ASPIRIN RESISTANCE AMONG PATIENTS WITH STABLE ISCHEMIC HEART DISEASE ASSESSED BY MEASUREMENT OF 11-DEHYDROTHROMBOXANE B2 IN URINE

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ABSTRACT

Background: **Aspirin is commonly used as antithrombotic agent to prevent primary and secondary cardiovascular events. A proportion of aspirin-treated patients was found to be resistant to its antithrombotic effect. This proportion varies widely in different studies ranging from 0 to 60% of aspirin treated patients.**

Aim**: To determine the prevalence of aspirin resistance among patients with stable ischemic heart disease through measurement of 11-dehydrothromboxane B2 in urine.**

Methods: **The level of 11-dehydroTXB2 in urine was measured by ELISA method using commercial kits (Wuhan company, China). Fifty three patients with stable ischemic heart disease (IHD) on 100 mg aspirin daily for not less than one month (recruited from the Teaching Hospital Consultation Clinics and 50 apparently healthy volunteers were involved in this study). Urine samples were taken in the morning from patients and volunteers and freezed until analysis for 11-dehydroTXB2 and urinary creatinine. Blood samples were used for measurement of different blood indices, lipid profile and glucose level. Absolute values of 11-dehydroTXB2 in pg/ml of urine and normalized values in pg/mg creatinine were used for analysis. Two cutoff points reported in the literature to be associated with increased incidence of clinical events were used to determine the prevalence of aspirin resistance.**

Results: **The level of 11-dehydroTXB2 in urine of normal subjects was 60.26±66.3 pg/mg creatinine, ranging from 11 to 379 pg/mg creatinine, while the level in patients with stable IHD on aspirin treatment 100mg orally, was 63.34 ± 61.27 pg/mg creatinine, ranging from 5 to 316 pg/mg creatinine. There was no statistically significant difference between males and females in the two groups. Associated diseases such as diabetes and hypertension, cholesterol level and different blood indices do not seem to be associated with altered levels of 11-dehydroTXB2. The prevalence of aspirin resistance in the present study was ranging from 1.8% to 7.5% for patients with stable ischemic heart disease.**

Conclusion: **the prevalence of aspirin resistance was ranging from 1.8% to 7.5% in patients with stable IHD. The level of 11-dehydroTXB2 in urine of apparently healthy subjects and patients with stable IHD on aspirin treatment 100mg orally was similar.**

INTRODUCTION

Clinical aspirin resistance is used to indicate the occurrence of cardiovascular events in patients on regular intake of aspirin at recommended doses.[1] This is called clinical aspirin resistance. While biochemical aspirin resistance is defined as the failure of aspirin to inhibit platelet function or thromboxane A2 production in vivo or in vitro.[2] Aspirin resistance could be pharmacokinetic resistance due to poor bioavailability, or true pharmacodynamic resistance where the enzyme cyclooxygenase is less sensitive to the inhibitory effect of aspirin.[3] A wide variation exists regarding the prevalence of aspirin resistance in patients with coronary artery disease.[4] Based on different studies, the prevalence of aspirin resistance has been reported to range from 5.5 to 60%.[5] It approaches zero in asymptomatic CVA/TIA patients and 34% in symptomatic patients.[6] The prevalence depends partly on the method used to determine aspirin resistance as well as the group of patients studied. In patients studied at post-operative stage or with more severe or unstable disease, the prevalence is increased compared to stable patients.[7]

This study is an attempt to determine the incidence of aspirin resistance in a sample of patients with stable ischemic heart disease from the south of Iraq.

PATIENTS AND METHODS

Patients with stable IHD consulting the cardiology clinic at Basrah Teaching Hospital for follow-up were recruited for this study during the period January 2010 to August 2010.

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Both male and female patients having stable IHD and aged 35 to 70 years were included. Aspirin 100 mg tablet was taken by all patients daily orally for not less than one month. No non-steroidal anti-inflammatory drug was taken with aspirin for at least one week before the study and no antiplatelet drug was taken with aspirin for two weeks before the study. The study protocol was approved by the college council and the ethical committee of Basrah college of Medicine. Blood was taken to measure lipid profile, glucose level, and platelet count. Platelet count was performed within one hour for the anticoagulated whole blood, while other tests were performed later on. Serum samples were freezed at approximately -10C°. Urine was the sample needed to perform enzyme-linked immunosorbent assay (ELIZA) test for 11-dehydroTXB2 level and for urinary creatinine level. All samples were collected in the morning and stored at approximately -10 C° until used. An ELISA kit to measure the level of 11-dehydroTXB2 in the urine was ordered from "Uscn Life Science Inc. Wuhan", China by a special request. The reagents and materials required for the 11-dehydroTXB2 test were provided with the kit. Kits were obtained from Biolabo, France for the determination of HDL and cholesterol, and Human, Germany for triglycerides (TG) and creatinine, and Plasmatec, UK for glucose. Platelet count, hemoglobin level, monocyte count, and other hematological tests were performed in the hematology laboratory of the teaching hospital by automated hematology analyzer *"Sysmex KX-21N"(Sysmex Corporation, Japan).* Apparently healthy volunteers (based on history and clinical examination) were also recruited for the study. Both males and females, age between 35-55 years, with no history of IHD or other chronic diseases and no non-steroidal antiinflammatory drug was taken for at least one week before or during the study, were included.

Test for compliance

The presence of aspirin in urine was tested using ferric chloride solution.[8] Briefly, one ml of urine was mixed with one ml of 9% ferric chloride solution in water. The appearance of purple color indicates the presence of aspirin in urine.

Statistical tests

The data were analyzed using statistical package for social sciences (SPSS) version 12. Analysis of variance (ANOVA) was used to find any significant difference between groups. Paired and unpaired t-test were used to test the significant differences between the two means. P-value less than 0.05 is considered statistically significant.

RESULTS

Level of urinary 11–dehydroTXB2 in apparently normal subjects

Urine samples from 50 apparently normal subjects (mean age 39.12±4.68 years, ranging from 31-53 years, 19 males and 31 females) were tested for 11–dehydroTXB2. The mean value was found to be 60.26±66.3 pg/mg creatinine, ranging from 11 to 379 pg/mg creatinine for normalized values and 82.10±101.94 pg/ml ranging from 24 to 709 pg/ml urine for absolute values.

Level of urinary 11–dehydroTXB2 in patients with stable ischemic heart disease (IHD) receiving once daily, 100 mg aspirin tablet

Fifty three patients with stable ischemic heart disease on treatments including oral aspirin tablet 100 mg once daily were tested for 11–dehydroTXB2 in their urine; their mean age 59.02±9.53 years (ranging from 41 to 75 years), 35 were males and 18 females. The duration of their aspirin treatment was more than one month (ranging from 1 month to 10 years). The characteristics of these patients are shown in (*table-1).* The average level of 11-dehydroTXB2 was 63.34±61.27 pg/mg creatinine, ranging from 5 to 316 pg/mg creatinine for normalized values and 76.58±64.06 pg/ml urine ranging from 27 to 372 pg/ml urine for absolute values *(table-2).*

Table 1. Characteristics of patients with stable IHD (n=53) on 100 mg aspirin orally

Characteristics	
Age (years), mean ± SD	59.02 ± 9.53
Male, n (%)	35 (66%)
Female, n (%)	18 (34%)
With Diabetes, n (%)	12 (22.6%)
With Hypertension, n (%)	26 (49%)
On statins, n (%)	23 (43.4%)
On ACE-I, n (%)	15 (28.3%)

Table 2. Levels of 11-dehydroTXB2 in apparently normal subjects and patients with stable IHD.

Subjects	Level in pg/mg creatinine	Level in pg/ml urine
Normal (n=50)	**60.26 (11-379)**	**82.10 (24-709)**
Patients with stable IHD (n=53)	**63.34 (5-316)**	**76.58 (27-372)**

The prevalence of aspirin resistance in this present study differs according to the cutoff point that have been reported in previous studies. For patients with stable IHD, the prevalence was 1.8% (cutoff point was 320 pg/ml, Thomson et al, 2009) *(Figure-1)*. Only one patient was found with a level of 11-dehydroTXB2 of 372 pg/ml. This patient was a male patient with no associated diseases. Thus, the prevalence between male patients was estimated to be 2.8%. According to the cutoff point reported by Eikelboom et al 2007; 193 pg/mg creatinine, the prevalence was 7.5% *(Figure-1)*. Four out of the 53 patients were found resistant to aspirin, 2 of them were males and 2 were females. One of the male patients was having a level of 316 pg/mg creatinine of 11-dehydroTXB2; his stable IHD was associated with hypertension and hyperthyroidism. He was on simvastatin, carbimazole, propranolol, and a combination of both hydrochlorothiazide plus amiloride *(Moduretic)* in addition to aspirin (SDI, Iraq), 100 mg daily. The other resistant male patient was non-hypertensive having the level of 220 pg/mg creatinine and was on metoprolol and simvastatin taken with aspirin SDI 100 mg daily. One of the female patients was having a level of 195 pg/mg and was taking carvedilol with aspirin (Sammara Drug Industry, SDI, Iraq) 100 mg daily with no associated diseases. The other female patient was with the level 250 pg/mg. She was hypertensive on atenolol with aspirin SDI 100 mg daily.

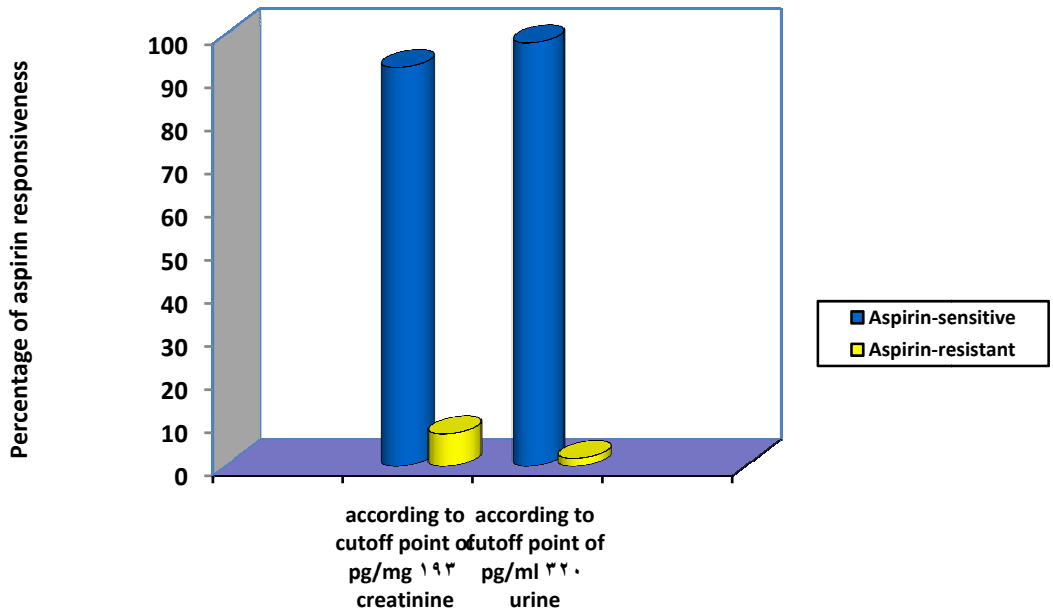

Fig 1. Prevalence of aspirin resistance according to two different cutoff points

When the level of 11-dehydro TXB2 in urine was compared according to different characteristics (gender, associated diseases- diabetes, hypertension, anemia, left ventricular dysfunction, type of drugs used- platelet count, monocyte count, triglyceride levels, the type of aspirin manufacture) in patients with stable IHD on aspirin 100mg treatment, no statistically significant differences were found.

DISCUSSION

There is a wide range in the reported prevalence of aspirin resistance world-wide, extending from 0% to 60 %.[1,9] This may depend on the method of platelet function assessment, definition of aspirin resistance and the characteristics of the populations tested.[10] In the present study on 53 patients with stable coronary artery diseases, the rate of aspirin resistance was found to range from 1.8% to 7.5% according to two cutoff points taken from two different studies (Thomson et al[11] and Eikelboom et al [12] respectively). These results are consistent with several other studies that had reported a close rate of aspirin resistance. A study by Tantry et al,[13] revealed that aspirin resistance in patients with coronary artery disease is rare when assessed by a method directly dependent on platelet COX-1. They studied 223 patients with IHD by using thromboelastograph platelet mapping, only one patient (0.4%) was resistant to aspirin treatment. In a study by Grundmann et al[14] on 53 patients with cerebrovascular disease taking 100 mg aspirin, the prevalence was 34% for symptomatic patients and 0% for asymptomatic patients by using PFA-100 system. Gum et al[15] studied aspirin resistance on 326 patients with stable cardiovascular disease taking aspirin 325 mg/day for ≥7 days. They reported that aspirin resistance was 5% measured by optical platelet aggregometry using ADP and arachidonic acid (AA) as agonists. Gurbel et al,[9] had studied the effect of aspirin dosing on platelet function in 125 patients with stable coronary artery disease (81, 162, 325 mg/day for 4 weeks). At any one dose, resistance to aspirin was 0% to 6% in the overall group by using AA in light transmittance aggregometry, whereas it was 1% to 27% by other methods (collagen and ADP-induced LTA, PFA-100). An incremental treatment effect of aspirin dosing on urinary 11-dehydroTXB_2 levels was, therefore, observed inpatients, with significant differences in levels

demonstrated between 81 mg and 325 mg. The mean levels of 11-dehydroTXB2 obtained in the present study after taking aspirin were 60 pg/mg creatinine in normal subjects and 63 pg/mg creatinine in patients group. This could possibly be explained in that the level of 11-dehydroTXB2 is elevated by the ischemic events and brought down by aspirin to normal levels. Numerous studies have shown that the level of 11-dehydroTXB2 increased in many diseases.[10,16-18] Urinary concentrations of 11-dehydroTXB2 were significantly higher among patients who developed stroke, myocardial infarction, or cardiovascular death than among those who remained free of these events.[12] Hypertensive patients in this study had a prevalence of aspirin resistance of 7.7% which is approximately similar to that observed in non-hypertensive patients (7.4%). However, Feher et al,[19] found a significantly higher prevalence of hypertension among aspirin-sensitive patients compared with that seen in aspirin-resistant patients. There was also a significantly higher rate of using beta-blockers and ACE-Is among aspirin-sensitive patients in their study. These drugs were speculated to exert an additive antiplatelet activity when combined with aspirin. In contrast, Chen et al,[20]found no difference in the use of beta-blockers, statins, or ACE-Is between aspirin resistant and sensitive groups. This study revealed that all aspirin resistant patients were on beta blockers and 2 of them were on simvastatin. No one of those 4 resistant patients was on ACE-I. Resistant patients in the present study were older than 55 years. Women had a slightly higher prevalence than men (11.11% of 18 females and 5.7% of 35 males, respectively). This might indicate that there is a relationship between age and sex with decreasing aspirin response. This finding is similar to that presented by Chen et al,[20] who found that older patients and women were more liable to be aspirin resistant. A recent meta-analysis on 2930 patients with cardiovascular disease, who were on aspirin (75-325 mg daily) alone or in combination with other antiplatelet therapy, found that aspirin resistance to be more prevalent in females.[21] Chen et al,[20] found that aspirin resistant patients were having low hemoglobin levels in comparison to aspirin sensitive patients. The present study showed that all aspirin resistant patients were having normal hemoglobin levels. No association between diabetes and aspirin resistance was found in our study. On the other hand, Fateh-Mughadam et al,[22] evaluated the prevalence of aspirin resistance in 172 patients with diabetes mellitus type 2 on chronic aspirin therapy. They found that thirty-seven (21.5%) of the type 2 diabetic patients were resistant to chronic aspirin therapy, 29(16.9%) semi-responders and 106(61.6%) were responders. The lack of such association in our study may be related to the small number of aspirin-resistant patients found. To conclude, the level of 11-dehydroTXB2 in urine of patients with stable ischemic heart disease on 100mg aspirin therapy is similar to that in normal subjects. The prevalence of aspirin resistance is ranging between 1.8 and 7.5% in patients with stable IHD.

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