Research

Prevalence of resistance to aspirin and clopidogrel in patients with stable coronary heart disease in Sri Lanka - A cross sectional study

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Abstract

Aspirin and clopidogrel resistance have prevalence rates of 17.3% and 68.8% respectively in the Sri Lankan population with stable coronary arterial disease. Dual antiplatelet therapy appears to be associated with least resistance as the responder rate is double that seen with aspirin or clopidogrel alone. Cause for the high prevalence of clopidogrel resistance needs further investigation.

Introduction

Atherothrombotic cardiovascular disease is an important category of non-communicable disease which causes significant morbidity and mortality in all societies in the modern world. Antiplatelet therapy, of which aspirin is most widely used, is an essential component of pharmacologic therapy given to patients with atherothrombotic disease. In recent times it has been the practice of many clinicians to use a combination of antiplatelet agents which have different modes of action. This is because laboratory and clinical data indicate that mono therapy with a single antiplatelet agent does not always achieve adequate platelet inhibition.
The combination of aspirin and clopidogrel has found justification by clinical trials and practice guidelines whereas the oral GP IIb / IIIa receptor blockers such as roxifiban have been discontinued due to lack of trial evidence of efficacy.

The main advantage of combined antiplatelet agents seems to be that any resistance to one agent would be countered by the action of the other, thus ensuring effective platelet inhibition.

There appears to be a normal distribution curve with reference to individual response to antiplatelet agents so that at a given dose the majority will respond satisfactorily to the drug but a small percentage will not respond to a level detectable by laboratory testing of platelet function or may show a 'hyper' responsiveness in which case platelet inhibition occurs above the average[1].

This latter phenomenon will probably present clinically with minor bleeds such as spontaneous bruising or echymotic patches. These patients will need downgrading of the antiplatelet dosage and conversion from dual to mono antiplatelet therapy. The non-responders will probably present clinically with recurrent vascular events. In this scenario increasing the aspirin dosage to 325mg daily and/ or clopidogrel dosage to 150mg daily is standard practice. Introducing more potent antiplatelet agents such as ticagrelor or prasugrel is a recent avenue of therapy.

The term aspirin / clopidogrel resistance has been used to categorize patients with hypo-responsiveness to antiplatelet agents.

In this context it must be pointed out that resistance may take two forms[2] – i.e. clinical and laboratory (biochemical), and that the two do not necessarily co-exist in all instances. This fact raises the problem of defining resistance in precise terms using numerical values derived from platelet function tests.

There may be significant differences in the genetic profile which lead to antiplatelet drug resistance in various racial groups and hence it would be helpful for the clinician to possess a background knowledge regarding the prevalence of antiplatelet resistance in the community from which his patients are drawn. This information could influence decision making regarding commencement of dual antiplatelet therapy and its duration.

Considering the increasing number of patients who are treated with coronary stenting the problem of antiplatelet agent resistance has gained importance in cardiac practice.

Buonamici et al found by multivariate analysis that clopidogrel non responsiveness predicted thrombosis of drug eluting stents[3].

Dual antiplatelet therapy is vital in preventing in-stent thrombosis. Significant resistance to dual anti platelet therapy could be fatal in certain individuals implanted with drug eluting stents.

Methods

Study population

This was a cross sectional study consisting of 571 subjects that was conducted at the Institute of Cardiology, National hospital of Sri Lanka.

Patients attending a single Cardiology clinic for three consecutive months were screened in order to select those who were on either aspirin, clopidogrel or both drugs for secondary prevention of ischemic heart disease. The inclusion criteria were one of the following:

(1) Confirmed acute coronary syndrome three months prior to recruitment.
(2) Stable angina confirmed by stress ECG / stress Echo or coronary angiography with significant occlusion of a major coronary artery or branch of over 70%.
(3) Coronary stenting with either bare metal stents (BMS) or drug eluting stents (DES).

Exclusion criteria

(1) Patients who have taken substances which could affect platelet function within the immediately preceding one month were excluded. Agents specifically questioned for were non-steroidal anti-inflammatory drugs,steroids ,tricyclic anti-depressants(TCA),anti-histamines, penicillins,cephalosporins,dipyridamole, proton pump inhibitors(PPI), aminophylline, alcohol and anticoagulants.
(2) Any hematological disorder.
(3) Elevated serum creatinine (>1mg%).
(4) Acute coronary syndrome within the past 3 months.

Clinical variables

A detailed questionnaire was administered by a single medical officer covering demographic details and clinical data.

The risk profile was included in the data collection sheet. Full details regarding the pharmacological agents prescribed were extracted from the clinical note sets. Past side effects were questioned in detail and side effect profiling was performed during the three months follow up.

All patients were administered a single generic brand of clopidogrel with >90% in the configuration at C7. The product used was from Torrent laboratories approved by the FDA.

Blood sampling and laboratory methods

Blood sampling was done after a 12hr overnight fast before consumption of daily medication. 20ml of venous blood was collected via the ante cubital vein into a plastic container with 3.2% trisodium citrate. Needles of 21g were used. Platelet rich plasma (PRP) was obtained by centrifuging at room temperature for 15 minutes at 3000 rpm. All samples were analyzed between 1-2 hours from collection and preparation of PRP.

Patients whose platelet counts were outside the limits of 200-400x10 / L were to be excluded from further evaluation of platelet function, but none in our study were in this category.

Light transmission aggregometry technique was selected for this study. The aggregation response was tested using Agg RAM system- 2004 by Helena laboratories, Texas, USA.

The agonists used were as follows:-

Arachidonic acid (AA)	-1mmol/L
Adenosine diphosphate (ADP)	-5µM/L
Adenosine diphosphate (ADP)	-20µM/L
Collagen	-2 µg/mL

Results and Statistical analysis

The responder status to aspirin and clopidogrel were defined by the follows cut off values:

Aspirin
20 µM ADP - <70%
and also AA - <20%
Clopidogrel
5 µM ADP - <50%
and also 20 µM ADP - <70%

If only one criterion was met the patients were taken to be semi responders to that particular drug.

These cut off points were selected as there appears to be a relationship between biochemical resistance and clinical resistance when antiplatelet agent resistance is defined based on these values. As collagen acts on receptors which are not involved in the pathways of aspirin and clopidogrel platelet reactivity. Collagen was not included in the determination of resistance.

Table 1: Responder status to antiplatelet agents.

Agent	Responders	Semi responders	Non responders
Aspirin	8 (15.4%)	35 (67.3%)	9 (17.3%)
Clopidogrel	6 (18.8%)	4 (12.5%)	22 (68.8%)
Aspirin & Clopidogrel	161 (33.1%)	191 (39.2%)	135(27.7%)

Table 2: Summary statistics for risk factors for CAD

Risk Factors for CAD	Yes		No	
	Frequency	%	Frequency	%
Diabetes mellitus	77	38.3%	124	61.7%
Hypertension	115	56.1%	90	43.9%
Dyslipidemia	113	55.4%	91	44.6%
Smoking (Current / Past History)	92	45.5%	110	54.5%
F/H of IHD	54	26.5%	150	73.5%

Table 3: Summary statistics for previous vascular events

Risk Factors for CAD	Yes		No	
	Frequency	%	Frequency	%
Cerebrovascular events	4	1.96%	200	98.04%
PVD	12	5.9%	192	94.1%
Heart Failure	23	11.30 %	181	88.70%
Cardiac arrhythmias	8	3.90%	195	96.10%

Discussion

In spite of aspirin being administered for secondary prevention, fresh thromboembolic ischemic events occur in more than half the patient population with ischemic heart disease[4].
Some investigators have suggested that patients who have a muted response to aspirin have a 3.5 times higher risk of death from a cardiovascular cause[5].

Therefore it is common practice to use dual antiplatelet therapy (DAPT) in the clinical scenario of acute coronary syndromes and stent implantation. Monotherapy is generally reserved for those manifesting excessive bleeding with DAPT.

The present study reveals the extent of antiplatelet agent resistance in the Sri Lankan population. The data highlights the importance of paying special attention to residual platelet activity in the ischemic patient cohort.

Aspirin resistance

Aspirin blocks the cox-I enzyme, the substrate of which is arachidonic acid. A non-responder to aspirin is defined as one showing a residual platelet aggregation of over 20% to arachidonic acid. Most studies vary in the cutoff point between 10-20%.

Lordkipanidze et al have reported that aspirin resistance varied considerably depending on the methodology of measuring platelet aggregation. They quote a range of 6.7%-59.5%[6]. The non-responders to aspirin were 17.3% in our study. In another smaller Sri Lankan study it was estimated to be 24.4%[7].

Clopidogrel resistance

Aggregation induced by ADP is the recommended test for clopidogrel efficacy.
Two concentrates of ADP were used in our study. The cut off points to diagnose clopidogrel resistance were influenced by studies correlating the residual ADP aggregation to clinical ischemic events. Hence platelet aggregation over 50% with 20µmol/L ADP and over 70% with 5µmol/L were selected as cut off points [8, 9].

The prevalence of clopidogrel resistance is variously estimated to range from 5-44% [10].Clopidogrel resistance in our study was high i.e. 68.8%. When the semi responders are excluded the absolute non-responders constitute 56.3% of the study population. It is a point worth investigation whether genetic polymorphism is responsible for the high rate of clopidogrel resistance seen in the Sri Lankan setting.

Resistance to dual antiplatelet therapy

Gori et al estimated that the combined resistance to aspirin and clopidogrel was approximately about 6%. In our study resistance to DAPT was 27.7%.

The ASCET [11] (Aspirin non-responsiveness and clopidogrel endpoint trial) included patients on a single antiplatelet therapy, namely aspirin 160mg daily or switched over to clopidogrel 75mg daily. There was no significant difference seen with monotherapy when either drug was used on the composite endpoint of unstable angina (UA), myocardial infarction (MI), ischemic stroke (CVA) and death.

Hence ASCET was a negative trial but it showed that the absolute reduction in the endpoints seen when aspirin non responders switched to clopidogrel, (compared with those who continued to be on aspirin) was not statistically significant. The negative results was probably because the trial was underpowered to demonstrate any significant difference. This trial is important as monotherapy is not encouraged by its results.

Factors affecting antiplatelet agent resistance

Aspirin failure, manifested as recurrent ischemic events is not due only to “true” aspirin resistance but also due to other factors such as smoking, chronic renal failure, inflammation and heparin administration[12,13,14].

A significant proportion of diabetics manifest aspirin resistance[15].

Patients with a higher ratio of TC/HDL-C seem to have an increased incidence of aspirin resistance[16]. Noncompliance too is an important factor in apparent aspirin failure, as most of these patients show a satisfactory response after observed aspirin ingestion[17]. Some investigators have reported a greater incidence of aspirin resistance in patients with metabolic syndrome[18]. Certain Korean investigators found that a low hemoglobin was associated with aspirin resistance and that high systolic and diastolic blood pressure were associated with clopidogrel resistance[19].

In the elderly obese patients population (mean age ± SD 66.5 ± 5.9) biochemical aspirin resistance was estimated to be 56.7% [20].

Ozben et al studied the aspirin resistance in 200 hypertensive patients. They found aspirin resistance in 25.6% patients with poorly controlled blood pressure whereas in those with satisfactory blood pressure control the value was 17.8%[21].

Thus it is clear that factors associated with residual platelet reactivity are numerous and that in different study populations different factors seem to be relevant. Hence the importance of studying the problem in the local patient cohort.

Of the 571 patients studied, complete data regarding co morbidities and coronary risk factors were available for 204 subjects which group was analyzed separately for any associated factors relating to antiplatelet resistance. (Table 4).

Our study data does not show significant association between antiplatelet resistance and any demographic factors or risk factors for atherosclerotic cardiovascular disease.

Table 4: Association between responders/semi responders / non-responders versus risk factors for CAD & previous vascular events.

Variable	Category	Responder		Semi Responders		Non-responders		p-value Chi square test
		Frequency	%	Frequency	%	Frequency	%	
Gender	Male	25	83.30%	84	84.00%	64	86.50%	0.877
	Female	5	16.70%	16	16.00%	10	13.50%	
Diabetes Mellitus	Yes	15	50.00%	33	33.70%	29	39.70%	0.261
	No	15	50.00%	65	66.30%	44	60.30%	
Hypertension	Yes	16	53.30%	55	55.00%	44	59.50%	0.788
	No	14	46.70%	45	45.00%	30	40.50%	
Hyperlipidemia	Yes	16	55.20%	54	54.00%	43	58.10%	0.863
	No	13	44.80%	46	46.00%	31	41.90%	
Smoking Status	Current Smoker	1	3.44%	3	3.03%	2	2.70%	0.828
	Ex-smoker	13	44.83%	38	38.38%	35	47.30%	
	Non smoker	15	51.72%	58	58.59%	37	50.00%	
Family History	Positive Family History	4	13.79%	29	29.00%	20	27.00%	0.253
	No Family History	25	86.21%	71	71.00%	54	73.00%	
Cerebrovascular events	Yes	0	0.00%	2	2.00%	2	2.70%	0.674
	No	29	100.00 %	98	98.00%	72	97.30%	
PVD	Yes	3	10.30%	4	4.00%	5	6.80%	0.411
	No	26	89.70%	96	96.00%	69	93.20%	
Heart Failure	Yes	5	17.20%	10	10.00%	8	10.80%	0.548
	No	24	82.80%	90	90.00%	66	89.20%	
Drug or Food Allergies	Yes	1	3.60%	3	3.00%	1	1.40%	0.723
	No	27	96.40%	96	97.00%	73	98.60%	
Bronchial Asthma	Yes	3	10.30%	13	13.10%	7	9.50%	0.74
	No	26	89.70%	86	86.90%	67	90.50%	

The prevalence rates of aspirin and or clopidogrel resistance found in our study are rather high but are comparable to findings elsewhere. The resistance prevalence values for western populations have been quoted before. The values for the Southeast Asian region too need to be mentioned.

Guha et al from Kolkata, India have reported antiplatelet resistance of 35% for aspirin, 72.5% for clopidogrel and 32.5% for dual therapy in patients with recurrent acute coronary syndrome (ACS). The corresponding values for the first ACS were 25.3%, 42.3% and 18.8% -i.e. considerably less than for patients with recurrent ACS[22]. Akhtar reported a prevalence of 12% for aspirin resistance in a cohort of Pakistani patients with stable coronary disease[23].

The findings of the PLATO trial, which studied ticagrelor, suggests that greater the resulting platelet inhibition greater is the clinical benefit. Thus a higher degree of residual platelet reactivity would be harmful. Hence excessive emphasis regarding the cut off values to diagnose antiplatelet resistance may be misplaced as residual platelet reactivity and clinical events could be considered to constitute a spectrum.

Platelet activation is mediated by multiple signaling pathways[24]. Aspirin acetylates a serine moiety in the cox-I system. The active metabolite of clopidogrel (which is a prodrug) finally inhibits the ADP mediated activation of the GPIIb/ IIIa complex.

However the sequence of events is modified and influenced by numerous other pathways acting laterally on the main signaling pathway so that escape avenues are available for platelets to be activated even if the antiplatelet agents are administrated appropriately.

Dose of antiplatelet agents

The present study used only 75mg of aspirin and 75 mg of clopidogrel. It has been suggested that a larger dose of aspirin (i.e. 325mg daily) would be more efficacious than a dose of 75-81mg daily in reducing the incidence of aspirin resistance[25]. However there is no evidence for this supposition. There may be single nucleotide polymorphisms of cox-I which make certain patients more or less sensitive or resistant to aspirin.

It is postulated by some workers that thromboxane A2 derived from macrophages is responsible for aspirin resistance[26]. This is sub served by cox-2 which is probably not suppressed by the 'baby aspirin' dose administered on a daily basis. In this subgroup of patients a higher dose of aspirin may be effective.

Clopidogrel resistance has been linked to genetic polymorphism with particular reference to regarding *CYP2C19* [27,28].

The PRINC (Plavix Response in Coronary Intervention) trial found that a higher loading dose (i.e. 1200mg) and maintenance dose (150mg) of clopidogrel were better than conventional doses for platelet inhibition[29].

Tailored therapy

Some studies provide evidence that tailored therapy is superior to blanket antiplatelet drug dosage[30,31].

Clinical impact

The present study highlights the importance of antiplatelet agent resistance in the Sri Lankan population, so that clinicians must be vigilant when selecting appropriate antiplatelet agents. In the context of platelet inhibition, monotherapy is more likely to lead to recurrent clinical events than DAPT.

However DAPT too cannot be considered to be universally efficacious in any given patient as significant non responder status to DAPT is found in our study. Hence in the high risk patient population with known aggressive atherothrombotic disease administering a more potent drug such as ticagrelor would make clinical sense. Increasing the dose of DAPT may be successful in some patients but it would not be a therapeutic avenue that would solve the non-responder problem significantly.

Limitations

This study investigated biochemical resistance alone. The follow up period was too limited for a study on clinical resistance.

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Conflict of interest

The authors report no conflicts of interest.

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