

UNIVERSITI TEKNOLOGI MARA

**DEVELOPMENT OF A POLYMERASE CHAIN
REACTION METHOD FOR DETECTION OF A
VARIANT OF VITAMIN K RECEPTOR, C1173T**

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ABSTRACT

Background: Warfarin is an oral anticoagulant indicated for thromboembolism disorders. Warfarin antagonizes vitamin K epoxide reductase specifically the VKORC1 subunit which is important in vitamin K recycle. However, the outcomes of warfarin among individuals are highly variable and one of the factors causing inter-individual variabilities is due to genetic polymorphism of VKORC1 1173C>T in intron 1. Patients with either heterozygous (1173CT) or homozygous (1173TT) mutation need lower average dose of warfarin to achieve therapeutic PT-INR.

Aim: The purpose of this study is to develop a convenient and effective polymerase chain reaction (PCR) based method to detect variant of VKORC1, C1173T.

Method: Optimization of direct-3-step polymerase chain reaction for detection of C1173T of VKORC1 was performed. The PCR was conducted under different annealing temperatures using TaKaRa PCR Thermal Cycler Dice™ Gradient model to determine the optimal annealing temperature for this method. DNA sequencing was also performed on five samples to validate the PCR method. The optimized method is then used to genotype twenty DNA samples which were selected randomly from the DNA pool.

Results: The PCR based method was successfully optimized according to the result of two positive controls. Genotyping of twenty randomly selected DNA samples showed all the samples were heterozygous for variant 1173CT.

Conclusion: Polymorphism at position 1173 in intron 1 of VKORC1 significantly affects patient's response to warfarin and bleeding occurs even at normal dose. Hence, this optimized PCR method can be utilized to genotype patient for C1173T variant prior to prescribing warfarin for personalized dose. Together with CYP2C9 genotyping, this approach can help to reduce the risk of bleeding without compromising the therapeutic effect of warfarin.

CHAPTER 1

INTRODUCTION

Pharmacogenomic is the study how the individual's pharmacological responses to drug are affected by genetic profile of the individual. Information derived from pharmacogenomic allows physician and pharmacist to predict and individualize the effective dose of a drug for its therapeutic and toxic effects prior to administration of the drug to a patient (Yin & Miyata, 2007).

Vitamin K is a fat-soluble vitamin. It serves as an essential cofactor that catalyzes carboxylation of glutamic acid residues on vitamin K-dependent proteins (Kimura *et al.*, 2006). Examples of the vitamin K-dependent proteins include the coagulation proteins such as factors II (prothrombin), VII, IX and X (Page *et al.*, 2002). Vitamin K undergoes a cycle of oxidation and reduction to maintain the level of vitamin K useful for carboxylation in the body. In the reaction, vitamin K is converted to vitamin K epoxide by gamma-glutamyl carboxylase before being recycled back to its reduced form, vitamin K hydroquinone and vitamin K by another enzyme known as vitamin K epoxide reductase (Page *et al.*, 2002).

Warfarin is an anticoagulant derived synthetically from coumarin. It is a chemical constituent found naturally in certain plants such as woodruff (*Galium*