

# Effect of hypoglycemic drugs on aspirin resistance in patients with diabetes mellitus

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**Abstract. – Background:** Aspirin reduces the odds of an arterial thrombotic event in high-risk patients. However, 10%-20% of patients with an arterial thrombotic event who are treated with aspirin have a recurrent arterial thrombotic event during long-term followup. Aspirin resistance has been described in some patient populations such as those with an acute coronary syndrome, ischemic stroke, percutaneous coronary intervention with drug-eluting stent, stent re-stenosis, and diabetes mellitus (DM). The aim of this study was to assess aspirin resistance and to compare it to the use of oral anti-diabetic drugs and insulin in patients with diabetes.

**Methods and Results:** Platelet aggregation was measured after aspirin treatment in 101 diabetic patients undergoing percutaneous coronary intervention. Two patient populations were included in the investigation: use of insulin (group 1) and use of oral anti-diabetic agents (OAD) (group 2) in diabetic patients. Platelet aggregation was determined using a multichannel Multiplate analyzer. Among group 1 patients, 4.7% were aspirin non-responders and among group 2 patients, 8.6% were aspirin non-responders. Statistical differences were not found between the groups ( $p = 0.359$ ).

**Conclusions:** This study demonstrated that there was no significant difference in aspirin resistance between type 2 diabetes mellitus patients on insulin treatment and type 2 diabetes mellitus patients on OAD treatment.

*Key Words:*

Aspirin, Platelets, Diabetes Mellitus.

## Introduction

Platelet activation and aggregation play an important role in the pathogenesis of arterial thrombosis. The most often used antiplatelet drug is aspirin (ASA), which irreversibly inhibits cy-

clooxygenase-1 activity in circulating platelets and consequently reduces the synthesis of thromboxane A<sub>2</sub>, a potent platelet activator. Aspirin is an oral antiplatelet drug that has been shown to reduce adverse clinical events across a wide spectrum of patients with atherothrombotic disease<sup>1</sup>.

Aspirin response is variable, and in some patients platelet aggregation is inhibited less than expected, which is often referred to as “aspirin resistance”. An impaired response to antiplatelet therapy with acetylsalicylic acid has been associated with stent thrombosis, major adverse cardiovascular events, recurrent ischemic events, recurrent myocardial infarction, stroke, and death despite patients’ compliance with regular intake of aspirin<sup>2</sup>.

Aspirin is widely prescribed worldwide for diabetes mellitus patients to prevent ischemic events. Diabetes mellitus (DM) is a well known risk factor for the development of atherosclerotic coronary artery disease (CAD). Platelets from diabetic subjects are less sensitive to aspirin<sup>2</sup>. Importantly, reduced sensitivity, or “poor response,” to aspirin has been associated with an increased risk of ischemic events<sup>1</sup>. The past decade has seen a surge of interest in identifying the etiology of biochemical antiplatelet resistance. Several studies have explored this phenomenon in diabetes mellitus patients<sup>3-5</sup>.

The physiological role of insulin in inhibiting platelet function could play a protective role in the prevention of thrombus formation and in the release of vasoactive mediators and chemotactic mitogenic substances, thus contributing to the reduction of pathological events such as thrombosis, hypertension and atherosclerosis.

The aim of the present study was to evaluate the utility of the Multiplate analyzer for the assessment of aspirin resistance on platelet function in patients with DM and compare it to the use of oral anti-diabetic drugs (OAD) and insulin.

## Patients and Methods

### Patients and Study Protocols

There were 101 consecutive DM patients enrolled in the study who were admitted undergoing elective coronary artery intervention and that received at least one ASA intake with a daily dose of 100 mg and 75 mg of clopidogrel in the 5 days before the intervention. Coronary interventions were performed according to current standard guidelines. All patients were enrolled and studied prospectively between March 2010 and July 2011. Patients were stratified according to the presence of type 2 diabetes, which was defined according to World Health Organization criteria. Diabetic patients were either on insulin or oral hypoglycemic medication, and were between the ages of 31 and 89 years old.

Two patient populations were included to investigate: use of insulin (group 1) and use of oral anti-diabetic agents (group 2) in diabetic patients.

**Group 1** consisted of 43 patients who used insulin. These patients were undergoing elective percutaneous coronary intervention and received at least one ASA intake with a daily dose of 100 mg and 75 mg of clopidogrel in the 5 days before the intervention.

**Group 2** was composed of 58 patients who used oral anti-diabetic agents. These patients were also undergoing elective percutaneous coronary intervention and received at least one ASA intake with a daily dose of 100 mg and 75 mg of clopidogrel in the 5 days before the intervention.

Diet-controlled diabetic subjects were not included. Those with a previous treatment with aspirin or clopidogrel were not included either. Subjects with liver disease, a gastrointestinal ulcer, pregnant subjects, those with a history of bleeding diathesis, a cerebrovascular event within 3 months of the intervention, major surgical procedure within one week prior to the intervention, and malignant paraproteinemias were rejected from the study as well. Patients with a platelet count  $< 100,000/\text{mm}^3$ , a hemoglobin count  $< 8$  g/dl, a history of heparin-induced thrombocytopenia, a contraindicated cerebrovascular event within 3 months, or a prothrombin time  $> 1.5$  times the normal control time were also not included.

Subjects were included if they were on a hypoglycemic treatment (oral anti-diabetic agents or insulin) for at least 1 year. Also, patients had

to display a symptomatic stable coronary artery disease and had to agree to the coronary intervention.

Blood samples for the aggregation tests were obtained from the antecubital vein, using a 21-gauge needle. Blood samples were collected in tubes containing 3.2% citrate. Platelet aggregation was measured by a Multiplate analyzer (MEA).

The institutional Review Boards approved the protocols, and written informed consent was obtained from each subject before enrollment. The study protocol was approved by the Ethics Committee of the Medical University of Dicle in accordance with the Declaration of Helsinki. The medical record of each patient was reviewed and demographic, laboratory, and platelet aggregation data were recorded.

### Platelet Aggregation

Platelet aggregation was determined using a multichannel Multiplate analyzer (MEA; Dynabyte, Munich, Germany) using arachidonic acid (ASPI-test, 0.5 mM). In the MEA test cell, 300  $\mu\text{l}$  of isotonic saline and 300  $\mu\text{l}$  of whole blood were combined and warmed for 3 minutes to a temperature of 37°C. The activator solutions (commercially available test reagents) were then added and aggregation was measured for 6 min. The impedance change between the sensor wires due to the attachment of the platelets was expressed in arbitrary aggregation units and platelet aggregation was quantified as the area under the aggregation curve (AUC, U). Normal range of AUC using hirudinized blood was 75-136 U in the ASPI-test that was performed in each of the 101 enrolled patients.

### Statistical Analysis

In patients with DM, paired 2-sample *t* tests and chi-square tests were used to compare platelet aggregation between oral anti-diabetic agents and insulin. Statistical analysis were performed using the Statistical package SPSS v15.0 (SPSS Inc.; Chicago, IL, USA). Student *t* tests were used for statistical analysis. All values were expressed as mean SD. A *p* value of  $< 0.05$  was considered statistically significant.

## Results

Of the 101 patients enrolled, 53 (52.5%) were male. The mean age was  $66 \pm 7$  years. Demographic data of all patient groups are shown in

Table I. Of the 101 patients, 7 showed an abnormal aggregation response and 94 showed a normal aggregation response. In the patient group of 43 subjects that used insulin, 4.7% (n=2 patients) displayed aspirin resistance. In the patient group consisting of 58 patients on OAD, 8.6% (n=5 patients) displayed aspirin resistance. However, statistical differences were not found between the groups ( $p = 0.359$ ) (Tables I and II).

Of the patients within the upper quartile of the ASPI area under the curve (AUC), 0-700 was considered the normal range, and those in the range of 700-1000 were considered to have drug resistance. Mean AUC induced by ASPI-tests were  $181.23 \pm 207.8$  in patients using insulin. Mean AUC induced by ASPI-tests were  $182.79 \pm 239.1$  in patients using OAD. Patients within the upper quartile of the ASPI area under the curve (AUC) were categorized as aspirin poor responders in group 1 with values of  $800 \pm 10$  and aspirin poor responders in group 2 with values of  $978 \pm 10$  (Figure 1). There were no significant differences in the baseline demographics and clinical characteristics (Table I). Additionally, there were no significant differences between aspirin resistance and blood parameters such as platelet counts, hematocrit, hemoglobin, mean platelet volume, sedimentation, C-reactive protein levels, urea, creatinine, sodium, potassium, ALT, AST, blood glucose, albumin, lipid parameters, and thyroid hormones. Meanwhile, there were no significant differences between aspirin resistance and HbA1c ( $p = 0.191$ ) (Table I).

## Discussion

Diabetes is commonly associated with accelerated atherosclerosis, resulting clinically in premature coronary artery disease, increased risk of cerebrovascular disease, and severe peripheral vascular disease<sup>6,7</sup>.

Platelet dysfunction can contribute to the increased risk of atherothrombotic complications in the diabetic population. Such altered platelet function has been revealed by hypersensitivity to be similar to aggregates observed in *in-vitro* studies<sup>11</sup>.

The present observational study compared two groups on hypoglycemic drugs in order to detect aspirin resistance in patients with DM. This randomized prospective study has confirmed the hypothesis that a comparison between oral anti-diabetic agents and insulin in treating diabetes mellitus must be taken into consideration regarding aspirin use. Although aspirin has been approved as an effective antithrombotic drug, this is the first study to report on its antiplatelet effect in diabetic patients and to demonstrate an association between aspirin resistance and use of hypoglycemic drugs. Importantly, reduced sensitivity, or “poor response,” to aspirin has been associated with an increased risk of ischemic events<sup>9,10</sup>. In the present study, ASPI-test platelet aggregation investigated aspirin administration in diabetic patients undergoing elective percutaneous coronary intervention. In the present study, 4.7% of 43 patients using insulin experienced aspirin resistance, and 8.6% of 58 patients using OAD experienced aspirin resis-

**Table I.** Patient characteristics at time of randomization.

Parameters	Patients group 1 (n = 43)	Groups group 2 (n = 58)	p
Age, years	67 ± 8	66 ± 7	NS
Sex, n (%)	22 (51%)	28 (48.2%)	
Male	21 (49%)	30 (51.8%)	NS
Female	25 ± 6	26 ± 5	NS
Body mass index, kg/m <sup>2</sup>	33 (69.5%)	37 (70%)	NS
Hypertension	39 (81%)	40 (75%)	NS
Hyperlipidemia			NS
LVEF 35% ≤	8 (16.7%)	11 (20%)	NS
35% ≥	40 (83.3%)	42 (80%)	NS
Smoker	23 (49.1%)	25 (49.2%)	NS
DM duration, years	8.51 ± 6.43	8.42 ± 7.52	NS
HbA1c, %	6.7 ± 6.25	7.46 ± 2.6	NS
Responder	41 (95.3%)	53 (91.4%)	NS
Nonresponder	2 (4.7%)	5 (8.6%)	NS

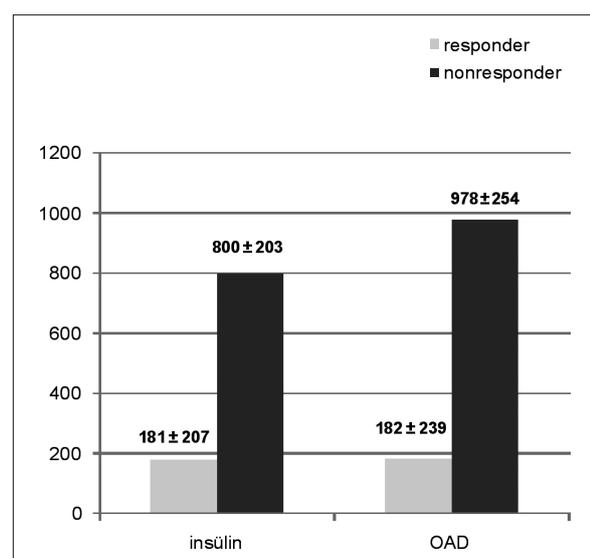
LVEF: Left ventricular ejection fraction.

**Table II.** Patient characteristics at time of randomization.

Aspirin	Group 1	Mean AUC induced by ASPI	Group 2	Mean AUC induced by ASPI
Responder	41 (87.5%)	128.9 ± 122.7	53 (90.6%)	136.6 ± 165.2
Poor responder	2 (4.7%)	856 ± 10	5 (8.6%)	866 ± 10
Total	43 (100.0%)		58 (100.0%)	

tance. However, statistical differences were not found between the two groups ( $p = 0.359$ ). In addition, there were no significant differences between aspirin resistance and blood parameters. Platelets from individuals with type 2 diabetes have been shown to have a reduced response to aspirin. The patient's body mass index (BMI) may be another contributing factor to the variability in platelet response to aspirin. Overweight patients (BMI of 25 kg/m<sup>2</sup>), due in part to their propensity to display insulin resistance, demonstrated a reduced antiplatelet effect with aspirin. The term *resistance* encompasses patients for whom aspirin does not achieve its pharmacological effect, and *failure of therapy* reflects patients who have recurrent events on therapy<sup>11</sup>.

In the present study the investigators did not find significant correlations between BMI and aspirin resistance. The reported rates vary between studies because of the techniques used to measure the extent of platelet aggregation and the presence of factors contributing to a greater baseline platelet reactivity<sup>12,13</sup>.

**Figure 1.** Aggregation values in both two groups.

Furthermore, the definition of non-responders has not been standardized. The question of whether increased doses of aspirin may overcome this resistance in non-responsive patients warrants further investigation.

Platelet function has typically been measured *in vitro*, in most instances, by light transmission aggregometry and this method is currently considered the gold standard. MEA is not a gold standard test although MEA is reliable. MEA provides a measurement of platelet aggregation in whole blood by monitoring changes in electrical impedance via disposable test cells. MEA is increasingly used for the monitoring of critically ill patients in the fields of cardiology, neuroradiology, and anaesthesiology.

Future investigations need to determine whether these patients are aspirin resistant, clopidogrel resistant, or both. Additionally, the possibility that drug-drug interactions between aspirin and OAD contribute to these events needs to be evaluated. Consistent definitions for aspirin resistance are needed that can be documented by reliable laboratory testing and associated with increased risk for thrombotic complications. In the future, measurement of antiplatelet drug efficacy with a point-of-care device and alternative antithrombotic strategies for "poor responders" could reduce the incidence of ischemic events. Current available data show that about 4% to 30% of patients treated with conventional doses of aspirin do not display adequate antiplatelet response.

### Study Limitations

Some inherent limitations existed in the present study. It should be mentioned that this study was observational in nature, comprising a relatively small sample size and, therefore, should not allow for definitive conclusions. These findings should be confirmed in a larger-scale study.

The measurements reflected the extent of platelet inhibition, and this single measure of platelet function may not be sufficient to diag-

nose aspirin resistance when optimal inhibition is required. It could be argued that measuring platelet aggregation is instrument dependent and laboratory dependent. Platelet function has been measured *in vitro*, in most instances, by light transmission aggregometry and this method is still considered as the gold standard. MEA is not considered a gold standard test. The investigators definition of aspirin resistance was arbitrary because there have not been extensive reports or consistent standards regarding this subject.

In conclusion, the study demonstrated that there was no significant difference between aspirin resistance among patients on insulin treatment and among patients on OAD treatment with type 2 diabetes mellitus. Aspirin resistance is a widely used term but has not been clearly defined. So far, it has been used to reflect failure of aspirin to achieve its platelet inhibiting effect.

## References

- 1) ANGIOLILLO DJ, FERNANDEZ-ORTIZ A, BERNARDO E, RAMÍREZ C, SABATÉ M, JIMENEZ-QUEVEDO P, HERNÁNDEZ R, MORENO R, ESCANED J, ALFONSO F, BAÑUELOS C, COSTA MA, BASS TA, MACAYA C. Platelet function profiles in patients with type 2 diabetes and coronary artery disease on combined aspirin and clopidogrel treatment. *Diabetes* 2005; 54: 2430-2435.
- 2) BONVINI RF, RENY JL, MACH F, ZELLER T, FONTANA P. Acute coronary syndrome and its antithrombotic treatment: focus on aspirin and clopidogrel resistance. *Curr Vasc Pharmacol* 2009; 7: 198-208.
- 3) BONVINI RF, RENY JL, MACH F, ZELLER T, FONTANA P. Acute coronary syndrome and its antithrombotic treatment: focus on aspirin and clopidogrel resistance. *Curr Vasc Pharmacol* 2009; 7: 198-208.
- 4) ANGIOLILLO DJ, BERNARDO E, RAMIREZ C, COSTA MA, SABATE M, JIMENEZ-QUEVEDO P, HERNANDEZ R, MORENO R, ESCANED J, ALFONSO F, BANUELOS C, BASS TA, MACAYA C, FERNANDEZ-ORTIZ A. Insulin therapy is associated with platelet dysfunction in patients with type 2 diabetes mellitus on dual oral antiplatelet treatment. *J Am Coll Cardiol* 2006; 48: 298-304.
- 5) ANGIOLILLO DJ, FERNANDEZ-ORTIZ A, BERNARDO E, RAMÍREZ C, SABATÉ M, JIMENEZ-QUEVEDO P, HERNÁNDEZ R, MORENO R, ESCANED J, ALFONSO F, BAÑUELOS C, COSTA MA, BASS TA, MACAYA C. Platelet function profiles in patients with type 2 diabetes and coronary artery disease on combined aspirin and clopidogrel treatment. *Diabetes* 2005; 54: 2430-2435.
- 6) KANNEL WB, MCGEE DL. Diabetes and cardiovascular risk factors: the Framingham study. *Circulation* 1979; 59: 8-13.
- 7) HAFFNER SM, LEHTO S, RONNEMAA T, PYORALA K, LAAKSO M. Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. *N Engl J Med* 1998; 339: 229-234.
- 8) MAK KH, MOLITERNO DJ, GRANGER CB, MILLER DP, WHITE HD, WILCOX RG, CALIFF RM, TOPOL EJ. Influence of diabetes mellitus on clinical outcome in the thrombolytic era of acute myocardial infarction. GUSTO-I Investigators. Global Utilization of Streptokinase and Tissue Plasminogen Activator for Occluded Coronary Arteries. *J Am Coll Cardiol* 1997; 30: 171-179.
- 9) BHATT DL. Aspirin resistance: more than just a laboratory curiosity. *J Am Coll Cardiol* 2004; 43: 1127-1129.
- 10) WANG TH, BHATT DL, TOPOL EJ. Aspirin and clopidogrel resistance: an emerging clinical entity. *Eur Heart J* 2006; 27: 647-654.
- 11) ANDERSEN K, HURLÉN M, ARNESEN H, SELJEFLOT I. Aspirin nonresponsiveness as measured by PFA-100 in patients with coronary artery disease. *Thromb Res* 2002; 108: 37-42.
- 12) MASON PJ, JACOBS AK, FREEDMAN JE. Aspirin resistance and atherothrombotic disease. *J Am Coll Cardiol* 2005; 46: 986-993.
- 13) SILLER-MATULA JM, LANG I, CHRIST G, JILMA B. Calcium-channel blockers reduce the antiplatelet effect of clopidogrel. *J Am Coll Cardiol* 2008; 52: 1557-1563.