Aspirin and clopidogrel resistance using the cone and plate(let) analyser in Indian patients with coronary artery disease

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ABSTRACT

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Received 11 August 2014 Revised 9 September 2014 Accepted 3 October 2014

Background Resistance to antiplatelet drugs is a wellknown entity. However, data for aspirin and clopidogrel resistance, and its clinical significance, in Indian patients are meagre.

Aims and objectives We sought to determine the prevalence of resistance to aspirin and clopidogrel in Indian patients with stable coronary heart disease (CHD), using the cone and plate(let) analyser (CPA) technology. Setting and design A single centre prospective study in a cohort of patients with stable CHD on chronic aspirin and clopidogrel therapy attending the cardiology outpatient clinic of a tertiary care hospital in Southern India.

Methods Platelet function was measured using the Impact-R device (DiaMed, Cressier, Switzerland). Resistance to aspirin and clopidogrel was measured in a cohort of 100 patients with stable documented CHD. Relation of antiplatelet resistance to various clinical comorbidities was also assessed.

Results Of the 100 patients, 85% were men, and 15% were above 65 years of age. 47% patients had diabetes, 29% of patients were hypertensive and 16% were smokers. Using the CPA, 12 patients (12%) were found to be resistant to aspirin and 19 patients (19%) were clopidogrel resistant. In addition, 10 patients (10%) were resistant to both aspirin and clopidogrel. There was no significant correlation between the presence of antiplatelet resistance and several baseline clinical variables, including age, sex, diabetes, hypertension and smoking.

Conclusions Resistance to aspirin and clopidogrel and dual antiplatelet resistance are prevalent in Indian patients, comparable with the prevalence worldwide. The CPA is a feasible assay to determine antiplatelet resistance.

INTRODUCTION

Antiplatelet drug therapy has become standard of care in the management of cardiovascular atherothrombotic disease including acute coronary syndromes and those undergoing percutaneous coronary interventions.¹² The efficacy of aspirin and clopidogrel in decreasing the risk of adverse cardiac events in patients with coronary heart disease (CHD) has been well established. In spite of this, recurrent atherothrombotic events continue to occur in some patients on chronic oral antiplatelet therapy. Variability in the response to aspirin and clopidogrel therapy has been well described.³⁻⁷ Non-responsiveness or 'resistance' to the effects of antiplatelet therapy has been studied in recent years

by a number of laboratory tests and has been linked to adverse cardiovascular clinical outcomes.8 5

CHD in Indian patients has a different epidemiological profile as compared with the Western population, with CHD occurring at an earlier age, tending to be more aggressive and extensive. Data regarding non-responsiveness to antiplatelet therapy among Indian patients, and its clinical consequences, are scarce and limited. The aim of the present study was to evaluate the prevalence of antiplatelet drug resistance in an Indian population with stable CHD using the cone and plate(let) analyser (CPA) technology.

Various platelet function tests have been devised to assess inborn or acquired abnormal platelet function and also the therapeutic response to antiplatelet agents. Light transmittance aggregometry (LTA) is the most commonly used platelet function test and may be regarded as the gold-standard test for assessing platelet function and also for comparing the efficacy of newer platelet function tests. Other tests include flow cytometry, urinary thromboxane levels, the PFA-100 system and the VerifyNow system, which uses light source to detect platelet aggregation. A relatively newer test on the horizon is the Impact-R device, which is based on the CPA technology. The device tests platelet adhesion and aggregation in anticoagulated whole blood under arterial shear conditions.

METHODS

Patients

We enrolled 100 patients with known CHD attending the outpatient clinic of a tertiary care hospital in South India. Inclusion criteria included those with stable angina with a positive stress test or documented coronary artery disease on a coronary angiogram, documented history of myocardial infarction (MI) (more than 1 month), history of percutaneous coronary intervention and/or coronary artery bypass graft surgery. All patients were on aspirin (enteric coated) 75-150 mg daily and clopidogrel 75 mg daily for a duration of at least 10 days.

Exclusion criteria included acute MI within 30 days of the test, any contraindication to aspirin or clopidogrel therapy, anaemia (Hb <10 g%), renal failure (creatinine >2.5 mg/dL), administration of ticlopidine, dipyridamole or other antiplatelet agents, current non-steroidal anti-inflammatory drug use, administration of heparin or other anticoagulants in the previous 24 h, family or personal history of bleeding disorders, platelet count



To cite: Koshy SK, Salahuddin S, Karunakaran B, et al. Heart Asia 2014:6:159-162. doi:10.1136/heartasia-2014-010568





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 $<150\ 000\ or >450\ 000$, history of myeloproliferative disorders and major surgical procedures in the last 1 week. All patients gave informed written consent, and the study was approved by the institutional ethics committee.

Measurement of antiplatelet resistance

The antiplatelet effect of aspirin and clopidogrel was assessed using the Impact-R device (DiaMed, Cressier Morat, Switzerland), which is based on the CPA technology. The device tests platelet adhesion and aggregation in anticoagulated whole blood under arterial shear conditions. The CPA technology is based on applying laminar shear force to whole blood on a polystyrene plate by a rotating cone, leading to platelet surface adhesion and aggregation. Samples of whole blood (130 µL) anticoagulated with sodium citrate were placed on polystyrene wells and subjected to flow at 1800 s^{-1} for 2 min using a special conical disc. After the surface is washed with phosphatebuffered saline and stained, samples were analysed by an image analyser. The results are expressed as a percentage of the well surface covered (SC) by platelets and as average size of the adherent aggregate particles. Blood samples were pretreated with agonists arachidonic acid (AA) and ADP, to assess specifically antiplatelet response to aspirin and clopidogrel, respectively.

Aspirin assay

For assessing resistance to aspirin, the blood sample was pretreated with AA (0.32 mM) under gentle mixing (10 rpm) for 1 min and then subjected to the regular Impact-R test. A result of a low SC suggests that the platelets do not respond to aspirin. A percentage SC of $\geq 2.5\%$ was considered as cut-off value indicating adequate response to aspirin, whereas a value <2.5% indicates resistance to aspirin.¹⁰ ¹¹

Clopidogrel assay

For assessing response to clopidogrel, the blood sample was pretreated with ADP (1.38 μ M) under gentle mixing (10 rpm) for 1 min and then subjected to the regular Impact test. A result of a low SC suggests that the platelets do not respond to clopidogrel. A percentage SC of \geq 2.8% was considered as cut-off value indicating adequate response to clopidogrel, whereas a value <2.8% indicated resistance.¹⁰ ¹¹

Statistical analysis

To ensure sample adequacy in the survey, we conducted power analysis to find the sample size required for a power that is >90%. Considering that previous studies have estimated an antiplatelet resistance of 5%–45% in patients taking aspirin and 4%–30% in patients taking clopidogrel,^{12–16} we took an estimated prevalence of 30% to calculate the sample size. Taking this into account the sample size obtained statistically was 98.

Categorical variables are expressed as frequencies and percentages. Differences between groups were assessed with the Fisher exact test for categorical variables. Unpaired t tests were used for comparison of normally distributed continuous variables between the 2 groups. A p value <0.05 was considered statistically significant. Statistical analysis was performed with SPSS V17.0 software (SPSS, Chicago, USA).

RESULTS

This study analysed the prevalence of resistance to aspirin and clopidogrel in 100 patients with CHD in an urban tertiary level care hospital in North Kerala in India. Majority of the patients (85%) were men and 15% of the patient population were above 65 years. The prevalence of diabetes in the total population was

Table 1 Baseline characteristics of all patients in the study (n=100)

	Total (n=100
Advanced age (>65 years)	15 (15%)
Male sex	85 (85%)
Diabetes	47 (47%)
Hypertension	29 (29%)
Smoking	16 (16%)
Family history of CHD	13 (13%)
Clinical characteristics	
Chronic stable angina	21 (21%)
History of recent NSTEACS	41 (41%)
History of recent STEMI	27 (27%)
Heart failure	11 (11%)
Drug intake	
β-Blocker	69 (69%)
Calcium channel blocker	20 (20%)
ACE inhibitor	71 (71%)
Aldosterone antagonist	20 (20%)
Statin	100 (100%)

CHD, coronary heart disease; NSTEACS, non-ST elevation acute coronary syndrome; STEMI, ST elevation myocardial infarction.

47%, and 29% were hypertensive. Sixteen per cent (16%) of the total population were smokers (table 1).

Using the Impact-R CPA, the percentage of patients with coronary artery disease having aspirin resistance was 12%, and clopidogrel resistance was found in 19% patients. Furthermore, 10% of patients showed resistance to the antiplatelet effect of both aspirin and clopidogrel (figure 1).

We also assessed the correlation of antiplatelet resistance with clinical variables, namely age, sex, diabetes, hypertension, smoking status, family history of coronary artery disease and concurrent medications. None of the clinical variables showed statistically significant correlation with antiplatelet resistance (table 2).

DISCUSSION

Significant heterogeneity exists among global studies reporting prevalence of aspirin and clopidogrel resistance. Lack of a gold-standard investigation and variability in the methods of assessing antiplatelet resistance are largely responsible for this heterogeneity. For aspirin, difference in prevalence in various studies may be due to variability in doses. In the case of clopidogrel, this difference can be attributed to variability in definitions, populations studied, laboratory methods and agonist doses within the same laboratory assay.⁷

Reported prevalence of aspirin non-responsiveness varies widely from 0% to 57%. In a systematic review by Hovens *et al*,¹⁷ the mean prevalence of aspirin resistance was 24%. In this analysis, studies using light aggregometry with AA as an agonist had a pooled prevalence of 6% (95% CI 0% to 12%), whereas in studies using point-of-care assays, the prevalence was higher at 26% (95% CI 21% to 31%). Similarly, studies evaluating prevalence of clopidogrel resistance have also showed considerable heterogeneity. In a study of 150 patients undergoing percutaneous coronary intervention, clopidogrel resistance using LTA was 24%.¹⁸ Buonamici *et al*¹⁹ studied 804 patients using ADP-induced platelet aggregation and reported a prevalence of 13%.

Studies from the Indian subcontinent and South East Asia, evaluating antiplatelet resistance, are scarce. Sadiq *et al*²⁰ showed



Figure 1 Distribution of patients with aspirin and clopidogrel resistance.

that nearly 42% of patients with CHD have inadequate response to aspirin. Akhtar *et al*²¹ studied aspirin resistance in 250 patients with stable CHD and showed that 12% of patients were resistant to aspirin. In the study conducted by Guha *et al*,²² in patients with acute coronary syndrome, 15.27% showed resistance to aspirin, 19.44% were resistant to clopidogrel and 12.5% were resistant to both. In another study involving patients with recurrent acute coronary syndromes, aspirin, clopidogrel and dual antiplatelet resistance were encountered, respectively, in 35%, 72.5% and 32.5% patients with recurrent acute coronary syndrome undergoing conservative management.²³

In our study population, aspirin resistance was found in 12% of patients, clopidogrel resistance in 19% of patients and 10% patients were found to be resistant to both antiplatelet drugs. The values obtained in our study are comparable with previous existing data. Only patients with stable CHD were included in our study. Whether differences exist between antiplatelet responsiveness in stable CHD and acute coronary syndromes is

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according	to antipla	telet re	esistance				
Table 2	Baseline of	clinical	characte	ristics o	of patient g	roups	,

	Aspirin resistant (n=12)	Clopidogrel resistant (n=19)	Dual resistant (n=10)
Advanced age (>65 years)	2 (16.7%)	5 (26.3%)	2 (20.0%)
Male sex	11 (91.7%)	16 (44.2%)	9 (90%)
Diabetes	4 (33.3%)	8 (42.1%)	3 (30%)
Hypertension	5 (41.7%)	10 (52.6%)	4 (40%)
Smoking	1 (8.3%)	2 (10.5%)	1 (10%)
Family history of CHD	2 (16.7%)	1 (5.3%)	1 (10%)
Clinical characteristics			
Chronic stable angina	4 (33.3%)	5 (26.3%)	4 (40%)
History of recent NSTEACS	3 (25%)	5 (26.3%)	2 (20%)
History of recent STEMI	4 (33.3%)	8 (42.1%)	4 (40%)
Heart failure	1 (8.3%)	1 (5.3%)	0 (0%)
Drug intake			
β-Blocker	8 (66.7%)	13 (68.4%)	8 (80%)
Calcium channel blocker	4 (33.3%)	5 (26.3%)	3 (30%)
ACE inhibitor	10 (83.3%)	15 (78.9%)	7 (70%)
Aldosterone antagonist	1 (8.3%)	5 (26.3%)	1 (10%)

Data are n (%) unless noted otherwise. There were no significant (p<0.05) differences between these groups.

CHD, coronary heart disease; NSTEACS, non-ST elevation acute coronary syndrome; STEMI, ST elevation myocardial infarction.



unclear. Differences also exist in platelet reactivity in different clinical conditions. Obesity, diabetes mellitus, hypercholesterolaemia, smoking and heart failure have been shown to be associated with increased degree of platelet reactivity.⁷ We could, however, find no statistically significant correlation between antiplatelet resistance and various clinical variables in our study.

Several assays exist to measure antiplatelet efficacy and resistance. These include flow cytometry, LTA, urinary thromboxane levels and several point-of-care tests like the VerifyNow assay. In an antiplatelet resistance study by Thomson *et al*²⁴ using urinary 11-dehydrothromboxane B2 levels as a surrogate marker for antiplatelet efficacy, aspirin resistance was found to be 38.1%. Between the major platelet function tests there has been poor correlation in determining the prevalence of aspirin resistance as illustrated by Lordkipanidzé et al^{25} in their study. The prevalence of aspirin resistance varied according to the assay used: 10.3%-51.7% for LTA using ADP as the agonist, 18.0% for whole blood aggregometry, 59.5% for PFA-100, 6.7% for VerifyNow Aspirin and 22.9% by measuring urinary 11-dehydrothromboxane B(2) concentrations. The rate of clopidogrel resistance is also dependent on the assay used. In a study of 70 patients receiving 150 mg clopidogrel after coronary stenting, the clopidogrel resistance was 13% with LTA using ADP as agonist, 39% with vasodilator-stimulated phosphoprotein assay and 33% with the VerifyNow P2Y12 assay.²⁶ Moreover, it has also been shown that aspirin resistance may not be a stable feature over time, may be transient and thus assessment at a single point of time may not hold true subsequently.^{27 28}

Compared with the various modalities of detecting antiplatelet resistance, the CPA used in our study assesses platelet function in physiological circumstances, is not time-consuming and can be easily done by trained lab personnel. Several studies have used the CPA to assess antiplatelet efficacy and have shown good correlation with currently accepted standards.^{29 30}

Antiplatelet therapy is the cornerstone in the primary and secondary prevention of acute and chronic coronary and cerebrovascular syndromes. Knowledge of the efficacy of these drugs is therefore of paramount importance, especially since there are alternatives like the new generation P2Y12 antagonists prasugrel and ticagrelor, which may be given to patients to overcome antiplatelet resistance. Our study shows that south Indian population also has a similar prevalence of individual and dual antiplatelet resistance. Further larger studies are needed to compare the efficacy of individual antiplatelet agents and to assess the effect of variables like the effect of diabetes, dyslipidaemia and smoking on antiplatelet resistance. The CPA may be a simple method to assess antiplatelet resistance, especially in

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patients with recurrent thrombotic events, stent thrombosis and other high risk population like diabetics.

STUDY LIMITATIONS

This study was done in a hospital setting on patients with stable CHD receiving treatment in the cardiology department. This selection bias limits the extent to which our results can be projected to the general population. Comparison of antiplatelet resistance using currently accepted assays like LTA and point-of-care tests like VerifyNow, with the Impact-R test used in our study, was not performed. Also, clinical outcomes of the patients with biochemical antiplatelet resistance were not studied in our study population.

CONCLUSIONS

Aspirin, clopidogrel and dual antiplatelet resistance is common in South India and is comparable with the values obtained globally. The prevalence of aspirin, clopidogrel and dual antiplatelet resistance in the study population was 12%, 19% and 10%, respectively. The CPA is a simple and feasible method to determine antiplatelet resistance. Further studies may be required to compare efficacy of this assay with currently accepted standards.

Contributors AF, AM and SKK were involved in conception and design of the study. SKK and SS were involved in acquisition, analysis and interpretation of data and drafting of the manuscript. AF, BK, JB, SYN and PVH were involved in critical review of the manuscript.

Competing interests None.

Patient consent Obtained.

Ethics approval Institutional Ethics Committee- Malabar Institute of Medical Sciences, Calicut, Kerala, India.

Provenance and peer review Not commissioned; externally peer reviewed.

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