INTRODUCTION

Warfarin is the most widely prescribed anti-coagulant for reducing cardiovascular thromboembolic disorders *(Daly and King, 2003)*. However initiation of therapy remains problematic because of inter-individual variability in degree of anticoagulation achieved in response to the same warfarin dose *(Petty et al., 1999)*.

Clinical factors, dermographic variables, and variations in two genes-cytochrome p450{family 2, subfamily C, polypeptide 9} (CYP2C9), and vitamin K epoxide reductase complex subunit 1(VKORC1) contribute significantly to the variability among patients in dose requirements for warfarin. It exerts its anticoagulant effect by reducing the regeneration of vitamin K from the vitamin K epoxide through inhibition of vitamin K epoxide reductase *(Anderson et al., 2007)*.

This protein is encoded by VKORC1 in which rare mutations associated with clotting factor deficiencies and warfarin resistance have been identified *(Rost et al., 2004)*. Most recently, genotype for several non-coding polymorphisms in this gene has

been shown to influence warfarin dose requirements *(Bodin et al., 2005)*. Ten VKORC1 single nucleotide polymorphisms, many tightly linked and 5 inferred halpotypes determine low, intermediate and high-dose requirements *(D'Andrea et al., 2005)*.

On the other hand the cytochrome P450 (CYP2C9) is a liver enzyme required for the oxidative metabolism of a large number of clinically important drugs including warfarin *(Rettie et al., 1992)*. A series of genetic polymorphisms have been described within the cytochrome P450 CYP2C9 locus *(Stubbins et al., ١٩٩٦)*.

Two gene variants, a substitution of a cystein for arginine at position 144 within the exon 3 (CYP2C9*2) and a substitution of leucin for an isoleucin at position ٣٥٩ within the exon ٧ (CYP٢C٩*٣), have been shown to impair hydroxylation of warfarin in vitro *(Steward et al., 1997)*.

These allelic variants of CYP2C9 code for enzymes within approximately 12% (CYP2C9*2) and ٥٪ (CYP٢C٩*٣) of the enzymatic activity of the wild-type genotype CYP2C9*1 *(Crespi and Miller, 1997)*.

Both variants have been associated with decreased warfarin dose requirements, more time to

achieve sTable dosing, a higher risk of bleeding during the initiation phase, and a significantly higher bleeding rate *(Scordo et al., 2002)*. Reduced warfarin dose requirements are dictated by the CYP2C9*2 and *3 genetic variants due to decreased S-warfarin metabolic clearance *(Aquilante et al., 2006).*

Genetic alterations are assessed by direct detection of abnormalities in genes or chromosomes using DNA-based tests or cytogenetic tests and other methods. Polymerase chain reaction (PCR). *(Holtzman and Watson, 1999)*, a process that involves the amplification of short segments of the genome, has revolutionized the field of molecular biology and genetics. Direct detection of alterations in genes involves extraction of genomic DNA from blood or tissue followed by amplification of the individual segments of the gene by PCR. The amplified fragment may be used in different ways to detect abnormalities *(Pergament, 2000)*.

AIM OF THE WORK

To assess the allelic frequencies and to investigate the relationship between “CYP2C9” and “VKORC1” genotype and warfarin dose requirements.

CORONARY HEART DISEASE

Coronary heart disease (CHD) is a leading cause of mortality in the world *(Amber et al., 2009)*. It refers to the consequences of coronary artery disease (CAD), its clinical presentation includes angina pectoris, unsTable angina, painful and silent myocardial infarction (MI), and CAD deaths *(Mittal, 2005)*.

Pathogenesis of coronary heart diseases:

Cardiovascular disorders are the principal clinical manifestation of atherosclerosis *(Virmani et al., 2000)*, which is a disease of the inner layer of medium sized muscular arteries (such as coronary and carotid arteries) and large elastic arteries such as aorta and iliac vessels *(Glass and Witztum, 2001)*.

Advances in basic science have established a fundamental role for inflammation in mediating all stages of atherosclerosis from initiation through progression and ultimately the thrombotic compli-cations of atherosclerosis (Fig. 1) *(Mittal, 2005)*.

It is difficult to identify the factors responsible for the initiation of the atheroma lesion and/or the order in which these factors contribute to plaque

formation. Nevertheless, it is known that endothelial dysfunction and high levels of circulating cholesterol, as oxidized (ox) LDL, play a key role in the proinflammatory process that triggers the first steps in the development of atherosclerotic plaques *(Shashkin, 2005)*.

Whatever the cause, these steps are characterized by an initial reversible accumulation of lipid-laden macrophages in the subendothelial space as a consequence of the increasing migration of blood-derived monocytes. These cells accumulate at focal points within the vascular wall of medium and small size arteries driven by chemokines and adhesion molecules produced by the damaged endothelium *(Sukhova et al., 2004)*.

Monocytes differentiate in situ into macrophages which express membrane receptors such as Toll-like receptors and scavenger receptors that participate in the clearance of oxLDL *(Michelsen and Arditi, 2006)*. Lymphocytes can also transmigrate and accumulate within the arterial wall from the very earliest stages. As the inflammatory process becomes chronic, smooth muscle cells also start to migrate from the media into the intima layer of the vessel, in response to

chemokines and aided by the release of membrane metalloproteinase (MMPs) that enable them to break through the elastic lamina into the subendothelial space *(Yan and Hansson, 2007)*.

Persistence of inflammation creates a vicious circle of cell migration, differentiation of smooth muscle cells, production of chemotactic and proinflammatory mediators, and cell death leading to vascular wall remodelling and formation of a new layer called neointima (Fig.1) *(Hoofnagle et al., 2006)*.

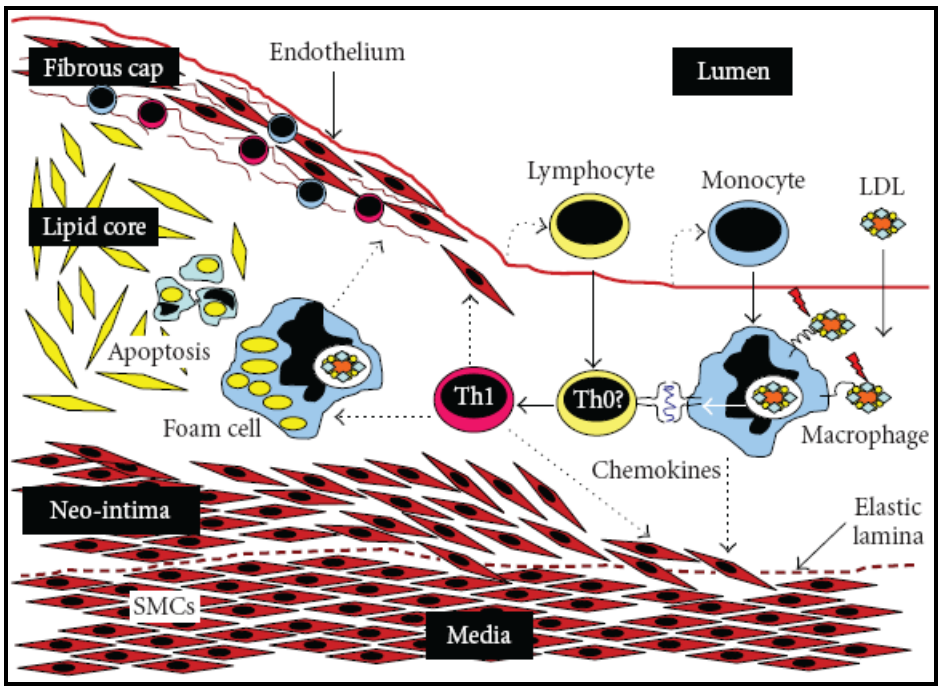

Fig. (1): Inflammatory basis of atherosclerotic plaque formation *(Hoofnagle et al., 2006)*.

Neointima formation is a complex phenomenon that occurs in response to vessel wall damage in which repair and injury mechanisms give birth to areas rich in proinflammatory cells and collagen deposition *(Hansson and Libby, 2006)*.

It is within these areas where a close contact between macrophages, dendritic cells (DCs), and lymphocytes has been reported. It has been speculated that the interaction between lymphocytes and DCs within the neointima is responsible for the development of local immune responses against exogenous and endogenous atherogenic antigens. These immune responses may contribute to cell death by apoptosis and accumulation of nondegradable cholesterol, contributing to the formation of the lipid core of the atherosclerotic plaque, However, the precise mechanisms involved are not yet clear *(Hansson and Libby, 2006)*.

Pathology of coronary heart disease:

The atherosclerotic lesions could be classified as types I to VI (Table 1), which range from minimal intimal change to changes associated with clinical manifestations (Fig. 2) *(Stary et al., 1995)*.

Table (1): Stary's classification of atherosclerosis

Type I	Isolate macrophage foam cells, no tissue injury
Type II	Fatty streak, foam cells lipid-laden smooth muscle cells under an intact endothelium.
Type III	Type II lesions with increased extracellular lipid and small lipid pool, microscopic evidence of tissue injury (preatheroma).
Type IV	Extensive lipid core, massive structural injury (atheroma)
Type V	Increased smooth muscle and collagen (fibroatheroma) Va, multiple lipid core; Vb, calcific; Vc, fibrotic.
Type VI	Thrombosis or hematoma Via, disruption of surface; VIb, hematoma; Vic, thrombosis.

(Stary et al., 1995)

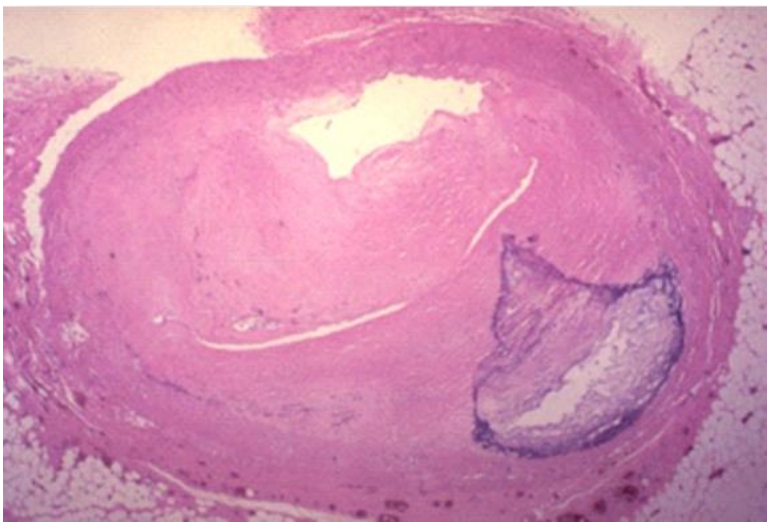

Fig. (2): Severe degree of narrowing in coronary artery. There is a large area of calcification on the lower right, appears bluish on this H&E stain *(Klatt, 2002)*.

Coronary heart disease risk factors:

Established risk factors:

١. *Cigarette smoking:*

Cigarette smoking has a clear cause-and-effect relationship with atherosclerotic disease, with the risk of myocardial infarction (MI) increasing with the number of cigarettes smoked *(Ockene et al., 2010)*.

Vasomotor dysfunction, alterations in thrombosis/fibrinolysis, and modification of lipids may mediate smoking-related vascular disease, with a central role of vascular inflammation and oxidative stress *(Ambrose and Barua, 2004)*.

Studies suggest that nicotine induces vasoconstriction, not only in coronary arteries but also in peripheral vessels, hypertension, pro-atherogenic effects, due to increase of platelet activation and fatty acids concentration, alterations of endothelial-cell shapes, as well as endothelial-cell proliferation *(Didilescu et al., ٢٠٠٩)*.

The use of tobacco products decreases high density lipoprotein cholesterol (HDL-C). Furthermore, tobacco smoke may adversely affect HDL metabolism and structure by modifying the activity of lecithin

cholesterol acyltransferase enzyme (LCAT). Due to the cardioprotective effect of HDL, these alterations may provide a mechanism by which cigarette smoking increases the risk for CAD *(Nagashima et al., 2007)*.

Smoking cessation alone can result in a 36% reduction in the relative risk of mortality in smokers who quit versus those who do not. The risk decreases rapidly after only 1 year of cessation, quitters have a lower relative risk of death from coronary heart disease (CHD) than do nonquitters, which decreases even further after 3yrs of cessation. Consequently, efforts to find effective treatments to enhance smoking cessation are of great importance. Psychosocial, pharmacological, and combined psychosocial and pharmacological intervention have been studied *(Ockene et al., 2010)*.

٢. *Dyslipidemia:*

Atherogenic dyslipidemia is a triad of elevated triglycerides, raised small LDL particles, and reduced HDL-C, and it is a risk factor for premature CHD. Typical patients have central obesity and insulin resistance and are physically inactive. Some patients with type 2 diabetes have atherogenic dyslipidemia. The management of atherogenic dyslipidemia includes

weight reduction in overweight patients and increased physical activity *(Mittal, 2005)*.

A- Familial Hypercholesteremia:

Familial hypercholesteremia (FH) has a strong association with premature atherosclerosis. The primary genetic defect is an autosomal dominant disorder in which a mutation is passed from a parent to roughly half the children. The defect leads to the production of a poorly functioning LDL receptor, LDL production is increased, and typically there are tendinous xanthomas, arcus and xanthelasma. The cholesterol level is about 345mg/dL. Untreated, the majority of male heterozygotes and half of the female heterozygotes will have a clinical CHD before the age of 60yrs *(Mittal, 2005)*.

Oxidized (ox) LDL has been detected in plasma of coronary heart disease (CHD) patients, which might play a key role in the generation of inflammatory processes in atherosclerotic lesions of all stages. It has also been shown that oxLDL is involved in the very early yet critical steps of atherogenesis, such as endothelial injury, expression of adhesion molecules, and leukocyte recruitment and retention, as well as foam cell and thrombus formation *(Meisinger et al., 2005)*.

B- Triglycriders:

Many epidemiological studies have reported associations between serum triglyceride concentrations and the risk of coronary heart disease (CHD), but their relevance to disease remains uncertain *(Sarwar et al., 2007)*.

In the largest and most comprehensive epidemiological assessment so far in Western populations, moderately strong associations were consistently observed between triglyceride concentrations and CHD risk, as well as moderately high levels of reproducibility in triglyceride values within individuals over time. These data renew the importance of further investigations to help assess the nature of any independent associations between triglycerides and CHD *(Sarwar et al., 2007)*.

٣. *Hypertension:*

Hypertension is defined as systolic blood pressure (BP) ≥140mmHg or diastolic BP ≥90mmHg or when the individual is taking an antihypertensive agent. There is a direct relationship between hypertension and coronary heart disease (CHD).The incidence of CHD among individuals with hypertension is equal to all other adverse outcomes combined *(Chobanian et al., 2003)*.

Severe hypertension may directly damage arterioles and cause atherosclerosis. The risk of cardiovascular events is increased two to three times in men and women with hypertension. It is estimated that 14% of deaths from CHD in men and 12% of deaths from CHD in women are due to hypertension *(Chobanian et al., 2003)*.

In people over the age of 50yrs, systolic BP of >140mmHg is a more important cardiovascular disease risk factor than diastolic BP. Beginning at BP ١١٥/٧٥mmHg, the CVD risk doubles for each increment of BP of 20/10mmHg *(Levy, 2004)*.

٤. *Diabetes mellitus:*

Diabetes mellitus is associated with a 2 to 4 fold increase in the risk of coronary heart disease, and among people with diabetes, about two thirds of deaths are due to cardiovascular disease, including ischemic heart disease, congestive heart failure, and stroke. The increase in mortality for patients with diabetes mellitus after myocardial infarction is seen both acutely and in a sustained manner and holds true for both men and women *(Goldfine and Beckman, ٢٠٠٨)*.

٥. *Obesity:*

The prevalence of overweight and obesity is increasing in most industrialized countries. A high risk of coronary heart disease is among the well-established adverse health effects associated with excess weight. Hypertension, hypercholesterolemia, and diabetes are among the clinical conditions that are important mediators of this association *(Jensen et al., 2008)*.

Thus, obesity is an appropriate target for primary prevention efforts because its modification has the potential to influence several important clinical conditions along the causal pathway. However, it is clear that achieving weight loss or preventing weight gain with aging is difficult for most individuals. Therefore, investigations of behavioral modifications that might reduce the impact of obesity on risk of morbidity and mortality could have potentially great public health impact *(Jensen et al., 2008)*.

٦. *Physical inactivity:*

Abdominal obesity and physical inactivity are recognized cardiovascular risk factors; although they are interrelated, studies have reported that their