

## CHAPTER IV

### RESULTS

Platelet aggregation test, serum thromboxane B<sub>2</sub>, serum insulin level and other clinical factors of all subjects were measured. Results of the study are presented in five parts which are (1) Patient characteristics; (2) Effect of aspirin on platelet aggregation; (3) Effect of aspirin on serum thromboxane B<sub>2</sub>; (4) Prevalence of aspirin resistance; and (5) Factors influencing platelet response to aspirin

#### 4.1. Patient Characteristics

The total number of patients who were eligible and agreed to participate in this study was 129. There were 97 diabetic patients who were taking chronic low-dose aspirin and 32 diabetic patients who were not taking any antiplatelet agents.

Basic characteristics of diabetic subjects in aspirin and non-aspirin group were shown in Table 1. Mean age of the subjects in aspirin group was  $58.7 \pm 9.0$  years old whereas in non-aspirin group was  $56.8 \pm 10.0$  years old. There were 54 women and 43 men in aspirin treated group, whereas in non-aspirin group, 20 were women and 12 were men. Patients in aspirin group were significantly heavier than in non-aspirin group ( $p=0.009$ ) but BMI was not statistically significant ( $p=0.056$ ). Blood pressure, fasting plasma glucose, lipid profile and hematological profile of both groups were comparable.

Health behaviors of the subjects were shown in Table 2. Most of the patients in both groups were not smoke or drink alcohol. Approximately half of both groups did not regularly exercise.

As shown in Table 3, aspirin was prescribed in diabetic patients who had hypertension and coronary artery diseases more than those who did not have these conditions. None of the patients in non-aspirin group had undergone cardiac intervention, cerebrovascular accident, peripheral vascular disease or foot ulcer.

Table 1 Characteristics of the subjects

Characteristics	Non-aspirin group (n=32)	Aspirin group (n=97)	p-value
Age (year)	56.8 ± 10.0	58.7 ± 9.0	0.327
Sex			0.502
Female	20 (62.5%)	54 (55.7%)	
Male	12 (37.5%)	43 (44.3%)	
Weight (kg)	62.87 ± 9.62	68.83 ± 13.31	0.009
Height (cm)	157.04 ± 7.21	159.21 ± 8.72	0.214
BMI (kg/m <sup>2</sup> )	25.56 ± 3.71	27.09 ± 3.97	0.056
Waist (cm)	86.97 ± 8.78	90.46 ± 11.04	0.112
Hip (cm)	97.77 ± 6.75	99.69 ± 7.46	0.206
Systolic BP (mmHg)	132.52 ± 10.31	131.77 ± 10.76	0.738
Diastolic BP (mmHg)	82.00 ± 8.38	80.49 ± 7.9	0.372
Duration of DM (year)	8.58 ± 12.71	6.53 ± 4.58	0.186
FPG (mg/dL)	140.19 ± 50.54	134.59 ± 31.28	0.458
HbA1c (%A1c)	7.82 ± 1.96	7.26 ± 1.118	0.130
Insulin (uIU/ml)	9.38 ± 3.90	11.56 ± 8.52	0.677
HOMA-IR	1.57 ± 0.67	1.89 ± 1.35	0.458
Total Cholesterol (mg/dL)	184.06 ± 36.83	173.79 ± 37.69	0.181
Triglyceride (mg/dL)	143.34 ± 10.13	142.71 ± 69.39	0.934
HDL (mg/dL)	43.34 ± 10.13	42.11 ± 12.46	0.614
LDL (mg/dL)	113.44 ± 32.51	104.90 ± 28.80	0.161
Hb (g/dL)	12.93 ± 1.42	12.96 ± 1.39	0.915
Hct (%)	39.01 ± 3.98	38.46 ± 4.09	0.509
Platelet (x1000)	278.78 ± 81.43	286.62 ± 71.03	0.603
MPV (fL)	8.51 ± 1.22	8.16 ± 8.72	0.078

Data represent in Mean ± SD. except for female represent in count (%).

\*\*  $p < 0.01$

Table 2 Health behaviors

<b>Characteristics</b>	<b>Non-aspirin group (n=32)</b>	<b>Aspirin group (n=97)</b>	<b>p-value</b>
Smoking			0.541
No	24 (77.4%)	66 (68.0%)	
Used to	5 (16.1%)	25 (25.8%)	
Current smoking	2 (6.5%)	6 (6.2%)	
Alcohol consumption			0.725
No	20 (64.5%)	55 (56.7%)	
Used to	8 (25.8%)	32 (33.0%)	
Current drinking	3 (9.7%)	10 (10.3%)	
Exercise			0.757
Rarely	16 (51.6%)	50 (51.5%)	
Sometimes	6 (19.4%)	24 (24.7%)	
Usually	9 (29.0%)	23 (23.7%)	

Data represent in count (%).

Table 3 Co-morbid conditions

<b>Disease</b>	<b>Non-aspirin group (n=32)</b>	<b>Aspirin group (n=97)</b>	<b>p-value</b>
Hypertension	16 (50.0%)	74 (76.3%)	0.005
Dyslipidemia	22 (68.8%)	72 (74.2%)	0.546
Coronary artery diseases	1 (3.1%)	27 (27.8%)	0.003
Cardiac intervention	0	10 (10.3%)	0.059
CVA	0	6 (6.2%)	0.150
PVD	0	1 (1.0%)	0.564
Foot ulcer	0	2 (2.1%)	0.413
Retinopathy	3 (9.4%)	15 (15.5%)	0.389

Data represent in count (%).

Concurrent medications used were shown in Table 4. Metformin, thiazolidinediones (TZD), statins, beta blockers, angiotensin converting enzyme inhibitors (ACEIs) and diuretics were more frequently used in aspirin group than in non-aspirin group. None of patients in non-aspirin group used nitrates or ranitidine.

Table 4 Medications

<b>Medication</b>	<b>Non-aspirin group (n=32)</b>	<b>Aspirin group (n=97)</b>	<b>p-value</b>
Sulfonylurea	22 (68.8%)	75 (77.3%)	0.330
Metformin	22 (68.8%)	84 (86.6%)	0.022
TZD	1 (3.1%)	17 (17.5%)	0.041
Alpha-glucosidase inhibitors	1 (3.1%)	5 (5.2%)	0.636
Statins	15 (46.9%)	71 (73.2%)	0.006
Fibrates	1 (3.1%)	7 (7.2%)	0.674
Beta-blockers	6 (18.8%)	43 (44.3%)	0.010
Ca Channel blockers	5 (15.6%)	29 (29.9%)	0.112
ACEIs	4 (12.5%)	58 (59.8%)	0.000
Diuretics	4 (12.5%)	29 (29.9%)	0.050
Nitrates	0	7 (7.2%)	0.118
Omeprazole	1 (3.1%)	9 (9.3%)	0.259
Ranitidine	0	3 (3.1%)	0.314

Data represent in count (%).

Dosage of aspirin prescribing in aspirin group are listed in Table 5, there were 75 patients (77.3%) taking 60 mg aspirin (Aspent-M<sup>®</sup>) once daily, 16 patients (16.5%) were taking 300 mg (Aspent<sup>®</sup>) once daily, 3 patients (3.1%) were taking 300 mg aspirin every other day, 2 patients (2.1%) were taking 60 mg aspirin 2 tablets a day and another patient was taking 100 mg aspirin (Cardiprin-100<sup>®</sup>).

Table 5 Dosage of aspirin

Dosage of aspirin	Number of patients	%
60 mg/d	75	77.3
100 mg/d	1	1.0
120 mg/d	2	2.1
150 mg/d	3	3.1
300 mg/d	16	16.5
Total	97	100

Of 97 patients in aspirin group, seventeen (17.5%) patients used aspirin as secondary prevention, whereas 80 (82.5%) patients used as primary prevention. Most (80.0%) of patients who used aspirin as primary prevention, received 60 mg/d aspirin. Among patients who use aspirin as secondary prevention, four (23.5%) of them received 300 mg/d aspirin and 11 (64.7%) patients received 60 mg/d aspirin. Dosage of aspirin was not significantly different between the group of primary and secondary prevention ( $p=0.293$ ) (Table 6).

Table 6 Dosage of aspirin in primary and secondary prevention

Dosage of aspirin	Primary prevention	Secondary prevention	p-value
	(n=80)	(n=17)	
60 mg/d	64 (80.0)	11 (64.7)	0.293
100 mg/d	1 (1.3)	0	
120 mg/d	2 (2.5)	0	
150 mg/d	1 (1.3)	2 (11.8)	
300 mg/d	12 (15.0)	4 (23.5)	

Data represent in count (%).

Table 7 Dosage of aspirin in cardiovascular disease

Conditions	n	Dosage of aspirin (mg/d)				
		60	100	120	150	300
CAD	27	17 (63.0)	0	0	3 (11.1)	7 (25.9)
Cardiac intervention	10	7 (70.0)	0	0	2 (20.0)	1 (10.0)
CVA	6	2 (33.3)	0	0	1 (16.7)	3 (50)

Data represent in count (%).

CAD = Coronary artery disease, CVA = Cerebrovascular accident

## 4.2. Effect of Aspirin on Platelet Aggregation

### 4.2.1 Platelet aggregation in diabetic patients with aspirin compared with diabetic patients with no aspirin.

#### 4.2.1.1 Platelet aggregation induced by arachidonic acid

Arachidonic acid is an aggregating agent investigating platelet function regarding effect of aspirin since mechanism of aspirin was to inhibit cyclooxygenase enzyme which convert arachidonic acid to prostaglandins including thromboxane. In this study, platelet aggregation was investigated in diabetic patients who were taking low-dose aspirin compared with diabetic patients who did not take aspirin or other antiplatelets. Mean platelet aggregation induced by arachidonic acid in non-aspirin group was  $79.34 \pm 7.34$  %, whereas arachidonic acid induced platelet aggregation in aspirin group was  $17.71 \pm 17.99$  %. Diabetic patients with aspirin had significantly lower level of mean platelet aggregation induced by arachidonic acid than diabetic patients with no aspirin ( $p < 0.001$ ) Figure 3 shows mean percentage platelet aggregation in the two groups.

Unlike patients in non-aspirin group, patients in aspirin treated group showed wide range of platelet aggregation. Although most of patients in aspirin group showed marked low percentage of platelet aggregation induced by arachidonic acid, some patients in this group still had platelet aggregation value as high as in non-aspirin group. There were 86 (88.66%) patients whose platelet aggregation less than 25%. Three (3.09%) persons had platelet aggregation induced by arachidonic acid between 25% and 65%. Another 8 patients had platelet aggregation value more than 65%. Figure 4 and 5 display distributions of platelet aggregation induced by arachidonic acid.



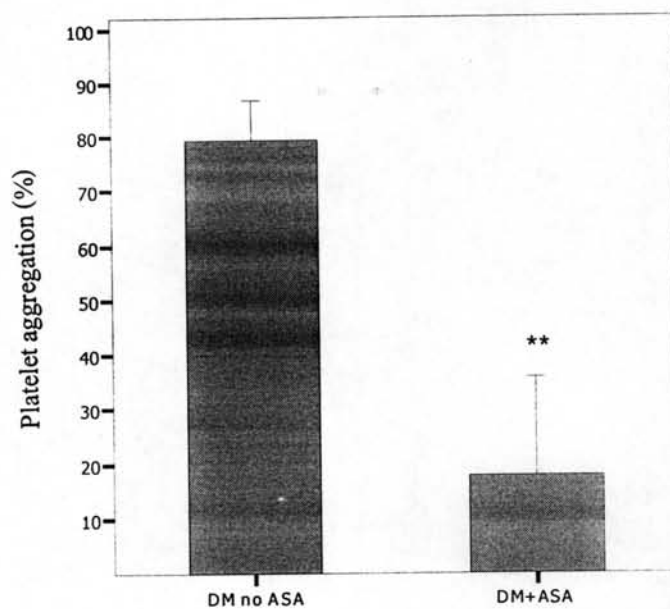


Figure 3 Mean % platelet aggregation induced by arachidonic acid in type 2 diabetic patients with no aspirin and with aspirin group. Bars show means. Error bars show SD.

\*\*  $p < 0.001$ .



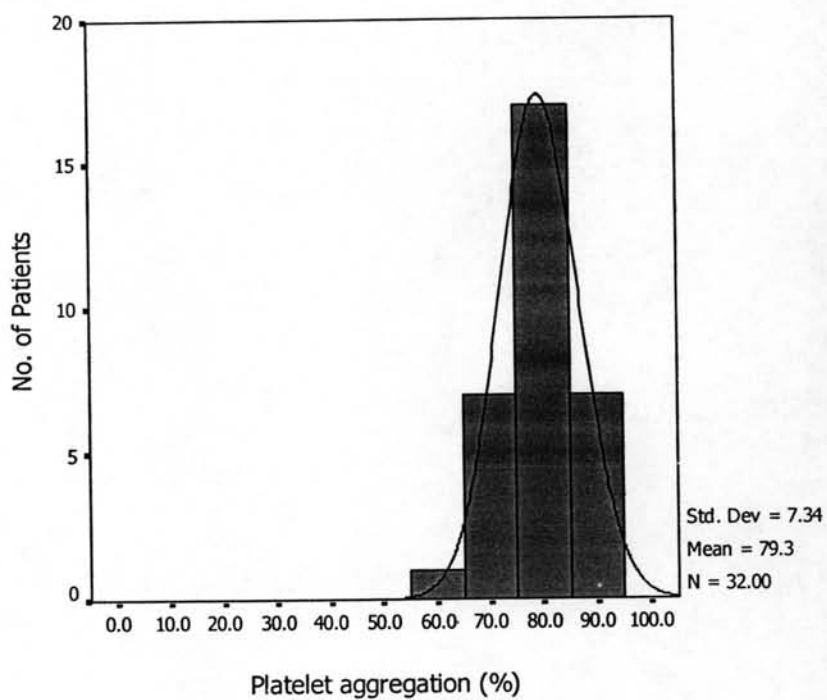


Figure 4 Distribution of platelet aggregation induced by arachidonic acid in diabetic patients with no aspirin

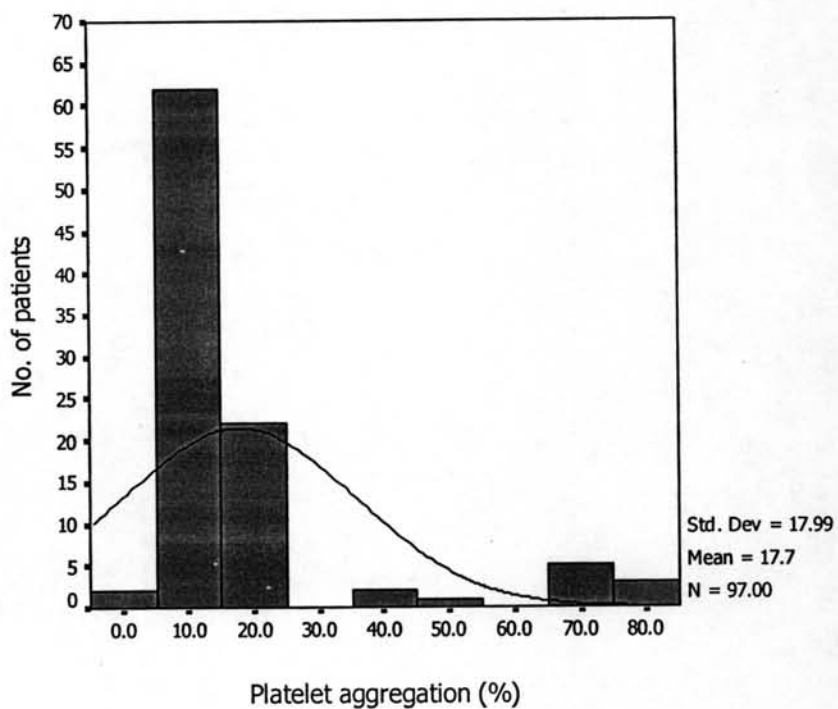


Figure 5 Distribution of platelet aggregation induced by arachidonic acid in diabetic patients with aspirin

#### 4.2.1.2 Platelet aggregation induced by ADP

Adenosine diphosphate (ADP) is used as an agonist of platelet aggregation *ex vivo* due to its vital role in human thrombus formation. Means of platelet aggregation induced by ADP were  $72.09 \pm 8.26\%$  and  $61.76 \pm 9.16\%$  in diabetic with no aspirin group and diabetic with aspirin group, respectively. Mean platelet aggregation in diabetic patients with aspirin show significantly lower than diabetic patients without aspirin ( $p < 0.001$ ). Figure 6 shows mean percentage of platelet aggregation induced by ADP in diabetic non-aspirin group and diabetic group.

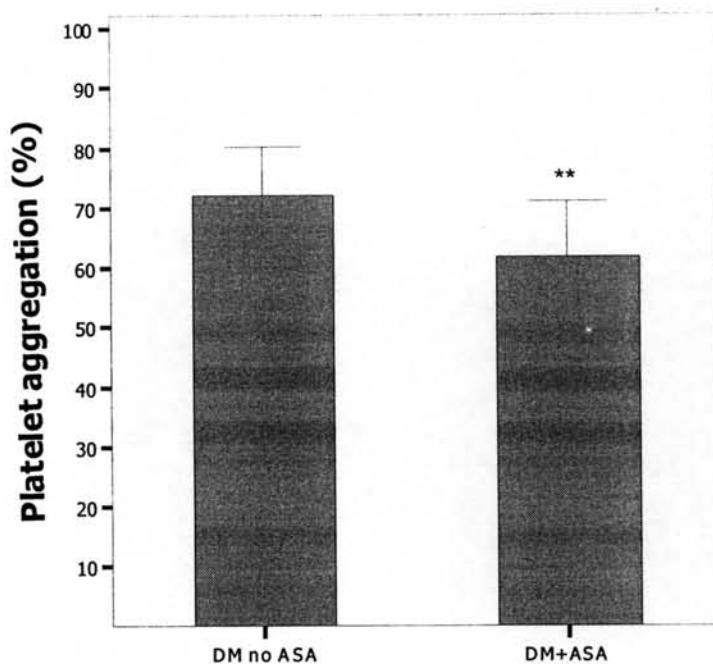


Figure 6 Mean % platelet aggregation induced by ADP in type 2 diabetic patients with no aspirin and with aspirin group. Bars show means. Error bars show SD.

\*\*  $p < 0.001$

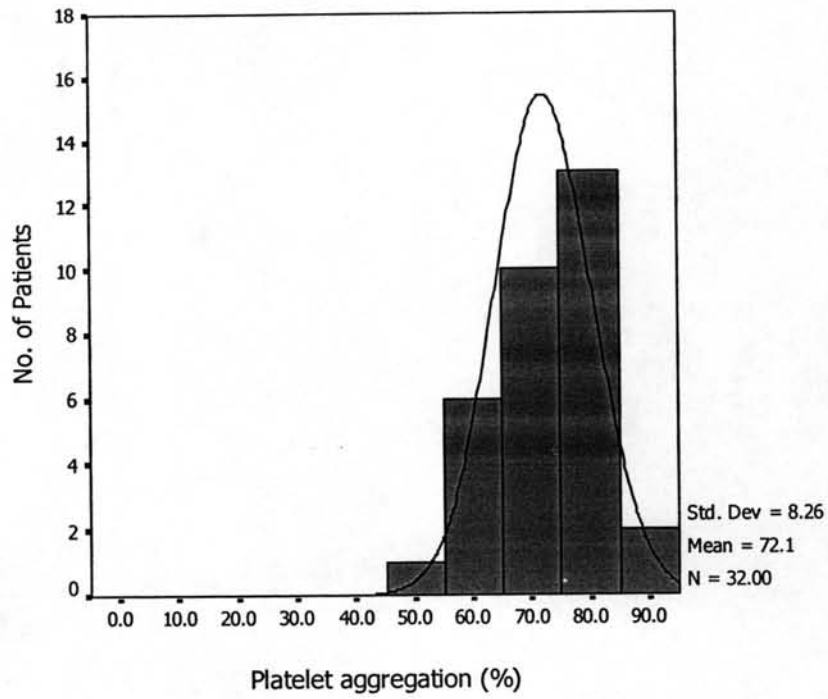


Figure 7 Distribution of platelet aggregation induced by ADP in diabetic patients with no aspirin

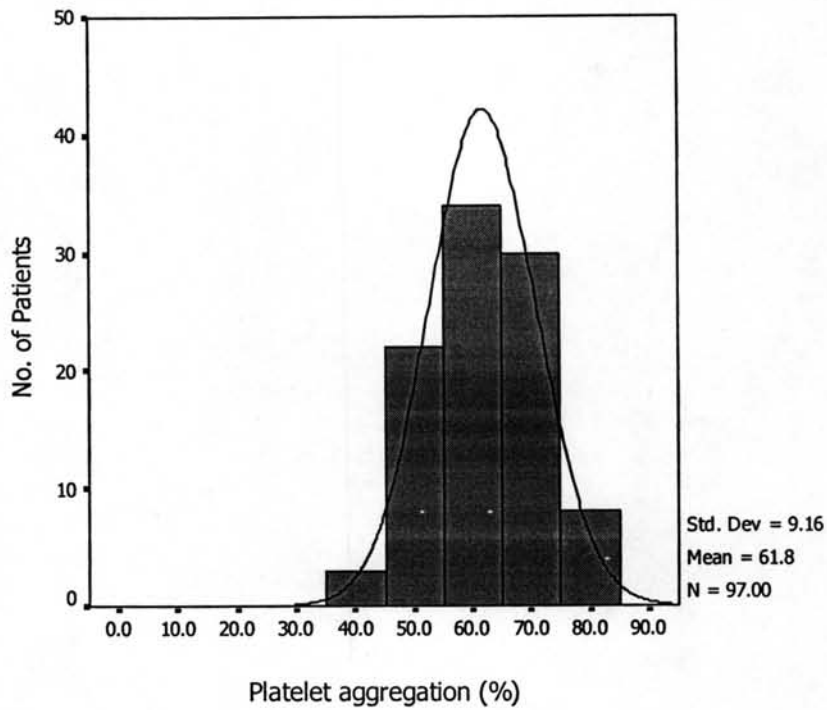


Figure 8 Distribution of platelet aggregation induced by ADP in diabetic patients with aspirin

#### 4.2.2 Effect of dosage of aspirin on platelet aggregation

##### 4.2.2.1 Effect of dosage of aspirin on platelet aggregation induced by arachidonic acid

In patients who were taking aspirin in all dose, mean platelet aggregation induced by arachidonic acid was significantly lower than those who did not received aspirin ( $p < 0.001$ ) (Table 8). However 300 mg/d aspirin did not show significantly lower platelet aggregation value than 60 mg/d aspirin. Histograms of arachidonic acid induced platelet aggregation in 60 mg/d aspirin and 300 mg/d aspirin were presented in Figure 9 and 10, respectively. Most of patients in 60-mg group had platelet aggregation less than 25%, however, there were 2 patients who had platelet aggregation between 35 and 45, and 8 patients had platelet aggregation between 65 and 85. Of 16 patients who received 300 mg/d aspirin, 15 had platelet aggregation  $\leq 20\%$ . Another one platelet aggregation induced by arachidonic was 45%.

Table 8 Aspirin dosage and platelet aggregation induced by arachidonic acid

Dose (mg/d)	N	Mean % aggregation	SD	Median	P25	P75
0	32	79.34	7.34	81.00	74.25	84.00
60	75	18.38 *	19.91	11.00	9.00	17.00
100	1	9.00	.-	9.00	-	-
120	2	18.00	1.41	18.00	-	-
150	3	12.67	2.52	13.00	-	-
300	16	15.31 *	8.79	13.00	9.50	17.25

\*.  $p < 0.001$  compared with non-aspirin group

P25 = 25<sup>th</sup> percentile, P75 = 75<sup>th</sup> percentile.

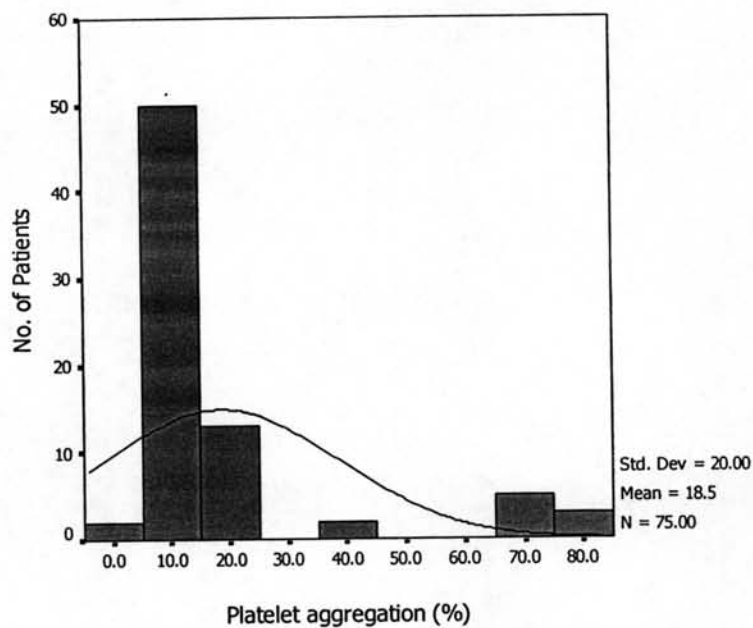


Figure 9 Distribution of platelet aggregation induced by arachidonic acid in 60 mg/d aspirin group.

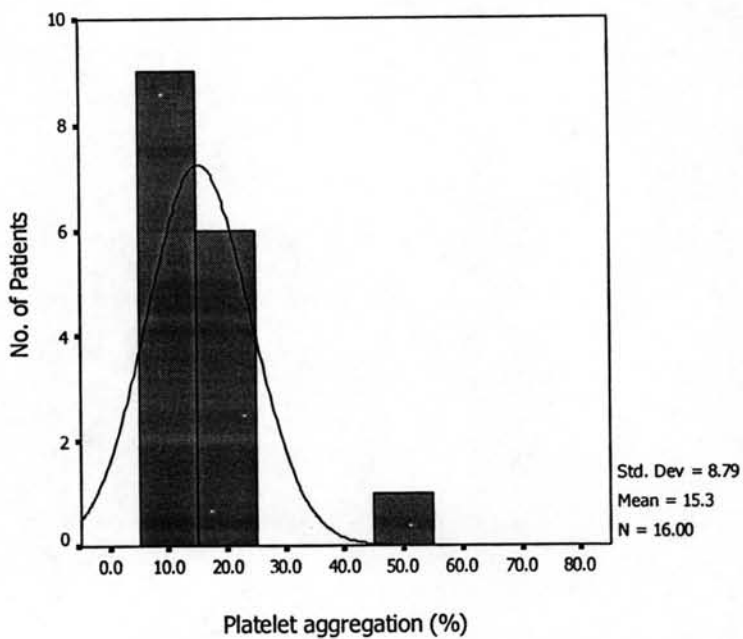


Figure 10 Distribution of platelet aggregation induced by arachidonic acid in 300 mg/d aspirin group.

#### 4.2.2.2 Effect of dosage of aspirin on platelet aggregation induced by

#### ADP

In patients who were taking aspirin in 60 mg/d and 300 mg/d aspirin, mean platelet aggregation induced by ADP was significantly lower than those who did not receive aspirin ( $p < 0.001$ ) (Table 9). However, no significantly difference of ADP induced platelet aggregation was found among doses of aspirin. Histogram of ADP induced platelet aggregation in 60 mg/d aspirin and 300 mg/d aspirin were presented in Figure 11 and 12, respectively.

Table 9 Aspirin dosage and platelet aggregation induced by ADP

Dose (mg/d)	N	Mean % aggregation	SD	Median	P25	P75
0	32	72.09	8.25	72.50	66.00	78.00
60	75	62.54 *	8.77	63.00	57.00	68.00
100	1	70.00	.-	70.00	-	-
120	2	66.00	5.66	66.00	-	-
150	3	50.67	10.60	49.00	-	-
300	16	60.00 *	10.54	56.50	50.25	72.25

\*.  $p < 0.001$  compared with non-aspirin group

P25 = 25<sup>th</sup> percentile, P75 = 75<sup>th</sup> percentile.

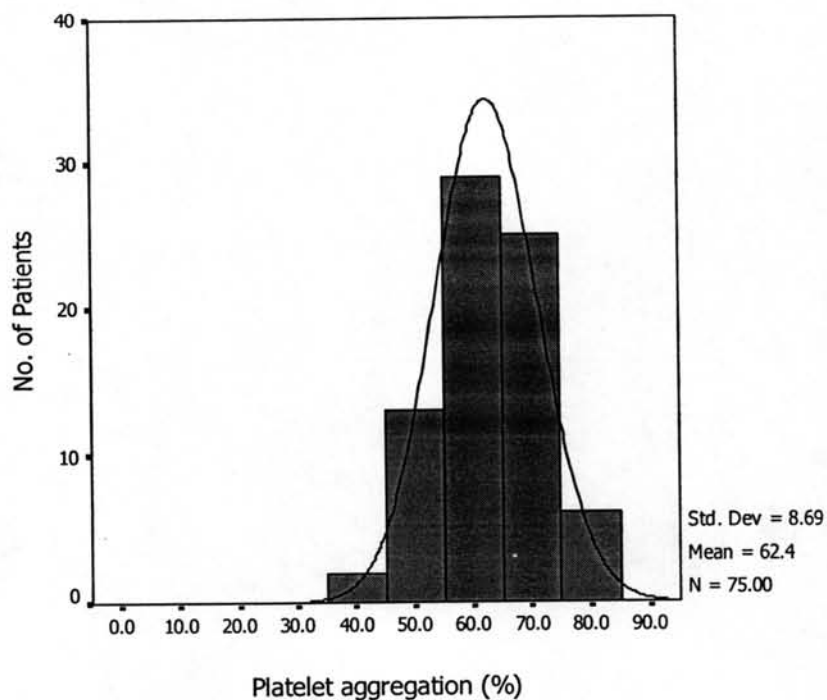


Figure 11 Distribution of platelet aggregation induced by ADP in 60 mg/d aspirin group.

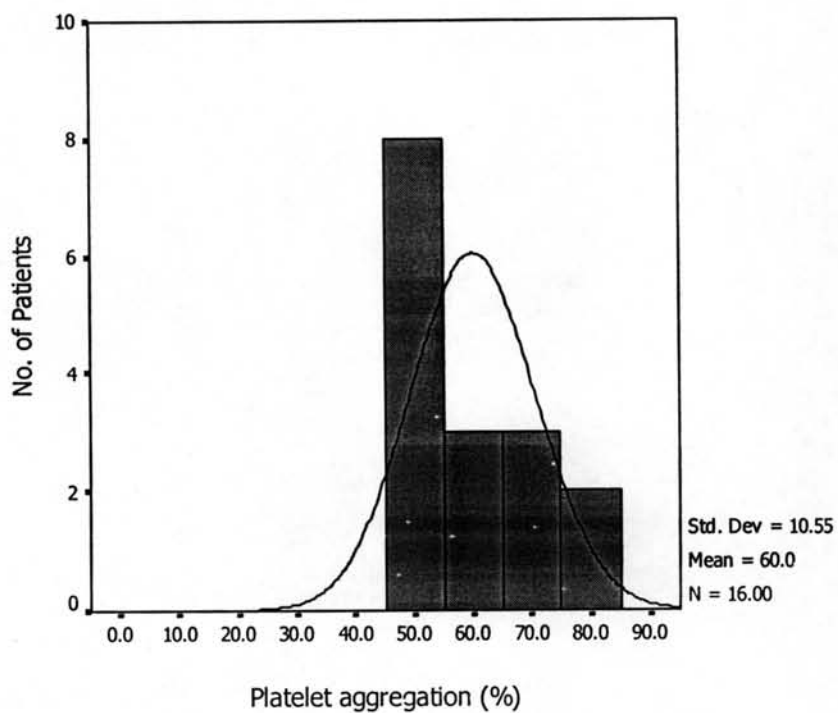


Figure 12 Distribution of platelet aggregation induced by ADP in 300 mg/d aspirin group.



### 4.3. Effect of Aspirin on Serum Thromboxane B<sub>2</sub>

#### 4.3.1 Serum thromboxane B<sub>2</sub> level in diabetic patients with aspirin compared with diabetic patients with no aspirin

The principal mechanism of aspirin is inhibition of cyclooxygenase enzyme, thus decreasing production of the potent platelet aggregation promoter, thromboxane A<sub>2</sub>. Thromboxane A<sub>2</sub> has a short half-life in serum and is rapidly converted into a stable metabolite, thromboxane B<sub>2</sub>. In this study, serum levels of thromboxane B<sub>2</sub> of 32 patients in non-aspirin group and 95 patients in aspirin group were measured. Since the data were not normal distribution (Figure 13 and 14), Mann-Whitney U test was used to compare thromboxane B<sub>2</sub> level in these two groups. Serum thromboxane B<sub>2</sub> level in diabetic patients taking aspirin was significantly lower than those in non-aspirin group (median = 0.189 ng/ml vs 5.526 ng/ml,  $p < 0.001$ ) (Figure 15).

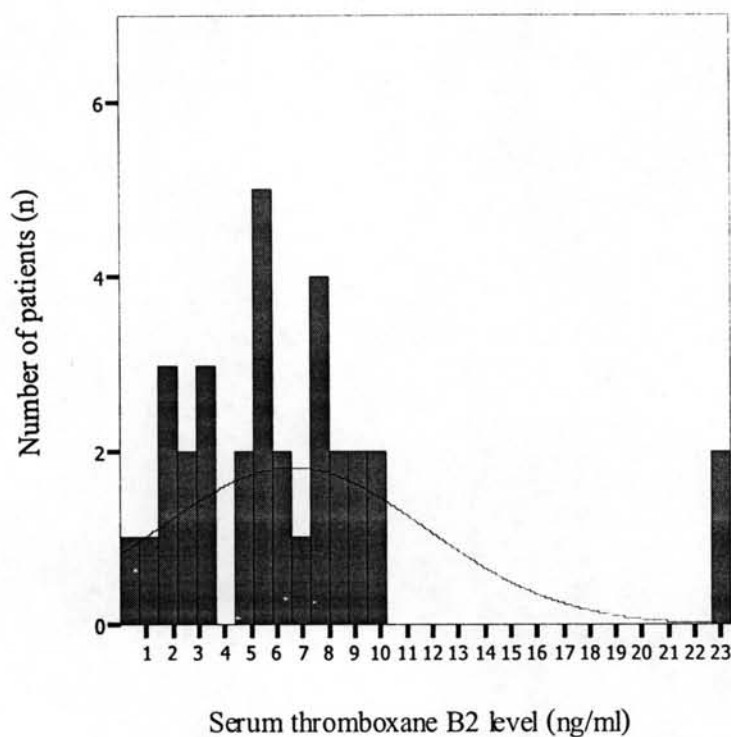


Figure 13 Distribution of serum thromboxane B<sub>2</sub> levels in diabetic patients with no aspirin.

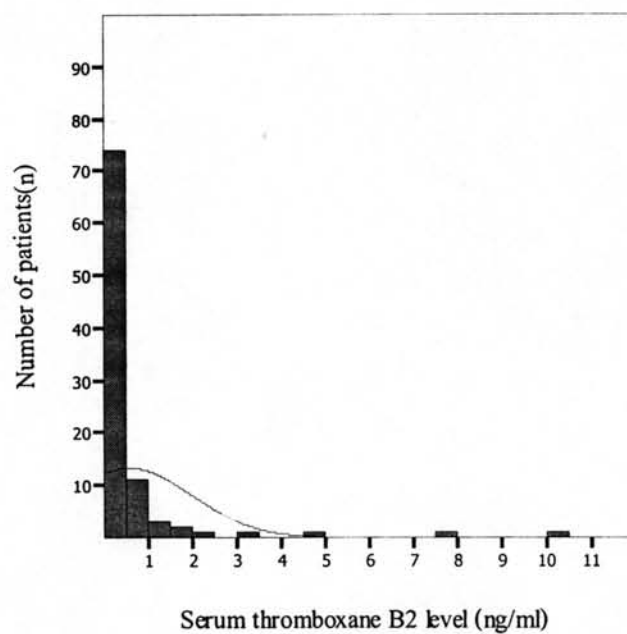


Figure 14 Distribution of thromboxane B<sub>2</sub> levels in diabetic patients with aspirin.

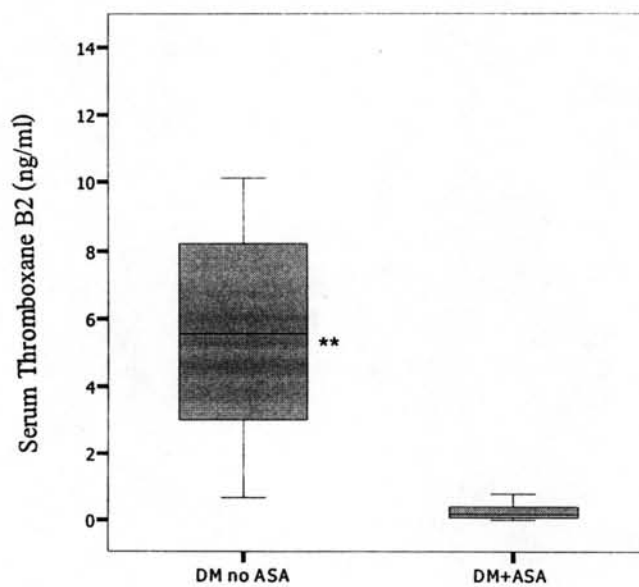


Figure 15 Serum thromboxane B<sub>2</sub> levels in type 2 diabetic patients with no aspirin and with aspirin group. Bars show medians. \*\*  $p < 0.001$

### 4.3.2 Effect of dosage of aspirin on thromboxane B<sub>2</sub>

Table 10 and Figure 16 illustrate serum level of thromboxane B<sub>2</sub> in different dosages of aspirin. Serum thromboxane B<sub>2</sub> level in patients with 300 mg/d aspirin was significantly lower than those in 60 mg/d (median = 0.046 ng/ml vs 0.274 ng/ml,  $p < 0.001$ ). The median reduction of serum thromboxane B<sub>2</sub> in 60 mg/d aspirin group and 300 mg/d aspirin group compared to those in non-aspirin group were 95.04% and 99.17%, respectively.

Table 10 Aspirin dosage and thromboxane B<sub>2</sub> levels

Dose (mg/d)	n	Serum thromboxane B <sub>2</sub> (ng/ml)				
		Mean	S.D.	Median	P 25	P 75
0	32	6.582	5.161	5.526	3.004	8.318
60	73	0.701	1.601	0.274 <sup>A</sup>	0.085	0.537
100	1	0.210	-	0.210	-	-
120	2	0.082	0.090	0.082	0.018	0.146
150	3	0.192	0.201	0.136	0.024	0.415
300	16	0.073	0.078	0.046 <sup>B</sup>	0.011	0.131

P 25 = Percentiles 25

P 75 = Percentiles 75

<sup>A</sup>  $p < 0.001$  compared with non-aspirin group value.

<sup>B</sup>  $p < 0.001$  compared with non-aspirin group value and 60 mg/d value.

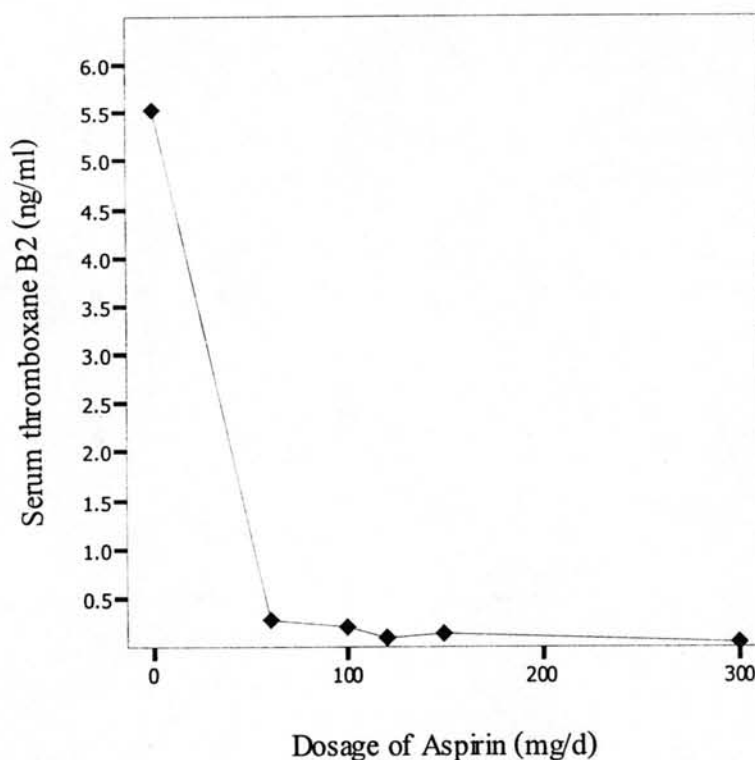


Figure 16 Median serum thromboxane B<sub>2</sub> level of type 2 diabetic patients who received different-dosages of aspirin.

#### 4.3.3 Association between serum thromboxane B<sub>2</sub> and platelet aggregation

##### 4.3.3.1 Association between serum thromboxane B<sub>2</sub> and arachidonic acid induced platelet aggregation

Pearson's correlations between serum thromboxane B<sub>2</sub> level and arachidonic acid induced platelet aggregation in type 2 diabetic patients with and with no aspirin were presented in Figure 17 and 18. In diabetic patients who were not taking aspirin, the platelet aggregation induced by arachidonic acid was not significantly correlated with serum thromboxane B<sub>2</sub> level ( $r = 0.219$ ,  $p = 0.229$ ). In aspirin treated group, serum thromboxane B<sub>2</sub> was markedly suppressed in most patients. A plot of percentage of platelet aggregation showed positively correlated with serum thromboxane B<sub>2</sub> level ( $r = 0.446$ ,  $p < 0.001$ ).

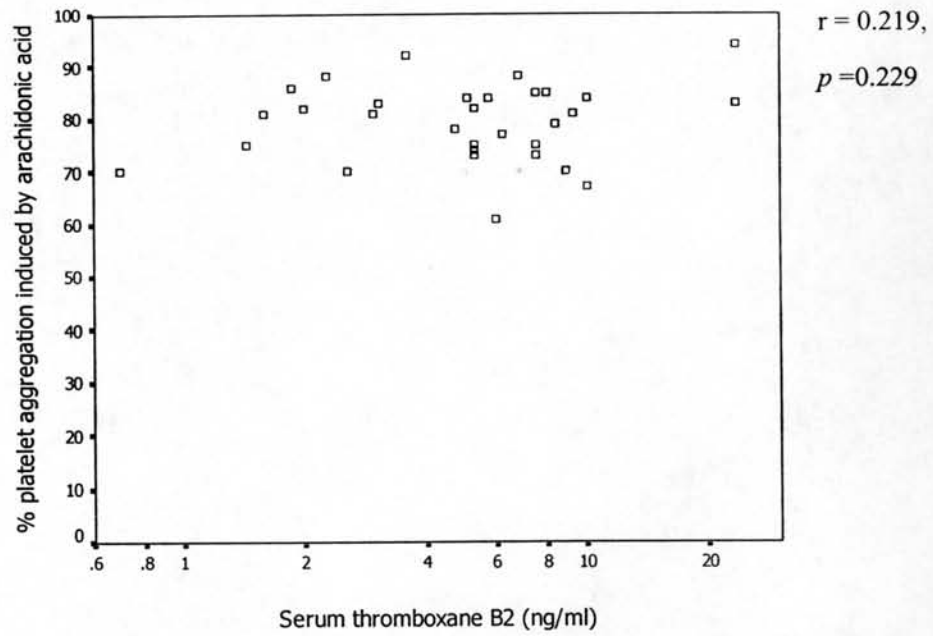


Figure 17 The relationship between serum thromboxane B<sub>2</sub> and arachidonic acid induced platelet aggregation in non-aspirin group.

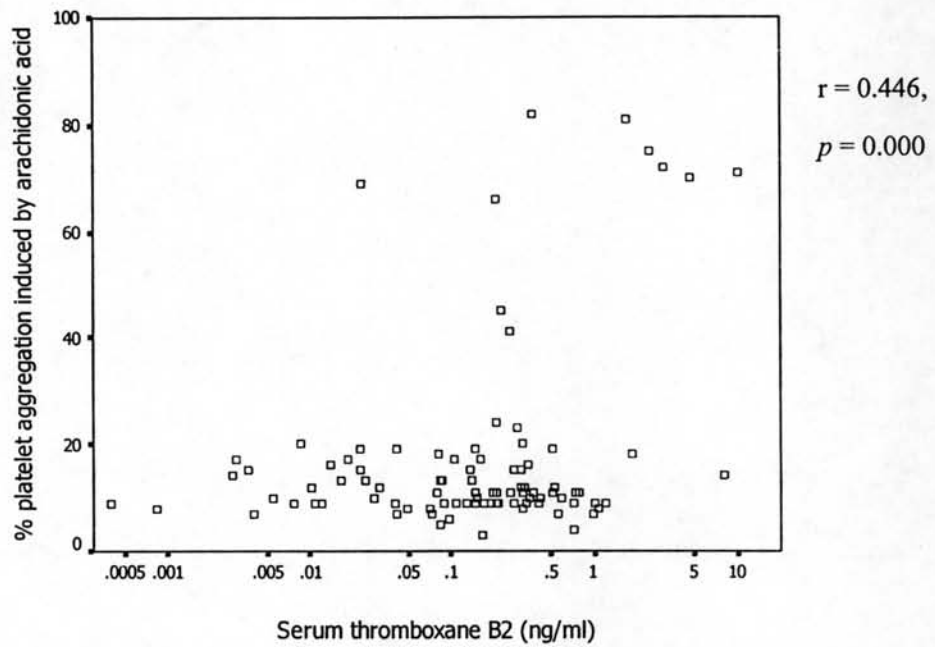


Figure 18 The relationship between serum thromboxane B<sub>2</sub> and arachidonic acid induced platelet aggregation in aspirin group.

#### 4.3.3.2 Association between serum thromboxane B<sub>2</sub> and ADP induced platelet aggregation

Pearson's correlations between serum thromboxane B<sub>2</sub> level and ADP induced platelet aggregation were shown in Figure 19 and 20. There was no correlation between serum thromboxane B<sub>2</sub> level and percentage of platelet aggregation induced by ADP in both non-aspirin group ( $r=0.343$ ,  $p > 0.05$  and aspirin group ( $r = 0.219$ ,  $p > 0.05$ ).

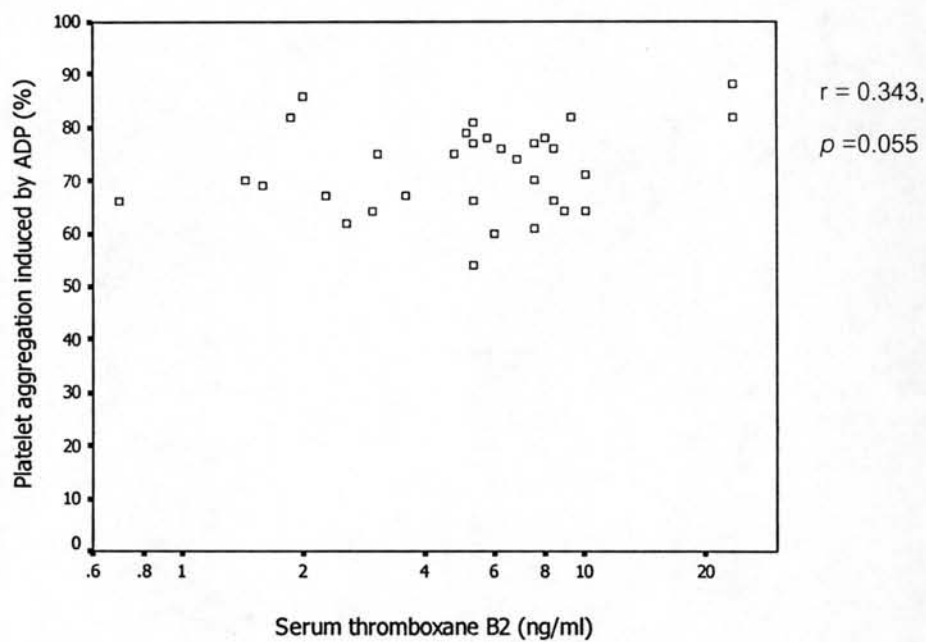


Figure 19 The relationship between serum thromboxane B<sub>2</sub> level and ADP induced platelet aggregation in non-aspirin group.

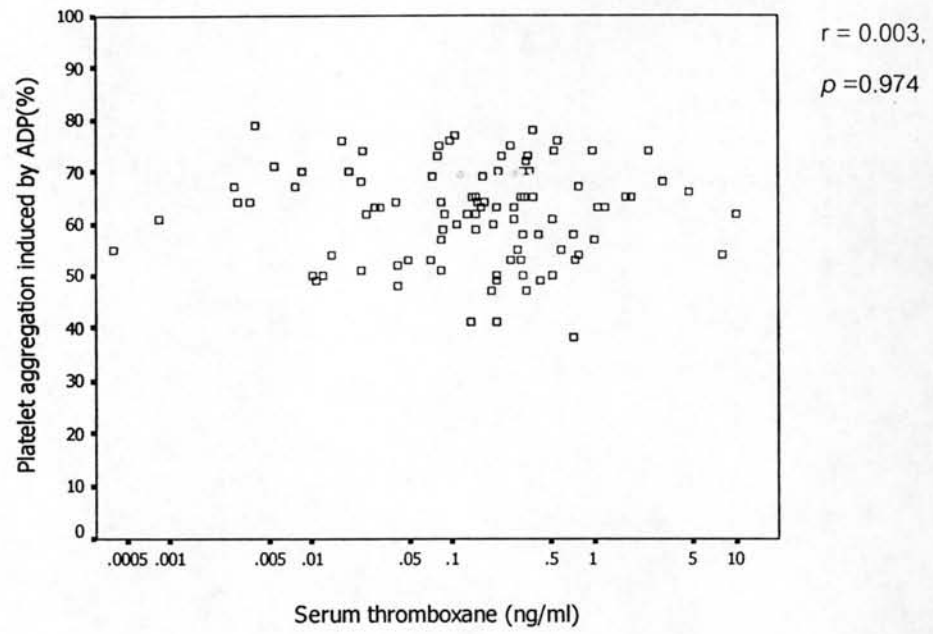


Figure 20 The relationship between serum thromboxane B<sub>2</sub> and ADP induced platelet aggregation in aspirin group.



#### 4.4. Aspirin Resistance

In this study aspirin resistance was defined as the patient whose platelet aggregation is  $\geq 20\%$  when induced with 1 mmol/l of arachidonic acid and platelet aggregation is  $\geq 70\%$  with 10  $\mu\text{mol/l}$  ADP. Aspirin semiresponders was defined as the patient whose platelet aggregation meet only one of the above criteria. And aspirin sensitive was defined as the patient whose platelet aggregation is  $< 20\%$  when induce with 1 mmol/l of arachidonic acid and platelet aggregation is  $< 70\%$  when induce with 10  $\mu\text{mol/l}$  of ADP.

##### 4.4.1 Frequency of aspirin resistance

Frequency of aspirin resistance in this study was displayed in Figure 21. Of the 97 diabetic patients who received aspirin, 6 (6.19%) was detected to be aspirin resistance, twenty five patients (25.77%) were aspirin semi responders and 66 (68.04%) were aspirin sensitives.

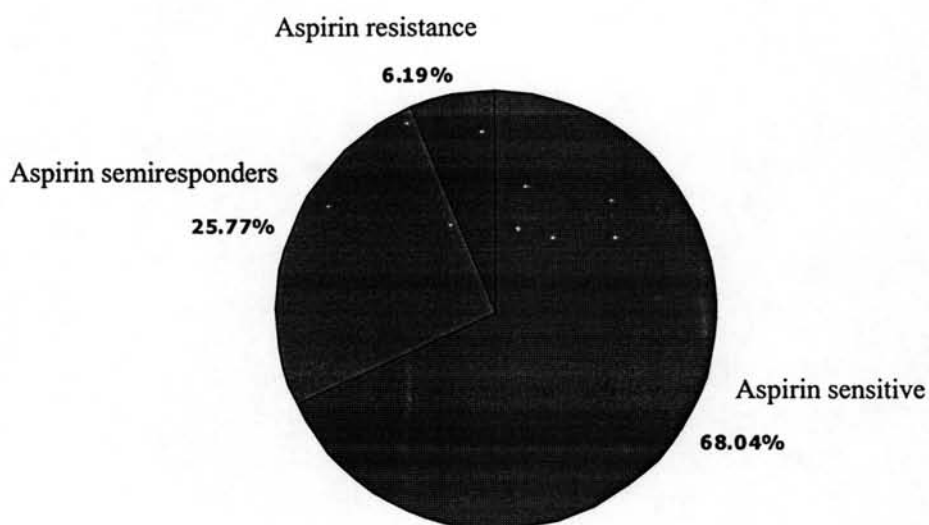


Figure 21 Frequency of aspirin resistance

#### 4.4.2 Patient characteristics of aspirin resistance

Comparisons of the demographic data among aspirin resistance, aspirin semiresponders, and aspirin sensitive groups are provided in Table 11-15. Of the 6 patients who were aspirin resistant, 5 were men; none of them had coronary artery disease, had history of CVA nor had undergone cardiac intervention. Although HDL cholesterol level of patients in aspirin resistant group seems to be lower than other groups, this was not statistically significant ( $35.67 + 5.35$  mg/dl vs  $42.54 + 12.68$  mg/dl,  $p=0.192$ ). Aspirin resistance was not related to age, gender, BMI, waist, hip, BP, smoking, exercise, co-morbid conditions, co-medications, Fasting plasma glucose, HbA1c, triglycerides, or dosage of aspirin slightly higher systolic blood pressure, although no significant differences were found.

Table 13 shows co-morbid conditions of the three groups. All of patients in aspirin resistant group had dyslipidemia but there was not significantly difference from the other groups ( $p>0.05$ ). None of aspirin resistant patients had coronary artery disease, cerebrovascular accident or had undergone cardiac intervention, but not statistically significant different. Diabetic microvascular complication including retinopathy was not different between these three groups.

Table 11 Demographic data of patients with aspirin resistant, aspirin semiresponder, and aspirin sensitive.

	Aspirin resistances (n=6)	Aspirin semi- responders (n=25)	Aspirin sensitives (n=66)	p Value
Age (year)	59.17 ± 9.97	59.58 ± 7.68	58.28 ± 9.58	0.710
Female	1 (16.7%)	16 (61.5%)	37 (56.9%)	0.113
BMI (kg/m <sup>2</sup> )	27.40 ± 3.16	27.16 ± 4.21	27.03 ± 3.99	0.910
Waist (cm)	98.93 ± 7.31	90.23 ± 12.27	89.75 ± 10.62	0.143
Hip (cm)	102.63 ± 5.23	99.75 ± 6.76	99.39 ± 7.91	0.604
SBP (mmHg)	137.00 ± 9.89	130.35 ± 9.93	132.05 ± 11.20	0.563
DBP (mmHg)	80.75 ± 8.46	79.81 ± 7.93	80.79 ± 7.98	0.925
Duration of DM (year)	6.50 ± 3.834	5.63 ± 3.831	6.88 ± 4.904	0.329

Data represent in Mean ± SD. except for female represent in count (%).

NS = Not significant

Table 12 Patient health behavior in aspirin resistances, aspirin semiresponders, and aspirin sensitives

		Aspirin resistance (n=6)	Aspirin semi- responders (n=25)	Aspirin sensitives (n=66)	p Value
Exercise	Usually	1 (16.7%)	6 (24.0%)	16 (24.2%)	0.373
	Sometimes	1 (16.7%)	3 (12.0%)	20 (30.3%)	
	Rarely	4 (66.6%)	16 (64.0%)	30 (45.5%)	
Smoking	Never	3 (50.0%)	21 (84.0%)	42 (63.6%)	0.160
	Used to	3 (50.0%)	4 (16.0%)	18 (27.3%)	
	Current	0	0	6 (9.1%)	
Alcohol consumption	Never	3 (50.0%)	17 (68.0%)	35 (53.0%)	0.243
	Used to	3 (50%)	4 (16%)	25 (37.9%)	
	Current	0	4 (16.0%)	6 (9.1%)	

Data represent in count (%) NS = Not significant

Table 13 Co-morbid conditions in aspirin resistances, aspirin semiresponders, and aspirin sensitives

	Aspirin resistance (n=6)	Aspirin semi- responders (n=25)	Aspirin sensitives (n=66)	p Value
Hypertension	4 (78.5)	18 (72.0)	52 (70.3)	0.378
Dyslipidemia	6 (100)	15 (57.7)	51 (78.5)	0.080
CAD	0 (0)	8 (30.8)	19 (29.2)	0.278
Intervention	0 (0)	1 (3.8)	9 (13.8)	0.279
CVA	0 (0)	0 (0)	6 (9.2)	0.110
Retinopathy	2 (33.3)	2 (7.7)	11 (16.9)	0.272

Data represent in count (%) NS = Not significant

Table 14 Co-medication in aspirin resistances, aspirin semiresponders, and aspirin sensitives

	<b>Aspirin resistance (n=6)</b>	<b>Aspirin semi- responders (n=25)</b>	<b>Aspirin sensitives (n=66)</b>	<b>p Value</b>
Sulfonylurea	6 (100)	17 (66.4)	52 (80)	0.099
Metformin	5 (83.3)	23 (88.5)	56 (86.2)	0.951
TZD	3 (50)	4 (15.4)	10 (15.4)	0.119
Statins	5 (83.3)	18 (69.2)	48 (73.8)	0.706
beta-blockers	2 (33.3)	11 (42.3)	30 (46.2)	0.715
CCB	0 (0)	8 (30)	21 (32.3)	0.226
ACEIs	1 (16.7)	8 (30.8)	32 (49.2)	0.154
Diuretics	2 (33.3)	5 (19.2)	10 (15.4)	0.756

Data represent in count (%)

NS = Not significant

Table 15 Biochemical parameters in aspirin resistances, aspirin semiresponders, and aspirin sensitives

Characteristics	Aspirin resistance (n=6)	Aspirin semi-responders (n=25)	Aspirin sensitives (n=66)	p-value
FPG (mg/dL)	132.83 ± 22.62	126.92 ± 23.17	137.82 ± 34.40	0.300
HbA1c (%A1c)	7.08 ± 0.7885	7.40 ± 1.40	7.22 ± 1.02	0.815
Insulin (uIU/ml)	16.60 ± 11.48	11.01 ± 9.10	11.30 ± 7.99	0.326
HOMA-IR	2.67 ± 1.77	1.76 ± 1.39	1.86 ± 1.30	0.325
TC (mg/dl)	165.00 ± 42.89	177.19 ± 31.02	173.25 ± 39.98	0.664
LDL (mg/dl)	103.17 ± 37.24	109.15 ± 25.26	103.35 ± 29.60	0.816
HDL (mg/dl)	35.67 ± 5.35	42.23 ± 10.48	42.66 ± 13.54	0.423
TG (mg/dl)	118.17 ± 66.01	142.19 ± 64.65	145.18 ± 72.01	0.635
Hemoglobin (g/dl)	14.15 ± 1.16	13.104 ± 1.29	12.795 ± 1.40	0.073
Hematocrit (%)	41.20 ± 3.72	39.06 ± 3.91	37.97 ± 4.12	0.147
Platelets (x1000)	279.50 ± 31.99	277.42 ± 77.18	290.95 ± 71.43	0.595
MPV (fL)	8.21 ± 0.26	8.39 ± 0.87	8.07 ± 0.88	0.266

Data represent in mean ± S.D., TC = Total cholesterol, NS = Not significant

<sup>^</sup> p-value comparing aspirin resistance and combined aspirin semiresponders with aspirin sensitives.

Table 16 Dosage of aspirin in aspirin resistances, aspirin semi-responders and aspirin sensitives

Dose (mg/d)	Aspirin resistance (n=6)	Aspirin semi-responders (n=25)	Aspirin sensitives	Sig. (2-tailed)
60	4 (66.7)	20 (76.9)	51 (78.5)	0.198
100	0	1 (3.8)	0	
120	0	2 (7.7)	0	
150	0	0	3 (4.6)	
300	2 (33.3)	3 (11.5)	11 (16.9)	

Data represent in count (%)

#### 4.4.3 Platelet resistant to agonists

##### 4.4.3.1 Platelet resistant to arachidonic acid

Platelets of aspirin treated patients that performed a maximal aggregation induced with 1 mmol/l arachidonic acid of > 20% was defined as aspirin resistant with arachidonic acid and patients that maximal aggregation of < 20% was aspirin sensitive with arachidonic acid. In this study, there were 82 (84.5%) patients that were aspirin sensitive with arachidonic acid and 15 (15.5%) patients that were aspirin resistant with arachidonic acid. Mean platelet aggregation in patients with aspirin resistant and aspirin sensitive with arachidonic acid are showed in Table 17.

Table 17 Statistics of platelet aggregation in patients with aspirin resistant and aspirin sensitive with arachidonic acid

	agonist	n	Mean	S.D.	Median	P25	P75
Sensitive with	AA	82	11.27	3.67	11	9	13.25
	ADP	82	61	9.05	62	53.75	67
Resistant with	AA	15	52.93	23.84	66	24	72
	ADP	15	65.93	8.92	67	62	74

AA = arachidonic acid. P25 = 25<sup>th</sup> percentile, P75 = 75<sup>th</sup> percentile.



Table 18 Demographics of aspirin resistant and aspirin sensitive with arachidonic acid

Characteristics	Resistant with AA (n=15)	Sensitive with AA (n=82)	p-value
Age (year)	59.20 ± 7.94	58.59 ± 9.29	0.810
Female	9 (60)	45 (54.9)	0.714
Weight (kg)	66.05 ± 12.48	69.36 ± 13.48	0.381
Height (cm)	156.45 ± 9.24	159.72 ± 8.59	0.184
BMI (kg/m <sup>2</sup> )	26.85 ± 3.55	27.14 ± 4.06	0.787
Waist (cm)	91.167 ± 10.73	90.32 ± 11.15	0.787
Hip (cm)	99.07 ± 6.16	99.81 ± 7.71	0.725
SBP (mmHg)	133.5 ± 11.2	131.5 ± 10.7	0.524
DBP (mmHg)	79.1 ± 9.2	80.7 ± 7.7	0.486
Duration of DM (year)	6.43 ± 3.86	6.55 ± 4.72	0.928

Data represent in Mean ± SD. except for female represent in count (%).

Table 19 Co-morbid conditions of 97 type 2 diabetic patients taking aspirin grouped by resistant to arachidonic induced platelet aggregation

Characteristics	Resistant with AA (n=15)	Sensitive with AA (n=82)	Sig. (2-tailed)
Hypertension	10 (66.7)	64 (78.0)	0.339
Dyslipidemia	10 (66.7)	62 (75.6)	0.525
CAD	1 (6.7)	26 (31.7)	0.060
Intervention	0 (0)	10 (12.2)	0.353
CVA	0 (0)	6 (7.3)	0.586
Retinopathy	3 (20)	12 (14.6)	0.697

Data represent in count (%)

Table 20 Medications of 97 type 2 diabetic patients taking aspirin grouped by resistant to arachidonic acid induced platelet aggregation

Characteristics	Resistant with AA (n=15)	Sensitive with AA (n=82)	p-value
Sulfonylureas	12 (80)	63 (76.8)	0.999
Metformin	14 (93.3)	70 (85.4)	0.685
TZD	5 (33.3)	12 (14.6)	0.131
Statin	12 (80)	59 (72)	0.753
Beta blockers	6 (40)	37 (45.1)	0.714
CCB	2 (13.3)	27 (32.9)	0.218
ACE inhibitors	4 (26.7)	37 (45.1)	0.183
ARB	3 (20)	14 (17.1)	0.723
Diuretics	3 (20)	14 (17.1)	0.723

Data represent in count (%)

\*  $p < 0.05$

Table 21 Clinical data of 97 type 2 diabetic patients taking aspirin grouped by resistant to arachidonic induced platelet aggregation

Characteristics	Resistant with AA (n=15)	Sensitive with AA (n=82)	p-value
FPG (mg/dL)	132.33 ± 26.49	135.00 ± 32.21	0.763
HbA1c (%A1c)	7.63 ± 1.61	7.19 ± 1.00	0.161
Insulin (uIU/ml)	12.54 ± 9.17	11.38 ± 8.44	0.629
HOMA-IR	2.04 ± 1.44	1.86 ± 1.34	0.645
TC (mg/dl)	177.40 ± 38.63	173.13 ± 37.72	0.689
LDL (mg/dl)	39.67 ± 11.59	42.56 ± 12.62	0.411
HDL (mg/dl)	110.27 ± 32.53	103.91 ± 28.17	0.435
Triglyceride (mg/dl)	132.33 ± 48.93	144.61 ± 72.59	0.531
Hemoglobin (g/dl)	13.08 ± 1.41	12.94 ± 1.39	0.707
Hematocrit (%)	38.41 ± 3.93	38.47 ± 4.14	0.956
Platelets (x1000)	276.07 ± 64.98	288.55 ± 72.29	0.534
MPV (fL)	8.38 ± 0.81	8.12 ± 0.87	0.284

Data represent in mean ± SD.

#### 4.4.3.2 Platelet resistant to ADP

When induced with 10 µmol/l ADP, aspirin treated patients whose platelets performed a maximal aggregation of > 70% were defined as aspirin resistant with ADP, regardless of arachidonic acid induced platelet aggregation. In this study, seventy five (77.3%) patients were aspirin sensitive with ADP and 22 (22.7%) patients were aspirin resistant with ADP. Mean platelet aggregations induced by arachidonic acid and ADP in patients with aspirin sensitive and aspirin resistant with ADP are presented in Table 22.

In ADP-resistances group, hemoglobin and hematocrit were significantly higher than that in ADP responders ( $p = 0.009$  and  $p = 0.005$ , respectively). There were no differences between groups of patient regarding age, sex, BMI, BP, blood sugar, lipid profiles and thromboxane B<sub>2</sub> level (Table 23- Table 26).

Table 22 Statistics of platelet aggregation in patients with aspirin resistant and aspirin sensitive with arachidonic acid

Subgroup	agonist	n	Mean	S.D.	Median	P25	P75
Sensitive with	AA	75	16.08	15.86	11	9	15
	ADP	75	58.28	7.26	60	53	64
Resistant with	AA	22	23.27	23.46	12.5	9	25.25
	ADP	22	73.64	2.74	74	70.75	76

AA = arachidonic acid, P25 = 25<sup>th</sup> percentile, P75 = 75<sup>th</sup> percentile.

Table 23 Demographic data of 97 type 2 diabetic patients taking aspirin grouped by resistant to ADP induced platelet aggregation

Characteristics	Resistant with ADP (n=22)	Sensitive with ADP (n=75)	p-value
Age (year)	60.0 ± 8.47	58.29 ± 9.24	0.440
Female	9 (60)	45 (54.9)	0.714
Weight (kg)	71.96 ± 13.48	67.86 ± 13.21	0.209
Height (cm)	162.43 ± 8.02	158.24 ± 8.75	0.048
BMI (kg/m <sup>2</sup> )	27.08 ± 3.64	27.09 ± 4.08	0.991
Waist (cm)	93.40 ± 11.51	89.62 ± 10.83	0.168
Hip (cm)	101.34 ± 6.10	99.22 ± 7.78	0.253
SBP (mmHg)	131.45 ± 9.28	131.87 ± 11.22	0.880
DBP (mmHg)	80.85 ± 7.04	80.39 ± 8.18	0.820
Duration of DM (year)	5.24 ± 3.42	6.90 ± 4.82	0.143

Data represent in mean ± SD.

Table 24 Co-morbid conditions of 97 type 2 diabetic patients taking aspirin grouped by resistant to ADP induced platelet aggregation

Characteristics	Resistant with ADP (n=22)	Sensitive with ADP (n=75)	p-value
Hypertension	10 (66.7)	64 (78.0)	0.339
Dyslipidemia	10 (66.7)	64 (78.0)	0.525
CAD	1 (6.7)	26 (31.7)	0.060
Intervention	0 (0)	10 (12.2)	0.353
CVA	0 (0)	6 (7.3)	0.586
Retinopathy	3 (20)	12 (14.6)	0.697

Data represent in count (%)

Table 25 Medications of 97 type 2 diabetic patients taking aspirin grouped by resistant to ADP induced platelet aggregation

Characteristics	Resistant with ADP (n=22)	Sensitive with ADP (n=75)	p-value
Sulfonylureas	12 (80)	63 (76.8)	0.999
Metformin	14 (93.3)	70 (85.4)	0.685
TZD	5 (33.3)	12 (14.6)	0.131
Statin	12 (80)	59 (72.0)	0.753
Beta blockers	6 (40.0)	37 (45.1)	0.714
CCB	2 (13.3)	27 (32.9)	0.218
ACE inhibitors	4 (26.7)	37 (45.1)	0.183
ARB	3 (20)	14 (17.1)	0.723
Diuretics	3 (20)	26 (31.7)	0.542
Omeprazole	0 (0)	9 (11.0)	0.347

Data represent in count (%)

\*  $p < 0.05$

Table 26 Clinical data of 97 type 2 diabetic patients taking aspirin grouped by resistant to ADP induced platelet aggregation

Characteristics	Resistant with ADP (n=22)	Sensitive with ADP (n=75)	p-value
FPG (mg/dL)	125.91 ± 20.15	137.13 ± 33.55	0.057
HbA1c (%A1c)	7.03 ± 0.86	7.33 ± 1.18	0.279
Insulin (uIU/ml)	13.02 ± 10.65	11.13 ± 7.82	0.362
HOMA-IR	2.07 ± 1.62	1.83 ± 1.26	0.394
TC (mg/dl)	172.27 ± 31.72	174.24 ± 39.45	0.831
LDL (mg/dl)	41.14 ± 7.81	42.40 ± 13.55	0.582
HDL (mg/dl)	103.91 ± 25.90	105.19 ± 29.75	0.856
Triglyceride (mg/dl)	133.55 ± 74.28	145.40 ± 68.17	0.484
Hemoglobin (g/dl)	13.63 ± 1.25 **	12.76 ± 1.37	0.009
Hematocrit (%)	40.57 ± 3.75 **	37.84 ± 4.00	0.005
Platelets (x1000)	276.77 ± 67.79	289.51 ± 72.14	0.463
MPV (fL)	8.30 ± 0.68	8.12 ± 0.91	0.399

TC = Total cholesterol

\*\* p < 0.01

#### 4.5. Factors Influencing Platelet Response to Aspirin

##### 4.5.1. Association between gender and platelet aggregation

As shown in Table 27, there was a higher percentage of platelet aggregation induced by arachidonic acid in female but this was not statistically significant. Diabetic women and diabetic men who were taking aspirin did not shown significant different of mean percentage platelet aggregation induced by ADP.

Table 27 Means percentage of platelet aggregation in different gender

Agonist	Gender	N	Mean	p-value
AA	Female	54	19.46 ± 20.09	0.592
	Male	43	15.51 ± 14.87	
ADP	Female	54	61.31 ± 8.58	0.285
	Male	43	62.33 ± 9.92	

Data represent in Mean ± SD. AA = arachidonic acid



#### 4.5.2 Association between other general demographic data and platelet aggregation

Table 28 demonstrates that demographic parameters including age, weight, height, BMI, waist, hip, blood pressure, and duration of DM were not correlated with platelet aggregation induced by arachidonic acid and platelet aggregation induced by ADP.

Table 28 Correlation coefficient of general demographic characteristics of patients and platelet aggregation

	AA		ADP	
	r	p-value	r	p-value
Age (year)	0.048	0.642	0.076	0.458
Weight (kg)	-0.117	0.262	-0.016	0.877
Height (cm)	-0.146	0.159	0.088	0.395
BMI (kg/m <sup>2</sup> )	-0.056	0.587	-0.098	0.340
Waist (cm)	-0.009	0.934	-0.014	0.895
Hip (cm)	-0.028	0.786	-0.044	0.671
SBP (mmHg)	0.109	0.317	0.041	0.972
DBP (mmHg)	0.004	0.972	0.081	0.456
Duration of DM (year)	0.038	0.719	-0.080	0.446

Data represent in Mean  $\pm$  SD. AA = arachidonic acid

### 4.5.3 Health behaviors and platelet aggregation

As shown in Table 29, exercise, smoking and alcohol consumption in diabetic patients with aspirin were not associated with platelet aggregation induced by arachidonic acid and ADP.

Table 29 Correlation coefficient of health behavior and mean aggregation

	n	AA		ADP	
		Mean	p-value	Mean	p-value
<b>Exercise</b>					
Usually	23	15.36 ± 13.26	0.457	61.04 ± 9.55	0.678
Sometimes	24	15.29 ± 12.98		60.79 ± 8.94	
Rarely	50	19.94 ± 21.57		62.56 ± 9.20	
<b>Smoking</b>					
Never	66	19.18 ± 19.46	0.384	61.88 ± 8.95	0.941
Used to	25	15.76 ± 15.58		61.76 ± 10.54	
Current smoking	6	9.67 ± 1.22		60.50 ± 5.99	
<b>Alcohol consumption</b>					
Never	55	20.78 ± 20.80	0.094	61.25 ± 9.03	0.812
Used to	32	15.25 ± 13.84		62.28 ± 8.98	
Current drinking	10	8.70 ± 2.95		62.90 ± 11.15	

Data represent in Mean ± SD.

#### 4.5.4 Co-morbid conditions and platelet aggregation

As shown in Table 30, diabetic patients who had coronary artery disease had mean platelet aggregation induced by arachidonic acid significantly lower than those who did not have coronary artery disease (12.41 + 4.39 % vs 19.76 + 20.68 %,  $p < 0.006$ ). Other co-morbid conditions, including hypertension, dyslipidemia, cardiac intervention, and diabetic retinopathy were not associated with platelet aggregation induced by arachidonic acid. However, no significant differences were found in other co-morbid conditions including hypertension, dyslipidemia, coronary artery disease and diabetic retinopathy. Patients who had undergone cardiac intervention had mean platelet aggregation induced by ADP significantly lower than those who did not have this condition (55.70% vs. 62.46% respectively,  $p = 0.026$ ). Likewise, diabetic patients taking aspirin who had history of cerebrovascular accident also shown significantly lower of mean % platelet aggregation induced by ADP than that who did not have history of CVA (54.50 + 9.09 % vs. 62.24 + 9.01% respectively,  $p = 0.044$ ).

Table 30 Correlation coefficient of co-morbid conditions and platelet aggregation induced by arachidonic acid and ADP

	AA		ADP	
	r	p-value	r	p-value
Hypertension	- 0.154	0.132	- 0.017	0.868
Dyslipidemia	- 0.081	0.432	- 0.101	0.327
Coronary artery disease	- 0.184	0.071	- 0.087	0.395
Cardiac intervention	- 0.121	0.236	- 0.225 *	0.026
CVA	- 0.068	0.511	- 0.205 *	0.044
Diabetic retinopathy	0.096	0.349	-0.030	0.774

\*  $p < 0.05$

#### 4.5.5 Co-medications and platelet aggregation

T-test of the mean percentage of platelet aggregation induced by arachidonic acid and concurrent medication used in diabetic patients who were taking aspirin was investigated and presented in Table 31. No medication was found to influence platelet aggregation in diabetic patients taking aspirin.

Table 31 Correlation coefficient of co-medication and platelet aggregation induced by arachidonic acid and ADP

	AA		ADP	
	r	p-value	r	p-value
Sulfonylurea	0.166	0.181	0.162	0.912
Metformin	-0.008	0.456	0.097	0.998
Thiazolidinediones	0.070	0.725	0.222	0.234
Statins	0.125	0.379	0.025	0.342
Beta-blockers	-0.082	0.419	-0.153	0.441
CCB	-0.156	0.063	-0.053	0.864
ACEIs	0.083	0.629	0.024	0.682
Diuretics	-0.072	0.458	-0.080	0.926

\*\*  $p < 0.01$

#### 4.5.6 Biochemical data and platelet aggregation

Association between platelet aggregation and biochemical data in diabetic patients including FPG, HbA1c, lipid profiles, hemologic profiles were investigated and provided in Table 32

Fasting plasma glucose did not show significant relationships with arachidonic acid and ADP induced platelet aggregation. But HbA1c in diabetic patients taking aspirin was significantly correlated with arachidonic acid induced platelet aggregation ( $r=0.203$ ,  $p<0.05$ ). Triglyceride was significantly correlated with platelet aggregation induced by ADP ( $r = -0.229$ ,  $p=0.024$ ), whereas total cholesterol, LDL and HDL did not demonstrate the relationships. Hemoglobin and hematocrit showed positive correlation with ADP induced platelet aggregation ( $r=0.256$ ,  $p=0.011$  and  $r=0.256$ ,  $p=0.011$ , respectively). Hemoglobin, hematocrit, platelets and mean platelet volume also did not show correlation with platelet aggregation induced by arachidonic acid.

Table 32 Correlation coefficient of blood glucose and platelet aggregation induced by arachidonic acid in asa group.

	AA		ADP	
	r	p-value	r	p-value
<b>Blood glucose</b>				
FPG	-0.023	0.823	-0.120	0.243
HbA1c	0.203 *	0.046	-0.002	0.984
<b>Lipid profiles</b>				
Total cholesterol	-0.024	0.818	-0.037	0.719
LDL	-0.023	0.827	-0.011	0.912
HDL	-0.005	0.960	0.088	0.389
Triglyceride	-0.114	0.265	-0.229 *	0.024
<b>Hematologic profiles</b>				
Hemoglobin	0.091	0.377	0.256 *	0.011
Hematocrit	0.040	0.698	0.256 *	0.011
Platelet count	-0.114	0.266	-0.100	0.332
MPV	0.183	0.079	0.058	0.575

Data represent in Mean  $\pm$  SD. \*  $p < 0.05$