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Influence of ABCB1 Gene Polymorphisms on Rivaroxaban Response and Hemorrhagic Events in Patients with Atrial Fibrillation in Iraq

A Thesis

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بِسْمِ اللَّـهِ الرَّحْمَـٰنِ الرَّحِيمِ

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صدق اللَّهُ العلي العظيم

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Dedication

To my loving family, especially my parents, who taught me the value of education since childhood and instilled in me the desire to reach for the sky. To my fiancée, for her love and encouragement, and for believing in me throughout this journey. My dear friends for their support especially, Ahmed Sabri.

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List of Abbreviations	
Abbreviations	**Full-Text**
ABCB1	ATP-Binding Cassette Subfamily B Member 1
ADRs	Adverse Drug Reactions
AF	Atrial Fibrillation
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
ANOVA	Analysis of Variance
APD	action potential duration
AS-PCR	Allele-Specific Polymerase Chain Reaction
AST	Aspartate Aminotransferase
AUC	Area under the curve
BMI	Body Mass Index
CBC	Complete Blood Count
CHA2DS2-VASc	Congestive heart failure, Hypertension, Age ≥75 years (2 points), Diabetes mellitus, Stroke/TIA/thromboembolism (2 points), Vascular disease, Age 65–74 years, and Sex category (female)
C_{max}	maximum plasma concentrations
CYP P450	Cytochrome P450
DAD	delayed after depolarization
DILI	drug-induced liver injury
DM	diabetes mellitus

DNA	Deoxyribonucleic Acid
DOACs	Direct Oral Anticoagulants
DVT	deep vein thrombosis
EDTA	Ethylenediaminetetraacetic acid
EHRA	European Heart Rhythm Association
ESC	European Society of Cardiology
FXa	factor Xa
GBD	Global Burden of Diseases
HB	Haemoglobin
HPLC	High Performance Liquid Chromatography
HTN	hypertension
HWE	Hardy-Weinberg equilibrium
INR	International Normalized Ratio
IQR	interquartile range
lncRNAs	Long non-coding RNAs
LTR	lifetime risk
LTR	long-term risk
NCBI	National Center for Biotechnology Information
NOAC	Novel Oral Anticoagulant
NVAF	non-valvular atrial fibrillation
PCR	Polymerase Chain Reaction

PE	pulmonary embolism
P-gp	P-glycoprotein
PLT	Platelet Count
PT	Prothrombin Time
RNA	Ribonucleic Acid
RyR2	ryanodine receptor 2
S.cr.	serum creatinine
SNP	Single Nucleotide Polymorphism
SPSS	Statistical Package for the Social Sciences
SR	sarcoplasmic reticulum
TBE	Tris-Borate-EDTA
UV	Ultraviolet radiation
VKA	Vitamin K Antagonists

Abstract

Background: Atrial fibrillation (AF) is a common cardiac arrythmia associated with significant morbidity and mortality due to thromboembolic events. Rivaroxaban, a direct oral anticoagulant (DOAC), is often prescribed to prevent strokes in patients suffering from non-valvular atrial fibrillation (NVAF). Rivaroxaban is a substrate for the transporter protein P-glycoprotein (ABCB1). however, the influence of genetic polymorphism on the pharmacokinetics and clinical outcomes of rivaroxaban in individuals with NVAF in Iraq remains largely unclear.

Aims of Study: This research examines the relationship between two single-nucleotide polymorphisms (SNPs) in the ABCB1 gene (rs4728709 G>A and rs4148738 C>T) with rivaroxaban response, steady-state plasma concentrations, and the incidence of bleeding events in patients suffering from atrial fibrillation (AF).

Patients and Methods: This cross-sectional study assesses 100 patients with atrial fibrillation (AF) receiving 20 mg rivaroxaban anticoagulation in Iraq between September 2024 and March 2025. Clinical data collected included demographics, comorbidities, and treatment adherence. Biochemical assays evaluated various coagulation parameters International Normalized Ratio (INR), Prothrombin Time (PT), liver and renal functions (Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP), Serum Creatinine (S.Cr), Blood Urea), a complete blood count (CBC), and the steady-state plasma concentrations of rivaroxaban using high-performance liquid chromatography (HPLC). Genotyping of ABCB1 SNPs was performed with allele-specific polymerase chain reaction (AS-PCR).

Results: This study involved 100 patients, including 45 males and 55 females, mostly over 46 years old, with a high obesity prevalence of 52%. The genotype distribution for rs4728709 was 72% GG, 13% GA, and 15% AA; for rs4148738, it was 26% CC, 39% CT, and 35% TT. Plasma rivaroxaban levels were markedly lower in homozygous mutants (AA for rs4728709 and TT for rs4148738) compared to wild-type genotypes (p=0.001). The

rs4148738 polymorphism had a significant association with bleeding incidents, whereas rs4728709 did not. Increased liver enzymes (ALT and AST) were observed in specific genotype groups, indicating the drug's potential effect on the liver based on genotype. Additionally, while no significant link was found between ABCB1 polymorphisms and the response to rivaroxaban treatment, there was a significant genetic effect on drug plasma concentrations and bleeding risk.

Conclusions: The polymorphisms rs4728709 and rs4148738 in the ABCB1 gene affect rivaroxaban plasma levels in Iraqi patients diagnosed with non-valvular AF however, the examined SNPs showed no significant impact on the response. The rs4148738 CT heterozygous variant exhibited a noteworthy association with increased risk for bleeding events.

Chapter One

Introduction

1.1. Atrial Fibrillation

It is a widely common persistent arrhythmias, impacting around 50 million people globally. The risk of stroke in these individuals is five times greater than in those who do not have this condition. This condition represents the most severe form of cardiac arrhythmia in the Western world countries, representing 40 percent of all heart rhythm disorders, it is associated with significant risks for strokes and systemic thromboembolic issues. (Abdrakhmanov et al., 2024).

Atrial fibrillation (AF) is the predominant form of supraventricular arrhythmias, affecting approximately 1% of the worldwide community. Its frequency significantly rises with age, potentially affecting about 10% of older adults (Linz et al., 2024). It is very common, about 1 in 3 to 5 individuals over 45 face a lifetime risk. From 2010 to 2019, the worldwide incidence of atrial fibrillation increased dramatically (Vinciguerra et al., 2024).

When abnormal electrical signals within the atria suddenly activate, they override the heart's normal pacemaker and interfere with the regulation of its rhythm, resulting in atrial fibrillation. This condition causes atrial muscle cells contracting abnormally with an accelerated rate. This may result in various symptoms like palpitations, tiredness, disorientation, dyspnea, and weakness (Brundel et al., 2022).

Etiology or disease persistence can be used to classify this condition. When AF is categorized by persistence, it can be separated into four forms. The first type is called paroxysmal AF, and it resolves either on itself or with intervention within seven days of beginning of onset. The second type is persistent AF, which continues for over a period of seven days, includes

episode which are terminated by cardioversion (whether it's pharmacological nor electrical) following a total of seven days. The third type is Long-standing, characterized as Continuous AF lasting exceeding twelve months if a rhythmic control approach is employed. Finally, Permanent AF will be recognized by both the individual and doctor, indicating no additional steps to recover or sustain sinus rhythm are planned (Kirchhof et al., 2016).

It primarily occurs in older adults (over 70 years) and people with lifestyle-related issues like high blood pressure, diabetes, and obesity (Hindricks et al., 2021). Atrial fibrillation is also found in certain patients with congenital heart disease, estimated at 4.7%. This condition, known as congenital AF, arises from a mix of embryonic defects possibly linked to genetic mutations and factors related to surgical treatment, both during and after the procedure (Waldmann et al., 2019).

Congenital AF begins at an earlier age compared to other types of AF and typically advances quickly from persistent to permanent AF (Teuwen & de Groot, 2017). About 15% of individuals with AF have family members affected by the condition, indicating a possible genetic predisposition. Numerous studies have highlighted the significant influence of genetic variants in the development of AF (Nattel & Dobrev, 2016).

Atrial fibrillation is rapidly emerging as a pressing public health concern, particularly in Western countries and specific areas of the East. Moreover, because AF is linked to serious problems like heart failure, stroke, and cognitive decline, as well as sudden cardiac arrest, it can significantly lower the quality of life for patients. It can lead to higher rates of morbidity and death as well as greater healthcare costs (Tucker et al., 2016).

Catheter ablation is the primary management of AF. Either the main arrhythmogenic substrate that predisposes people to arrhythmias or the trigger that initiates AF is supposed to be eliminated by this technique. Heat via radiofrequency ablation or freeze via cryoablation is used in the process. Catheter ablation has been the primary method for treating AF because it seems better successful compared to anti-arrhythmic medicines at preserving rhythm of sinus (Stabile et al., 2006).

Pharmacotherapies for AF available today, dating back to the 1960s, focus on channels of ions and fail for preventing 85% of patients' AF from starting or getting worse. This failure of drugs to target the underlying molecular reasons of AF is probably the cause for their lack of efficacy. Furthermore, anti-AF medication use is limited because of the possibility of serious or even fatal side effects (Hindricks et al., 2021).

The response of a certain patient to atrial fibrillation therapy is frequently unpredictable. Although novel druggable targets implicated in the aetiology of AF have been identified, the use of these discoveries in clinical drug trials remains limited. The lack of curative therapy for atrial fibrillation correlates with the insufficient understanding of its pathophysiology in individual patients (Brundel et al., 2022).

Investigation is currently concentrating on understanding the molecular and electrical factors that underlie its progression to improve its therapy and diagnostics. Experimental and medical studies on atrial fibrillation (AF) demonstrate that electropathology plays a crucial role as a catalyst for AF. Electropathology refers to electrical conduction abnormalities and resultant contractile dysfunctions that result from molecular alterations in the atrial

tissue that induce morphological alterations, including myolysis, dilation, and fibrosis, which resulting in the onset and persistence of atrial fibrillation. Key pathways for potential molecular alterations including protein homeostasis, stress signaling, and inflammasome activation, leading to compromised calcium handling in cardiomyocytes, intricate electrical activation patterns, and consequently, contractile failure. Furthermore, key regulators in these pathways are potential targets for medication and diagnosis, enabling mechanism-based and personalized treatment of atrial fibrillation (AF) (J. Li et al., 2021; N. Li & Brundel, 2020; van Marion et al., 2021).

1.2. Epidemiology

The Global Burden of Diseases, Injuries, and Risk Factors Study (GBD) illustrates the data estimated global population affected by AF reached 55.4 million in 2022 (Mensah et al., 2023). Atrial fibrillation demonstrates a growing prevalence and incidence as age increases (Schnabel et al., 2015). The main other causes of risk for AF are myocardial infarction (MI), heart failure (HF), tobacco use, alcohol consumption, body weight index, elevated blood pressure, hypertrophy of the left ventricle, and sex (Kornej et al., 2020; J. Zhang et al., 2021).

A detailed analysis has recently been performed on the worldwide epidemiological of AF in relation to social and economic and geographic risk factors, age, sex, and genetics (Kornej et al., 2020; J. Zhang et al., 2021).

Factors like sex, age, race, and socioeconomic position affect the prevalence of AF, which varies by region (Fig. 1). Regardless of race or socioeconomic status, men are more likely than women to have atrial fibrillation, both in terms of incidence and prevalence.

The likelihood of incident AF is increased by a familial history or being Caucasian. "Familial AF," or a family previous diagnosis of AF, is linked to about 40% increased likelihood of getting AF for the first time (Brundel et al., 2022). Among a cohort of elderly patients with significant atherosclerosis, over 20% have a family history of AF, which correlates to a boost likelihood of cardiovascular events (CVEs) and mortality (Pastori et al., 2020).

The prevalence of the condition (AF) differs by ethnic origin and race, with Caucasians exhibiting an increased probability than Black, Asian, or Hispanic populations. In the 1990s, the lifetime risk (LTR) for atrial fibrillation has been reported as 1 in 4 for Caucasian adults aged 40 and older, according to statistics from the United States and European. After ten years, this risk seems to be increasing to 1 in 3 for Caucasian adults aged over 45. Conversely, lower LTRs have been documented for African American and Chinese individuals, estimated at about 1 in 10 and 1 in 5 for those aged 40 and over, respectively (Vinciguerra et al., 2024).

Atrial fibrillation, correlates with a higher likelihood for death and disability worldwide. In 2017, six million disability-adjusted life years were lost, approximately 0.24 percent of the total worldwide disability-adjusted life years (Lippi et al., 2021).

The number of cases of the condition correlates with regions that have a high sociodemographic index, which is determined by factors such as mean income per individual, education levels, as well as overall rates of fertility. Additionally, it is linked to typical lifestyle-associated cardiovascular causes and coexisting health disorders (Allan et al., 2017; J. Zhang et al., 2021).

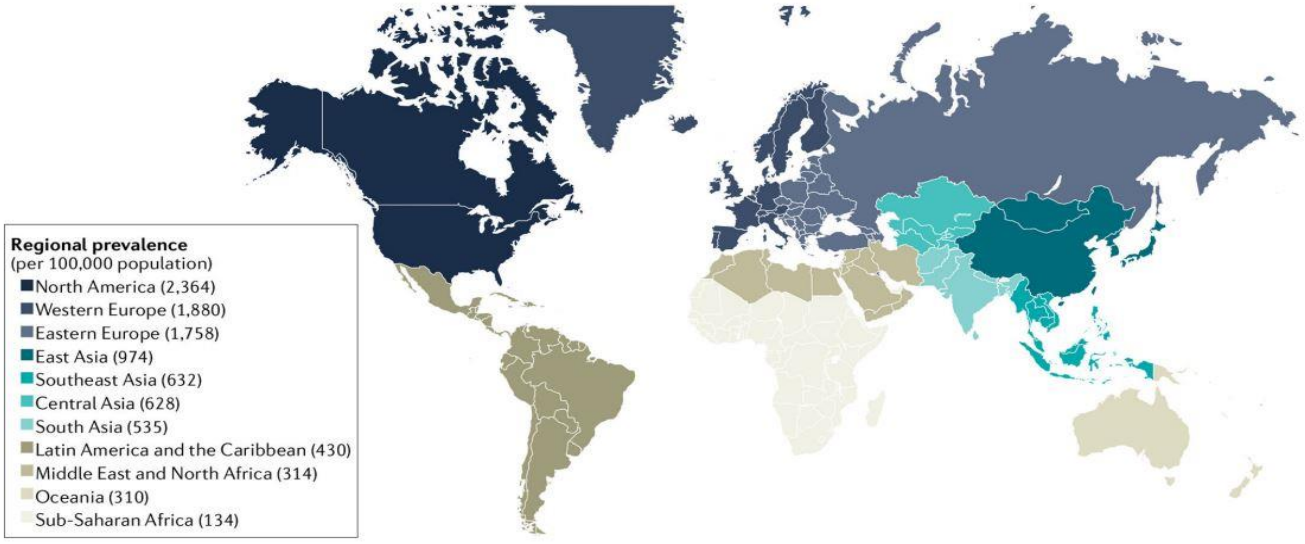

Figure. (1.1): Worldwide prevalence of atrial fibrillation. The number of events of atrial fibrillation (AF) per 100,000 people is the regional prevalence. The information comes from the GBD 2019 (Mensah et al., 2023) .

Besides ethnicity, race or sex, the long-term risk (LTR) was approximately one to five for people possessing the ideal risk factor profile. This likelihood increases by one to three when at least one increased risk factor was present. Consequently, tackling changeable risk factors such as elevated blood pressure, a history of diabetes, sleep apnea, hyperlipidemia, with lifestyles risks (like excessive alcoholic drinks consumption, tobacco use, and insufficient physical exercise) is vital to avoiding both a new episode and ongoing atrial fibrillation (AF) (Staerk et al., 2018).

Personal habits associated with an increased risk of AF are low carbohydrate and high-fat diets, sleep apnea, excessive alcohol consumption, and either a sedentary lifestyle or excessive activity (Chung et al., 2020).

The frequency of atrial fibrillation is about 1% in individuals of Asian descent, compared to around 2% in white individuals. However, Asian individuals face a significantly greater overall disease burden due to their larger aging population. By 2050, approximately an estimated (5.2) million

males while (3.1) million females aged over 60 in China are expected to be affected by AF, which represents a prevalence that is roughly 2.3 times higher than in USA (Tse et al., 2013; J. Zhang et al., 2021).

This difference can likely be attributed to the increasing prevalence of chronic diseases, such as elevated blood pressure, metabolic syndrome, and diabetes, which are linked to city living and changes in diet in the Chinese community (Tse et al., 2013).

Enhanced genetic testing for AF will illuminate ethnicity-specific genetic variants associated with AF and support the creation of genetic risk assessments in standard clinical practice. Understanding the fundamental causes of risk factors influencing AF may promote innovative strategies for better AF management.

1.3. Pathophysiology

1.3.1. Electro-Pathology as a Fundamental Etiology of Atrial Fibrillation

The basic idea of atrial fibrillation is that its episodes are induced by ectopic activity. Ectopic activities denote spontaneously depolarizations of atrial myocardium arising from outside to the (SA) node at frequencies exceeding the sinus rhythm rate (Brundel et al., 2022).

1.3.2. Electricity and Calcium Remodeling in Atrial Cardiomyocytes

It has been linked to alterations to the electric remodeling of myocardial ion channels including Ca2+ and K+ channel.(Nattel et al., 2007).

For those who have atrial fibrillation, ectopic activity predominantly originates from the pulmonary veins. because unlike most veins in the body, the pulmonary veins have muscular walls and can generate electrical impulses in addition the left and right pulmonary veins are typically associated with specific anatomical locations in the left atrium, with the veins forming electric links to atrial tissue so this anatomical relationship is crucial in understanding their role in AF initiation. Furthermore, in patients with AF, the atrial myocardium undergoes remodeling, which leads to the creation of substrates for arrhythmias. Therefore, this remodeling can enhance the vulnerability of the atria to the ectopic impulses generated by the pulmonary veins, making AF more likely to occur and persist (Alves, 2024).

Experimental research indicates that early after depolarization and delayed after depolarization (DAD) may contribute to ectopic activity, potentially initiating atrial fibrillation (AF). The experimentally identified molecular causes responsible for these irregular activities involves an elevation in diastolic release of calcium by the sarcoplasmic reticulum SR. Calcium ions are released by permeable ryanodine receptors 2 (RyR2) calcium release channels, altering ions channels functioning and resulting in a reduction of action potential duration (APD) (Nattel et al., 2020).

1.3.3. Molecular Defects in Electro-Pathology

With recent results, identifying underlying molecular causes of electrophysiological anomalies could significantly improve the management of personalized atrial fibrillation diagnostics and therapies. Understanding the molecular anomalies may facilitate the advancement of mechanism-based, potentially more efficacious, atrial fibrillation therapeutics. Examination of

human tissue of atria samples has demonstrated that atrial fibrillation induces persistent morphological degeneration in atrial cardio-myocytes defined by a destruction of mitochondrial, cytoskeletal, and sarcomeric systems, correlated with the development of autophagosomes, indicating of the induction of autophagic protein breakdown and modifications in chromatin structure. Consequently, the molecular paths implicated within those morphological alterations involve impaired Proteostasis, stimulation of stress signaling, instability in the genome, and inflammatory activation. Understanding a function of essential regulators in those molecular paths is Initial steps towards creating innovative test instruments with therapeutics which particularly target electric and contraction disorders, potentially aiding in the management of atrial fibrillation at various stages (N. Li & Brundel, 2020b; D. Zhang et al., 2019).

1.4. Diagnosis and Screening

Atrial fibrillation has numerous risks, including a twofold increase in myocardial infarction, a fivefold raising the level of strokes and cardiovascular disease, as well as dementia and cognitive decline. The presence of the aforementioned circumstances correlates with increased mortality relative to each condition individually. Incident atrial fibrillation is linked to a 3.0-fold and 2.5-fold increased likelihood of non-cardiac or abrupt cardiac mortality, respectively. Over nearly three decades, global deaths related to atrial fibrillation significantly increased, from an average of (117,038) loss of life in 1990 to (315,337) by 2019. The average life-years lost to atrial fibrillation throughout a decade has increased considerably (Vinciguerra et al., 2024).

Individuals with this condition could be asymptomatic or present with symptoms including palpitations, syncope, chest discomfort, or tiredness. Atrial fibrillation is currently categorized based on medical presentation and length of episodes as paroxysmal, persistent, long-standing chronic, or permanent atrial fibrillation. Importantly, early diagnosis of atrial fibrillation (AF) aims to identify AF in individuals presenting AF related signs and symptoms. However, evaluation promotes testing even asymptomatic people (Euro WHO: A Short Guide, n.d.2022). A recently published meta-analysis involving four controlled randomized studies REHEARSE-AF, SCREEN-AF, LOOP, and STROKESTOP with 35,836 individuals revealed that atrial fibrillation screenings have been linked with a reduction in strokes incidence compared to no screenings (McIntyre et al., 2022). The diagnosis of atrial fibrillation necessitates the documenting of an episode lasting over 30 seconds, recorded with 12 lead electrocardiogram or single lead. For uncommon episodes, long-term recordings utilizing devices like as Holter monitors are essential (Kirchhof et al., 2016).

1.5. Management

Atrial fibrillation, is a multifaceted illness necessitating extensive therapy involving many treatment decisions about effective thromboprophylaxis, symptom alleviation, prevention of AF development, and the identification and management of concurrent cardiovascular risk factors and comorbidities (Potpara et al., 2021). The primary objective of atrial fibrillation treatment is to eradicate AF attacks, reestablish rhythm of sinuses restore atrioventricular synchronization, with enhance the atrial contribution to the stroke volume. The 2020 ESC recommendations for diagnosing and treating atrial fibrillation (AF) require that patient

management starts with the verification of the arrhythmia, subsequently followed by patient assessment through the 4S-AF framework, which include the risk of stroke (CHA2DS2VASc score), severity of symptoms (EHRA symptom score), AF burden degree (Self-terminating, persistent, paroxysmal, and permanent), along with substrates degree (comorbidities, structural heart disease, ageing). An integrated care strategy utilizing the AF Better Care 'ABC' holistic approach is advised (Hindricks et al., 2021). The "ABC" approach consists of 'A' (Avoiding stroke/anticoagulant), 'B' (better management of symptoms through patient-centered, symptom-directed decisions regarding rate or rhythm for control), and 'C' (Optimization of cardiovascular risk and comorbidities, incorporating changes in lifestyle, psychologically morbidity, and patient's values/preferences, as proposed by numerous standards) (Rivera et al., 2023).

1.5.1.'A' Avoiding Stroke: The Significance of Anticoagulant Therapy

Atrial fibrillation increases the risk of stroke five times. However, that risk may not be uniform and depends on the presence of additional risk factors for stroke. Established and confirmed risk factors for stroke are being utilized as differentiation systems for the risk of stroke, a CHA2DS2VASc score becoming the one most widely recommended in standards. This score, in conjunction with a CHADS2 and 'ABC' scores, offers most accurate predictions for ischemic stroke events (Borre et al., 2018). The CHA2DS2-VASc score is a classical scoring system constructed to assess the thromboembolic risk and guide anticoagulant therapy in patients with atrial fibrillation and considers variables Such as congestive heart failure (1 score), high blood pressure (1 score), aging (≥75 years: 2 scores; 65–74 years: 1

point), diabetes (1 score), strokes (2 scores), vascular disorders (1 score), and female sex (1 score), all of which are easy to calculate (Fang et al., 2022) . Based on the ESC guidelines on AF, oral anticoagulation is recommended or preferred for people with one or more stroke risk factors (i.e. a CHA_2DS_2-VASc score of ≥1 in males, or ≥2 in females) (Sulzgruber et al., 2021).

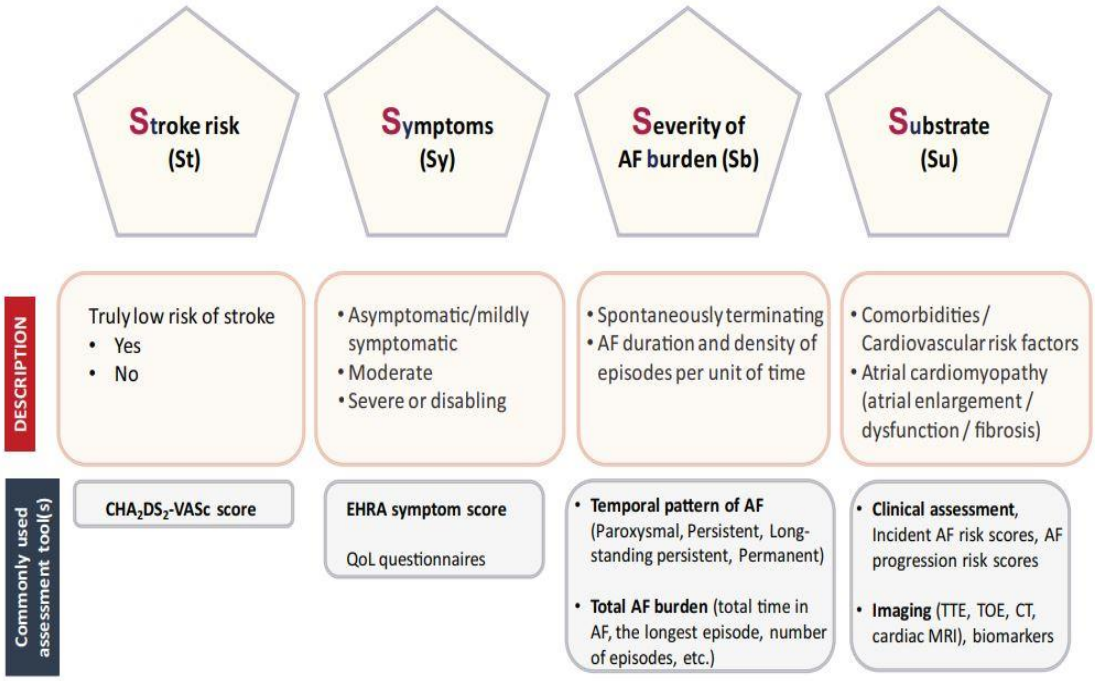

Figure. (1.2): The 4S-AF scheme for characterization of patients with atrial fibrillation. AF, atrial fibrillation; CT, computed tomography; EHRA, European Heart Rhythm Association; MRI, magnetic resonance imaging; QoL, quality of life; TOE, transesophageal echocardiogram; TTE, transthoracic echocardiogram.(Potpara et al., 2021)

The latest recommendations simplify the decision-making process, prescribing that the standard protocol is to administer an oral anticoagulant for preventing strokes, except in cases where a person is classified as 'low risk'. In that circumstance, anticoagulant medicine will be not advised (Andrade et al., 2020). Patients classified as 'low risk' are defined as those

possessing a CHA2DS2VASc score within zero in males or as for females, exhibiting an annual strokes incidence that is less than one percent., which is a limit for initiating anticoagulant therapy. Preventing stroke has consistently been essential in the treatment of this condition. Most guidelines favor for non-VKA oral anticoagulants, also known as direct oral anticoagulants, owing to their superior safety, effectiveness, and convenience compared to Vitamin K antagonists, leading to an increasing utilization in healthcare settings (Hohnloser et al., 2019).

1.5.2.'B' Improved Symptom Management: Regulation of Rate and Rhythm

Presently, pharmacologic anti-arrhythmic treatment for atrial fibrillation (AF) focus on rhythm or rate management. Anti-arrhythmic drugs target ions channel, demonstrating poor effectiveness. Moreover, many of these treatments are related to serious and possibly fatal adverse reactions (De Vecchis et al., 2019). The principal reason for the limited effectiveness of existing anti-arrhythmic treatments is due to inadequate understanding about mechanisms governing electropathology and atrial fibrillation in particular individuals.

1.5.3.'C' Cardiovascular Risk Factor and Comorbidity Management

Since numerous triggers for atrial fibrillation were reversed, minimizing the changeable hazards may be beneficial for the prevention as well as the treatment of AF. Diverse lifestyles alterations can reduce atrial fibrillation. Ignoring methodological concerns, the PREDIMED trial, an

important Spanish study on the Mediterranean diet, revealed that a diet supplemented with extra-virgin olive oil reduced the risk of atrial fibrillation. The following PREDIMAR study is presently assessing a comparable approach for preventive measures (Barrio-Lopez et al., 2020).

Although evidence regarding the effects of plant-based diets on patients with atrial fibrillation is insufficient, these diets lower the possibility and prevalence of diabetes, obesity, high blood pressure, obstructive sleep apnoea and inflammation. Also preventing atherosclerosis and coronary artery disease events. Thus, this diet is thought to lower the risk of atrial fibrillation by minimizing established risk factors associated with it (Brundel et al., 2022).

The AF patient community has acknowledged the potential efficacy of magnesium supplementation in preventing and/or treating atrial fibrillation (AF). Therefore, magnesium is crucial for cardiac function, and inadequate nutritional supply is associated with a 50% heightened chance of developing new-onset atrial fibrillation (AF). A meta-analysis of 20 randomized controlled trials indicates that routine magnesium supplementation minimizes the occurrence of post-operative atrial fibrillation (Chaudhary et al., 2019).

Recent studies indicate that consistent moderately vigorous activity (up to 150 minutes per week) reduces the burden of atrial fibrillation (AF) and improves symptoms as well as quality of life (QOL) for individuals. However, too much physical activity may increase the likelihood of acquiring AF. The specific pathogenic pathways that increase the risk of atrial fibrillation from excessive exercise are not yet discovered, but may include atrial hypertrophy, inflammation, and autonomic instability (Buckley et al., 2020).

1.6. Rivaroxaban

The development of direct oral anticoagulants (DOACs), exemplified by rivaroxaban, represents a significant advancement in medicine (Chen et al., 2020). Rivaroxaban, the first oral direct inhibitor of factor Xa (FXa), has been approved for both the prevention and treatment of DVT (deep vein thrombosis) and PE (pulmonary embolism), in addition to reducing the possibility of stroke and embolism in non-valvular atrial fibrillation (Schwarb & Tsakiris, 2016).

The past decade has witnessed a substantial increase in the therapeutic use of rivaroxaban in anticoagulant therapy for various compelling reasons. Direct oral anticoagulants (DOACs) have been progressively adopted in clinical practice, particularly rivaroxaban, whose utilization rate increased from 0.13% to 13.87% between 2011 and 2014 (Alalwan et al., 2017).

Nonvalvular atrial fibrillation (NVAF) is a prevalent arrhythmia that can elevate the risk of ischemic stroke by approximately fivefold. Fatal bleeding and cerebral hemorrhage are less prevalent in patients taking rivaroxaban compared to those treated with warfarin (Patel et al., 2011).

Direct oral anticoagulants (DOACs) such as rivaroxaban offer numerous advantages over warfarin, including a more rapid onset of action, faster therapeutic effect, consistent pharmacokinetic and pharmacodynamic profiles, reduced incidence of food and medication interactions, no longer requiring routine INR monitoring, and improved patient satisfaction. Rivaroxaban is increasingly preferred over warfarin as an oral anticoagulant due to warfarin's narrow therapeutic index, interactions with food and drugs

affecting CYP isoenzyme pathways, the necessity for frequent laboratory testing, and its heightened bleeding risk profile (Schwarb & Tsakiris, 2016).

Despite the lack of routine coagulation monitoring for DOACs, the incidence of severe bleeding episodes has occasionally been documented in individuals using these medications. Rivaroxaban ranked among the top ten pharmaceuticals linked to emergency department visits due to adverse medication events in the United States during 2013-2014 (Shehab et al., 2016).

In addition, the interindividual variation in the pharmacokinetics of rivaroxaban among individuals with non-valvular atrial fibrillation (NVAF) has been shown to be considerably large (Hori et al., 2012; Xu et al., 2012).

1.6.1. Physical and Chemical Properties

Rivaroxaban is 5-chloro-N-([(5S)-2-oxo-3-[4-(3-oxomorpholin-4-yl)phenyl]-1,3-oxazolidin-5-ylmethylthiophene-2-carboxamide (Figure. 3) (Perzborn et al., 2005). Possesses a molecular mass of 435.88 and it is defined within the Bio Pharmaceutical Classification System (BCS) to be a low-solubility, high-permeability substance (Class two). Rivaroxaban, having a log P value of (1.5), shows intermediate lipophilicity, demonstrated by its low to moderate affinities for tissues around the body (Perzborn et al., 2005).

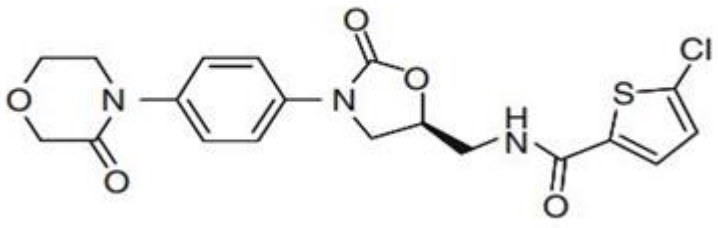

Figure. (1.3): Chemical structure of rivaroxaban (Perzborn et al., 2005)

1.6.2. Pharmacodynamics

Rivaroxaban directly and reversibly inhibits Factor Xa in a highly selective and competitive manner, exhibiting over 10,000-fold selectivity for Factor Xa compared to other related serine proteases, thereby interrupting both the intrinsic and extrinsic pathways of the blood coagulation cascade in a dose-dependent manner. Consequently, this activity inhibits the progress of the coagulation cascade via the last common pathway, thereby inhibiting thrombin production (Samama, 2011).

Factor Xa exists in both circulating and clot-bound forms. Rivaroxaban unlike indirect Factor Xa inhibitors such as fondaparinux or heparin, this drug directly suppresses both free and clot-bound Factor Xa, along with prothrombinase activity, hence prolonging clotting durations (Mujer et al., 2020)

1.6.3. Pharmacokinetic

1.6.3.1. Bioavailability, Absorption, and Biopharmaceutical Properties

Rivaroxaban levels within human blood were quantified using a high-performance liquid chromatography-tandem mass spectrometry technique. The technique facilitated the quantification of a broad spectrum of plasma levels (0.50 to 500 micrograms per liter) with excellent inter-assay precision (96.3 to 102.9%) (Rohde, 2008).

Rivaroxaban was rapidly absorbed, achieving maximum plasma concentrations (C_{max}) 2–4 hours post-administration of a single dose. The oral bioavailability of a twenty milligram a tablet had been (66%) in fasting terms.

Absorption arrived at completion if a twenty-milligram rivaroxaban tablet took in at meals, leading to a 39% increase in the mean AUC. When administered orally with food, the AUC and C_{max} values for a whole as well as crushed for 20-milligram pills were comparable. However, a crushed pill suspended in water and delivered via a tube into the gastrointestinal tract, coupled with a liquid meal, resulted in comparable values for AUC whereas an (18%) decrease in C_{max}. The heightened absorption in the fed state may be associated with the lipophilicity and restricted aqueous solubility of rivaroxaban (Mueck et al., 2014).

1.6.3.2. Protein Binding and Distribution

The medication exhibits a strong affinity for plasma proteins, predominantly albumin, with the unbound fraction generally ranging from 5% to 8%. The volume of distribution is around 50L, reflecting its low to moderate affinity for peripheral tissues (Weinz et al., 2005).

1.6.3.3. Metabolism and Excretion

The excretion of rivaroxaban occurs through two routes, mostly via the kidney route (66%) and, to a lesser extent, through the fecal/biliary routes (28%), as determined by measuring rivaroxaban-associated radioactivity in humans (Weinz et al., 2009).

According to in vitro and in vivo drug interaction studies, the P-glycoprotein (P-gp, ABCB1) and breast cancer resistance protein (BCRP, ABCG2) are the carriers linked to the active renal secretion of rivaroxaban (Gnoth et al., 2011).

Fifty-seven percent of the original oral dosage was shown to undergo metabolic breakdown. Rivaroxaban was metabolized by cytochrome P450 enzymes (CYP3A4/5, CYP2J2) as well as by CYP-independent processes. CYP3A4 contributed 18%, while CYP2J2 contributed 14% to the overall elimination of rivaroxaban. Along with the oxidative biotransformation, (14%) of the total rivaroxaban elimination was due to non-CYP-mediated hydrolysis of the amide bonds. No significant or active metabolites were identified in plasma, with unchanged rivaroxaban representing the predominant component in human plasma (89% of total AUC), indicating a negligible presence of metabolites (Lang et al., 2009).

In younger individuals, the clearance of rivaroxaban from plasma has a half-life ranging from 5 to 9 hours, while in older adults, it extends from 11 to 13 hours (Bratsos, 2019).

1.6.3.4. Drug–Drug Interactions

Research on the potential impact of medications that may affect rivaroxaban's metabolism and renal excretion showed that the concurrent administration of potent CYP3A4 and P-gp/BCRP inhibitors (such as azole antimycotics or HIV protease inhibitors) significantly increases rivaroxaban exposure and its pharmacodynamic effects, resulting in the recommendation to avoid coadministration. Conversely, the concurrent use of rivaroxaban with medications known to strongly stimulate CYP3A4 and P-gp, such as rifampicin, carbamazepine, and phenytoin, may reduce rivaroxaban exposure and the risk of thrombosis. Lastly, careful attention is needed when taking medications that have known pharmacodynamic interactions, such as

antiplatelet medications, additional anticoagulants, NSAIDs, and selective serotonin or noradrenaline reuptake inhibitors (Wieland & Shipkova, 2019).

1.7. Pharmacogenetic Aspects

Genetic variations among individuals in drug-metabolizing enzymes and transporters influence the effectiveness and safety of various medications. Pharmacogenomics, a crucial component of precision medicine, examines individual responses to medications based on genomic information, facilitating the assessment with particular genetic variations that influence drug response (Ahmed et al., 2016).

Genomic variations among individuals occur around every 300-1000 nucleotides, with over 14 million single nucleotide polymorphisms (SNPs) dispersed throughout the human genome (Roden & George Jr, 2002) .

Innovations in pharmacogenomics in recent years have significantly enhanced our understanding of variations in interindividual drug response behaviors. Variability in individual reactions within a large patient group may clarify why a treatment shown to be effective in certain patients often fails to yield satisfactory outcomes in others. Furthermore, therapy failure in affected individuals may result in severe adverse effects or even mortality, that indicative of personal differences in medication safety and effectiveness (Pirmohamed, 2006).

The drug response of individual patients is primarily influenced by the pharmacokinetic and pharmacodynamic characteristics of administered medications, which are directly or indirectly affected by polymorphisms in drug-metabolizing enzymes and transporters. Various groups exhibit distinct

allele frequencies in genes associated with drug-metabolizing enzymes and transporters. Precision medicine integrates molecular and clinical data to fully explain the underlying causes of diseases and to create therapies that enhance patient outcomes (Roden & Tyndale, 2013).

Multiple genetic factors account for 20% to 95% of the diversity in medication responses among individuals (Tang & Endrenyi, 1998). Moreover, individual differences in responses due to genetics are often permanent. Mutations in the coding regions of genes may lead to alterations in gene expression or protein structure, thereby causing variations in both the quantity and quality of proteins. Genetic polymorphisms in drug transport genes can affect the pharmacokinetic properties of a medication, consequently influencing its plasma levels and concentrations in target organs (Ma & Lu, 2011).

1.7.1. ABCB1 Gene

The ABCB1 gene, or multidrug resistance 1 (MDR1), encodes P-glycoprotein (P-gp), which is present on the surface of epithelial cells. It inhibits intestinal absorption, protects the fetus and brain from xenobiotic exposure, and promotes renal and hepatobiliary excretion. P-glycoprotein is expressed in a polarized fashion within the plasma membrane of cells in barrier and excretory organs, serving protective and eliminatory roles. It significantly contributes to first-pass clearance of orally ingested medications, thereby restricting their bioavailability by effluxing pharmaceuticals from the lumen-exposed epithelia in the small intestine as well colon, in addition to from the bile-exposed canaliculi in the liver. It removes substrates that reside in systemic circulation via the urine-facing portion of the brush border

membrane in the proximal tubule of the kidneys and subsequently through biliary excretion (Hodges et al., 2011).

1.7.2. Literature Review

The distribution of certain allelic variations seems to be dependent upon ethnicity. The SNP rs1045642 (3435C>T) is prevalent in Asians, with frequencies of 60%-72%, whereas it is less common in Caucasians, with frequencies ranging from 34%-42% (Ahmed et al., 2016).

Numerous genetic variations that could potentially influence the efficacy of rivaroxaban therapy in vivo have been proposed. For the first time, one previous study identified that the ABCB1 rs4148738 and rs4728709 gene polymorphisms significantly influenced the C_{trough}/D of rivaroxaban in individuals with atrial fibrillation. Ninety-five individuals participated in this study and 9 gene loci were analyzed. The dose-adjusted trough concentration ratio (C_{trough}/D) of rivaroxaban was significantly lower in the homozygous mutant type compared to the wild type at the ABCB1 rs4148738 locus, and the mutant type also exhibited a significantly lower ratio than the wild type at the ABCB1 rs4728709 locus (Wu et al., 2023).

An additional investigation identified over 100 polymorphisms of ABCB1, with rs2032582 (C.2677G>T) and rs1045642 (C.3435C>T) demonstrated to influence rivaroxaban metabolism. SNPs (C.2677G>T) and (C.3435C>T) demonstrate linkage disequilibrium and are commonly reported as haplotypes. The prevalence of the TT haplotype in the Caucasian population is approximately 25–40%. A recently published case study reveals those patients homozygous for haplotype (C.2677G>T; TT and C.3435C>T;

TT) may exhibit elevated plasma levels, C_{max}, prolonged half-life, and an increased risk of bleeding events (Kanuri & Kreutz, 2019).

Another study demonstrates that polymorphisms at the rs1128503 locus are associated with serum concentrations of rivaroxaban in individuals of Mongolian descent. They found no significant connection was between polymorphisms at the rs1128503 locus and bleeding episodes (Wang et al., 2021).

Moreover, another study revealed that being an AC or CC for rs1045642 is attributed to a considerably higher rivaroxaban level in participants using rivaroxaban. That is to say, rs1045642 is a remarkable predictor of rivaroxaban metabolism. Thus, the study concluded that identifying rs1045642 before drug administration might decrease ADRs (adverse drug reactions). Although further studies adjusted for potential confounders are strongly suggested (Mardi et al., 2023).

The study reported by Alridha et al. shows that drug plasma levels, maximum concentration, half-life, and risk of hemorrhage were higher in mutant homozygous (MHM) carriers of the haplotypes (rs2032582 and rs1045642). It is likely advisable to screen for these genetic variants, especially when rivaroxaban is being used in a renally impaired patient or concomitantly with an enzyme inhibitor. The association of ABCB1 SNPs and prothrombin variant in patients undergoing total hip replacement and total knee replacement while receiving rivaroxaban as VTE prophylaxis is noted. The prothrombin variant was significantly associated with the genotypes of ABCB1 SNP (rs1045642) (Alridha et al., 2022).

A recent case study indicated that patients homozygous for the ABCB1 haplotype (c.2677G>T; T/T and c.3435C>T; T/T) may exhibit elevated plasma levels, C_{max} values, half-lives, and an increased risk of bleeding problems (Nakagawa et al., 2021).

A study involving 216 Chinese patients with non-valvular atrial fibrillation (NVAF) assessed the use of rivaroxaban for anticoagulation therapy. Plasma concentrations of rivaroxaban were measured using a validated ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) method. Genotyping for seven SNPs in four genes was performed using Sanger dideoxy DNA sequencing. The analysis focused on the relationships between genotype variants, the occurrence of hemorrhagic events, and the timing of bleeding. The ABCB1 2677G (rs2032582) variation showed a strong association with the dose-adjusted peak concentration of rivaroxaban in plasma (C_{max}/D). Carriers of the ABCB1 G allele exhibited higher rivaroxaban C_{max}/D levels compared to non-carriers. This suggests that the ABCB1 2677G (rs2032582) genetic variant significantly influences both the rivaroxaban C_{max}/Dose and the frequency of hemorrhagic events (D. Zhang et al., 2022).

The use of DOACs has become more prevalent over the past two years in Iraq. However, their related pharmacogenomics study is limited. Further studies are required to explore the role of genes in anticoagulant therapy. Individual pharmacogenomics will be accurate and rational for individuals receiving anticoagulation treatment, and personalized therapeutic interventions will continue to develop.

1.8. Aims of The Study

The study aims to:

1. Investigate the association between two single-nucleotide polymorphisms (SNPs) in the ABCB1 gene (rs4728709 G>A and rs4148738 C>T) with rivaroxaban response.

2. Evaluate the impact of these SNPs on steady-state plasma concentration, and the incidence of bleeding events in patients suffering from atrial fibrillation (AF).

Chapter Two

Patients, Materials, and Methods

2.1. Research Methodology and Patients

It is cross-sectional research. The study happened from early September 2024 to late March 2025 and focused on evaluating two SNPs in individuals with Atrial Fibrillation. One hundred Iraqi patients who had nonvalvular atrial fibrillation and started on anticoagulant medication were included in this study. All patients who were enrolled were administered rivaroxaban 20mg once daily for a minimum duration of three months.

2.1.1. Patient Criteria

2.1.1.1. Inclusion Criteria

1. Aged above eighteen years

2. Confirm diagnoses of nonvalvular atrial fibrillation and the necessity for anticoagulant medication.

3. Capable and willing for providing informed consent.

2.1.1.2 Exclusion Criteria:

1. Confirm diagnostic of valvular atrial fibrillation (existence of prosthetic heart valves and hemodynamically severe mitral stenosis).

2. Elevated risk of hemorrhage

3. Recent stroke within one month or any prior occurrence of hemorrhagic stroke

4. Recognized non-cardiovascular diseases linked to unfavorable prognosis

5. Renal impermeant

6. Elevated liver function above twice its usual value, or any recognized hepatic illness linked to coagulopathy.

7. Impaired swallowing

8. Individuals concurrently utilizing DOACs alongside drug categories such as CYP3A4 and P-gp inhibitors (amiodarone, verapamil, diltiazem, quinidine, ticagrelor, and clarithromycin), as well as CYP3A4 and P-gp inducers (rifampicin, carbamazepine, phenobarbital, phenytoin, pantoprazole, and atenolol).

9. Patients having a history of hypersensitivity or established contraindications to rivaroxaban.

10. Females who are either pregnant or breastfeeding.

2.1.2. Collection of Clinical Data:

The collected data contained the patient's age, sex, weight, height, duration of treatment, compliance, patient education level, smoking habits, and whether the patient had diabetes mellitus (DM) or hypertension (HTN). Biochemical assays measured the International Normalized Ratio (INR), Prothrombin Time (PT), complete blood count (CBC), liver and kidney functions, serum creatinine, urea, AST (Aspartate Aminotransferase), ALP (Alkaline Phosphatase), ALT (Alanine Aminotransferase), and plasma drug concentration. Symptoms of thrombosis and adverse effects of rivaroxaban included less serious issues such as vaginal bleeding, gum bleeding, and epistaxis, as well as more severe concerns like gastrointestinal bleeding, hematuria, intracranial bleeding, and additional symptoms such as rash, nausea, and anaphylaxis. This information was obtained from medical records

with informed consent and directly from the patients in the local language. Everyone involved was aware of the study's objectives and aim. They were informed that the study seeks to examine the relationship between ABCB1 polymorphisms with response and bleeding events in patients using rivaroxaban. The genetic aspects of the study were also explained, including the analysis of their DNA to detect specific polymorphisms. All this information was provided in simple, non-technical language to the patients.

2.1.3. Sample Collection:

Blood Sample Collection:

A venous blood sample was drawn approximately 5 ml from each patient. Three ml collected in a tube containing ethylene diamine tetra acetate (EDTA). This was initially for CBC and then for DNA extraction and SNP detection. These samples were preserved at (−20°C) prior analysis. Additionally, another 2 ml collected in a sodium citrate tube for INR and PT analysis and approximately 0.5 ml in a gel tube for kidney and liver function tests analysis.

2.1.4. Body Mass Index Determination:

The body mass index (BMI) was calculated by dividing the patient's weight in kilograms by the square of their height in meters, using the formula: BMI (kg/m^2) = weight (kg) / height^2 (m^2) (Eknoyan, 2007).

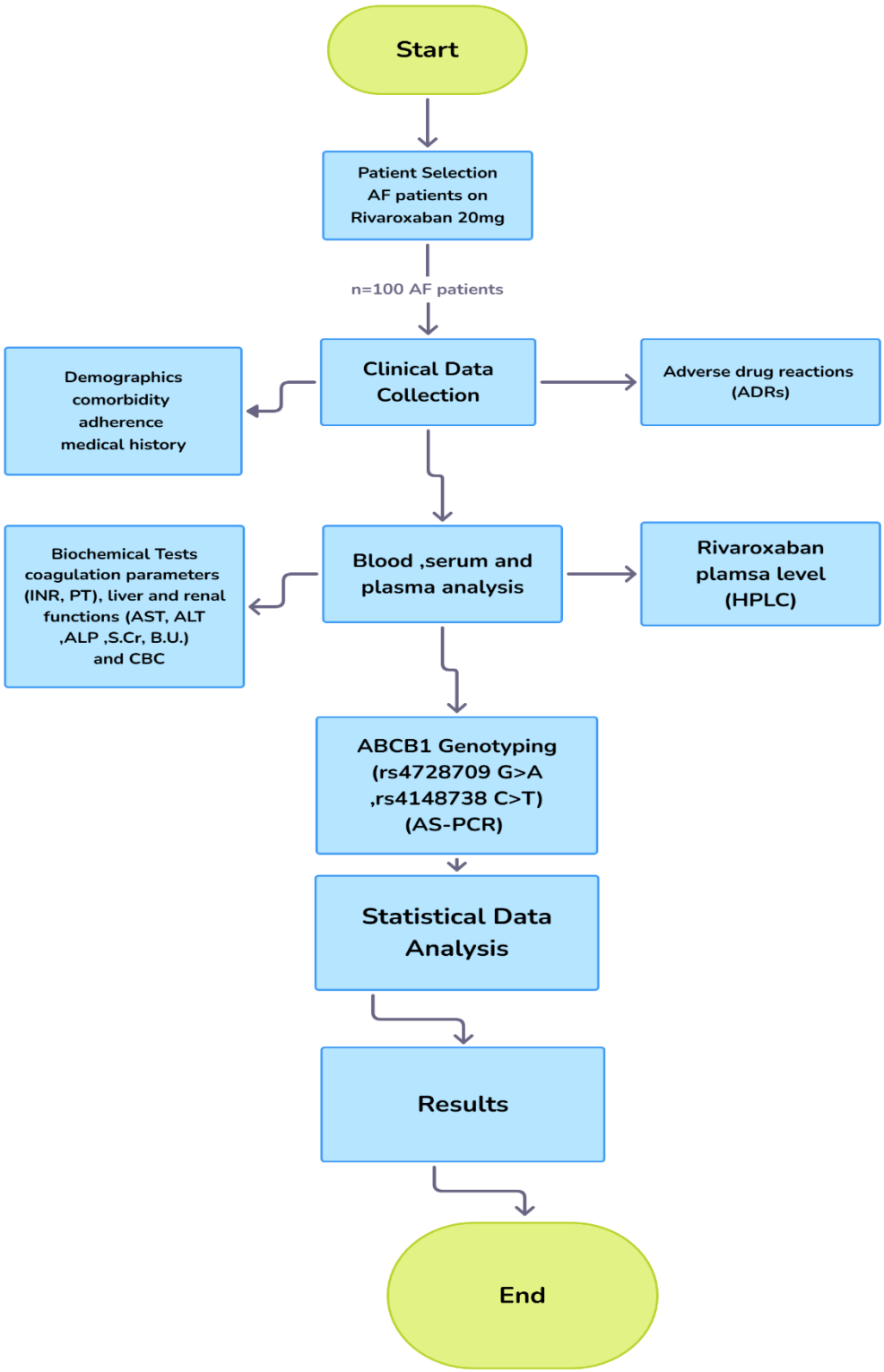

Figure (2.1): Flow chart of study design

2.2. Materials:

2.2.1. Instruments:

Table 2.1 provides a detailed list of all the instruments and equipment utilized in the present research, along with the manufacturers and countries of origin.

Table (2.1): Instruments, Manufacturers, and Countries of Origin

Instrument	Manufacturer	Countries of Origin
Centrifuge	SIGMA	Germany
Digital camera	Canon	England
Distillator	GFL	Germany
Electrophoresis apparatus	Techin me	England
Hood	Lab Tech	Korea
Hot plate stirrer	Lab Tech	Korea
Micropipette	SLAMED	Japan
PCR- apparatus	TECHINE	England
Refrigerator	Hitachi	Japan
Sensitive balance	AND	Taiwan
UV-trans illuminator	Syngene	England
Vortex mixer	Human Twist	Germany

2.2.2. Kits and Chemicals:

Table 2.2 outlines all the chemicals and kits utilized in this study, including their manufacturers and countries of origin.

Table (2.2): Chemicals, Kits, Manufacturers, and Countries of Origins

Chemicals and kits	Manufacturer	Countries of Origin
Agarose	Intron	Korea
DNA extraction kit	geneaid	Taiwan
DNA ladder	Bioneer	Korea
Ethanol 70%	SDI	Iraq
Isopropanol	SDI	IRAQ
Nuclease free water	Intron	Korea
PCR master mix	geneaid	Taiwan
Primer set tubes	Macrogen	Korea
Red safe nucleic acid stain	Infobio	India
TBE buffer	Bioneer	Korea

2.3. Methods

2.3.1. Biochemical Assay Methods

2.3.1.1. Prothrombin Time (PT) and International Normalize Ratio (INR) Measurement

PT, INR was determined using wondfo instrument according to the manufacturer's instructions.

2.3.1.2. Complete Blood Count (CBC) Measurement

CBC was determined using mindray (BC-30S) instrument according to the manufacturer's instructions.

2.3.1.3. Renal and Liver Functions Test

LFTs and RFTs were determined using cobas (C111) from Rouch according to the manufacturer's instructions.

2.3.1.4. Analytical Method of Plasma Concentration

Plasma levels of rivaroxaban have been measured using high-performance liquid chromatography (HPLC) (Altınöz et al., 2013).

A standard stock solution of RIV (1000 µg/mL) was formulated in a combination of acetonitrile and water (80:20 v/v). Standard working solutions (50, 100, 200, 300, 400, 500 µg/mL) were formulated by diluting the stock solution in the mobile phase. The stock solution was stored at +4 °C, where it remains stable for at least one month. Standard solutions were generated daily by diluting the stock solution with the mobile phase. A 500 µL sample was transferred to a 10 mL volumetric flask, and 8 mL of diluent (ACN:Water (80:20 v/v)) was subsequently added. The solution was sonicated for 30 minutes, filtered using a 0.45 µm membrane filter, and then diluted with the mobile phase to a final volume of 100 µL before to injection into the HPLC apparatus. The conditions for HPLC were as follows: the SYKAM model from Germany was employed to determine Rivaroxaban, utilizing a C18-ODS separation column (250 mm x 4.6 mm). The column temperature was sustained at 40 °C, and the flow rate was 1.2 mL/min during isocratic elution using an ACN:Water (55:45 v/v) mixture. The injection volume was 100 µL,

with UV detection at 249 nm. Peak identification was confirmed using retention time comparison.

2.3.2. Genotyping

2.3.2.1. DNA Extraction

The DNA extraction kit GEB100 from Geneaid was used, and the following Protocol Procedure was done (Geneaid™, 2024).

1. RBC Lysis: 900 µl of RBC Lysis Buffer and 300 µl of whole blood were added into a 1.5 ml microcentrifuge tube and mixed by inversion. Then, incubate for 5 minutes at room temperature before centrifuging at 3,000 x g for 5 minutes to form a leukocyte (white blood cell) pellet. Carefully remove the supernatant, retaining approximately 50 µl of residual buffer and leukocyte pellet, and vortex the tube until the leukocyte pellet is completely resuspended in the residual buffer.

2. Lysis: 300 µl of Cell Lysis Buffer is Added to the tube and mixed by vortexing. Incubate at 60ºC for at least 10 minutes to ensure the sample lysate is clear and homogeneous, inverting the tube every 3 minutes during incubation.

3. Protein Removal: 100 µl of Protein Removal Buffer is mixed into the sample lysate, vortexed for ten seconds, and centrifuged at 14,000 to 16,000 x g for three minutes to produce a rigid, dark brown protein pellet.

4. DNA Precipitation: the supernatant Transferred to a new, clean 1.5 ml microcentrifuge tube, 300 µl of isopropanol was added, then thoroughly mixed by gently inverting 20 times. Centrifuged at 14,000 to 16,000 x g for 5 minutes, with careful attention the supernatant discarded, and 300 µl of 70% ethanol added to wash the pellet. Centrifuge at 14,000 to 16,000 x g for 3

minutes, with careful attention the supernatant discarded, and allow the pellet to air-dry for 10 minutes.

5. DNA Rehydration: Finally, 100 µl of DNA Hydration Buffer is added, gently vortexed for 10 seconds, and incubated at 60ºC for 5-10 minutes to dissolve the DNA pellet. During incubation, the bottom of the tube is tapped to promote DNA rehydration.

2.3.2.2. Allele Specific Polymerase Chain Reaction (AS-PCR).

2.3.2.2.A. Primers Preparation:

To conduct the Polymerase Chain Reaction (PCR) and amplify the ABCB1 gene variants rs4148738 and rs4728709, specific primers were used. These primers were custom-designed using Primer-BLAST software and were obtained as lyophilized products from Macrogen, Korea. The lyophilized forward and reverse primers reconstituted in nuclease-free water to prepare a stock solution with a concentration of 100 pmol/µl (refer to Table 2.3). For the working solution, dilute 10 µl of the stock solution for each primer with 90 µl of nuclease-free water to achieve a concentration of 10 pmol/µl. The primers were stored at -20 °C until needed.

Table (2.3): Dilution of primer set tubes.

Primer set tubes	Dilution volume
rs4148738 -forward C allele	250 µL
rs4148738 -forward T allele	250 µL
rs4148738 -reverse	250 µL
rs4728709- forward G allele	250 µL
rs4728709- forward A allele	250 µL
rs4728709- reverse	250 µL

Table (2.4): Nucleotide sequence of primer set tubes.

Primer set tubes	Nucleotide sequence	Product length	Reference
rs 4148738 Forward C allele Forward T allele Reverse common	 5- TGAGGGGAGGAACTAAAACCC-3 5- TGAGGGGAGGAACTAAAACCT-3 5- AGACACCTCAAACTTGGCCC-3	260	Current study
rs 4728709 Forward G allele Forward A allele Reverse common	 5- ATTTAGCCCATCTGAGTCCAG-3 5- ATTTAGCCCATCTGAGTCCAA-3 5- ACAGTGCCTGAAACACCCTA -3	275	Current study

2.3.2.2.B. Optimization of Polymerase Chain Reaction (PCR) Conditions:

To optimize the PCR conditions, multiple trials were conducted to determine the optimal annealing temperature, concentrations of DNA and primers, and an ideal total number of amplification cycles. Table 2.5 shows the details of the PCR components used for each amplified fragment, while Table (2.6, 2.7) outlines the optimized PCR programs.

2.3.2.2.C. Conducting the Polymerase Chain Reaction:

Setting Up the PCR:

PCR was started by mixing the reaction components at the specified concentrations. Following that, the PCR was executed using the optimized protocols provided in the tables below.

Table (2.5): PCR tube components and their volume.

Components	Volume (µL)
Forward primer	1.5 µL
Reverse primer	1.5 µL
DNA	3 µL
PCR master mix	12.5 µL
Nuclease free water	6.5 µL
Total volume	25 µL

Table (2.6): Optimized PCR program for rs4148738.

Step	Temperature/ °C	Time	Cycles number
Initial denaturation	95	5 minutes	1
denaturation	95	25 seconds	35
Annealing	60	30 seconds	
Extension	72	1 minute	
Final extension	72	7 minutes	1

Table (2.7): Optimized PCR program for rs4728709.

Step	Temperature/ °C	Time	Cycles number
Initial denaturation	95	5 minutes	1
denaturation	95	25 seconds	35
Annealing	59	30 seconds	
Extension	72	1 minute	
Final extension	72	7 minutes	1

2.3.2.3. Agarose Gel Electrophoresis

Preparing the Gel:

1. First, 1.5 grams of agarose powder took and mixed it with 100 milliliters of TBE buffer (pH 8). Heat the mixture until the agarose was completely dissolved.
2. Then, the solution stirred well to ensure it was perfectly mixed and free of any bubbles. It resulted in a nice, clear solution.
3. After that, the solution let cool down to a temperature of around 50 to 60 °C.
4. Ten µL of the ethidium bromide stain was added into the gel.
5. To create wells for loading the PCR product samples, a comb placed at one end of the tray.
6. The agarose solution poured into the tray and left it to solidify at room temperature for about 30 minutes. Once it was solid, the comb removed carefully.

7. The gel set up in a gel electrophoresis tank, adding TBE buffer until it covered the gel by about 3-5 millimeters.
8. Five microliters of DNA ladder loaded into one well and 5 microliters of each PCR product into the remaining wells.
9. The voltage of the electrophoresis device adjusted to create an electrical field of 5 volts per centimeter between the cathode and anode.
10. Once the run was complete, a UV transilluminator used and set to 360 nm to visualize the bands. Finally, capture an image of the gel using a digital camera.

2.4. Statistical Analysis:

The collected data of the present study were collected from patients' sheets and analyzed through the Statistical Package for the Social Sciences (IBM SPSS version 26). The data were presented as frequencies and percentages or mean and standard deviation in appropriate tables and graphs. Independent t-test, as well as ANOVA test with post hoc analysis were used to find out the possible association between the related variables of the current study for normal distributed data. Furthermore, Mann Whitney U test and Kruskal Wallis test were executed with Bonferroni correction for non-normal distributed data and the data presented as median (IQR). Besides, correlation test was done to detect the association among some variable in the study. Multiple response test was done to evaluate the percent of responses for symptoms. Multiple linear regression was conducted to achieve the impact of genetic variation on the parameter under the study. Statistical association was considered significant when p value equal or less than 0.05 (P value ≤ 0.05).

Chapter Three

Results

3.1. Socio-demographic Characteristics of Study Patients:

This study involved heterogeneous group of samples with several socio-demographic and health conditions. Patients were 45% male and 55% female. Nearly half were older adults (49% were >60 years and 42% were 46–60 years old), whereas 9% were 30–45 years old.

The BMI categorization indicated that 52% of participants were obese, 35% were overweight, and 13% were of healthy weight. There were no participants with underweight.

In terms of duration of treatment, 85% of the patients have been treated between 3 and 42 months, and 15% for more than 42 months. Smoking habits, 84% of participants were non-smokers, and 15% were smokers. The participants were examined in terms of compliance with the treatment, which indicated that 89% had good compliance, 1% had fair compliance, and 10% had poor compliance.

Of the participants, 36% had diabetes mellitus and 64% did not have it. There was also a high prevalence of hypertension, which occurred in 85% of the study population. However, 15% were normotensive.

Bleeding incidence was 9%, with bleeding events being relatively rare, and 91% had no bleeding. Participant educational levels varied greatly, with 86% illiterate, 13% diploma graduates, and only 1% master's degree holders.

Table (3.1): Descriptive statistics of the demographic characteristics of the studied patients (n=100)

Variable		N	Percent
Sex	Male	45	45
	Female	55	55
Age	30-45	9	9
	46-60	42	42
	>60	49	49
BMI	Underweight	0	0
	Healthy weight	13	13
	Over weight	35	35
	Obese	52	52
Duration of treatment (months)	3-42	85	85
	>42	15	15
Smoking	no smoke	84	84
	smoker	15	15
Compliance	Good	89	89
	Fair	1	1
	Bad	10	10
Diabetes mellitus	Yes	36	36
	No	64	64
Hypertension	Yes	85	85
	No	15	15
Patient education	Illiteracy	86	86
	Diploma	13	13
	Master	1	1
Bleeding event	Yes	9	9
	No	91	91

3.2. Biochemical Parameters

3.2.1. Description of Biomarkers of The Studied Patients According to Gender

The Mann-Whitney U test was used to examine nonnormally distributed variables and determine whether there is a difference between male and female groups regarding the tested biochemical parameters. Serum

creatinine is statistically different in males (median=0.90) than in females (median=0.80), p=0.001. An independent sample t-test was conducted on normally distributed variables to determine whether there was a difference between male and female groups regarding the studied biochemical parameters. Hemoglobin was significantly higher in males (13.47±1.54) compared to females (12.63±1.49), p=0.007.

Table (3.2): Description of biomarkers of the studied patients according to Gender (n=100)

Parameter	Gender	Median	IQR or SD	P value
BMI	Male	29.38	5.02	0.355
	Female	31.14	9.36	
INR	Male	1.20	0.27	0.478
	Female	1.20	0.20	
PT	Male	13.70	2.30	0.992
	Female	13.60	2.90	
PLT	Male	216.00	75	0.410
	Female	213.00	78	
S.Cr.	Male	0.90	0.2	**0.001***
	Female	0.80	0.3	
ALP	Male	88.00	42	0.390
	Female	86.00	49	
ALT	Male	24.00	14.1	0.275
	Female	23.00	13.0	
Hb	Male	13.47	1.54	**0.007***
	Female	12.63	1.49	
B.U.	Male	37.64	9.17	0.665
	Female	38.53	10.79	

AST	Male	29.18	10.89	0.351
	Female	27.13	10.82	

Mann-Whitney U-test was used, with a significant p-value of less than 0.05. Results are presented as median with IQR. (BMI, INR, PT, PLT, S.Cr, ALP,ALT) are non-normal distributed parameters
An independent t-test with a significant p-value of less than 0.05 was used. Results are presented as mean ± SD. (HB, B.U., AST) are normal distributed parameters
BMI (Body Mass Index kg/m²) INR (International Normalized Ratio) (Prothrombin Time Seconds) PLT (Platelet Count $10^3/\mu L$) S.Cr. (Serum Creatinine mg/dL) ALP (Alkaline Phosphatase U/L), ALT (Alanine Aminotransferase U/L), Hb (Hemoglobin g/dL), B.U. (Blood Urea mg/dL), and AST (Aspartate Aminotransferase U/L).

3.2.2. Description of Laboratory Biomarkers of The Studied Patients According to The Duration of Treatment

The Mann-Whitney U test was used to examine if there is a difference between the two groups according to duration of treatment regarding the tested biochemical parameters. ALP is statistically different in a group with duration time less than 3.5 years (median=84.00), rather than a group with duration time more than 3.5 years (median=143.00), p=0.015. So, the ALP would differ between the two treatment durations, and the ALP will be higher in patients with longer than 42 months.

Table (3.3): Description of laboratory biomarkers in the examined patients based on treatment duration (n=100)

Parameter	Duration of treatment (months)	Median	IQR or SD	P value
INR	3-42	1.20	0.24	0.900
	>42	1.22	0.25	
PT	3-42	13.70	2.50	0.750
	>42	13.60	2.40	
PLT	3-42	212.00	76	0.156
	>42	240.00	132	
S.Cr.	3-42	0.900	0.3	0.743
	>42	0.900	0.3	
ALP	3-42	84.00	36	**0.015***
	>42	143.00	119	
ALT	3-42	24.00	13.9	0.372
	>42	20.00	10.0	

Hb	3-42	12.98	1.49	0.745
	>42	13.16	1.99	
B.U.	3-42	37.74	10.21	0.360
	>42	40.33	9.08	
AST	3-42	27.99	11.00	0.895
	>42	28.40	10.30	

Mann-Whitney U-test was used, with a significant p-value of less than 0.05. Results are presented as median with IQR. (BMI, INR, PT, PLT, S.Cr, ALP,ALT) are non-normal distributed parameters
An independent t-test with a significant p-value of less than 0.05 was used. Results are presented as mean ± SD. (HB, B.U., AST) are normal distributed parameters

3.2.3. Description of Laboratory Biomarkers of The Studied Patients According to Age

Kruskal-Wallis test used for nonnormally distributed variables and computed to compare the median of studied biochemical parameters with age categorical groups. No significant difference was found between age groups.

A one-way analysis of variance (ANOVA) was performed for normally distributed variables to compare the biochemical parameters across different patient age groups. This analysis revealed that the Hb of patients with A group (14.51±1.20) is statistically significant over B and C groups (13.04±1.48), p= 0.009; (12.71±1.56), p= 0.001, respectively. The analysis shows that hemoglobin levels decrease with age. Group A (30-45 years) has a higher hemoglobin level in comparison to Group B (46-60 years) and Group C (>60 years).

Table (3.4): Description of laboratory biomarkers of the examined patients categorized by age (n=100).

Parameter	A:30-45(years)	B:46-60 (years)	C:>60 (years)	MC	P value
INR	1.20 (0.26)	1.20 (0.28)	1.20 (0.24)	NS	0.783
PT	13.1 (2.45)	13.70 (2.68)	13.60 (2.35)	NS	0.737
PLT	186.00 (81)	220.00 (76)	209 (71)	NS	0.754
S.Cr.	0.900 (0.3)	0.900 (0.2)	0.800 (0.3)	NS	0.849
ALP	88.00 (33)	91.00 (56)	83.00 (45)	NS	0.417

ALT	25.00 (15.0)	24.60(15.3)	21.00 (13.7)	NS	0.196
Hb	14.51±1.20	13.04±1.48	12.71±1.56	A vs B A vs C	**0.009*** **0.001***
B.U.	33.44±5.36	37.12±9.13	39.86±11.15	NS	0.148
AST	28.08±13.18	29.50±11.04	26.80±10.30	NS	0.502

Kruskal-Wallis test had been used with a significant p value <0.05. Results are described as median with (IQR).

ANOVA test had been used with a significance p value < 0.05. Results are described as mean ± SD

3.2.4. Description of Laboratory Biomarkers of The Studied Patients According to BMI

The Kruskal-Wallis test was calculated to compare the BMI categorization groups with the median investigated biochemical markers. The groups did not significantly differ from one another. The biochemical parameters and BMI categorization groupings of patients were compared by applying a one-way analysis of variance test (ANOVA). The groups showed no significant differences from each other.

Table (3.5): Description of laboratory biomarkers for the examined patients categorized by BMI (n=100).

Parameter	A:18.5-24.99	B:25-29.99	C:>30	MC	P value
INR	1.20 (0.28)	1.17 (0.25)	1.20 (0.24)	NS	0.595
PT	14.00 (2.81)	13.20 (2.20)	13.95 (2.70)	NS	0.765
PLT	198.00 (61)	227.00 (80)	211.50 (79)	NS	0.241
S.Cr.	0.900 (0.4)	0.900 (0.2)	0.800 (0.3)	NS	0.737
ALP	79.00 (29)	94.00 (52)	85.50 (42)	NS	0.178
ALT	26.00 (13.2)	23.00 (11.0)	21.00 (15.5)	NS	0.933
Hb	12.95±1.53	13.21±1.39	12.89±1.69	NS	0.656
B.U.	36.03±8.99	37.12±9.13	38.67±10.72	NS	0.199
AST	28.23±9.32	29.15±11.48	27.27±10.89	NS	0.733

Kruskal-Wallis test had been used with a significant p value <0.05. Results are described as median with (IQR).

ANOVA test had been used with a significance p value < 0.05. Results are described as mean ± SD

3.2.5. Description of Laboratory Biomarkers of The Studied Patients According to SNP (rs4728709)

Kruskal-Wallis test was used to compare the patients with regard to the studied biochemical factors according to different genotypes (GG, GA, AA) of SNP (rs4728709). Alanine aminotransferase (ALT) in homozygous AA was high (30.00±12.0), which is highly significant from the ALT in wild GG (20.00±11.0) with a p-value of 0.005.

There is a significant difference in concentration in wild GG, which is very high compared to the homozygous AA group. The concentration significantly differs between GG (464.35±340.0) and AA (144.00±24.90), p = 0.001.

An analysis of variance was applied to compare differences between genotype category groups (GG, GA, AA) for the studied biochemical parameters. A statistically significant difference in AST in wild GG vs. homozygous AA exists. The AST level is lower (26.64±10.42) in wild GG than in homozygous AA (33.93±12.80), p = 0.018.

Table (3.6): Description of laboratory biomarkers for the examined patients according to SNP (rs4728709) (n=100).

Parameter	GG	GA	AA	MC	P value
INR	1.20 (0.25)	1.30 (0.15)	1.15 (0.31)	NS	0.417
PT	13.65 (2.37)	14.40 (1.90)	13.10 (3.37)	NS	0.690
PLT	211.00 (78)	226.00 (136)	213.00 (59)	NS	0.742
S.Cr.	0.900 (0.3)	0.800 (0.3)	0.800 (0.3)	NS	0.472
ALP	85.00 (42)	82.00 (63)	93.00 (37)	NS	0.475
ALT	20.00 (11.0)	24.00 (11.0)	30.00 (12.0)	GG vs AA	**0.005***
Conc.	464.35 (340.03)	153.70 (313.85)	144.00 (24.90)	GG vs AA	**0.001***
Hb	13.11±1.57	12.72±1.78	12.78±1.38	NS	0.584
B.U.	37.29±10.36	39.77±7.5	40.37±10.40	NS	0.400
AST	26.64±10.42	29.07±8.98	33.93±12.80	GG vs AA	**0.018***

Kruskal-Wallis test had been used with a significant p value <0.05. Results are described as median with IQR.

ANOVA test had been used with a significance p value < 0.05. Results are described as mean ± SD

3.2.6. Description of Laboratory Biomarkers of The Studied Patients According to SNP (rs4148738)

Kruskal-Wallis test was used to compare the patients regarding the studied biochemical factors according to different genotypes (CC, CT, TT) of SNP (rs4148738). Another significant statistical difference was between wild CC (483.20±175.83) and homozygous TT (144.60±14.10), besides heterozygous CT (471.90±317.60), compared to homozygous TT (144.60±14.10) concentration, with a p-value of 0.001 for each group. The concentrations in the homozygous TT genotype are considerably lower than those in the wild CC and heterozygous CT genotypes. This suggests that this feature has a very strong genetic influence.

Table (3.7): Description of laboratory biomarkers of the studied patients according to SNP (rs4148738) (n=100).

Parameter	CC	CT	TT	MC	P value
INR	1.08 (0.30)	1.23 (0.20)	1.27 (0.25)	NS	0.090
PT	12.85 (2.75)	14.00 (1.50)	13.70 (2.80)	NS	0.152
PLT	221.50 (60)	198.00 (79)	239.00 (98)	NS	0.124
S.Cr.	0.800 (0.3)	0.900 (0.2)	0.900 (0.3)	NS	0.431
ALP	93.50 (50)	83.00 (32)	86.00 (48)	NS	0.404
ALT	26.10 (16.0)	24.00 (14.1)	20.00 (11.0)	NS	0.383
Conc.	483.20 (175.83)	471.90 (317.60)	144.60 (14.10)	CC vs TT CT vs TT	**0.001*** **0.001***
Hb	13.06±1.63	12.76±1.48	13.41±1.38	NS	0.608
B.U.	38.36±10.94	37.62±9.43	38.20±5.95	NS	0.847
AST	28.00±14.5	23.00±11	26.00±15.8	NS	0.579

Kruskal-Wallis test had been used with a significant p value <0.05. Results are described as median with (IQR).

ANOVA test had been used with a significance p value < 0.05. Results are described as mean ± SD

3.3. Outcomes of the Amplification Reaction

3.3.1. Genotyping for rs4728709 (G>A) Genetic Polymorphism:

The genetic variant rs4728709 (G > A) produced a unique band of 275 base pairs in molecular size. The amplicon size was determined by comparison with a DNA ladder ranging from 100 to 1500 base pairs. As shown in figure (3.1):

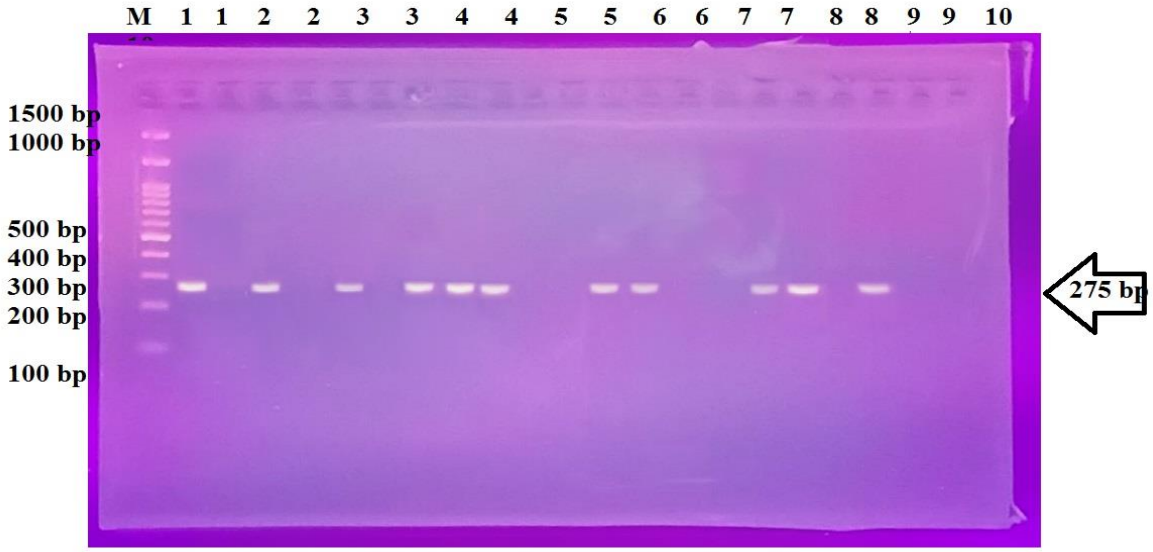

Figure (3.1.): Genotyping of rs4728709 genetic polymorphism. The agarose gel electrophoresis for identifying rs4728709 G > A SNP using AS-PCR. M: DNA ladder, lanes 1, 2, 3, 5, 7 and 9 represent the wild type (GG), lanes 4 represent the heterozygous mutant type (GA), and lane 6 and 8 shows the homozygous mutant type (AA).

3.3.1.A. Distribution of Allele Frequencies of rs4728709 Polymorphism (G > A) among the patients

The patients were categorized based on three genotypes of the ABCB1 gene rs4728709 (G>A) polymorphism: wild-type homozygous (GG), heterozygous mutant (GA), and homozygous mutant (AA). According to the

table (3.8), there were 72 GG genotypes, representing 72% of the population, 13 GA genotypes, representing 13% of the population, and 15 AA genotypes, representing 15% among 100 patients.

Table (3.8): Distribution of gene polymorphisms for SNP rs4728709 genotype in patients (N=100)

The variables		The frequency	Percentage
Genotype SNP rs4728709	GG wild	72	72
	GA heterozygous	13	13
	AA homozygous	15	15

3.3.2. Genotyping of rs4148738 (C > T) Genetic Polymorphism:

The rs4148738 (C > T) gene polymorphism resulted in a distinct band measuring 260 base pairs. The size of the amplicon was established by comparing it against a DNA ladder ranging from 100 to 1500 base pairs. As shown in figure (3.2):

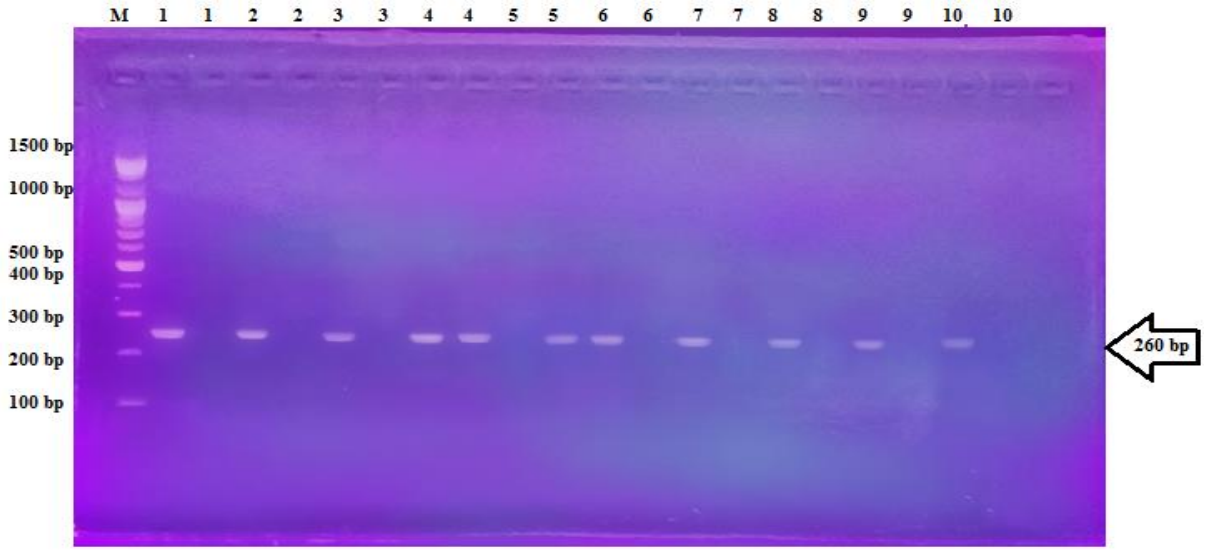

Figure (3.2.): Genotyping of rs4148738 genetic polymorphism. The agarose gel electrophoresis for identifying rs4148738 C > T SNP using AS-PCR. M: DNA ladder, lanes 1, 2, 3, 6, 7, 8,9 and 10 represent the wild type (CC), lanes 4 represent the heterozygous mutant type (CT), and lane 5 shows the homozygous mutant type (TT).

3.3.2.A. Distribution of Allele Frequencies for the rs4148738 Polymorphism (C > T) Among Patients

The patients were classified into three genotypes for the *ABCB1* gene rs4148738 (C >T) polymorphism: wild-type homozygous (CC), heterozygous (CT), and homozygous mutant (TT). According to the table (3.9), the wild CC genotypes represented 26% of the population, the heterozygous CT genotypes represented 39%, and the homozygous TT genotypes represented 35% of the population, among 100 patients.

Table (3.9): Distribution of gene polymorphisms for the SNP rs4148738 genotype in 100 patients.

Variables		Frequency	Percentage
Genotype SNP rs4148738	CC wild	26	26
	CT heterozygous	39	39
	TT homozygous	35	35

3.3.3. Cross tabulation for SNP rs4728709 and SNP rs4148738

Patients were categorized into nine genotype groups based on the cross-tabulation of two single nucleotide polymorphisms (SNPs): rs4728709 and rs4148738 in the ABCB1 gene. The distribution was as follows: CC-GG (15 patients), CT-GG (31 patients), TT-GG (26 patients), CC-GA (5 patients), CT-GA (4 patients), TT-GA (4 patients), CC-AA (6 patients), CT-AA (4 patients), TT-AA (5 patients), as shown in table (3.10).

Table (3.10): Cross tabulation for SNP rs4728709 and SNP rs4148738

Parameter		SNP rs4148738		
SNP rs4728709		CC	CT	TT
	GG	15	31	26
	GA	5	4	4
	AA	6	4	5

3.3.4. Hardy-Weinberg Equilibrium (HWE) for Genotypes of Studied Patients

3.3.4.A Hardy-Weinberg Equilibrium (HWE) for ABCB1 rs4728709 Genotypes among Patients

Chi-square test examined patients for Hardy-Weinberg equilibrium of SNP (rs4728709). Genotypes' distributions deviate from Hardy–Weinberg equilibrium (X^2 = 37.80, P < 0.001), indicating potential biological or population-level factors influence on this locus.

Table (3.11): Hardy–Weinberg equilibrium for SNP (rs4728709) in patients.

Variable			Frequency	Percentage	Alleles n (%)		Hardy–Weinberg equilibrium X^2 test
Genotype	GG wild	Observed	72	72	G	A	
		expected	61.62	61.62			
	GA hetero	Observed	13	13	157 (78.5)	43 (21.5)	**X^2 = 37.8067 P<0.001* (S)**
		expected	33.76	33.76			
	AA homo	Observed	15	15			
		expected	4.62	4.62			

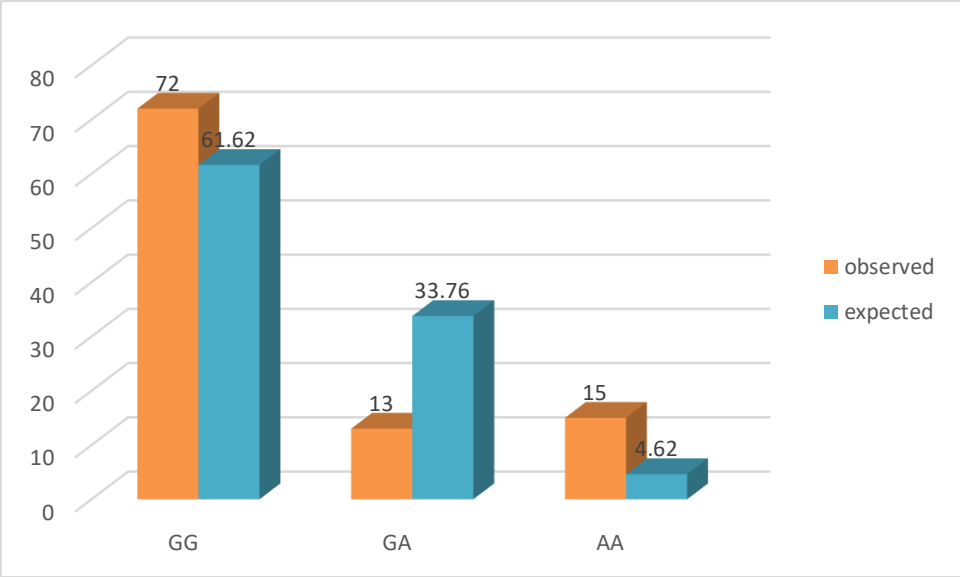

Figure (3.3): Hardy–Weinberg equilibrium for SNP (rs4728709) among individuals.

3.3.4.B Hardy-Weinberg Equilibrium (HWE) for ABCB1 rs4148738 Genotypes in Patients

Chi-square test examined patients for Hardy-Weinberg equilibrium of SNP (rs4148738). Genotypes' distributions deviate from Hardy–Weinberg equilibrium (X^2 = 4.5638, P < 0.0327), indicating potential biological or population-level factors influence on this locus.

Table (3.12): Hardy–Weinberg equilibrium for SNP (rs4148738) in patients.

Variable			Frequency	Percentage	Alleles n (%)		Hardy–Weinberg equilibrium X^2 test
Genotype	CC wild	Observed	26	26	C	T	
		expected	20.7	20.7			
	CT hetero	Observed	39	39	91 (45.5)	109 (54.5)	**X^2 = 4.5638 P<0.0327* (S)**
		expected	49.6	49.6			
	TT homo	Observed	35	35			
		expected	29.7	29.7			

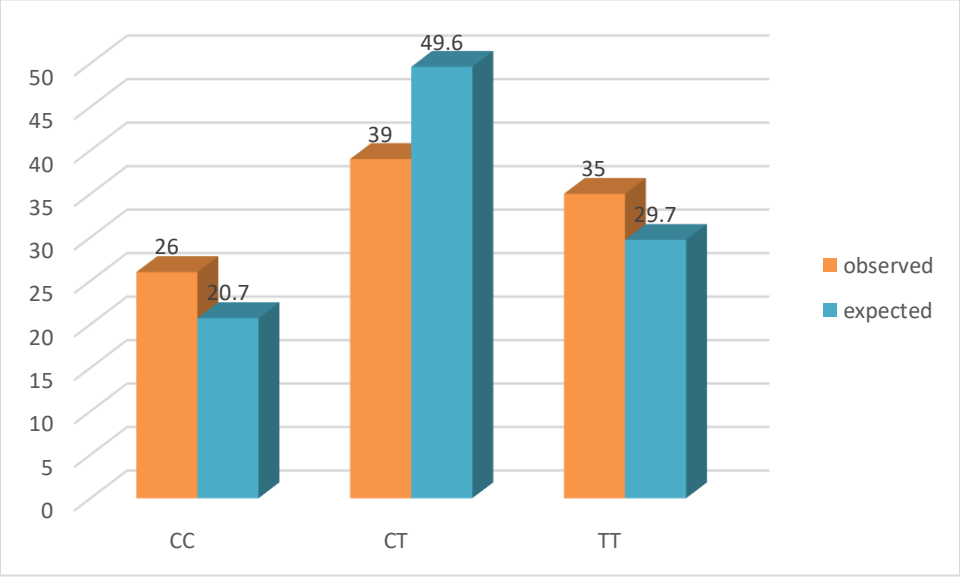

Figure (3.4): Hardy-Weinberg equilibrium for SNP (rs4148738) in individuals.

3.3.5. Multiple Linear Regression Model for Genotypes of Studied Patients

3.3.5.A. Multiple Linear Regression Model for ABCB1 rs4728709 Genotypes Among Patients

A multiple linear regression model was employed to predict concentration levels using demographic and genetic variables. The model accounts for approximately 14.6% of the variance in concentration levels (Adjusted $R^2 = 0.146$), indicating a small effect size. The model demonstrates overall statistical significance ($p < 0.001$), suggesting that the predictor variables collectively exhibit a significant relationship with concentration.

In this model, age and BMI are not significant predictors of concentration levels. Genetic polymorphisms, specifically single-nucleotide polymorphisms (SNPs), exhibit substantial and statistically significant effects.

Individuals with the GA genotype exhibit approximately 115 units lower concentration levels than those with the GG genotype, which serves as the reference group. Individuals with the AA genotype show a more pronounced reduction of approximately 188 units than those with the GG genotype.

The findings indicate that SNP genotype significantly affects concentration levels, potentially through changes in the studied compound's expression, metabolism, or excretion**.**

Table (3.13): **Multiple linear regression model predicting concentration levels according to demographic and genetic variables for ABCB1 rs4728709 genotypes.**

R	R Square	Adjusted R Square	Std. Error of the Estimate	Model P value
0.425	0.181	0.146	158.69	**<0.001***

Predictor variable	Unstandardized Coefficients		P value
	B	Std. Error	
Age	0.125	1.628	0.939
BMI	-4.035	2.695	0.138
GA – GG (Ref)	-115.612	49.004	**0.020***
AA – GG (Ref)	-188.439	45.340	**<0.001***

3.3.5.B. Multiple Linear Regression Model for ABCB1 rs4148738 Genotypes Among Patients

A multiple linear regression model was employed to predict concentration levels using demographic and genetic variables. The model accounts for approximately 46.9 % of the variance in concentration levels (Adjusted R^2 = 0.469), indicating a moderate effect size. The model demonstrates overall statistical significance ($p < 0.001$), suggesting that the predictor variables collectively reveal a significant relationship with concentration.

In this model, age and BMI are not significant predictors of concentration levels. Genetic polymorphisms, specifically single-nucleotide polymorphisms (SNPs), exhibit substantial and statistically significant effects.

Individuals with the TT genotype exhibit approximately 268 units lower concentration levels than those with the CC genotype, which serves as the reference group.

The findings indicate that SNP genotype significantly affects concentration levels, potentially through changes in the studied compound's expression, metabolism, or binding**.**

Table (3.14): Multiple linear regression model predicting concentration levels based on demographic and genetic variables for ABCB1 rs4148738 genotypes

R	R Square	Adjusted R Square	Std. Error of the Estimate	Model P value
0.700	0.490	0.469	125.165	**<0.001***

Predictor variable	Unstandardized Coefficients		P value
	B	Std. Error	
Age	0.569	1.267	0.654
BMI	0.821	2.120	0.699
CT – CC (Ref)	-27.954	31.711	0.380
TT – CC (Ref)	-267.998	32.615	**<0.001***

3.3.6. Analysis of Adverse Drug Reactions (ADRs) by Multiple Responses

The prevalence and impact of the ADRs are summarized in the table (3.15). Gum bleeding was reported in some individuals, seven patients (26.9% of all ADR responses and 43.8% of cases). Rash occurred at the same rate, with seven patients affected. Epistaxis occurred in 4 patients, representing 15.4% of ADR reports and 25.0% of cases. The most frequently reported ADR of nausea was observed in 8 patients, accounting for 30.8% of ADR responses and 50.0% of occurrences of nausea.

Twenty-six patients reported at least one ADR, sometimes more than one symptom at the same time. This overlap reached a total of 162.5%, reflecting the diverse responses of patients to therapy.

Table (3.15): Adverse drug reaction multiple responses.

Adverse reaction	N	ADR responses	percentage of cases
Gum bleeding	7	26.9%	43.8%
epistaxis	4	15.4%	25.0%
rash	7	26.9%	43.8%
Nausea	8	30.8%	50.0%
Total	26	100.0%	162.5%

3.3.7. Association of Bleeding Events Among Genotypes

Chi-square test is utilized to assess the association and compare bleeding events across genotypes. For rs4728709, bleeding occurred among all genotypes (GG, GA, AA), but the difference was not significant ($p = 0.337$). In contrast, for rs4148738, bleeding was significantly associated with genotype, particularly in the heterozygous CT, showing a strong significant association ($p < 0.001$).

Table (3.16): Association of genetic variation and bleeding events.

SNP	Genotype	Bleeding	No-bleeding	P value
rs4728709	GG	7	65	0.337
	GA	2	11	
	AA	0	15	
rs4148738	CC	0	26	**<0.001***
	CT	9	30	
	TT	0	35	

3.3.8. Analysis of Biomarkers Correlations by Spearman's rho

A Spearman correlation analysis was performed to investigate the relationships among demographic and biochemical parameters. The INR and PT demonstrate a significant positive correlation ($p = 0.833$, $P < 0.001$), indicating that elevated INR values are closely linked to prolonged PT, which reflects coagulation dynamics. A notable positive correlation exists between PLT and ALP ($p = 0.382$, $P < 0.001$), suggesting a potential interaction

between platelet activity and alkaline phosphatase levels. Serum creatinine exhibits a strong correlation with blood urea (p = 0.554, P < 0.001), indicating a dependence of renal function on these two markers. ALT show a significant positive correlation with AST (p = 0.629, P < 0.001), underscoring their function as liver enzymes that frequently vary together in hepatic conditions. Hemoglobin (HB) display a negative correlation with age (p= -0.203, P= 0.043) as well BMI (p= -0.212, P= 0.034), indicating that older individuals and those with elevated BMI may have reduced hemoglobin levels. A moderate negative correlation is noticed between ALT and blood urea (p = -0.264, P = 0.008), suggesting potential alterations in metabolic function that may influence liver and kidney interactions.

Table (3.17): Correlation Matrix.

	Age	BMI	D.Rx	INR	PT	PLT	S.Cr.	ALP	ALT	HB	B. UREA	AST
Age	1.000											
BMI	-.183	1.000										
D.Rx	.142	-.019	1.000									
INR	.068	.030	.086	1.000								
PT	.109	-.009	.044	.833**	1.000							
PLT	-.052	.019	.120	-.040	-.028	1.000						
S.Cr.	-.013	-.110	-.016	.092	.143	-.248*	1.000					
ALP	-.082	-.120	.199*	.036	.013	.382**	.075	1.000				
ALT	-.189	-.058	-.028	-.021	-.067	-.167	-.180	.016	1.000			
HB	-.203*	-.212*	-.075	.028	.062	-.105	.250*	-.058	.128	1.000		
B. UREA	.142	-.056	-.029	.036	.163	-.210*	.554**	-.076	-.264**	.055	1.000	
AST	-.117	-.140	-.037	.057	-.015	-.001	-.246*	-.035	.629**	.072	-.276**	1.000

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Chapter Four

Discussion

4. Discussion

Atrial fibrillation (AF) constitutes 40% of all cardiac arrhythmias and it is linked to a significant risk of stroke and systemic thromboembolic events. Rivaroxaban is a direct oral anticoagulant (DOAC) demonstrated to avoid strokes in people having non-valvular atrial fibrillation (AF). This study delineates the socio-demographic characteristics of the patient, analysis biochemical parameters, and conducts a molecular examination of two SNPs in the ABCB1 gene. Interindividual variability in response to the medication rivaroxaban may be partly attributed to variation in genes encoding the transporter p-gp (Kanuri & Kreutz, 2019). Nevertheless, information about the impact of genetic variants on pharmacological responses remains controversial. Consequently, this is a cross-sectional study to elucidate the association between genetic variants and rivaroxaban outcomes, highlighting the importance for future genetic analysis for rivaroxaban.

4.1. Socio-Demographic Data

The sample of the study included 100 patients who had non-valvular atrial fibrillation (AF) and were treated with the anticoagulant rivaroxaban. The patients were of both sexes, with 45% males and 55% females. Of the study population, 49% were aged over 60 years, 42% were aged 46 to 60 years, and 9% were aged 30 to 45 years. According to the BMI decile, 13% had a healthy weight, 35% were overweight, and 52% were obese. On treatment duration, 85% of patients had a duration between 3 and 42 months, and 15% above 42 months.

In the results of the study, there was a relation between patients' sex and Serum creatinine values. Serum Creatinine is significantly higher in males

(median=0.90) than females (median=0.80), p=0.001 for these patients, which agrees with (Yim et al., 2023), they found approximately 98 % of the total body creatinine is contained in the muscles and serum creatinine levels are in proportion to muscle mass. Moreover, Hemoglobin was higher in males (13.47±1.54) than in females (12.63±1.49), p=0.007. In the females, the decline pattern was reported by (Su et al., 2023) as the underlined drop in erythropoiesis due to iron loss or blood loss in this menstruating female cycle which is a very important factor in this variation, or on the contrary, an increase in the functioning of erythropoiesis due to androgens and greater muscle mass of men.

4.2. Biochemical Findings

Alkaline Phosphatase is statistically distinct in the group with a duration length of less than 3.5 years (median = 84.00) compared to the group with a duration exceeding 3.5 years (median = 143.00), p = 0.015. Drug-induced liver injury (DILI) associated with DOACs is infrequent, occurring in 0.1%-1% of cases. Rivaroxaban has been linked to a greater occurrence of drug-induced liver injury (DILI) compared to other direct oral anticoagulants (DOACs). According to (Karki et al., 2023) the mechanism of liver harm caused by rivaroxaban is likely idiosyncratic and may be immunologic. However, extended use and prolonged exposure may elevate the risk of liver damage, even infrequently, due to the cumulative metabolic load on the liver. The majority of DILI instances are benign and resolve upon the discontinuation of the causative medication. The hepatotoxicity of rivaroxaban is infrequent, though it has been reported to induce acute liver failure.

This study showed that hemoglobin (Hb) of group A patients (14.51±1.20) is significantly higher than B (13.04±1.48) (p= 0.009) and C group (12.71±1.56) (p= 0.001). There is a decrease in the Hb content with age, with Group A (30-45 years) having its highest hemoglobin content ($p<0.05$) compared to Group B (46-60 years) and Group C (above 60 years).

Hemoglobin concentration began to decline at the peak age. Our findings support prior work of Warren et al., who observed that Hemoglobin decreased with age in males, and this reduction may reflect a gradual loss of androgens, which in turn seems to stimulate enhanced production of erythropoietin and RBC (Warren et al., 2022). The decreasing trend in females might be linked to a steady decline found in the expression of androgens and weight loss with age, in the amount of iron intake and where females might have lost more blood due to menstruation (Mirza et al., 2018). As organ function deteriorates, bone marrow activity typically declines, accompanied by iron, vitamin B12, and folate malnutrition, diminished immune function, prolonged medication effects, other systemic diseases, and even malignancies. Consequently, the elderly are more susceptible to anemia, with hemoglobin levels decreasing as age increases (X. Wu et al., 2015).

Regarding the efficacy (response) outcomes, the assessment of coagulation indicators INR and PT across various genotypes for the SNPs rs4728709 and rs4148738 shows no statistically significant changes among the examined patients. This indicates that these genetic variations do not significantly influence coagulation status as evaluated by these parameters. Additionally, Yi Ma and his colleagues presented findings on efficacy outcomes, including PT and INR levels, and reported no significant

correlation observed between ABCB1 rs4148738 or rs4728709 and PT levels across different studies (Y. Ma et al., 2024). A second investigation revealed that rs4148738 had no significant correlation with PT (Prothrombin Time), INR (International Normalized Ratio), or bleeding incidents in patients (D. Zhang et al., 2022).

The study demonstrates that there is no association between the patients' BMI and their biochemical parameter levels. A P-value > 0.05 is considered statistically insignificant.

4.3. Molecular Analysis

4.3.1. Polymorphisms of the ABCB1 Gene

The impact of genetic factors on pharmacological responses is complicated, with variations in genetic structure considered primary contributors to individual differences. In addition, genetic polymorphisms in Various enzymes involved in metabolism, transporters, receptors, and other pharmacological targets implicated in vivo delivery of drugs lead to variations in therapeutic efficacy and toxicity. Furthermore, p-glycoprotein (P-gp) serves as a crucial transporter for numerous drugs in vivo. Therefore, the activity and expression of P-glycoprotein (P-gp) influence the absorption, distribution, metabolism, and elimination of medications. In the same way, research indicates that polymorphisms in the ABCB1 gene may alter its activity and expression, leading to variability in therapeutic efficacy and adverse effects among individuals. Rivaroxaban is a metabolic substrate of P-glycoprotein thus, mutations within the ABCB1 gene could influence the relationship between dose and response as well as the bleeding risk associated with rivaroxaban (Wang et al., 2021).

The ABCB1 gene rs4728709 (G > A) and the ABCB1 gene rs4148738 (C > T) were amplified using polymerase chain reaction (PCR). For the ABCB1 gene rs4728709 (G > A), the results prove that the G allele was the predominant allele, while the A allele was the recessive allele in the population we studied. For the ABCB1 gene rs4148738 (C > T), the T allele is the predominant allele, whereas the C allele is the recessive allele. These results are consistent with findings perversely reported in the Chinese population (F. Zhang et al., 2022). Distributions of genotypes are deviated from Hardy–Weinberg equilibrium, indicating the influence of potential biological or population-level factors on this locus.

4.3.2. Role of ABCB1 Gene Polymorphism on Liver Enzyme Levels

Alanine aminotransferase (ALT) in the mutant homozygous AA group was high (30.00±12.0), which is highly significant from the ALT in the wild GG group (20.00±11.0) with a p-value of 0.005. A statistically significant difference in AST in wild GG vs homozygous AA exists. Aspartate aminotransferase (AST) level is lower (26.64±10.42) in wild GG than in mutant homozygous AA (33.93±12.80), p=0.018.

ABCB1 is primarily involved in the first-pass clearance of orally taken pharmaceuticals, thereby reducing their bioavailability by effluxing medicines from the lumen-facing epithelia of the small intestine and colon, as well as from the bile-facing canaliculi of the liver. The disposition of drugs mediated by ABCB1 is affected by the modification of ABCB1 gene expression and/or ABCB1 activity via multiple ways. The overexpression of these transporters on plasma membranes results in enhanced efflux and

reduced intracellular accumulation of numerous medicines, culminating in suboptimal therapeutic response (Robert,2007).

According to dbSNP (Database SNP) from NCBI (National Center for Biotechnology Information), the SNP rs4728709 is located within an intron (non-coding regions). As reported by (Maan et al., 2021) Intronic SNPs do not directly alter protein-coding regions. Nevertheless, they can significantly affect gene function by influencing RNA alternative splicing, genomic imprinting, regulating gene expression via lncRNAs (Long non-coding RNAs), transcription enhancers, chromatin looping, programmed cell death, and premature stop codons. Consequently, these effects contribute to modifications in the gene expression of P-glycoprotein without altering the protein sequence.

Individuals having the AA genotype of rs4728709 frequently exhibit altered P-glycoprotein expression or activity. P-glycoprotein is vital for drug efflux, inhibiting the buildup of harmful substances in hepatic cells. P-glycoprotein is expressed in the canalicular membrane of hepatocytes within the liver. Anyway, hepatic P-glycoprotein expression levels are around seven times lower than those in the gut (Köck & Brouwer, 2012). When the activity of P-glycoprotein is compromised, drugs and metabolites may accumulate within liver cells, leading to an increased concentration of harmful substances and subsequently resulting in cellular damage to the liver. The liver elevates ALT and AST levels when inflamed, stressed, or injured. In individuals undergoing treatment with liver-metabolized pharmaceuticals (e.g., rivaroxaban), the rs4728709 AA genotype might hinder the clearance of these

medications, resulting in drug-induced liver damage (DILI). These may manifest as high blood levels of ALT and AST.

4.3.3. Role of ABCB1 Gene Polymorphism on Plasma Concentration

This study is the first to assess how various gene polymorphisms influence the steady-state trough concentration of rivaroxaban and bleeding incidents in patients with NVAF in Iraq. Our findings were concentration of rs4728709 is statistically significant between wild GG (464.35±340.03) and homozygous AA (144.00±24.90), p=0.001, while for rs4148738, the concentrations in the homozygous TT genotype (144.60±14.10) are considerably lower than those in the wild CC (483.20±175.83) and heterozygous CT genotypes (471.90±317.60). These findings were similar to a previously reported study by (T. Wu et al., 2023).

They discovered for the first time that the ABCB1 rs4148738 and rs4728709 gene polymorphisms significantly influence the C_{trough}/D of rivaroxaban in patients with non-valvular atrial fibrillation. Specifically, the results indicated that the ABCB1 SNP rs4148738 (P=0.039) and SNP rs4728709 (P=0.029) notably affected the C_{trough}/D levels of rivaroxaban. For ABCB1 rs4728709, multiple comparisons revealed that the homozygous mutant type exhibited a significantly lower C_{trough}/D compared to the wild type (GA vs. GG, P=0.032). Similarly, for ABCB1 rs4148738, the homozygous mutant type was associated with a significantly reduced C_{trough}/D compared to the wild type (TT vs. CC, P=0.033). However, for ABCB1 rs4148738 there was no significant differences were found in C_{trough}/D between the

heterozygous mutant type and either the homozygous or wild type (CT vs. TT, P=0.611; CT vs. CC, P=0.242).

Previous studies have found four SNPs of the ABCB1 gene (rs2032582, rs1045642, rs4148738, and rs1128503) with elevated plasma levels of rivaroxaban and an increased risk of hemorrhagic events (Abdrakhmanov et al., 2024). While another study found, ABCB1 (rs1045642 and rs4148738) polymorphisms did not influence rivaroxaban pharmacokinetics in patients aged 80 years and older with NVAF (Sychev et al., 2022). In contrast to current study findings Fedina and his colleagues found that in carriers of the genotype CT+TT ABCB1 (rs4148738) C>T, the concentration of rivaroxaban in blood plasma was statistically significantly higher (Fedina et al., 2023). The results of the study indicated that variations in the P-glycoprotein produced by the ABCB1 gene may influence the plasma concentrations of rivaroxaban.

The multiple linear regression models for ABCB1 rs4728709 and rs4148738 genotypes show important results regarding their crucial roles in the concentration influence on patients in the study. Both of them were statistically significant with $p < 0.001$, which proves the tight connection between genetic variation and drug plasma concentration. Conversely, their effect sizes differ, and rs4728709 has only 14.63% of variance, while rs4148738 accounts for 46.93%, confirming the greater role of rs4148738.

It is noteworthy that age and BMI were not significant predictors, suggesting that genetically determined factors play a more critical role in determining that concentration. For patients with genotype rs4728709, the plasma concentration of rivaroxaban in heterozygous GA carriers is approximately 115 units lower ($p = 0.020$) than in the wild GG reference

group. Additionally, individuals with the AA genotype show a reduction of 188 units compared to those with the GG genotype ($p < 0.001$). While in patients with the rs4148738 genotype, homozygous TT genotype carriers demonstrated the most significant reduction in concentration, decreasing by 268 units compared to wild-type CC individuals ($p < 0.001$).

In vitro studies suggest that several SNPs in the ABCB1 gene may correlate with P-gp expression and function alterations (Hodges et al., 2011). The findings highlight the functional consequences of the ABCB1 rs4728709 and rs4148738 polymorphisms, likely through the modulation of gene expression, transporter efficacy, by reduced mRNA stability or altered splicing efficiency, potentially resulting in reduced P-glycoprotein levels. In these situations, diminished drug efflux may result in elevated intracellular accumulation and consequently greater plasma concentration. Therefore, adjusting rivaroxaban dosing based on genotyping results is an option. Extensive population studies are needed to clarify the clinical implications of genotyping for this medication.

In evaluating the relationship between ABCB1 polymorphisms and bleeding events in patients undergoing anticoagulant therapy, certain findings confirm other studies, but others disagree. These variations may be attributed to differing study designs, population characteristics, and drugs examined. The results revealed that the distribution of ABCB1 rs4728709 polymorphisms did not show a significant association with bleeding events. In contrast, the association between the rs4148738 genotype and bleeding was highly significant, particularly for the heterozygous CT genotype, which was markedly significant for these individuals. Several studies have shown a link

between ABCB1 C > T polymorphisms and a heightened chance of bleeding. Research conducted by Mardi et al. (Mardi et al., 2023) indicated that patients with the TT genotype, linked to diminished P-gp expression, had elevated plasma levels of DOACs, contributing to an elevated probability of hemorrhage. Multiple investigations indicate that there is no significant relationship among the rs4148738 C > T mutation and clinical effects. A Study conducted by Cosmi et al. (Cosmi et al., 2019) has demonstrated no notable differences in bleeding probability across different ABCB1 genotypes in people taking rivaroxaban. A reasonable reason for the different outcomes is due to the expression of P-glycoprotein may influence rivaroxaban absorption, distribution and excretion, owing to differing levels of dependence on this transporter. The gene polymorphism of ABCB1 may result in either an elevation or reduction of P-glycoprotein expression. Variations in study populations may account for the inconsistencies. Parallel reports indicate the role of the ABCB1 gene (rs4148738) polymorphism; for instance, Wu et al. demonstrated that polymorphisms in the ABCB1 gene showed no correlation with bleeding events when using rivaroxaban (Wu et al., 2023). The current study had a small sample size, and the links between gene polymorphisms and bleeding events require validation in studies with larger samples.

4.4. Correlations of Biomarkers

The results show a significant positive relationship between prothrombin time (PT) and the international normalized ratio (INR); thus, as PT increases, the INR also rises correspondingly. This relationship is anticipated since the INR is the standardized representation of PT, computed through a specific formula (Dorgalaleh et al., 2021). Serum creatinine and

blood urea commonly rise together as both indicate renal function, primarily showing the kidneys' efficiency in filtering waste from the blood. Pandya reported similar findings in their study, confirming a significant linear relationship between serum urea and creatinine levels (Pandya, 2016). Alanine Aminotransferase and AST (Aspartate Aminotransferase) typically increase together because both are enzymes released from injured liver cells. Both enzymes are present in large quantities in hepatocytes; therefore, liver damage from conditions such as hepatitis, fatty liver, or toxins results in a similar elevation in their blood levels. This result is similar to that of a study that found a significant positive correlation between AST/ALT with prothrombin time in Chronic Liver Disease (CLD) subjects without cirrhosis (Karim et al., 2015).

As mentioned earlier, hemoglobin has a negative correlation with increasing age and BMI. This is obviously due to the gradual loss of androgens, which seems to stimulate enhanced production of erythropoietin and red blood cells (RBC). As organ function deteriorates, bone marrow activity typically declines, accompanied by malnutrition of iron, vitamin B12, and folate, diminished immune function, prolonged medication effects, other systemic diseases, and even malignancies.

Platelet is positively correlated with ALP, which are frequently associated with liver injury or inflammation in response to tissue damage. In a previous study, Platelet Count showed an important association with liver infection than non-infected livers, and this is consistent with other studies that reported platelet count is a reliable marker for liver inflammation. Furthermore, the same study reported significant ALT, AST, ALP and GGT

elevations were found in hepatitis B virus (HBV) patients compared with non-infected healthy controls (Ali, 2019). Rivaroxaban is metabolized in the liver, and the influence of ABCB1 gene polymorphism may cause drug accumulation, potentially resulting in drug-induced liver injury. Additionally, age-related increases in osteoporosis and bone turnover have been associated with elevated alkaline phosphatase (ALP) levels. One study reported that serum calcium concentrations were significantly lower in post-menopausal women compared to their pre-menopausal counterparts, while ALP levels were modestly elevated (Tirtha et al.,2013).

The negative link between ALT and urea levels in patients treated with rivaroxaban could indicate a complex relationship between liver and kidney functions. Elevated ALT levels point to liver damage, while low urea levels may either signify stable renal function or reduced urea production resulting from hepatic injury. Rivaroxaban may induce metabolic changes that inversely affect liver and kidney functions. Consequently, liver stress or damage from the medication may lead to increased ALT levels, while urea decreases either if renal function remains intact or if protein synthesis decreases due to liver damage (Karki et al., 2023). Recent studies indicate that drug-induced liver injury associated with rivaroxaban has become more frequently reported, likely due to its rising prescription rates. A study by Baig and his colleagues found that a case suggests the potential causal link between acute liver failure and rivaroxaban usage. Therefore, physicians should be vigilant when prescribing rivaroxaban, closely monitoring liver function tests, particularly in elderly patients with acute congestive heart failure (Baig et al., 2015).

The usage of DOACs has grown in frequency over the past two years in Iraq. However, their associated pharmacogenomics research is limited, and larger population studies are needed to investigate the significance of genes in anticoagulant treatment. Along with the advancement of pharmacogenomics, the ongoing clarification of the molecular mechanisms of anticoagulant agents will involve genes identified in various ethnic and geographic communities, supported by the development of genome-wide association research. Pharmacogenomics for individuals promises to offer precise and logical guidance for patients undergoing anticoagulant therapy, while personalized therapeutic interventions continue to develop.

In general, data is limited regarding the impact of gene polymorphisms on the response and plasma concentration. The majority of research focuses on the ABCB1 gene, leading to somewhat conflicting findings. This study examined two genetic loci linked to rivaroxaban transporter.

The results of this study are expected to promote further exploration of how variations in genetics influence the bioavailability and clinical outcomes of rivaroxaban.

The present study serves as a basis for upcoming studies on genetic variables among the creation of a bleeding scenario for rivaroxaban for individuals having non-valvular AF, aimed at predicting bleeding risk and minimizing adverse effects in clinical practice, thereby facilitating the individualized and rational use of medications.

Conclusion

The following conclusions are obtained from the study:

1. The ABCB1 gene polymorphisms rs4728709 and rs4148738 variants are associated with plasma concentrations of rivaroxaban in individuals from Iraq.

2. A significant correlation exists between the rs4148738 locus and bleeding events. However, no significant correlation was found between the rs4728709 locus and bleeding events

3. Neither rs4728709 nor rs4148738 showed a significant influence on the overall clinical response to rivaroxaban therapy in a sample of Iraqi atrial fibrillation patients.

Recommendations

1. Physicians should carefully monitor liver function tests when initiating rivaroxaban in patients, particularly elderly individuals with acute congestive heart failure.
2. Making future investigations on larger populations to gain a deeper understanding of the clinical importance of genetic analysis for this drug.
3. Investigate the genetic influences of other SNPs including (rs2032582, rs1045642, rs4148738, and rs1128503) associated with the ABCB1 gene to better illustrate their impact on rivaroxaban response in patients with AF.
4. focuses on particular genetic polymorphisms related to other metabolizing enzyme genes that affect rivaroxaban's pharmacokinetics (e.g., CYP3A4/5 and CYP2J2).

Limitations of The Study

The current cross-sectional study included 100 Iraqi patients. We suggest conducting similar studies with a larger sample size in the same target populations. Regarding the response, we are unable to analyze the factor Xa level.

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Appendixes

Thi -qar Governorate
Thi-qar Health Directorate
Nasiriyah heart hospital

محافظة ذي قار
دائرة صحة ذي قار
مستشفى الناصرية للقلب

العدد : ق/ ٤٨٤٨

التاريخ : ١٠ / ١٠ /٢٠٢٤

إلـى/ الاقسام كافة

م/ تسهيل مهمة

تحية طيبة ..

لامانع لدينا من تسهيل مهمة الباحث طالب الماجستير(حيدر خضير جليل) لتسهيل مهمة بحثيه الموسومين

(Influence of ABCBI Gene Polymorphism on Rivaroxaban Response and Hemorrhagic Events in Patients with Atrial Fibrillation in Iraq)

(Impact of CYP3 A4 Gene polymorphism on the Response of rivaroxaban in Iraqi Atrial Fibrillation Patients)

للفترة من ٢٠٢٤/١٠/١ ولغاية ٢٠٢٥/٥/١ .

للتفضل بالاطلاع ... مع الاحترام .

الدكتور
عقيل سلمان صالح
مدير مستشفى الناصريـة للقلب
١٠ / ١٠ /٢٠٢٤

نسخة منه إلى/
* الموارد البشرية
انوار

Ministry of Higher Education
and Scientific Research
University of Karbala
College of Pharmacy
Department of Postgraduate Studies

جمهـــورية العـــراق
وزارة التعليم العالي والبحث العلمي
جامـــعة كربلاء
كلـــية الصيـــدلة
شعبة الدراسات العليا

Issue No.:
Date:

العدد: د.ع/ ٦ / ٥٦٦
التاريخ: ١٠ / ٢ / ٢٠٢٥

الى/ شركة بايونير للصناعات الدوائية

م/تسهيل مهمة

تحية طيبة ..

يرجى تفضلكم بالموافقة على تسهيل مهمة طالب الدراسات العليا / ماجستير/ الادوية والسموم في كليتنا **(حيدر خضير جليل)** لغرض اكمال اجراءات بحث الماجستير الموسوم:

Influence of ABCB1 Gene Polymorphism on Rivaroxaban Response and Hemorrhagic Events in Patients With Atrial Fibrillation in Iraq

شاكرين تعاونكم معنا مع التقدير....

أ.م.د. محمد ابراهيم رسول
عميد كلية الصيدلة
٢٠٢٥ / ٢ / ١٠

نسخة منه الى:
- مكتب السيد العميد ، للتفضل بالاطلاع .
- مكتب معاون العميد للشؤون العلمية .
- شعبة الدراسات العليا للحفظ مع الاوليات .
- فرع الادوية والسموم .
- الصادرة .

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Ministry of Higher Education
and Scientific Research
University of Karbala
College of Pharmacy
Department of Postgraduate Studies

جمهـــورية العـــراق
وزارة التعليم العالي والبحث العلمي
جامـــعة كربلاء
كلـــية الصيـــدلـــة
شعبة الدراسات العليا

Issue No.: العدد: د.ع/ 6 / 2189
Date: التاريخ: 25/ 9 / 2024

الى/ دائرة صحة ذي قار / مستشفى الناصرية للقلب

م/تسهيل مهمة

تحية طيبة ..

يرجى تفضلكم بالموافقة على تسهيل مهمة طالبي الدراسات العليا / ماجستير/ الادوية والسموم في كليتنا (**حيدر خضير جليل**) و (**محمد راضي عباس**) لغرض اكمال اجراءات بحثي الماجستير الموسومين:

- **Influence of ABCB1 Gene Polymorphism on Rivaroxaban Response and Hemorrhagic Events in Patients With Atrial Fibrillation in Iraq**

- ***Impact of CYP3A4 gene polymorphism on the response of rivaroxaban in iraqi Atrial Fibrillation patients***

شاكرين تعاونكم معنا مع التقدير....

أ.م.د. جمال علي عاشور
معاون العميد للشؤون العلمية
2024 / 9 /

مركز الناصرية للقلب
Nasiry heart center
د. عقيل سلمان الخفاجي
جراحة القلب

نسخة منه الى:
- مكتب السيد العميد ، للتفضل بالاطلاع .
- مكتب معاون العميد للشؤون العلمية .
- شعبة الدراسات العليا للحفظ مع الاوليات .
- الصادرة .

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Ministry of Higher Education
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Department of Postgraduate Studies

جمهـــورية العـــراق
وزارة التعليم العالي والبحث العلمي
جامـــعة كربلاء
كلـــية الصيـــدلـــة
شعبة شؤون الدراسات العليا

Issue No.: العدد: د.ع/6/ ٤٧١
Date: التاريخ: ٧ / ١ / 2025

الى / هيئة البحث العلمي / مركز التطبيقات الصناعية وتكنلوجيا المعلومات
م/ تأييد

تحية طيبة...

نؤيد لكم بأن **(ص. حيدر خضير جليل رعيد)** هو احد طلبة الدراسات العليا / ماجستير/الادوية والسموم/ المرحلة البحثية لكلية الصيدلة للعام الدراسي 2024-2025 ومستمر بالدوام وبناءً على طلبه زود بهذا التاييد .

...مع التقدير...

أ.م.د. جمال علي عاشور
معاون العميد للشؤون العلمية
والدراسات العليا
2025 / 1 /

نسخة منه الى:
- مكتب السيد العميد .. للتفضل بالاطلاع.
- مكتب معاون العميد للشؤون العلمية .. للتفضل بالاطلاع.
- شعبة شؤون الدراسات العليا .. للحفظ مع الاوليات.
- الصادرة.

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NO.26……

-Demographic

Name	Gender	Age	Weight	Height	BMI	Duration Rx	DM	HTN	Smoking
	male	65	127	190		6 months	No	Yes	no

Compliance	Good
Patient Education	primary

-Clinical

PT	INR	CBC	RFT	LFT	plasma concentration
14	1.35	HB:14.3 PLT:218	Creatinine:0.7 Urea:26	AST:51 ALT:24 ALP:83	488ng/ml

-Adverse drug reactions (ADRs)

symptoms of thrombosis	V.Bleeding	Gum bleeding	epistaxis	GIT bleeding	brain hemorrhage	Rash	Nausea	anaphylax
no	no	no	yes	no	no	no	no	no

-Genetic analysis

Gene	SNP	Polymorphism
ABCB1	RS 4728709	GG
ABCB1	RS 4148738	CT

الملخص

المقدمة: يعرف الرجفان الأذيني بأنه اضـطراب قلبي شـائع يؤدي إلى معدلات مرتفعة من المراضـة والوفيات نتيجةً للانسـدادات التخثرية. يُوصـف عقار ريفاروكسـابان، وهو مضـاد تخثر فموي مباشـر، للوقاية من السـكتات الدماغية لدى مرضـى الرجفان الأذيني غير الصـمامي. حيث انه لا يزال تأثير العوامل الوراثية في الحركية الدوائية والنتائج السـريرية لريفاروكسـابان لدى المصـابين بالرجفان الأذيني غير الصـمامي في العراق غير محددة بشكل غير كاف.

هدف الدراسـة: هدفت الدراسـة إلى فحص العلاقة بين اثنين من تعدد الأشـكال النوكليوتيدية المفردة (rs4728709 G>A and rs4148738 SNPs ABCB1) (C>T والأستجابة عقار ريفاروكسابان، ومستويات العلاج في البلازما بعد الاستقرار، وحدوث الاعراض النزفية لدى المرضـى الذين يعانون من الرجفان الأذيني (AF). بالإضافة إلى ذلك، نقترح بروتوكولًا مخصصًا لإدارة العلاج بمضادات التخثر.

المرضـى والاسـاليب: دراسـة مقطعية تقيم المرضـى الذين يعانون من الرجفان الأذيني (AF) والذين يتلقون عقار ريفاروكسـابان في الفترة بين سبتمبر ٢٠٢٤ ومارس ٢٠٢٥ في العراق. جمعت البيانات السريرية والتي شملت المعلومات الديموغرافية، والأمراض المصاحبة، والامتثال للعلاج. قيمت الاختبارات الكيميائية الحيوية لمختلف معايير التخثر (INR، PT)، ووظائف الكبد والكلى (AST، ALT، ALP، S.Creatinin، Urea)، وعدد كريات الدم الكامل (CBC)، وتراكيز البلازما بعد الاسـتقرار لريفاروكسـابان باستخدام الكروماتوغرافيا السائلة عالية الأداء (HPLC). أجريت تحاليل الجينات لتعدد الأشـكال النوكليوتيدية لجين ABCB1 باسـتخدام تفاعل البوليميراز المتسـلسـل الخاص بالأليل (AS-PCR).

النتائج: شـملت هذه الدراسـة ١٠٠ مريض، ٤٥ ذكراً و٥٥ أنثى، وكان مع أكبر من ٤٦ عاماً، مع انتشـار مرتفع للسـمنة بنسـبة ٥٢٪. وكانت توزيع الجينات للمتغير الجيني rs4728709 كالتالي: ٧٢٪ GG، ١٣٪ GA، و١٥٪ AA؛ وللـ rs4148738 :٢٦٪ CC ,٣٩ % CT ,٣٥٪ TT. كانت مسـتويات عقار ريفاروكسـابان في البلازما أقل بشكل ملحوظ في الأفراد الذين يحملون الجينات المتنحية (AA للـ rs4728709 وTT للـ rs4148738) مقارنـةً بـالجينـات السـائدة (p=0.001). كـان للمتغير الجيني

rs4148738 ارتباط كبير بحوادث النزيف، بينما لم يظهر rs4728709 أي ارتباط. لوحظت زيادة في إنزيمات الكبد (ALT وAST) في مجموعات جينية معينة، مما يشير إلى تأثير محتمل للعقار على الكبد بناءً على الجينات. علاوة على ذلك، بالرغم من عدم العثور على ارتباط كبير بين تعدد الأشكال الجينية لـــ ABCB1 واستجابة العلاج بالريفاروكسابان، إلا أن هنالك تأثيراً جينياً كبيراً في تركيز البلازما للعقار ومخاطر النزيف.

الاستنتاج : أستنتجت الدراسة أن تعدد الأشكال الجينية rs4728709 و rs4148738 في جين ABCB1 يؤثر على مستويات عقار ريفاروكسابان في بلازما للمرضى العراقيين الذين شخصوا بمرض الرجفان الأذيني غير الصمامي، خصوصاً rs4148738 حيث يظهر ارتباطًا ملحوظًا باعراض النزيف.

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فرع الادوية والسموم

تأثير تعدد الاشكال الجيني لـجين ABCB1 على الاستجابة لعقار ريفاروكسابان والأحداث النزفية لدى مرضى الرجفان الأذيني في العراق

رسالة
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كجزء من متطلبات نيل درجة الماجستير في علم الادوية والسموم

بواسطة
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ميلادي ٢٠٢٥ ١٤٤٧هجري