UNIVERSITI TEKNOLOGI MARA

THE IMPLICATION OF THE POLYMORPHISM OF *COX-1*, *UGT1A6*, AND *CYP2C9* AMONG CARDIOVASCULAR DISEASE (CVD) PATIENTS TREATED WITH ASPIRIN

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AUTHOR'S DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of University Teknologi MARA. It is original and the result of my own work, unless otherwise indicated or acknowledged as referenced work. This thesis has not been submitted to any other academic or non-academic institution for any other degree or qualification.

I, hereby acknowledge that I have been supplied with the Academic Rules and Regulations for Postgraduate, Universiti Teknologi MARA, regulating the conduct of my study and research.

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ABSTRACT

Acetylsalicylic acid (ASA) or aspirin is a pro-drug of salicylates which has significant effect in reducing the cardiovascular events. Potential polymorphic enzymes responsible for the pharmacokinetic variations of ASA include cyclooxygenase-1 (COX-1), UDP-glucuronosyltransferase (UGT1A6) and P450 (CYP) (CYP2C9). The main objective of this study is to determine the types and frequencies of variants of genes encoding *COX-1 (A-842G), UGT1A6 (UGT1A6*2*; *A541G* and *UGT1A6*3*; *A522C*) and *CYP2C9* (*CYP2C9*3*; *A1075C*) in the three major ethnic groups in Malaysian population (Malays, Chinese and Indian). The relevance of those polymorphisms in patients with cardiovascular disease was investigated. The project was approved by relevant Research Ethics Committee. A total of 165 patients with cardiovascular disease who were treated with 75-150 mg daily dose of aspirin and 300 healthy volunteer participants were recruited. DNA was extracted from the blood samples and genotyped for the single nucleotide polymorphisms (SNPs) using allele specific polymerase chain reaction (AS-PCR). All statistical analysis was performed using SPSS software version 2.0. p-values ≤ 0.05 were considered statistically significant. The association between genotype and adverse effect of aspirin therapy was estimated using odd ratio (OR). For *UGT1A6*, the pair-wise of both SNPs linkage disequilibrium (LD) was computed using Haploview. The variants frequencies for *UGT1A6*2*,**3* and *CYP2C9*3* were 22.83%, 30.0% and 6.50%, respectively; while *COX-1 (A-842G)* was absent. We found that the genotype for both polymorphisms in *UGT1A6* and *CYP2C9*3* were significantly different between Indians, Malays and Chinese ethnic. The level of bilirubin and triglyceride of patients with different genotypes of *UGT1A6* and *CYP2C9*3* were significantly different (p-value < 0.05), respectively. In addition, *CYP2C9*3* was found associated with gastritis events with odd ratio (OR) 6.8 (95 % Cl: 1.39 – 33.19; $P = 0.033$). SNPs of *A541G* and *A522C* among the Indians show strong linkage disequilibrium with D' value of 1.0. This study had identified significant association of genetic polymorphisms of *UGT1A6* and *CYP2C9*3* with gastritis event, level of bilirubin and triglyceride. Screening of patients with defective genetic variants could be of relevance in the clinical setting in identifying patients at risk of aspirin induced adverse effect. However, a iiiandomized, clinical study of bigger sample size would be needed before it is translated to clinical routine.

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CHAPTER ONE
INTRODUCTION

1.1 INTRODUCTION

Acetylsalicylic acid (ASA) or aspirin is a pro-drug of various forms of salicylates which has anti-inflammatory activities (Wu, 2000). This non-steroidal anti-inflammatory drug (NSAIDs) is the most commonly used antiplatelet in prevention or treatment of cardiovascular disease, also as an over-the-counter medication for the treatment of pain and inflammation. It has been shown that aspirin therapy reduced the risk of vascular death and the risk of non-fatal myocardial infarction and stroke (Hennekens et al., 1997).

Aspirin (75-150 mg, daily) has been found to reduce risk of vascular events by approximately 32% in patients with cardiovascular disease (CVD) (Baigent et al., 2002). However, a substantial number of people still suffer from cardiovascular events, although they were already on aspirin treatment (Eikelboom *et al.*, 2002). For instance in Malaysia, although aspirin are available, CVD is still the foremost cause of mortality. This phenomenon may be due to inter-individual variability in pharmacokinetic (PK) or pharmacodynamic (PD) resulting in different drug responses (Agúndez et al., 2009). Genetic variations have been shown to influence the efficacy of various drugs including aspirin. Those polymorphic enzymes potentially affecting PK-PD of aspirin include cyclooxygenase-1 (COX-1), UDP-glucuronosyltransferase 1A6 (UGT1A6) and P450 (CYP2C9) enzyme.

COX-1 is an enzyme that is directly and irreversibly inhibited by aspirin which consequently decrease the formation of precursors of prostaglandins and thromboxanes. Genetic variants in *COX-1* gene were suggested to modulate arachidonic acid-induced platelet aggregation and serum thromboxane B2 (TXB2) levels in patients treated with aspirin. Moreover, the polymorphic UDP-glucuronosyltransferase (UGT1A6) and P450 CYP2C9 enzyme is reported to be associated with altered enzyme function which affect aspirin metabolism and efficacy. All these polymorphic enzymes could modulate the therapeutic effect of aspirin in treating or preventing CVD. These polymorphic enzymes are believed to cause increase risk of adverse effect. It is tempting to reason that; the carriers of variant