CHAPTER I

INTRODUCTION

1.1. Rationale and Significance of the Problem

Cardiovascular complications such as myocardial infarction (MI), cerebrovascular accident (CVA), and cardiovascular death are the leading cause of morbidity and mortality in patients with type 2 diabetes mellitus (T2DM) [1]. In a prospective cohort study, non-diabetic subjects with prior coronary heart disease had a 7-year event rate of approximately 18.8%, whereas diabetic subjects without prior coronary heart disease had an event rate of approximately 20% [2]. This result demonstrates that diabetic patients carry the same level of risk of acute coronary events as do non-diabetic patients with previous acute myocardial infarction. Current U.S. federal guidelines recommend that patients with diabetes be treated just as aggressively as patients without diabetes who have already experienced a coronary heart disease event.

Platelets play an important role in the pathogenesis of cardiovascular complications in diabetic patients. Increased platelet activity is critically involved in the increased thrombogenic potential among diabetic patients [3]. Diabetic platelets are larger, have a greater number of glycoprotein (GP) IIb/IIIa receptors and aggregate more readily to known agonists in vitro than platelets from non-diabetic patients [4]. Moreover, platelets in diabetic patients with and without complications and even in newly diagnosed have been demonstrated hyperaggregation as well. Platelets from people with diabetes are often hypersensitive in vitro to platelet aggregating agents [5]. A major mechanism is increased production of thromboxane, a potent vasoconstrictor and platelet aggregator [6]. Investigators have found evidence *in vivo* of excess thromboxane release in T2DM patients with cardiovascular disease. To diminish platelet aggregation in order to prevent acute coronary syndrome, antiplatelet therapy is usually prescribed.

Acetylsalicylic acid (Aspirin, ASA) is the most widely used and least expensive antiplatelet agent in existence. Low concentrations of aspirin irreversibly acetylate platelet cyclooxygenase, an enzyme responsible for the formation of eicosanoids including thromboxane A_2. Because thromboxane A_2 promotes platelet aggregation, the inhibition of cyclooxygenase by aspirin decreases thromboxane generation in platelets, and therefore platelet aggregability, throughout the platelet's lifetime [7]. Presently, aspirin is considered the treatment of choice for secondary prevention in patients who have evidence of the large vessel disease and has been considered for primary prevention in adults at high risk of cardiovascular events. The antithrombotic Trialists' Collaboration compiled a meta-analysis of 65 trials using aspirin in high-risk patients and found 22% odds reduction in vascular events in the aspirin-treated groups [8]. Aspirin is also a very effective therapy for patients suffering an acute myocardial infarction. As demonstrated by the Second International Study of Infarct Survival (ISIS-2) trial, acute aspirin administration reduced mortality by 22%, a comparable (and importantly additive) effect to thrombolytic therapy [8]. Aspirin also has an important role in primary prevention of cardiovascular events. The Physician's Health Study demonstrated a 44% reduction in the incidence of a first myocardial infarction in middle-aged men treated with aspirin compared with placebo over a 5-year follow-up period [9].

Several studies have demonstrated a beneficial role for antiplatelet therapy with aspirin in primary or secondary prevention of coronary heart disease in patients with diabetes. The antithrombotic Trialists' Collaboration compiled a meta-analysis of 65 trials using aspirin in high-risk patients and found 22% odds reduction in vascular events in the aspirin-treated groups [10]. The diabetic substudy in this meta-analysis demonstrated a significant reduction in cardiovascular events for those diabetic patients treated with aspirin [10]. Subgroup analysis of a diabetic cohort of the US Physician's Health Study demonstrated a reduction in the myocardial infarction rate from 10.2% for the placebo-treated group to 4.0% for the aspirin treated group [11].

Nowadays, the American Diabetes Association (ADA) recommended aspirin (75-162 mg/day) as a secondary prevention strategy in diabetic patients with a history of myocardial infarction, vascular bypass procedure, stroke or transient ischemic attack and angina.

ADA also recommended to use 75-162 mg/day aspirin as a primary prevention in T2DM patients at increased cardiovascular risk, including those who are > 40 years of age or who have additional risk factors (family history of cardiovascular disease, hypertension, smoking, dyslipidemia, or albuminuria) [12].

Nevertheless, aspirin therapy in diabetic patients showed less benefit than the general patient population did. A meta-analysis of 287 trials of antiplatelet therapies before 1997 [13] demonstrates that, in a total of 5,126 patients with known diabetes, the overall reduction in incidence of vascular events in the control group versus the antiplatelet therapy group was only 7% (standard error 8%), which is far less than the 22% average. Recently, the Primary Prevention Project (PPP) study [14] that non-diabetic people had a 41% reduction in heart disease-related death with aspirin versus 10% in diabetic patients. The investigators suggested that the antiplatelet effects of aspirin in diabetic patients are overwhelmed by aspirin-insensitive mechanisms of platelet activation.

Recent data suggest that antiplatelet effects of aspirin may not be equivalent in all patients. Measurements of platelet aggregation, platelet activation, bleeding time, and thromboxane metabolites have confirmed wide variability in patients' responses to aspirin therapy. Based on this clinical and laboratory evidence of absent responses to aspirin treatment in some patients, the concept of aspirin resistance has emerged. Several studies have been reported aspirin resistance in various populations. However, since there was no standard definition of aspirin resistance, frequency of aspirin resistance is highly variable due to the method used to assess aspirin resistance and population of study. In one study, the frequency was 5.2% in patients with stable cardiovascular disease [15]. In another study of patients with congestive heart failure, frequency of aspirin resistance was 56.8% [16].

Patients with non-response to aspirin have been reported to associate with the risk of major cardiovascular events. Prospective study of Gum et al.[17] has reported that patients with stable cardiovascular disease whose platelets were not responded to 325 mg aspirin had a three-fold risk of death, myocardial infarction, or stroke compared with the patients who were aspirin responders. Nevertheless, the association was found only with patients who were defined aspirin resistance by optical platelet aggregometry, a conventional method. They also measure

platelet activation by Platelet Function Analyzer (PFA-100), a newer, point of care device. But aspirin resistant defined by optical aggregation and PFA-100 showed poor correlation. Prevalence of aspirin resistance defined by PFA-100 was two-fold higher than by optical platelet aggregation. Moreover, aspirin resistant patients by PFA-100 were not associated with the further outcomes. These studies indicated that point of care devices may overestimate prevalence of aspirin resistance and the optical platelet aggregation should be used to measure aspirin resistance due to its ability to predict risk of cardiovascular events. Recently, two studies reported that prevalence of aspirin resistance in patients with T2DM were 21.5% and 16.2% by point of care methods, PFA-100 and Rapid Platelet Function Assay, respectively.

There are several possible mechanisms to explain the reduced sensitivity of blood platelets to aspirin. These proposed mechanisms deal with alternate pathways of platelet aggregation that may not be blocked by aspirin, dose-related resistance, and receptor polymorphisms. In diabetes, there are many factors involved in increase platelet reactivity that may be a reason of aspirin resistance in DM. *In vitro* study of Watala et al.[18] demonstrated that reduced response of platelets in diabetic subjects to aspirin was associated with a higher level of HbA1c, lower concentration of HDL-cholesterol and a higher total cholesterol concentration. HbA1c levels, indicator of the extent of glycation in diabetes, are directly linked to the concentration of plasma glucose, platelet activation measured as platelet surface expression of CD62P and CD63, and cardiovascular disease. For increased HbA1c level in every 1%, there is a 24% increase of risk of cardiovascular disease and total mortality[19]. Insulin level and insulin resistance in T2DM have also been reported that have effect on platelet function. Platelets have a functional insulin receptor and insulin can reduce platelet function both *in vitro* and *in vivo* at physiological concentrations [20]. But this beneficial action is impaired in obesity and in obese T2DM who were insulin resistance [21].

To date, there is no data to determine aspirin resistant in type 2 diabetic patients by the conventional method, optical platelet aggregation. This study was conduct to assess platelet aggregation in Thai patients with T2DM who were taking 60-300 mg/d aspirin by optical platelet aggregation using arachidonic acid and ADP as agonists. The aims of this study were to evaluate frequency of aspirin resistance and to clarify factors associated platelet response to

aspirin in T2DM. In addition, platelet aggregation and serum thromboxane B_2 level in patients who received different dosages of aspirin were compared to define the relationships.

1.2. Objectives

1. To determine frequency of aspirin resistance in type 2 diabetic patients treated with aspirin.
2. To identify association of different dosages of aspirin in type 2 diabetic patients on platelet aggregation and serum thromboxane B_2.
3. To clarify clinical factors related to type 2 diabetes that effect on the reduction of antiplatelet effect of aspirin.

1.3. Scope of the Study

1.3.1 The population in this study is type 2 diabetic patients who attended Diabetic Clinic and General Medicine Clinic, Ramathibodi hospital during the time of study.

1.3.2 Variables in this study consist of

1.3.2.1. Dependent variable: Platelet aggregation

1.3.2.2. Independent variables:

1.3.2.2.1 Demographics: age, gender, body mass index (BMI), waist, hip, health behaviors, co-morbid conditions, and co-medications.

1.3.2.2.2. Plasma lipid: high density lipoprotein (HDL), low density lipoprotein (LDL), triglyceride (TG), and total cholesterol (TC)

1.3.2.2.3. Blood glucose: fasting plasma glucose (FPG) and glycosylated hemoglobin (HbA1c)

1.3.2.2.4. Insulin level and Insulin resistance (HOMA-IR)

1.3.2.2.5 Hematologic profile: platelet count, hemoglobin (Hb), hematocrit (Hct), mean platelet volume (MPV)

1.3.2.2.6. Dose of aspirin

1.3.2.2.7. Serum thromboxane B_2 level

1.3.2.3. Control variables:

1.3.2.3.1. Insulin therapy

1.3.2.3.2. Other drugs affecting platelets: other NSAIDs, other antiplatelets, heparin, and warfarin.

1.3.2.3.3. Liver or renal dysfunction

1.3.2.3.4. Bleeding disorders

1.3.2.3.5. Alcohol consumption

1.3.2.3.6. Cigarette smoking

1.4. Operational definition

Platelet aggregation:	An ability of platelet to link to another platelet to form platelet aggregates after induced by ADP and arachidonic acid *in vitro*. The method to assess platelet aggregation in this study is optical aggregometry.
Low dose aspirin:	Dose of aspirin for primary and secondary therapy to prevent cardiovascular events in diabetic and non-diabetic patients. The dosages used are ranged from 60 to 300 mg/day
Aspirin resistance:	Failure of aspirin to inhibit platelet aggregation *in vitro*. With 10 uM ADP, a mean aggregation is > 70% and a mean aggregation is > 20% with 0.5 mg/ml arachidonic acid.
Insulin resistance:	An impaired biological response to insulin assessed by using the computer model of homeostatic model assessment (HOMA), a method for assessing β-cell function and insulin sensitivity from plasma glucose and insulin concentration. The computer model will give a value for insulin sensitivity expressed as HOMA-%S (where 100% is normal). The insulin resistance is the reciprocal of HOMA-%S.
Type 2 Diabetes mellitus:	Non-insulin-dependent diabetes mellitus, diagnosed by American Diabetes Association (ADA) criteria which are;