Original Article

Prevalence of Aspirin Resistance in Diabetic Patients and its Associated Factors

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Abstract

***Background*: Aspirin resistance has posed a major dilemma in the prevention of cardiovascular disease and stroke. There have been many factors that have been associated with aspirin resistance. Among these factors, the inflammatory processes of diabetes and glycaemic control have been significantly associated with aspirin resistance. Our study evaluated the prevalence of aspirin resistance and its associated factors.**

***Methods:* This was a cross-sectional, interventional study, which was implemented from October to November 2012 at the Hospital Universiti Sains Malaysia (HUSM). Sixty-nine patients with diabetes who were taking aspirin were enrolled. The glycosylated haemoglobin (HbA1c) and C-reactive protein (CRP) levels were measured in these patients. The thromboelastography (TEG) level was measured using a TEG machine by a trained technician employing standard methods. The variables obtained were analysed for prevalence of aspirin resistance, HbA1c, CRP, and TEG level. The Chi-square test (and Fisher exact test where applicable) were used to evaluate the associations between aspirin resistance with glycaemic control (HbA1c) and inflammatory markers (CRP).**

***Results:* The prevalence of aspirin resistance was 17.4% (95%; CI 9.3, 28.4). Glycaemic control (HbA1c) and inflammatory markers (CRP) were not associated with aspirin resistance. Aspirin resistance was prevalent in our study population and was comparable to other studies. The mean HbA1c in the aspirin-resistant group was 8.9%, whereas the mean HbA1c in the aspirin-sensitive group was 8.6%.**

***Conclusion:* There was no significant difference in HbA1c between the two groups. There was no significant association between CRP levels and aspirin resistance.**

Keywords: *aspirin resistance, thromboelastography, diabetes mellitus*

Introduction

Aspirin resistance has been recognised in patients with cardiovascular disease (CVD) and stroke, particularly in the patients who also have diabetes. Aspirin resistance has been associated with poor clinical outcomes. In Germany, researchers reported that the prevalence of aspirin resistance in their population with diabetes was 21.5% (1). Another study mentioned that 17.6% of 18 subjects with aspirin resistance had diabetes (2). Meanwhile, in China, 4.3% of patients with Type 2 diabetes were found to be resistant to aspirin therapy using the light transmission aggregometry (LTA) method, whereas 31 patients (22.1%) were found to be aspirin resistant using the thromboelastography (TEG) method (2). The studies that have been completed in patients with diabetes have shown that there was a higher occurrence of aspirin resistance in this population (4).

Aspirin resistance in patients with diabetes also has an association with multiple risk factors such as glycaemic control and inflammation. There have been reports that found that glycaemic control was not associated with aspirin resistance in patients with diabetes (1,5). However, these results have been contradicted by a different study (6). Few other studies have also supported the belief that poor glycaemic control was associated with a higher prevalence of aspirin resistance (7–9).

On the other hand, few studies have looked at the association between inflammation and aspirin

resistance in patients with diabetes. They found that the stroke group that was aspirin-resistant had higher levels of IL-6 than the stroke group that was aspirin-sensitive. The inflammatory mediators, such as P-selectin, IL-6, CD40 ligand (CD40L), and its soluble form (sCD40), were higher in patients with diabetes compared with patients without diabetes (11–13). Another study also reported that high levels of P-selectin and IL-6 had been associated with adverse cardiovascular outcomes and the development of coronary events in subjects with diabetes who did not have established CVD (14).

There have been few postulated mechanisms for aspirin resistance in patients with diabetes, including platelet dysfunction, endothelial dysfunction, gene dysmorphisms, and the pro-inflammatory and prothrombotic states in patients with diabetes.

Thus, the purpose of this study was to determine the prevalence of aspirin resistance in the Malaysian population, particularly in the groups of people with diabetes. This study also evaluated the risk factors that were associated with aspirin resistance, including glycaemic control (through glycosylated haemoglobin (HbA1c)) and inflammatory markers (through C-reactive protein levels).

Materials and Methods

The study was a cross-sectional report performed from October to November 2012 in the NeuroMedical Specialist Clinic, Hospital Universiti Sains Malaysia (HUSM), Health Campus.

Participants

The total number of patients required for this study was 70 patients (with a study power of 80%), considering 10% of dropped out. However, we only enrolled 69 patients with diabetes. All patients who were diabetic, older than 18 years of age, and were receiving aspirin therapy with follow-up at Klinik Pakar Perubatan, HUSM were included. All of these patients underwent systematic randomised sampling.

Aspirin therapy included all forms of acetylsalicylate acid, such as aspirin (non-enteric coated form) or cardiprin (enteric-coated form), regardless of the duration of treatment or dosage.

The exclusion criteria were patients with clinically active bleeding, a history or current symptoms suggestive of gastroesophageal reflux or active peptic ulcer disease, a history of recent gastrointestinal bleeding within the prior 3 months, recent intracranial bleeding within the previous year, a history of aspirin allergy, patients who were taking other types of antiplatelet agents, a history of bleeding disorders, a history of haematological disorders, concomitant use of anticoagulant drugs, a platelet count less than 100×10^3/L, or a patient receiving any type of traditional medicine.

This study was approved by the Human Research Ethical Committee USM on 23rd October 2012.

Laboratory investigation

Blood samples were collected from each patient 2 hours after the participants received a 300 mg aspirin bolus that maintained the antiplatelet effect in all subjects, including those who were noncompliant. The blood samples were sent out for the thromboelastography (TEG) studies. The TEG studies were performed by a trained medical lab technician, who employed the standard TEG method. The coefficient of variation for clot strength was 4% (8).

The term aspirin-sensitive was defined as the occurrence of less than 50% platelet aggregation after stimulation with 1 mmol/l AA as measured by TEG two hours after the infusion of 300 mg of aspirin therapy (15). The term aspirin resistance was defined as the occurrence of more than 50% platelet aggregation after stimulation by 1 mmol/l AA as measured by TEG two hours after the infusion of 300 mg of aspirin therapy.

The latex agglutination method was used to measure C-reactive protein (CRP) to screen for positivity. When CRP was found to be positive, the level was further assessed using an immunoturbidimetric assay with mg/L units. The CRP level was graded as positive when the CRP level was ≥ 10 mg/L. The CRP level was considered negative when the CRP level was < 10 mg/L.

Statistical analysis

The variables obtained were analysed for the prevalence of aspirin resistance and their associated factors. Chi-square tests (Fisher's exact test where applicable) were used to evaluate the association of HbA1c and CRP levels with aspirin resistance. Multiple logistic regressions were also employed to analyse other risk factors that may have been associated with aspirin resistance.

Results

There were 69 patients recruited for this study. The mean age at presentation was 61-year-old. Majority of the patients were Malays (84.1%).

There were more male patients (63.8%) than female patients (36.2%). Of the 69 patients, 52 of them took aspirin as their antiplatelet agent; 46 of these 52 patients took an aspirin dose of 150 mg (66.7%). Approximately 10% of this study population were active smokers. The demographic data and baseline characteristic of the participants are summarized in Table 1, 2, and 3.

Twelve patients were found to be aspirin resistant in this study. The prevalence was 17.4% (SD 0.38) (95% CI 0.08, 0.27%) of all patients with diabetes in this study.

There were no significant association of aspirin resistance with age, blood pressure, fasting blood sugar level, HbA1C, triglyceride level, high density lipoprotein (HDL) cholesterol levels, low density lipoprotein (LDL) cholesterol levels, CRP levels, and serum creatinine levels (Table 4).

Discussion

The prevalence of aspirin resistance in our 69 subjects with diabetes was 17.4%. There have been few studies that have evaluated the prevalence of aspirin resistance; however, these studies have been performed in different populations, and used other methods to measure aspirin resistance. These factors may be the reason why there were discrepancies in the prevalence of aspirin resistance in the other studies.

In one study in Germany that used platelet function analyser (PFA), the researchers reported that the prevalence of aspirin resistance in the population with diabetes was 21.5% (1). Another study was completed in patients with stable CVD, and reported that the prevalence of aspirin resistance was 5.5% and 9.5% using the optical aggregation and the PFA method, respectively. Out of 18 subjects with aspirin resistance, 17.6% had diabetes (2). One study from China reported that 4.3% of patients with Type 2 diabetes were found to be resistant to aspirin therapy using the LTA method and 22.1% were found to be aspirin resistant using TEG (15). We can see that the prevalence of aspirin resistance was around 20% in these previous studies (1,15), which was not very different from the results of our study. However, a significant number of patients have aspirin resistance at our centre. This group of patients may need an additional dose or type of antiplatelet

Table 1: Demographic data of patients (n = 69)

Variables	Frequency, n (%)	Mean (SD)
Age (year)	–	61 (7.6)
Races		–
Malay	58 (84.1%)	
Chinese	10 (14.5%)	
Indian	1 (1.4%)	
Gender		–
Male	44 (63.8%)	
Female	25 (36.2%)	
Types of aspirin		–
Cardiprin (enteric coated)	17 (24.6%)	
Aspirin(non-enteric coated)	52 (75.4%)	
Aspirin dosage(mg)		–
75 mg	6 (8.7%)	
100 mg	17 (24.6%)	
150 mg	46 (66.7%)	
Ischemic heart disease	34 (49.3%)	–
Hypertension	56 (81.2%)	–
Hyperlipidemia	43 (62.3%)	–
Ischemic stroke	11 (15.9%)	–
Smoking	7 (10.1%)	–

agent or may need to change their antiplatelet agent. We may also need to measure aspirin resistance in patients who have had recurrent cardiovascular events as well as recurrent strokes. Multicentre involvement may decrease the bias from the local setting. The inclusion of multiracial participants instead of predominantly Malay may also better reflect our national population.

Association of risk factors with aspirin resistance

Previous studies had suggested that there were risk factors that were associated with aspirin resistance, such as gender, age, presence of metabolic syndrome, dose of aspirin, amount of glycaemic control (HbA1C and fasting blood sugar), cholesterol levels, and inflammatory markers (1,15). In our study, we studied two variables that had been associated with aspirin resistance: glycosylated haemoglobin (HbA1C) and CRP level.

Association of glycaemic control with aspirin resistance

In one study, aspirin resistance had been significantly associated with an HbA1c ≥ 8% (P = 0.002) and obesity (BMI ≥ 30 kg/m^2; P = 0.01) (9). Another study reported different results with no association between glycosylated haemoglobin and aspirin resistance (5).

In this study, we also analysed fasting blood sugar which was the other parameter for glycaemic control. We also found no significant association with aspirin resistance (Table 5). Our results contradicted the results in a different study, which demonstrated that a fasting blood glucose level > 5.6 mmol/L (P = 0.045) significantly affected aspirin resistance (7).

Table 2: Baseline characteristic of patients (n = 69)

Variables	Frequency, n (%)
Systolic BP (mmHg)	
< 130	11 (15.9)
130–139	25 (36.2)
140–149	12 (17.4)
150–159	8 (11.6)
160–169	9 (13.0)
170–179	3 (4.3)
> 180	1 (1.4)
Diastolic BP (mmHg)	
< 80	27 (39.1)
80–89	34 (49.3)
> 90	8 (11.6)
HbA1C (%)	
> 7	53 (77.7)
CRP (mg/L)	
> 10	4 (5.8)
LDL-C (mmol/L)	
> 2.0	51 (73.9)
Triglycerides (mmol/L)	
> 1.7	16 (23.2)
Fasting blood sugar (mmol/L)	
> 6.0	54 (78.3)

Abbreviations: BP = Blood pressure; HbA1C = Glycosylated haemoglobin; CRP = C-reactive protein; LDL-C = Low density lipoprotein cholesterol.

Table 3: Demographic data of patients in aspirin resistance and aspirin sensitive group, (n = 69)

Variables	Aspirin resistance, (n = 12)	Aspirin sensitive, (n = 57)	*P* value
	N (%)/mean (SD)		
Age (year)	59 (5.0)	61.42 (8.0)	0.191[a]
Gender			
Male	7 (58.3)	37 (64.9)	0.746[c]
Female	5 (41.7)	20 (35.1)	
Races			
Malay	10 (83.3)	48 (84.2)	> 0.950[d]
Non-Malay	2 (16.7)	9 (15.8)	
Smoking			
Smoker	2 (16.7)	5 (8.8)	0.596[d]
Non-smoker	10 (83.3)	52 (91.2)	
Types of aspirin (ASA)			
Aspirin (non-enteric coated)	9 (75.0)	43 (75.4)	> 0.950[d]
Cardiprin (enteric coated)	3 (25.0)	14 (24.6)	
Dosage of aspirin (mg)			
75 mg	0 (0.0)	6 (10.5)	0.691[d]
100 mg	3 (25.0)	14 (24.6)	
150 mg	9 (74.0)	37 (64.9)	
DM duration (year)	10.0 (9.5)*	10.0 (13.0)*	0.695[b]
Duration of aspirin used (year)	8.5 (5.8)*	6.0 (6.0)*	0.904[b]
Related diseases			
Ischemic Heart Disease			
Yes	5 (41.7)	29 (50.9)	0.562[c]
No	7 (58.3)	28 (49.1)	
Hypertension			
Yes	9 (75.0)	47 (82.5)	0.685[c]
No	3 (25.0)	10 (17.5)	
Hyperlipidemia			
Yes	8 (66.7)	35 (61.4)	> 0.950[d]
No	4 (33.3)	22 (38.6)	
Stroke			
Yes	3 (25.0)	8 (14.0)	0.390[d]
No	9 (75.0)	49 (86.0)	

Abbreviation: ASA = Acetylsalicylates acid.
[a]independent sample t-test.
[b]Mann-Whitney test.
[c]Chi-square test.
[d]Fischer's exact test.
*median (interquartile range, IQR).

Table 4: Univariate analysis of the association of various risk factors with aspirin resistant and aspirin sensitive group (n = 69)

Variables	Aspirin resistance	Aspirin sensitive	*P* value
	Mean (SD)		
Age	59 (5.0)	61.4 (8.0)	0.191[a]
Fasting Blood Sugar (FBS) (mmo/l)	9.7 (4.3)	8.5 (3.9)	0.353[a]
% Glycosylated Hemoglobin (HbA1C)	8.9 (2.3)	8.6 (2.2)	0.833[a]
Triglycerides (TG) (mmo/l)	1.1 (0.8)*	1.2 (0.8)*	0.486[b]
High Density Lipoprotein Cholesterol (HDL-C) (mmo/l)	1.2 (0.2)	1.2 (0.3)	0.804[a]
Low Density Lipoprotein Cholesterol (LDL-C) (mmo/l)	2.8 (1.0)	2.7 (1.1)	0.797[a]
Serum Creatinine (mmol/l)	10.0 (9.5)*	10.0 (13.0)*	0.695[b]
C-Reactive Protein (CRP) level (mg/l)	0.00 (0.00)	0.00 (0.00)	0.066[b]

[a]independent sample *t* test.
[b]Mann-Whitney test.
*median(interquartile range, IQR).

Table 5: Univariate analysis of the association of HbA1C, CRP positivity, blood pressure, LDL-C and TG level with both aspirin resistant and aspirin sensitive group (n = 69)

Variables	Aspirin resistant, n = 12	Aspirin sensitive, n = 57	*P* value
	n (%)		
CRP level			
Positive	2 (16.7)	2 (3.5)	0.137[a]
Negative	10 (83.3)	55 (96.5)	
HbA1c (%)			
≥ 7	10 (83.3)	43 (75.4)	0.718[a]
< 7	2 (16.7)	14 (13.2)	
Systolic BP (mmHg)			
≥ 130	11 (91.7)	47 (82.5)	0.674[a]
< 130	1 (8.3)	10 (17.5)	
Diastolic BP (mmHg)			
≥ 80	6 (50.0)	36 (63.2)	0.518[a]
< 80	6 (50.0)	21 (36.8)	
Triglyceride(mmol/l)			
≥ 1.7	2 (16.7)	14 (1.7)	0.718[a]
< 1.7	10 (83.3)	43 (75.4)	
LDL-C(mmol/l)			
≥ 2.0	11 (91.7)	40 (70.2)	0.163[a]
< 2.0	1 (8.3)	17 (29.8)	

[a]Fisher's exact test.
Abbreviations: BP = Blood pressure; LDL-C = Low density lipoprotein cholesterol, CRP- C = Reactive protein; HbA1c = Glycosylated haemoglobin.

Association of inflammatory markers with aspirin resistance

There was a study that regarded the interrelationships between aspirin resistance and the inflammatory marker, interleukin-6 (IL-6). It showed that aspirin resistance was independently associated with IL-6 (P = 0.005) (10). Other researchers reported that the CRP level was not associated with aspirin resistance (16). Those findings were similar to our study, where there was no association between aspirin resistance and CRP levels and positivity (Table 5). Another study that used high-sensitivity CRP (hsCRP) as an inflammatory marker showed that hsCRP was significantly associated with aspirin resistance (7). Another inflammatory marker that had been studied was homocysteine. Homocysteine was shown to be a significant risk factor for aspirin resistance by TEG (15).

Conclusion

The prevalence of aspirin resistance in patients with diabetes in our centre was 17.4%. The mean HbA1c in the aspirin-resistant group was 8.86%, whereas the mean HbA1c in the aspirin-sensitive group was 8.62%; there was no significant difference between the two groups. There was no significant association between the HbA1c levels and aspirin resistance or between the CRP levels and aspirin resistance. We also found no association in aspirin resistance and other risk factors such as age, aspirin use, types of aspirin, aspirin dosage, or other comorbidities in our study.

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Conflict of Interest

None.

Funds

None.

Authors' Contributions

Conception and design:NHH, SAH, SB, JMA, WMWB
Analysis and interpretation of the data, drafting of the article, critical revision of the article for the important intellectual content: NHH, SAH, SB
Final approval of the article, provision of study materials or patient, statistical expertise, obtaining of funding, administrative, technical or logistic support, collection and assembly of data: NHH, SAH, SB, JMA, WMWB

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