standard for the monitoring of oral anticoagulation therapy to adjust the dose, different factors most importantly the inter-individual genetic variation limits its effective use in the clinics.

Recommendations

Due to the narrow window of safe warfarin therapy and limitation of monitoring parameters by different factors, plasma warfarin level monitoring is essential in some situations. This is of particular importance in patients whose INR is difficult to be achieved in the ideal target and fluctuation is common problem. In addition, since co-medications give a significant change in the plasma concentration, necessary cautions have to be taken like determining warfarin plasma concentrations to check toxicity. Studies with wider scope of determining the different factors and individual metabolic activities may help in strengthening the findings of this study. Further genotyping studies also need to be conducted to rule out practicability of genetic variation and importance of adopting pharmacogenetic treatment algorithm as it was done in some Asian countries like China and India.

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Annex-I: Data abstraction form for the research

"Association of plasma warfarin level with monitoring parameters among patients treated at Hematology Referral Clinic, Tikur Anbessa Specialized Hospital"

Name of Institution: TikurAnbessa Specialized Hospital

Department: Internal Medicine - Hematology Unit

Name: _____ Card No._____ Code: _____

Age: _____ Sex: _____ Date of Exam: _____

Wt. _____ (Kg) Height: _____

Dose of Warfarin (mg) _____ INR: _____

Indication for Warfarin: _____

Disease condition for Warfarin Indication: _____

Other Co-Morbid conditions;

_____
_____
_____
_____
_____

Other Medications in Use:

_____
_____
_____
_____
_____

Annex-II: Participant Information Sheet and Consent form

The following information sheet is intended to give necessary information about the research "Association of plasma Warfarin level with monitoring parameters among patients treated with Warfarin" to possible participants. The research study is to be conducted as part of MSc thesis for Pharmacology program under the School of Medicine, AAU.

We would like to invite you to take part in our research study. Before you decide we would like you to understand why the research is being done and what it would involve for you. One of our team members will go through the information sheet with you and answer any questions you have. We would suggest this should take about 10-20 minutes.

Part I provides the purpose of the study and detailed information about the conduct of the study.

Part II includes written consent form for signature.

Ask us if there is anything that is not clear. Take time to decide whether or not you wish to take part.

Study title: Association of serum warfarin level with maintenance dose among patients within the normal internationalized normalization ratio range

PART - I

1. The purpose of the study

Warfarin is a drug useful for the prevention and treatment of thromboembolic diseases. While it is effective, regulating warfarin dosing is challenging because of the drug's narrow therapeutic range. Just like other drugs, Warfarin has side effects. Yet outside the narrow therapeutics range; supra-therapeutic treatment puts patients at risk of bleeding and similarly sub-therapeutic treatment may not protect against the thromboembolic complications

Clinical factors, drugs, foods and also genetic makeup are major factors that influence the functions of Warfarin on the body and the function of the body on Warfarin, and thus its dose requirements. However, variations in genes account for the greater percentage of the variability in dosing of warfarin which can usually be reflected by the serum drug level due to their role in drug metabolism.

The purpose of this study is to determine the serum Warfarin level (which can reflect the genetics role if confounding factors are blinded) and its association to the variability of maintenance dose. Since the role of genotype of Ethiopians in the dose requirement is not clearly known, the study anticipates in giving scientifically sound reasoning for variable dose requirement of this narrow therapeutic range medication, Warfarin, based on the serum drug level findings blinding other confounders. Thus, it can be important in knowledgeable treatment of patients with regard to the possible factors in the warfarin dose variation.

2. How participants are selected and choose to participate

You have been chosen to participate in the study because you fulfill the eligibility criteria of the study which are being in the adult age group (>18 Years old) treated at the department of internal medicine at TASH-AAU and taking Warfarin for at least 6 weeks; also you are not in the exclusion criteria. For the study purpose around total of fifty seven patients will participate.

It is up to you to decide to join the study. If you agree to take part, we will then ask you to sign a consent form. You are free to withdraw at any time without giving a reason. This would not affect the standard of care you receive.

3. **What requires during participation**

If you agree to participate, there will be no more than one visit for the study purpose and we will gain access to your personal demographic information, medication information, collect 5ml (1 tsp) blood sample and perform serum warfarin level determination test from the blood sample. No other blood test will be performed.

Genetic test will not be done at this point of time. But this could be possible in another consecutive research for which you will be notified and asked to sign a separate consent form if you would like to participate again.

4. **The possible benefits and risks of taking part**

We cannot promise the study will help you but the information we get from this study will help improve the treatment of people with Warfarin.

During participation in the study, the possible risks incurred by participants include pain at the site of injection for withdrawing blood, bruising or some kind of blood mass at the injection site.

5. **Confidentiality of participation**

If you consent to take part in this study, the records obtained while you are in this study as well as related health records will remain strictly confidential at all times. The information will be held securely on paper and electronically. Your name will not be passed to anyone else outside the research team. You will be allocated a code number, which will be used as to identify you.

Your records will be available to people authorized to work on the research study. By signing the consent form you agree to this access for the current study.

At the end of the study your data will be securely archived for a minimum of 5 years. Arrangements for confidential destruction will then be made.

6. **Who reviewed the study and what will happen if participation is withheld**

This study has been reviewed and given favorable opinion by Research Ethics Committee of the Institutional Review Board at CHS, AAU.

If you withdraw from the study, we will destroy all your identifiable samples, but we will need to use the data collected. Information collected may still be used. Any stored blood that can still be identified as yours will be destroyed if you wish.

7. **What will happen to the results of the research study?**

The results of the study will be available after it finishes and will usually be published in a medical journal or be presented at a scientific conference. The data will be anonymous and none of the participants involved in the study will be identified in any report or publication. Should you wish to see the results, or the publication, please ask the research team.

If you decide you would like to take part then please read and sign the consent form (**Part II)**. You will be given a copy of this information sheet and the consent form to keep. A copy of the consent form will be filed with the study records.

Thank you for taking the time to read this information sheet and to consider this study.

Part II - Study Participant Consent Form

Association of plasma Warfarin level with monitoring parameters among patients treated at hematology referral clinic, Tikur Anbessa Specialized Hospital

Gediwon Negash (BPharm), Getnet Yimer (MD, MSc, PhD) and Abdulaziz Abubeker (MD)

I have been given information about the research entitled " Association of serum warfarin level with maintenance dose among patients within the normal internationalized normalization ratio range " and discussed the research project with the research team member. The research is to be conducted Gediwon Negash as part of his MSc Thesis supervised by Dr. Getnet Yimer and Dr. Abdulaziz Abubeker in the department of Internal Medicine at Tikur Anbessa Specialized Hospital, Addis Ababa University.

I have been advised of the potential risks and burdens associated with this research, which include pain at the site of injection for withdrawing blood, bruising or some kind of blood mass at the injection site. I also have had the opportunity to ask any questions I may have about the research and my participation.

I understand that my participation in this research is voluntary, I am free to refuse to participate and I am free to withdraw from the research at any time. My refusal to participate or withdrawal of consent will not affect my treatment in any way /my relationship with the Department of Internal Medicine or my relationship with the Addis Ababa University.

If I have any enquiries about the research, I can contact Gediwon Negash (0913011010), Dr. Getnet Yimer or Dr. Abdulaziz Abubeker at the departments if I have any concerns or complaints regarding the way the research is or has been conducted. I can also contact the Ethics Officer, Institutional Review Board on.

By signing below I am indicating my consent to (Please tick circles) I understand that the data collected from my participation will be used for thesis research and I consent for it to be used in that manner.

- ◯ Give personal demographic information,
- ◯ Give information of concomitant medications I am taking, and
- ◯ Give blood sample for the analysis of my plasma warfarin level.

_____	_____	_____
Full Name	Telephone and email ID	Signature

Annex-III የምርምር ተሳትፎ መረጃ እና የተሳትፎ ማረጋገጫ

የዚህ ፅሁፍ አላማ ሰለ "በደም ውስጥ የሚገኝ የዋርፋሪን መድሀኒት መጠን እና ታካሚዎች የሚወስዱትን መጠን ግንኙነት" ጥናትላይ ያተኮረ መረጃ ላይ ፅፌ ተሳታፊን በቂ መረጃ መስጠት ነው። ጥናቱ የሚካሄደው በፋርማኮሎጂ ትምህርት የአዲስ አበባ ዩንቨርሲቲ የህክምና ትምህርት ክፍል ማስተርስ ድግሪ የመመረቂያ ፅሁፍ አካል በመሆን ነው።

በዚህ ጥናት ላይ እነዲሳተፉ እንጋብዛለን። ለመሳተፍ ከመወሰኖ በፊት ግን ጥናቱ መካሄድ ያስፈለገበትን ምክንያት እንዲሁም ለምን እርሶን ሊያሳትፉ እንደሚያስፈልግልና ስረዳዎት እንፈልጋለን። ከጥናቱ ባለሙያዎች መካከል አንድ ሰው አብሮት ይህን የመረጃ ዶሴ ያስረዳዎታል። ማንኛውንም ጥያቄ ካለዎት እንዲጠይቁን እናበረታታለን። ከ10 - 20 ደቂቃ ያህል ያስፈልገናል።

ክፍል I - ስለ ጥናቱ አላማ እንዲሁም የጥናቱን ባህሪ በተመለከተ በቂ መረጃ ያስረዳናል።

ክፍል IIየተሳትፎ ፈቃደኝነት ማረጋገጫ ፎርም ይይዛል። ግልጽ ያልሆነለትን ማንኛውም ጥያቄ ይጠይቁን። የተሳትፎ ፈቃደኝነቶን ለመወሰን በቂ ጊዜ ይውሰዱ።

የጥናቱን እና ታካሚዎች ሚወስዱትን መጠን ግንኙነት"

ክፍል 1: የምርምር ተሳትፎ መረጃ

1. የጥናቱ ዓላማ

ዋርፋሪን ከደም መርጋት ምክንያት የሚመጡ በሽታዎች ለመከላል እና ለማከም የሚያገለግል መድኃኒት ነው። መድኃኒቱ ውጤታማ ቢሆንም በመድኃኒቱ ጠባብ የውጤታማና የህክምና መጠን ምክንያት የአወሳሰድ መጠኑን ለመቆጣጠር አስቸጋሪ ነው። እንደማንኛውም መድኃኒት ዋርፋሪን አላስፈላጊ የጎንዮሽ ጉዳዮች አሉት። ከጠባቡ የህክምና መጠን ውጪ ሲሆን ከተፈቀደው መጠን በላይ ሲሆን የደም መፍሰስ ችግር ከተፈቀደው መጠን በታች ሲሆን ከደም መርጋት ጋር የተያያዙ ችግሮችን ያስከትላል።

ክሊኒካዊ ምክንያቶች፣ ሌሎች መድኃኒቶች፣ አንዳንድ ምግቦች እንዲሁም የዘር ዓይነት የመድኃኒቱን በሰውነት ላይ ያለውን ውጤትና ሰውነቱ በመድኃኒቱ ላይ ያለውን ውጤት በቀላሉ ይለውጡታል። ስለዚህም ዳግም የመድኃኒቱን አወሳሰድ መጠን በቅርብ መከታተል ያስፈልጋል። ከነዚህም ክያቶች ውስጥ ደግሞ በተለይ የዘር አይነት ለዚህ የቅርብ ክትትል አስፈላጊነት እና አወሳሰድ መጠን መቀያየር ትልቁን አስተዋጽኦ ይይዛል። የዚህም ምክንያት የዘር አይነት ሰውነት በመድኃኒት ላይ ያለውን ውጤት በቀላሉ መቀየር ስለ ሚችል ነው። ያም በሰውነት ደም ውስጥ በሚገኘው የመድኃኒት መጠን ይገልጻል።

የዚህ ጥናት አላማ ዋርፋሪንን በሚወስዱ ታካሚዎች ደም ውስጥ ያለውን መድኃኒት መጠን እና የመድኃኒቱን አወሳሰድ መጠን ልዩነቶች መካከል ያለውን ግንኙነት ማጥናት ነው። ይሄንንም ሌሎች ለዚህ ግንኙነት አስቸጋሪ የሚሆኑ ምክንያቶችን በመቆጣጠር ያደርጋል። የኢትዮጵያዊያኖች የዘር ዓይነት በዚህ የመድኃኒት አወሳሰድ መጠን ላይ ያለውን ተጽእኖ በተመለከተ ጥናቶች ግልጽ ስላልሆኑ ከላይ በተጠቀሰው መልኩ ከጥናቱ ውጤት ሳይንሳዊ የሆነ የዘር ዓይነትን ተጽእኖ ግምት ይሰጣል። ስለዚህም ይህ ጥናት የዋርፋሪን አወሳሰድ መጠን ልዩነት ማምጣት የሚችሉ ምክንያቶች ከግምት ያስገባ የህክምና ሁኔታ እንዲፈጠር ይረዳል።

2. ተሳታፊዎች እንዴት እንደ ሚመረጡና እንደሚሳተፉ ማስረዳት፡-

በዚህ ጥናት እንዲሳተፉ እርሶን የመረጥንበት ምክንያት በጥናቱ ተሳትፎ መስፈርት መሰረት ከአዋቂዎች የዕድሜ ክልል (ከ18 ዓመት በላይ) መሆኖ፣ በውስጥ ደዌ ህክምና ክፍል ታካሚ በመሆኖ እና ዋርፋሪንን በትንሹ ከ6 ሳምንት በላይ በመውሰዶ እንዲሁም እንዳይሳተፉ እርሶን

የሚከለክሉ መስፈርት ስላልተገኘ ው። በጥናቱም ላይ በአጠቃላይ ወደ ሃምሳ ሰባት ተሳታፊ ታካሚዎች እንዲካተቱ እንፈልጋለን።

3. በተሳትፎ ውስጥ የሚያስፈልጉ ነገሮች

ለመሳተፍ ከወሰኑ በጥናቱ ውስጥ አንድ ጊዜ እናገናኛዎት እና የሚከተሉት ከእርሶ እንጠይቃለን። እነዚህም እርሶን በተመለከተ አጠቃላይ የሰውነት መረጃ፣ የሚወስዱቸው መድኃኒቶች ካሉስማቸውን እንዲሁም 5 ሚሊ ሊትር ወይንም አንድ ሙሉ የሻይ ማንኪያ ደም ናቸው። ከዚያም በኋላ የወሰድነው ደም በውስጡ ምን ያህል የዋርፋሪን መጠን እንዳለ እናጠናለን። ሌሎች የደም ላብራቶሪ ጥናቶች አይደረጉበትም።

በዚህ የጥናት እቅድ የዘር አይነት ጥናት አይካሄድም ሆኖም ወደፊት በተከታታይ ጥናት ማድረግ ሊስያስፈልግ ፈቃደኛ ከሆኑ እናሳውቆት እና ሌላ የፈቃደኝነት ማረጋገጫ እንዲፈርሙ እንጠይቆታለን።

4. በጥናቱ ላይ መሳተፍ ምክንያት ሊኖሩ የሚችሉ ጥቅሞች እና ጉዳዮች

በዚህ ጥናት እርሶ የሚገኙት ቀጥተኛ ጥቅም መኖሩን እናረጋግጥሎትም ቢሆንም ግን ከዚህ ጥናት ዋርፋሪን ለሚወስዱ ታካሚዎች የህክምና መሻሻልን የሚያመጣ መረጃ ይሰጠናል። በጥናቱ ጊዜ ልኑር የምትሉ ጉዳዮችም ደም በሚወስደው የሰውነት ክፍል የህመም ስሜት፣ የመቅላት እና ደም የመቋጠር ሁኔታዎች ናቸው።

5. የተሳትፎን ምስጢር ምልከታ

በጥናቱ ለመሳተፍ ከወሰኑ እርሶን በተመለከተ ማንኛውም መረጃ እንዲሁም የጤንነት ሁኔታ መረጃም በሚስጥርነት ተይዘው ይቆሉ። የተሰበሰበውም መረጃ ደህንነቱ ተጠብቆ በወረቀት እና በኤሌክትሮኒካዊ መዝገብ ማለትም በኮምፒውተር ይቆል። ስምዎትንም ከጥናቱ ባለቤቶች በቀር ለማንም አይተላለፍም። ስለዚህም እርሶን መለየት የምንችልበት የሚስጥር ቁጥር እንጠቀማለን።

የእርስዎ ማንኛውም ሪከርድ ለጥናቱ ባለሙዎች ብቻ ታልፎ ይሰታል። የተሳትፎ ማረጋገጫውን በመፈረም የጥናቱ ባለሙያዎች የሰጡትን ናሙና መረጃ እንዲጠቀሙ ያቅዳል።

ጥናቱ ከተጠናቀቀም በኋላ የሰጧቸው መረጃች ደህንነቱ በተጠበቀ መልኩ በትንሹ ለ5 ዓመታት እንዲቀመጡ ይደረጋል። ከዚያ በኋላ ምስጢሩ በተጠበቀ ሁኔታ የሚወገድበትን መንገድ ያመቻቻል።

6. ጥናቱ ስለ መግምገሙ እና የጥናቱ ተሳትፎ ቢቋረጥ ምን እንደ ሚደረግ

ይህ ጥናት በአዲስ አበባ ዩንቨርሲቲ የጤና ሳይንስ ኮሌጅ የምርምርና ጥናት ስምግባር መርማሪ ቦርድ ኮሚቴ ተፈትሾና አስፈላጊው አስተያየት ተሰጥቶበታል። ከጥናቱ በራ ፈቃድ ቢያቋርጡ ወይም አለመፈለጉን ቢገልጹ ከእርሶ የተወሰዱ ናሙናዎች ቢኖሩ በአግባቡ እንዲወገዱ ይደረጋል። የተወሰዱት ሌሎች መረጃዎች ግን እንጠቀምባቸው ማንናውም የተቀመጠ የደም ናሙና ግን እንደ እርሶ ፈቃድ ማወገድ ይቻላል።

7. ከጥናቱ የሚገባው ውጤት አያያዝ

ጥናቱ እንደ ተጠናቀቀ ከጥናቱ የሚገኙት ውጤቶች በህክምና የጥናቶች ሪከርድ እንዲወጣ እንዲሁም በሳይንሳ ኮንፈረንሶች ላይ እንዲቀርብ ይደረጋል። መረጃቹ ስም አልባ እንዲሆኑና ተሳታፊዎቹ እንዳይጠቀሱ በጥንቃቄ ይደረጋል። የጥናቱ ውጤት ማግኘት ቢፈልጉ ወይም ሪከርዱን ማየት ቢፈልጉ የጥናቱን ባለሙያዎች የመጠየቅ መብት አለዎት።

ከጥናቱ ለመሳተፍ ከወሰኑ ከዚህ በመቀጠል ክፍል 2 ላይ የሚገኘው የጥናት ተሳትፎ ማረጋገጫ እንዲፈርሙ እንጋብዞታለን። ይህን የተሳትፎ ማስረጃ ቅጸ እና ተሳትፎ ማረጋገጫ አንድ ኮፒ እንሰጠታለን። ሌላው የተሳትፎ ማረጋገጫ ኮፒ ከቀሪው የጥናቱ ሪከርድ ጋር ይቀመጣል። ጊዜ ወስደውና ይህን መረጃ ስላነበቡ እና ለመሳተፍ ስላሰቡ በቅድሚያ እናመሰግናለን።

ክፍል 2: የጥናት ተሳትፎ ማረጋገጫ ፎርም

በደም ውስጥ የሚገኝ የዋርፋሪን መድኃኒት መጠን እና ታካሚዎች የሚወስዱት መጠን ግንኙነት

ጌዲዎን ነጋሽ (BPharm) ጌትነት ይመር (MD, MSc, PhD) አብዱላዚዝ አቡበከር (MD)

"በደም ውስጥ የሚገኝ የዋርፋሪን መድሀኒት መጠን ታካሚዎች የሚወስዱትን መጠን ግንኙነት" በሚል ጥናት ላይ የተሰጠውን መረጃ አግኝቻለሁ። ጥናቱን ለመመረቂያ ፅሁፍነት የሚሰራው ጌድዮን ነጋሽ ሲሆን በዶ/ር ጌትነት ይመር እንዲሁም ዶ/ር አብዱላዚዝ አቡበከር አማካሪነት በጥቁር አንበሳ ሆስፒታል የአዲስ አበባ ዩንቨርሲቲ የውስጥ ደዌ ህክምና ክፍል ነው። ስለ ጥናቱ ተወያይተንበታል።

በጥናቱ ጊዜ ሊኖሩ ስለሚችሉ ጥቅሞች እንዲሁም ጉዳዮች ማለትም ደም በሚወሰድ ጊዜ ሊኖር ስለሚችል የህመም ስሜት፣ የደም መቋጠር ወይም መቅላት ሁኔታች ተነግሮኛል። ጥንቱን በተመለከተና ስለተሳትፎ ያሉኝን ጥያቄዎች እንድጠይቅና እንድረዳ እድሉ ተሰጥቶኛል።

የኔ ተሳትፎ በፈቃደኝነት ላይ የተመሰረት ስለ መሆኑ ተረድቻለሁ። አለመሳተፍም ሆነ ከጥናቱ ማቋረጥ መብቴ እንደሆነ ጠንቅቄ አውቃለሁ። አለመሳተፍም ሆነ ጥናቱን ማቋረጫ በህክምናዬ ላይ ወይም በህክህምና ክፍሉና በትምህርት ክፍሉ ጋር ግንኙነት ላይ ተጽእኖ ኤደርግም።

ማንኛውም ጥያቄዎች እና ቅሬታዎች ቢኖሩት ጌድዮን ነጋሽን በ0913011010 ወይም ዶ/ር ጌትነት ይመርንና ዶ/ር አብዱላዚዝ አቡበከር በትምህርትና ህክምና ክፍሉ መጠየቅ እችላለሁ። ካልፈለኩም የምርምርና ስነምግባር መርማሪ ቦርድ ኮሚቴ መጠየቅ እችላለሁ። በፊርማዬ ከስር የተጠቀሱትን (ክቦቹን "√" ምልክት ያድርጉባቸው) መፍቀዴንና በመመረቁ ጥናቱ ላይ መሳተፍ መፍቀዴን አመለክትላሁ።

- ራስ ተኮር አጠቃላይ የሰውነት መረጃዎቸች ለመስጠት
- ሌሎች የምወስዳቸውን መድኃኒቶች ስም ለመስጠት
- 5 ሚሊ ሊትር /አንድ ሙሉ የሻይ ማንኪያ ደም ለመስጠት

ሙሉ ስም	ስልክ ወይም ኢሜይል	ፊርማ