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Prevalence of Aspirin Resistance in Patients of Chronic Kidney Disease

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Abstract

Background: Chronic renal failure (CRF) is associated with increased risk of cardiovascular morbidity and mortality. Antiplatelets especially low dose aspirin becomes mandatory for prevention of primary and secondary prevention of cardiovascular events in patients of CKD. The aim of this study was to explore the prevalence of aspirin resistance in CRF.

Methods: A prospective clinical study was conducted in which 130 patients suffering from CRF with concomitant cardiovascular risk factors were recruited and were on aspirin (100 mg daily) for 4 weeks. Aspirin non-responder status was identified by PFA-100 system.

Results: Aspirin resistance was detected in 53 patients undergoing hemodialysis, 32 patients with stage 3-4 CKD and 22 controls. The frequency of aspirin resistance was significantly higher in the CRF group compared with controls (34.7% vs. 16.9%, $P < 0.001$) and in hemodialysis patients (46.1%) compared with stage 3-4 CKD patients (24.6%, $P < 0.001$) and controls (16.9%, $P < 0.001$). Multivariate analysis revealed female sex (odds ratio (OR) = 2.201; 95% confidence interval (95% CI), 1.173 - 4.129; P = 0.014), hemodialysis (OR = 3.636; 95% CI, 1.313 - 10.066; P = 0.013) and HDL cholesterol (OR = 0.974; 95% CI, 0.950 - 0.999; P = 0.043) as independent predictors of aspirin resistance in this cohort of patients.

Conclusions: Patients with CRF have higher frequency of aspirin resistance. This might further increase the risk of cardiovascular morbidity and mortality in these patients.

Keywords: Chronic renal failure; Aspirin resistance; PFA-100

Introduction

Low dose aspirin has been implicated to attenuate the risks of myocardial infarction, stroke, and vascular related deaths in patients with cardiovascular disease on long term use [1]. Still a significant number of patients on low dose aspirin as antithrombotic therapy have major adverse vascular related events each year [2]. In most cases lack of patient compliance, low responsiveness or resistances to aspirin fail to reach the therapeutic goal. Though there is no universally accepted definition of aspirin resistance (AR) , in clinical terms it is characterized by occurrence of a thrombotic episode despite treatment with aspirin, in pharmacological terms it means insufficient pharmacological inhibition of platelet cyclooxygenase-1 (COX-1) derived thromboxane formation with subsequent insufficient inhibition of platelet function by standard antiplatelet dose of aspirin or a phenomenon where the expected inhibition of platelet responses is not obtained as evaluated by different biological tests [3]. The degree of inhibition of platelet aggregation can be assessed by a variety of tests, such as optical platelet aggregation, PFA-100, expression of platelet surface receptor [4, 5].

Chronic kidney disease (CKD) has emerged as a major public health problem of primary importance especially in developing countries [6]. The presence of CKD is one of the most potent known risk factors for cardiovascular disease (CVD). Individuals with CKD have a 10- to 20-fold greater risk of cardiac death, mainly because of the high risk for coronary heart disease and other cardiovascular complications which virtually coexists with diabetes, hypertension, obesity, lipid abnormalities, hyperaggregability state and endothelial dysfunction [7, 8]. CKD in the presence of other co-morbidities like type 2 diabetes mellitus and hypertension can lead to early progression to end stage renal disease (ESRD or stage V CKD), which confers a greater risk for CVD morbidity and mortality. Cardiovascular events are the leading cause of

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Table 1. Baseline Demographic Data and Clinical Characteristics of Patients Participated in the Study

Parameters	Mean ± S.D
Age (years)	65.03 ± 9.02
Male : Female	99:27
BMI (kg/m^2)	27.78 ± 4.30
Patients with Diabetes	67
Patients with Hypertension	96
Patients with Hyperlipidemia	56
Smoker	37
Systolic Blood Pressure (mmHg)	147.6 ± 17.4
Diastolic Blood Pressure (mmHg)	93.1 ± 7.7
Fasting Blood Sugar (mg/dL)	115.89 ± 19.96
HDL (mg/dL)	54.42 ± 9.98
LDL (mg/dL)	157.53 ± 25.82
TGL (mg/dL)	166.35 ± 31.25
TC (mg/dL)	210.2 ± 21.73
Fibrinogen (g/L)	2.16 ± 0.36
Creatinine Clerance (mL/min)	35.50 ± 12.02

premature death in patients with

CKD even before their progression to ESRD [9], therefore antiplatelets especially low dose aspirin becomes mandatory for prevention of primary and secondary prevention of cardiovascular events in patients of CKD. Studies have indicated that the efficacy of aspirin in primary prevention of cardiovascular events for type 2 diabetic patients was reduced [10], and metabolic syndrome patients were prone to develop cardiovascular disease because of platelet hyperactivity [11] and conferred a higher risk of long term MACCE (Major Adverse Cardiac and Cerebral Events) in patients with CAD [12]. There is a paucity of studies to evaluate the clinical efficacy and resistance of low dose aspirin in individuals of CKD, a surprise omission despite of number of studies on Aspirin Resistance in many clinical states. In this study we evaluate the prevalence of aspirin resistance in CKD patients.

Methods

Patients

All 130 patients were prospectively enrolled between February 2009 and October 2009. The patients presenting to the outpatient clinic for follow-up treatment of CKD were recruited in this study. Concomitant cardiovascular risk factors of these patients were patients with Type 2 Diabetes Mellitus (fasting blood sugar of ≥ 126 mg/dL), hypertension (defined as systolic blood pressure ≥ 140 mmHg and diastolic blood pressure ≥ 90 mmHg and/or antihypertensive treatment), hypercholesterolemia (blood cholesterol levels ≥ 200 mg on diet and /or treatment with statins), smoking (currently smoking), obesity (body mass index > 30 kg/m^2) and family history (first degree relatives with symptomatic coronary heart disease). Exclusion criteria included ingestion of an-

Table 2. Comparison Between Aspirin Sensitive and Aspirin Resistant Group to 100 mg Aspirin Taken Daily for 4 Weeks

Parameters	Aspirin Sensitive (n = 87)	Aspirin Resistance (n =39)	P value
Age (years)	64.37 ± 9.12	65.70 ± 8.99	0.57
Male : Female	70:17	29:10	0.69
BMI (kg/m^2)	26.27 ± 3.57	29.30 ± 4.49	0.005*
Diabetes	42	25	0.1
Hypertension	64	36	0.03*
Hyperlipidemia	34	22	0.05*
Smoker	22	15	0.20
SBP (mmHg)	146.42 ± 18.32	148.76 ± 16.92	0.83
DBP (mm Hg)	92.71 ± 7.01	93.57 ± 8.4	0.43
Fasting Blood Sugar (mg/dL)	108.67 ± 21.16	123.11 ± 17.01	0.01*
HDL (mg/dL)	55.25 ± 8.09	53.38 ± 11.89	0.69
LDL (mg/dL)	147.2 ± 21.13	167.87 ± 34.07	0.05*
TGL (mg/dL)	162.27 ± 34.13	171.43 ± 27.63	0.43
TC (mg/dL)	255.27 ± 36.48	210.20 ± 21.73	0.61
Fibrinogen (g/L)	2.01 ± 0.32	2.31 ± 0.40	0.04*
Creatinine Clerance (mL/min)	39.88 ± 10.87	31.13 ± 13.83	0.05*

tiplatelet drugs or other non-steroidal anti-inflammatory drugs, administration of heparin or low-molecular-weight heparin within 24 h before enrolment; major surgical procedure within one week before enrolment; family or personal history of bleeding disorders; platelet count < 150000/μL or > 450000/μL; haemoglobin < 8 g/dL, history of myeloproliferative disorders; or history of drug-induced thrombocytopenia, patients with end stage renal disease (ESRD) and patients on dialysis.

Study design

The present study is a 4- week, prospective clinical study conducted in a single centre. The study was carried out by Department of Pharmacology and Department of Clinical Nephrology in Sir Sunderlal Hospital, Banaras Hindu University, Varanasi, a tertiary care government hospital. The study was approved by Institute Ethical Committee and procedures followed in this study are in accordance with the ethical standard laid down by ICMR's Ethical guidelines for biomedical research on human subjects (2006). A written informed consent was taken from all the patients participated in the study after explaining the patient's diagnosis, the nature and purpose of a proposed treatment, the risks and benefits of a proposed treatment (aspirin), alternative treatment and the risks and benefits of the alternative treatment. All patients were on regular aspirin treatment with 100 mg daily. Compliance on aspirin was determined by pill count method at follow-up. Baseline values were measured before administration of Aspirin and compared with values measured after

4 weeks of treatment.

Laboratory measurements

Blood samples were obtained from each subject by antecubital venipuncture in the fasting state in the morning between 8 and 10 a.m., 2 - 4 hours after daily aspirin intake. Citrated blood (0.129 M trisodium citrate in dilution 1:10) was used for PFA-100 analysis (Platelet Function Analyzer, Dade Behring, Germany) and 4.5 mL blood were collected in EDTA (ethylene-diamine-tetraacetic acid) tubes for platelet count and hematocrit determination. All analyses were performed within 1 - 2 hours after blood collection.

Lipid profile

Total cholesterol, HDL cholesterol and triglyceride levels were measured enzymatically on a Hitachi 911 autoanalyzer (Hitachi, Japan). LDL cholesterol level was determined using the Friedewald formula.

Platelet function analysis

The effect of aspirin was assessed using the platelet function analyser (PFA-100) system (Dade-Behring International, Germany). PFA-100 system is a novel platelet function test that enables rapid, simple, and reproducible quantitative assessment of in vitro platelet aggregation and is used for identification of aspirin nonresponder status [13-15]. PFA-100 contains a microprocessor controlled instrument/test cartridge system that measures in vitro bleeding time. This test is based on a highly sensitive and specific in vitro system for the assessment of platelet aggregation in small samples of citrated whole blood. It uses a disposable test cartridge coated with either collagen or epinephrine or with collagen and adenosine diphosphate (ADP). The instrument aspirates citrated whole blood under a constant vacuum condition at a high shear stress of 5000 - 6000 s^{-1} through a capillary tube and a precisely defined aperture in the membrane that mimics microcapillary system of human circulation. Time to complete occlusion of the aperture is defined as the closure time (CT). Normal reference ranges of closure time in our laboratory are 85 - 165 sec for collagen/epinephrine-coated membrane (EPI). Since aspirin has a prolonged EPI closure time in a dose-dependent fashion, aspirin resistance was defined as EPI closure time of < 186 sec. All tests were repeated twice.

Safety analysis

Safety was assessed in terms of reported adverse experiences and vital signs, which were measured at baseline and at the end of the study. All reported adverse drug reactions were graded according to The National Cancer Institute Common Toxicity Criteria (CTC) and compared between aspirin sensitive and resistant groups.

Statistical analysis

Data were analyzed using the SPSS statistical software package (15.0 for Windows). Results were expressed as mean ± standard deviation for continuous data or as percentages and numbers for categorical data. Continuous variables with normal distribution and unequal distribution were analyzed with the Student's t-test. The odds ratios (OR) and 95% confidence intervals (CI) were calculated. $P < 0.05$ was considered as statistically significant.

Results

The baseline characteristics of the patients are listed in Table 1. The mean age of patients was 65.03 ± 9.02 years. No. of male patients were 101 and female patients were 29. The mean BMI of patients was 27.78 ± 4.30 kg/m^2, 67 patients were diabetic, 96 patients were hypertensive, 56 patients had hyperliidemia and 37 patients were chronic smokers. Of a total 130 patients enrolled in the study group 4 patients were lost to follow up. Total of 39 patients were aspirin resitant (Table 2). On comparison to Aspirin sensitive group, aspirin resistant group had higher BMI of 29.30 ± 4.49 kg/m^2 in comparison to aspirin sensitive group (26.27 ± 3.57 kg/m^2) ($P < 0.005$). There was significant no. of hypetensives in aspirin resistant group (92%) in comparison to aspirin sensitive group (73%) ($P < 0.03$) and significant no. of hyperlipidemic patients in aspirin resistant group (39%) in comparison to aspirin sensitive group (56%) ($P < 0.05$). The mean fasting blood glucose level was significantly higher in aspirin resistant group (123.11 ± 17.01 mg/dL) in comparison to aspirin sensitive group (108.67 ± 21.16 mg/dL) ($P < 0.01$). The mean LDL level in aspirin resistant group (167.87 ± 16.92) was significantly higher ($P < 0.05$) in comparison to aspirin sensitive group (147.2 ± 21.13). The fibrinogen level in aspirin resitant group (2.31 ± 0.4) was significantly higher than the aspirin sensitive patients (2.01 ± 0.32) ($P < 0.04$). Creatinine clearance as calculated by the Cockrauft Gault Formula in aspirin resistant group (31.13 ± 13.83 mL/min) was significantly lower ($P < 0.05$) in comparison to aspirin sensitive patients (39.88 ± 10.87 mL/min).

Discussion

Our results show that up to 30.95% of patients with CKD were aspirin resistant as measured by PFA-100 and is in concordance with the aspirin resistance reported in previous studies as done by PFA-100 [16].

CKD is recognized as independent risk factor for car-

diovascular events and disease more so with comorbid conditions like diabetes mellitus, hypertension, obesity, hypercholesterolemia and endothelial dysfunction [7, 8] and low dose aspirin becomes essential for primary and secondary prevention of cardiovascular events.

Aspirin resistance is a phenomenon where the expected inhibition of platelet responses is not obtained as evaluated by different biological tests [3] and inability to inhibit thromboxane A2 biosynthesis in vivo and other patient related noncompliance and other factors. Interestingly, in a systematic review done by Krasopoulos et al, the relationship between aspirin resistance and a history of renal impairment was observed [17]. The reason for high aspirin resistance in CKD may be due to abnormality of platelet arachidonic acid metabolism [18] which may lead to altered thromboxane synthesis which is a key factor for the development of resistance to aspirin, considered to be due to the increased activity of phospholipase A2 in the platelets of patients with uraemia [19].

Studies done by Abe et al and Asano et al on animal models have shown that thromboxane play a detrimental role in physiological function of kidney and thromboxane receptors exist in renal vasculature and nephron segments [20, 21]. TXA2 plays a key role in the regulation of renal haemodynamics mainly acting in conjunction with angiotensin II. TXA2, in addition to angiotensin II and arginine–vasopressin constrict larger vessels within the renal vascular tree via activation of a rho-associated kinase pathway [22] and enhanced production of thromboxane in the kidney has been demonstrated in several diseases including lupus nephritis, ureteral obstruction and nephrotoxic renal injury [23-25]. In patients with CKD have higher TXA2 synthesis as compared to that of prostaglandin I_2.

In this study Body Mass Index, fasting blood glucose, LDL, Fibrinogen level was significantly higher in aspirin resistant patients in comparison to aspirin responders.

A study done by Ertugrul et al in Type 2 diabetic patients body mass index, fasting blood glucose levels were significantly positively correlated with aspirin resistance and diabetics were more likely to be aspirin resistant than nondiabetics and 100 mg low dose aspirin increased aspirin resistance by 26% in diabetics than 300 mg aspirin [26]. Diabetes mellitus and hyperglycemia are associated with platelet activation and platelet reactivity [27-29]. Hyperlipidemia patients had poor responsiveness to aspirin therapy as studied by Friend et al. and this may explain the possible causes of aspirin resistance in CKD patients.

This study has some limitations. Inclusion of a small number of patients in both groups was the major limitation. Platelet function was evaluated with only one method. Aspirin resistance was defined only biochemically, but not clinically. It was not a clinical follow-up study.

In conclusion, aspirin resistance was high in patients with CKD in this study. Aspirin resistance is higher especially in CKD patients with high body mass index, increased fasting blood glucose, LDL, fibrinogen levels and decreased Creatinine clearance. Percentage of diabetics, hypertensive and smokers were more in aspirin resistant patients so it will be better to scan this group of patients for aspirin resistance as it seems that CKD patients with comorbid conditions are more prone to aspirin resistance and therapeutic options and strategies should be looked for increasing aspirin dose, alternate or addition of antiplatelet therapies.

Conflict of Interest

NIL.

Financial Interest

NIL.

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