CHAPTER III

METHODOLOGY

3.1. Subjects

3.1.1 Aspirin-treated type 2 diabetic patients

The population of this study was type 2 diabetic patients. who were treated at diabetic clinic, family medicine clinic and heart clinic in Ramathibodi hospital. The subjects were recruited from consecutive patients appointed for follow-up treatment between December 3, 2005 and December 28, 2006.

Inclusion criteria were as follows:

- Was diagnosed to have type 2 diabetes by the physicians
- Had taking low dose (60-325 mg/d) aspirin for at least 2 week with well compliance.
- Age between 30-80 years.

Exclusion criteria included:

- Insulin therapy;
- Use non-steroidal anti-inflammatory drugs (NSAIDs), ticlopidine, clopidogrel, dipyridamole or warfarin for 2 weeks prior to the study;
- Administration of heparin or low-molecular-weight heparin within 24 h before the study;
- Family or personal history of bleeding disorders;
- Platelet count < 150 x 103/μl;
- Hemoglobin < 8 g/dl;
- Malignancy;

- Had major surgery within 1 week before enrollment;
- Smoking within 1 day prior to the examination;
- Alcohol taking within 2 days prior to the examination;
- AST and/or ALT > 3 times of the upper limit;
- Serum creatinine > 3 mg/dl.

3.1.2 Control subjects

Type 2 diabetic patients with above inclusion and exclusion criteria but were not prescribed with aspirin were included into the study as a control group to be compared with aspirin-treated type 2 diabetic patients.

Written inform consent was obtained from all subjects. The study protocol was reviewed and approved by the Ethics Committee of the Faculty of Medicine, Ramathibodi hospital, Mahidol University, Bangkok.

3.2. Data Collection

Patients were called at one week and 2 days before the appointment to confirm about their appointment and to remind them to take their medicine daily, and do not forget to fast during the night before and in the morning of the test. Patients were interviewed on the day of recruitment, the day that a phone call was made at the day of the test to check for the compliance of aspirin.

Demographic data, concomitant medication and co-morbid conditions were recorded. Blood samples were obtained 24 $\pm$ 2 hours after the administration of the last dose of aspirin. Thirty milliliters (ml.) of blood were drawn from antecubital vein with butterfly scalp vein needle and were transferred into the following tubes:

1. 2 ml of blood were transferred into a plastic tube containing sodium fluoride for blood sugar determination,

2. 3 ml of blood were transferred into each of two plastic tubes containing EDTA for hemoglobin A1c (HbA1c) and complete blood count (CBC) determination,

3. 6 ml of blood were transferred into a plastic tube for determination of serum lipid profile,

4. 10.8 ml of blood were transferred into a plastic tube containing 1.2 ml of 3.2% sodium citrate for platelet aggregation study,

5. The rest of the blood samples were collected into a plastic tube for determination of serum thromboxane B_2. Samples were centrifuged at $4^{\circ}C$ for 10 minutes at 3000 rpm, and the serum was transferred into microtubes and storage at $-80^{\circ}C$ until analyzed.

3.3. Laboratory Assays

Platelet function was assessed by optical platelet aggregation using a Chrono-log Lumi-Aggregometer (model 560 Ca, Chronogog, Inc.). Platelets in platelet rich plasma (PRP) were stimulated with 1 mmol/l of arachidonic acid and 10 umol/l of ADP. Aggregation was expressed as the maximal percent change in light transmittance from baseline, using platelet-poor plasma as a reference. Each sample was analyzed in duplicate. All platelet aggregation tests were performed within two hours after blood collection. Detail of the determination of platelet aggregation is described in Appendix A.

Serum TXB_2 level was determined by enzyme immunoassay (EIA) technique (Thromboxane B_2 EIA Kit, Cayman chemical Co., cat no. 519031.1). Detail of the determination of serum level TXB_2 of is described in Appendix B.

Serum insulin level was analyzed by radioimmunoassay at laboratory of endocrinology unit, Department of medicine, Ramathibodi hospital. HOMA-IR was calculated from HOMA 2 model software program (Diabetes Research Laboratories, Oxford, U.K.) Fasting plasma glucose, HbA1c, lipid profile and complete blood count were sent to analyzed at the central lab of Ramathibodi hospital.

3.4 Data Analysis

Patient characteristics and laboratory data were presented as mean $\pm$ S.D. Gender, health behaviors, co-morbid conditions and co-medication were summarized by frequencies and percentages. Serum TXB_2 level was summarized by medians and interquartile (IQ) ranges. Student's t test was used to compare characteristics and clinical data of diabetic with aspirin group and diabetic with no aspirin group. Comparisons of gender, health behaviors, co-morbid conditions and co-medication between the 2 groups were done by Chi-square test. Comparison of serum TXB2 level in aspirin group and non-aspirin group was performed by Mann-Whitney U test. Analysis of variance (ANOVA) technique and Tukey post hoc comparison were used to compare platelet aggregation and clinical data between dosages of aspirin and between aspirin resistance, aspirin semiresponders and aspirin sensitive. The relationship among platelet aggregation and patient characteristics were evaluated by Pearson product moment correlation coefficient. A two tailed p value of < 0.05 was considered statistically significant. Data was analyzed using computer programs SPSS for windows (Statistical Package for Social Science for windows) version 11.5.