Original Article

Measurement of platelet function to determine the prevalence of aspirin non-responsiveness among Saudi type II diabetic patients

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ABSTRACT

Introduction: Aspirin is widely used as either a primary or secondary preventive measure in of cardiovascular events however, platelets from diabetic patients are less responsive to aspirin and are unable to protect themselves from thrombotic events.

Objective and Method: 180 diabetic patients were enrolled for measuring their platelet aggregation. The aim was to evaluate the prevalence of aspirin non-responsiveness among Saudi type II diabetic patients. Serum glucose level and other clinical data were collected to find out the possible determinant of reduced platelet sensitivity to aspirin.

Results: The prevalence of aspirin non-responsiveness was 9.44%. A significant correlation between aspirin test and each of fasting blood sugar, HbA_1c, cholesterol and platelet count was observed. In contrast, there was no correlation among aspirin non-response, body mass index, age or hypertension.

Conclusion: The relationship between the levels of glucose in the blood and aspirin resistance relates the importance of controlling blood glucose in diabetic patients to guarantee better aspirin action. Regular examining of type II diabetic patients to determine the sensitivity of platelet to the antiplatelet therapy is necessary to protect them from the risks of cardiovascular complications.

Keywords: Aspirin, diabetes, platelet, thrombosis

INTRODUCTION

Diabetes mellitus

Diabetes mellitus (DM) is a major health problem worldwide, especially in Saudi Arabia.[1] Its incidence is increasing rapidly.[2] Study in Saudi Arabia has shown that the prevalence of DM is about 23.7% in adults.[1] DM is considered as the major risk factor for cardiovascular disease including heart ischaemic disease, stroke, high blood pressure, atherosclerosis and heart failure.[3] In fact, it has been reported that about 75% of type II diabetic patients will die from complications attributed to atherosclerosis.[4] Diabetic patients are at a higher risk for recurrent events of coronary heart disease than non-diabetic patients, e.g., in type II diabetic patients, the incidence of myocardial infarction was 3.2 times and stroke was 4.2 times greater compared to age-matched non-diabetic patients.[5]

Many studies concluded that in diabetic patients there are abnormalities of coagulation screening tests

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(shorter prothrombin times and activated partial thromboplastin times) with increased activation markers (prothrombin activation fragment 1 + 2) compared to non-diabetics.[4] Also in diabetic patients, as reported by several studies, there is an increase in the level of clotting factors such as kallikrein, factor XII, factor XI, factor VIII and von Willebrand factor levels.[4]

Aspirin

Aspirin was developed by Hoffmann in 1898. Aspirin was then used as an antipyretic and anti-inflammatory agent. In 1960s, aspirin emerged as a vital agent that prolonged the bleeding time and inhibited platelet aggregation. Evidence indicates that therapy with aspirin also resulted in a 25% reduced risk of non-fatal myocardial infarction, non-fatal stroke, vascular death in high-risk patients, arterial hypertension or diabetes.[1,6-9]

Aspirin inhibits prostaglandin synthesis by irreversible inactivation (acetylation) of cyclooxygenase where an acetyl group is covalently attached to a serine residue in the active site of the cyclooxygenase-1 enzyme (COX-1), thereby leading to decreased thromboxane A2 (TXA_2) production in platelets.[10] Aspirin also inhibits COX in vascular endothelial cells and reduces the production of prostaglandin, which has an antithrombotic action. The inhibitory effects of aspirin are permanent due to the inability of platelet to resynthesize a new COX enzyme. The major advantage of aspirin, apart from being cheap, is that it does not require any monitoring. Compared to other anticoagulants, it also has a low risk of bleeding and can be orally administered.

Studies on aspirin non-responsiveness

Non-responsiveness is the inability of aspirin to reduce platelet production of TXA_2 resulting in platelet activation and aggregation. There has been no agreement on the definition of aspirin non-responsiveness despite the regular wide consumption of aspirin. It also leads to thrombotic complications even after being prescribed as an antithrombotic therapy.[11,12]

Aspirin's non-responsiveness can be assessed using a variety of platelet function tests such as light transmission aggregometry and whole blood aggregometry platelet function analyser-100 (PFA-100). Aspirin non-responsiveness in diabetic patient was first hypothesised in 1986 by DiMinno *et al*., who showed that there was a high rate of platelet turnover in diabetic patients, relating to more young platelets entering the blood stream.[13,14] Later, some studies found high proportion of non-responders to aspirin in obese insulin-resistant diabetic patients, compared with control (insulin-sensitive).[15,16]

Some studies concluded that aspirin therapy decreases the risk of ischaemic events by 22%, but the reduction in the diabetic patients was only 7%.[17] In the 2005 study conducted by Fateh-Moghadam *et al*., about 21.5% of patients with type II diabetes under chronic aspirin therapy were resistant and about 16.9% were partially resistant to aspirin using the PFA-100 system.

Moreover, inappropriate dosing of aspirin and the presence of alternative pathways independent of arachidonic acid that may activate platelets or block COX-1 against acetylation by certain non-steroidal anti-inflammatory drugs or due to the genetic changes in the COX-1 protein[18] leads to non-responsiveness in diabetes.[19]

Another study concluded that the decrease in platelet sensitivity to aspirin in diabetic patients is due to poor metabolic control.[20,21] A different study suggested that high blood glucose results in glycosylation of platelet protein making them less accessible to acetylation which further leads to significantly impaired platelet response to acetyl salicylic acid (ASA) in diabetic patients.[22]

An *in vitro* study has reported that there is a direct effect of blood glucose level and aspirin concentration on platelet adhesion protein expression, and that may explain the reduced response to aspirin in diabetic patients as measured by the expression of the two adhesion proteins, GP IIb–IIIa and P-selectin.[9]

Other studies on type II DM also conclude that hyperglycaemia changes the bioavailability of nitric oxide (NO) in platelets by decreasing NO synthase activity, thus resulting in ASA-insensitive thromboxane biosynthesis; besides, in type II diabetic patients, there is an increase in platelet activation through decrease in the number and affinity of insulin receptor in platelets.[15,20]

Moreover, recent studies have suggested that there is a decrease in COX-1 sensitivity and thromboxane B_2 (TxB_2) production in both type II and type I diabetic patients under chronic aspirin therapy and there is a correlation between TxB2 production and inhibitory action of aspirin.[23,24]

Another study observed that the there was a reduction in the response of platelets in diabetic patients to aspirin by 14% using whole blood impedance aggregometry and 79% using PFA-100.[21]

The underlying mechanism for this is still largely debated, but scientists believe that hyperglycaemia in

diabetic patients is a determinant of reduced platelet sensitivity to the inhibitory action of aspirin.

OBJECTIVE AND METHOD

Subjects

180 type II diabetic male patients who were on aspirin (81 mg/day) for at least 7 days were inducted in the study in the Diabetes Centre of King Abdul-Aziz University Hospital and Prince Salman Ibn Abdul-Aziz Hospital, Riyadh. Written consent was obtained from each participant before inclusion in the study. All patients were males, and their age ranged between 37 - 82 years. The risk factors of these patients included hypertension (defined as systolic blood pressure ≥140 mmHg and diastolic blood pressure ≥90 mmHg, hypercholesterolaemia (blood cholesterol levels ≥5 μmol) and obesity (body mass index [BMI] >30 kg/m^2, BMI defined as weight in kilograms divided by the square of height in meters).[25]

Exclusion criteria

- Patients with a platelet count <150.000/μl or >450.000/μl and haemoglobin level <8 g/dl
- Those taking any other antiplatelet drugs such as Plavix (clopidogrel), ticlopidine, dipyridamole or other non-steroidal anti-inflammatory drugs
- Those on oral anticoagulation therapy
- Those who underwent treatment using unfractionated heparin or low-molecular-weight heparin
- Those who had any blood disease, such as family history of bleeding disorders

Controls

50 healthy male volunteers, who had not been on aspirin or non-steroidal anti-inflammatory drugs for the last 10 days. These controls were used to establish normal ranges for platelet aggregation.

Collection of blood samples

Venous blood was collected by antecubital venipuncture, directly into vacutainer tubes containing lithium heparin for platelet analysis using multiplate analyser (MPA), and other samples were collected in a tube containing ethylene diamine tetraacetic acid for platelet count and for measuring haemoglobin level. To avoid spontaneous platelet activation, venipuncture and blood collection was performed and the initial blood was drawn in plain tube for measuring the serum glucose level and cholesterol level.

All samples were gently mixed by inversion and kept at room temperature. Samples for platelet aggregation were examined without delay 0.5 - 3 h after blood collection to minimize any artificial activation of platelets by prolonged stasis.

Measuring platelet aggregation using multiple electrode aggregometry

Measuring platelet aggregation in whole blood was determined using a new generation impedance aggregometer (Multiplate analyser, Dynabyte Medical, Munich, Germany).

Aggregation was triggered using 20 μl of arachidonic acid (0.16 mM, ASPItest, Dynabyte, Munich, Germany). The ability of platelets to adhere to the metal sensors in the test cuvette was detected. The adhesion and aggregation of platelets was detected by measuring the impedance change. The resistance change was transformed to arbitrary aggregation units (AUs) and plotted against time. The area under the aggregation curve was used to quantify the aggregation response and expressed in units (1U corresponds with 10 AU min). The results registered by the two sensors provided two aggregation curves.

Statistics

The data were analysed using the SPSS Chicago, USA (version 18) software. Mann–Whitney U-test was used to calculate mean rank in each of age, BMI, fasting blood sugar (FBS), cholesterol, HbA1C, plt and Hb for responders and non-responders. Logistic regression analysis was used to determine the expected predictor for aspirin resistance in diabetic patients. $P < 0.05$ was considered significant.

Ethics

The research protocol and use of clinical samples for this study were approved by the Institution Review Board of College of Medicine and King Khalid University Hospital and informed consent was obtained from all the patients.

RESULTS

Results of control group

5 percentile of aggregation values of the aspirin test for 50 healthy blood donors was 62.45 AU, which was selected as the cutoff for non-responders in patients taking aspirin.

Patients on chronic aspirin therapy showing higher aggregation values than the cutoff (62.45 AU) were classified as non-responders. Patients with aspirin test <62.45 AU were defined as aspirin responders.

Result of patient group

The percentage of responders to aspirin was 90.56% (163/180) and non-responders was 9.44% (17/180) [Figure I].

DISCUSSION

Determining the prevalence of aspirin non-responsiveness

Many patients are still at risk of cardiovascular events despite the regular ingestion of aspirin. There are a lot of disagreements on the type of diagnostic method used to determine the prevalence of aspirin non-responsiveness.[10]

To our knowledge, this is the first study to show the prevalence of aspirin non-responsiveness among diabetic patients in Saudi Arabia where DM is considered a major health problem.[1] The main result of this study is the prevalence of aspirin non-responsiveness among 180 Saudi type II diabetic patients, which was found to be 9.44% using the MPA (ASPItest).

Previous studies have demonstrated the percentage of non-responders among type II diabetic patients using the PFA-100 system to be 21.5%.[21,25] Another study observed that there was a reduction in the response of platelets in diabetic patients to aspirin by 14% using whole blood impedance aggregometry and 79% using PFA-100.[21]

Moreover, another study using multiplate analyser for chronic cardiovascular patients on chronic aspirin therapy found the rate of non-responder to be 4% using ASPItest compared to the 6.6% of non-response of aspirin using another instrument (PFA-100).[26]

Factors that might contribute to the aspirin non-responsiveness

Many variables affect platelet sensitivity towards aspirin. Some studies suggested an association of aspirin non-responsiveness with smoking, hypertension, hypercholesterolaemia and age.[25] In our study, we observed a positive significant correlation between aspirin test and each of FBS, HbA1c, cholesterol and platelet count in a group of total 180 Saudi type II diabetic patients [Table 1]. In contrast, there was no correlation among aspirin non-response, BMI, age or hypertension. Furthermore, in the present study, we also found that there were statistically significant differences between the responders and non-responders, in each of the FBS, HbA_1C and platelet count. In contrast, there were no significant differences noted in age, BMI, cholesterol or Hb.

By using logistic regression analysis, we found that age, FBS and cholesterol were to be the expected predictors for aspirin resistance in diabetic patients, and these findings agree with those of Fateh-Moghadam *et al.*, who reported that age was an independent predictor for aspirin resistance in diabetic patients due to the increased platelet turnover in younger people.

In the current study, correlation among FBS, HbA1c and higher aggregation in ASPItest, despite the regular ingestion of aspirin in type II diabetic patients, suggests that hyperglycaemia may play a significant role in the reduced platelet sensitivity to aspirin in type II diabetic patients [Figure 2]. These findings agree with those of Pulcinelli *et al.*, (2009), who reported that an increase in either fasting plasma glucose or HbA_1c levels is associated with reduced platelet sensitivity to aspirin by measuring TxB_2 production in diabetic patients.

In addition, our findings agree with the report by Keating *et al.*, (2003), who suggested that there are significant associations between the altered platelet function and the glucose level by measuring platelet glycoprotein IIb/IIIa and P-selectin receptors expression on the platelets of patients with and without DM using flow cytometry.

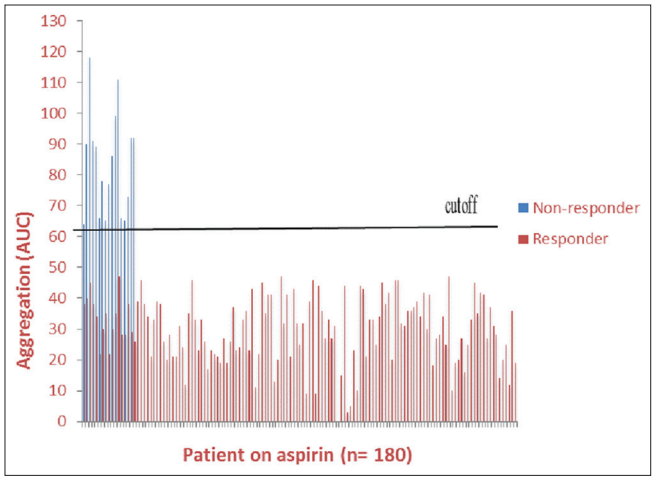

Figure 1: Distribution of the aggregation values of 180 diabetic patients (responder vs. non-responder). Red columns represent aggregation values of the non-responders that lie above the cutoff value

Table 1: Relationship between 180 evaluated diabetic patients' and age, HbA_1c, FBS, BMI, blood pressure and cholesterol level

	All patients (*n* = 180)	Non-responder (*n* = 17)	Responder (*n* = 163)	*P*
Age (years), mean ± SD	56.27 ± 9.28	55.35 ± 6.29	57.11 ± 9.66	0.924
HbA_1c (%), mean ± SD	7.984 ± 1.62	8.72 ± 1.99	7.859 ± 1.58	0.045*
FBS (mmol/l), mean ± SD	8.45 ± 3.36	10.04 ± 4.47	8.037 ± 3.082	0.034*
BMI (kg/m^2), (mean±SD, *n*)	29.01 ± 4.60 (63)	28.21 ± 4.79 (4)	28.727 ± 4.386 (59)	0.237
Hypertension, *n* (%)	76 (42.22)	6 (35.29)	70 (42.94)	0.412
Hypercholesterolaemia (mmol/l), *n* (%)	34 (18.88)	7 (41.17)	27 (16.56)	0.318

*Coefficient is significant at <0.05 level. BMI: Body mass index, FBS: Fasting blood sugar, SD: Standard deviation

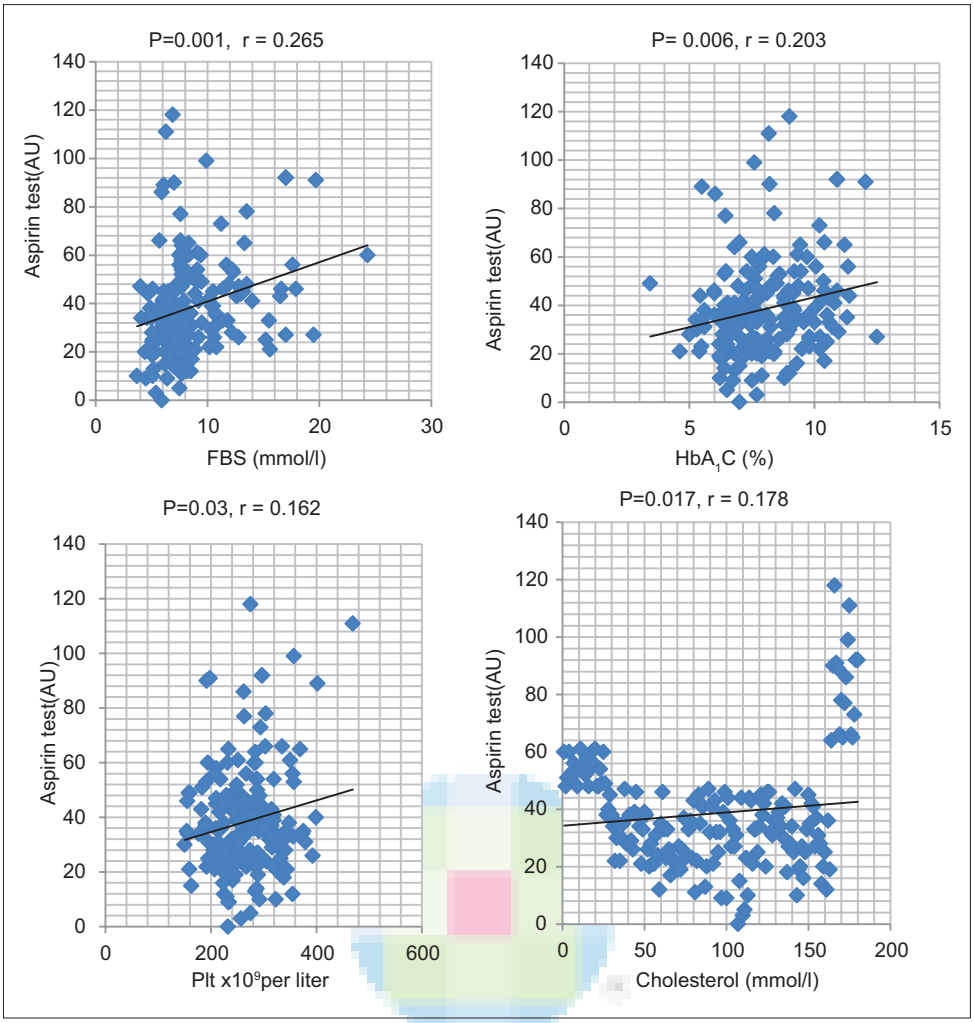

Figure 2: Correlations between the aggregation values of aspirin test and results of other variables in diabetic patients

CONCLUSION

Type II diabetes is a significant health problem in Saudi Arabia and is considered a high risk for cardiovascular disease. It is, therefore, important to do platelet function tests on diabetic patients who are on aspirin therapy to find out those who do not respond to aspirin; thereby, getting no benefit from the drug. Such patients should be treated with different antiplatelet drugs to protect them from cardiovascular diseases and cerebrovascular events.

In addition, our finding of the relationship between the levels of glucose in the blood and aspirin resistance highlights the importance of controlling blood glucose in diabetic patients to guarantee better performance of aspirin action.

Furthermore, regular examining of type II diabetic patients to determine the sensitivity of platelet to the antiplatelet therapy is necessary to protect them from the risk of cardiovascular complications.

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Conflicts of interest

There are no conflicts of interest.

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