Original Article

CORONARY INTERVENTION AND CLOPIDOGREL RESISTANCE –A LOCAL EXPERIENCE

Bilal S. Mohydin [a*], Faiza Bashir [b], Sajjad Ahmad [a], Ayesha Hameed [c], Shabana Azim [c], Bilal Zakariah Khan [a]

ABSTRACT:

BACKGROUND: *Dual antiplatelet therapy (DAP) is the cornerstone managing coronary artery disease in the current era of coronary interventions. The pattern of resistance to Clopidogrel in our population is not known. The objective of this study was to assess the frequency of resistance / hypo-responsiveness to Clopidogrel after percutaneous coronary intervention (PCI) in Pakistani population.*

METHODS: *The study was done at Punjab Institute of Cardiology over a period of 5 months in 2012-13. After informed consent, patients who underwent PCI 4 weeks earlier were enrolled. All patients had recieved 600mg of Clopidogrel 4 to 6 hours before PCI and were taking Aspirin 75mg and Clopidogrel 75mg twice daily in addition to their routine medicine. Venous blood samples of all selected patients were collected and P2Y12 blockade analysis test was performed. The test has a "Closure Time" measured in seconds. Patients were labelled, on the basis of this Time, as resistant (closure time < 106 seconds), hypo-responsive (closure time 106 to 224 seconds), and responsive (closure time ≥ 225 seconds). Demographic data and coronary risk factors wre noted for all patients.*

Results: *Fifty patients (38 men and 12 women) were studied. Fifteen (30%) patients were resistant to Clopidogrel, 5 (10%) were hyporespnsive and 30 (60%) were fully responsive as per closure time criteria. None of them suffered a clinically evident coronay event during 4 weeks post PCI.*

CONCLUSION: *More than 1/3rd of our post PCI patients are likely to be resistant or hypo-responsive to Clopidogrel.*

KEY WORDS: *Antiplatelet, Clopidogrel, P2Y12.*

(J Cardiovasc Dis 2013;11(1):14-18)

INTRODUCTION

Coronary angioplasty is many times essential after Acute Coronary Syndrome (ACS). Today, the standard of care requires that all patients post ACS must be prescribed DAP (Aspirin and Clopidogrel) unless there is a contraindication. This is essential for the antithrombotic management, which plays a key role in ACS care. (1, 2, 3) Literature suggests that nearly 4 to 30% of patients are resistant to clopidogrel (4). Presently we do not know what level of Clopidogrel induced platelet inhibition will prevent atherothrombotic events. Neither is there a definite association between low responsiveness to clopidogrel and thrombotic events. (5,6) In light of these facts the need to prescribe more potent atherothrombotic drugs like Prasugrel, which carries a higher risk of stroke becomes debatable (7). The most commonly prescribed thienopyridine antiplatelet is Clopidogrel but we do not have significant local data regarding resistance to it in our patients. This descriptive study was done to find clopidogrel resistance / hypo-responsiveness in our patients.

Platelets form a monolayer by adhering to collagen and von Willebrand factor at the site of plaque rupture (8). This results in their activation and they release secondary agonists like thromboxane A_2 and adenosine diphosphate (ADP), and along with thrombin, which has been generated by the coagulation cascade more platelets are recruited and activated leading to atherothrombosis. It is because of this that antiplatelet therapy is so

[a] Department of Cardiology, Punjab Institute of Cardiology, Lahore-Pakistan
[b] Hematology Section, Department of Pathology, Services Institute of Medical Sciences, Lahore.
[c] Hematology Section, Chughtai's Lahore Lab, Lahore.

* Corresponding author:
Email: drbilalmohydin@hotmail.com

essential in the management of ACS and after PCI.

ANTIPLATELET AGENTS

Thromboxane A2 (a prothrombotic and vasoconstrictor substance) production from platelets is inhibited by aspirin by irreversibly acetylating cyclooxygenase (COX)(9). It is thus extremely useful not only for managing ACS, Stroke, and Peripheral Arterial Disease in both short and long term, but also post PCI to reduce the frequency of ischemic complications after angioplasty. Unfortunately despite the action of aspirin, patients still have atherothrombosis. It is because of this that more potent antiplatelet agents, like glycoprotein IIb/IIIa inhibitors and thienopyridines have been developed.

Thienopyridines irreversibly inhibit ADP by binding to the P2Y12 receptor on the platelet surface. This results in inhibiting platelet activation, degranulation, and aggregation. Clopidogrel is a thienopyridine antiplatelet agent. It is a pro-drug that is activated in the liver by hepatic cytochrome P450 (CYP450) to generate an active metabolite (10). Only a small proportion of clopidogrel undergoes metabolism by CYP450; it is mostly hydrolyzed by esterases to an inactive carboxylic acid derivative that accounts for 85% of clopidogrel-related circulating compounds. The effect of clopidogrel is time and dose dependent. Maximum platelet inhibition is 50% to 60%. Loading dose of 300mg to 600mg gives a maximum inhibition in 4 to 24 hours, whereas a daily dose of 75 mg without a loading dose gives a maximum steady-state level in 4 to 7 days.

The place of clopidogrel in the cardiology armamentarium has been well established by the CAPRIE (11) and CURE (2) trials. Furthermore the CREDO (12) trial compared and demonstrated that a loading dose of clopidogrel 300mg given more than 6 hours before PCI along with maintenance dose of 75mg daily, compared with only maintenance dose of 75mg daily, found significant reduction in early events in the loading dose group. It has also been seen that in a low risk PCI, a loading dose of Clopidogrel 600mg is sufficient and no additional advantage is gained by adding an intravenous glycoprotein IIb/IIIa inhibitor to reduce early post ischemic PCI events.

CLOPIDOGREL RESISTANCE

Aspirin treatment failure raised the question that some patients may be resistant to it. Evidence for aspirin resistance was noted in patients with prior stroke. Those with aspirin resistance were more likely to have a recurrent cerebro-vascular event than those who had no resistance within 2 years. (13). To identify the failure to achieve a pharmacological effect, one must be able to measure it reliably. Several assays are available to measure platelet function and effects of antiplatelet agents (14). A commonly used test of platelet function measures platelet aggregation by light transmittance (optical aggregometry) in platelet-rich plasma in response to an agonist (arachidonic acid, ADP, collagen, epinephrine, or a thrombin receptor–activating peptide). This mechanism allows monitoring of different drug effects by allowing choice of agonist (e.g., ADP for thienopyridines). Because of inter- and intra-patient variability, standardized responses are not meaningful, and results are often reported as a percentage of a baseline value. Other methods include the cone and plate(let) analyzer,(15) a rapid test that measures whole blood platelet aggregation under conditions of high shear stress.

Clopidogrel resistance is dose and time dependent and there is variability in response. The key clinical question is "what role does resistance to an agent play in failure of therapy". In a study by Gurbel et al, (5) 96 patients undergoing elective coronary stenting were monitored before and at multiple time points after standard clopidogrel therapy (300-mg loading dose followed by 75 mg daily). Clopidogrel resistance, empirically defined as <10% reduction in aggregation in response to 5 ìmol/L ADP compared with pretreatment values, was seen in 63% of patients at 2 hours, 31% at 24 hours, 31% at 5 days, and 15% at 30 days.(5) Patients with the highest pretreatment values had the least antithrombotic protection over the first 5 days.(5) In another report, Muller et al(16) defined

Table-1: Demographic and Important Clinical Characteristics the Study Populations.

Characteristic		**Result** n(%) N=50
Gender	Male	38(76%)
	Female	12(24%)
Smoker		11(22%)
Post PCI Smoker		5(10%)
Diabetes Mellitus		16(32%)
Clopidogrel Responsiveness (Closure Time in seconds)	Non-responsive (<106)	15(30%)
	Hypo responsive (106-224)	5(10%)
	Responsive (≥ 225)	30(60%)

Table-2: Association of Diabetes mellitus and clopidogrel resistance with respect to gender.

		Male N=38	Female N=12	P-value
Diabetes Mellitus	Non-responsive	4(10.52%)	2(16.67%)	0.9254
	Hypo responsive	3(7.89%)	1(8.33%)	
	Responsive	5(13.15%)	1(8.33%)	
Non Diabetes Mellitus	Non-responsive	7(18.42%)	2(16.67%)	0.0409
	Hypo responsive	1(2.63%)	2(16.67%)	
	responsive	18(47.36%)	4(33.33%)	

non-responders as those with <10% reduction in platelet aggregation to ADP and semi-responders as those with 10% to 29% reduction 4 hours after 600-mg clopidogrel load, as no additional effect was seen with this treatment regimen at 24 hours. This study found that to 5 ìmol/L ADP, 5% were non-responders and 9% were semi-responders, and to 20 ìmol/L ADP, 11% were non-responders and 26% were semi-responders. (16) Although not designed to evaluate clinical outcomes, an intriguing finding in this study was that 2 patients (out of 105 tested) developed sub acute stent thrombosis, and both met the definition of clopidogrel nonresponse.

Resistance to clopidogrel can be due to various extrinsic and intrinsic reasons. A few of the extrinsic factors are: inappropriate or under-dosing, drug-drug interaction e.g. clopidogrel and Omeprazole, clopidogrel and Atorvastatin (17,18,19), variable absorption of the pro-drug, clearance of active metabolites, quality of drug, the excipient used and the manufacturing technique of the pill. Regarding intrinsic factors variability, the P2Y12 receptor number variability, increased release of ADP and up regulation of other platelet activation pathways are a few of those.

MATERIALS AND METHODS

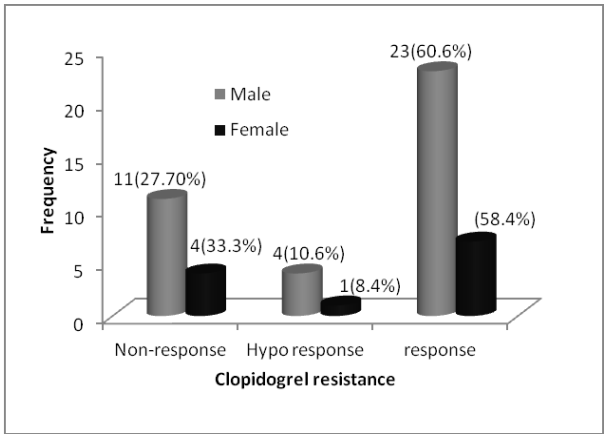
This descriptive study was done at Punjab Institute of Cardiology, Lahore, Pakistan over a period of 5 months in 2012-13. After informed consent, patients who underwent PCI 4 weeks earlier were enrolled. All patients had recieved 600mg of Clopidogrel 4 to 6 hours before PCI and were taking Aspirin 75mg and Clopidogrel 75mg twice daily in addition to their routine medicine. Patients who took the drug irregularly, or used any other drug likely to interact with Clopidogrel, like Omeprazole, were excluded. Venous blood samples of all selected patients were collected in vaccutainers containing 3.2% buffered sodium citrate and P2Y12 blockade analysis test was run on Siemens Innovance PFA 200 analyzer. In this equipment we used a specific single test cartridge to access P2Y12 blockage by clopidogrel. The test measures "Closure Time" in seconds, a possible alternative or supplement to the old bleeding time. Patients were categorized, on the basis of this Closure Time, as resistant (closure time < 106 seconds), hypo-responsive (closure time 106 to 224 seconds), and responsive (closure time ≥ 225 seconds). Demographic data and coronary risk factors wre noted for all patients.

Statistical analysis was performed with SPSS Version 20.0. Categorical variables like gender, smoking, diabetes and clopidogrel resistance were reported as frequencies and percentages. Chi-Square test (Fisher exact test) was applied to observe the association of clopidogrel resistance with respect to gender. P value d" 0.05 was considered significant. Test was applied as two tailed.

RESULTS

A total of 50 patients (38 men and 12 women) were studied. Age of the study population ranged from 38 to 70 years. Sixteen (32%) patients had Diabetes Mellitus and 11 (22%) were smoker; 5 (10%) patients continued smoking post PCI. On the basis of Closure Time value, 15 (30%) patients were found to be resistant to Clopidogrel, 5 (10%) were hyporespnsive and 30 (60%) were fully responsive (Table 1). Males who were non diabetic were found to have significantly high rate of responsiveness to Clopidogrel as compare to females of the same group (Table 2). Gender alone, however, had no effect on Response to Clopidogrel

Figure-1: Graphical distribution of clopidogrel resistance according to gender.



(Figure 1). None of our patients reported any symptom suggestive of ischemia during 4 weeks post PCI despite a high rate of in vitro resistance to Clopidogrel.

DISCUSSION

This study realed that prevalence of Clopidogrel resistance is quite high in Pakistani population. Post PCI antiplatelet medication is prescribed to prevent atherothrombosis. In these fifty patients only 60% were responders while 30% were resistant and 10% were hypo-responders. Of these 40% patients none of them in the study period had symptoms suggestive of ischemia. Clinically all the patients were stable on dual antiplatelet medication (DAPT). These results indicate that P2Y12 blockade assay (an in vitro assessment) does not guide us regarding clinical status of the patients .

Gurbel et al (5) evaluated platelet function by optical platelet aggregometery in response to ADP. Of the 96 patients studied those who had the highest pretreatment platelet reactivity, were most at risk, as they remained most reactive at 24 hours after treatment.

Muller et al (16) also showed that the 2 patients who developed stent thrombosis both fulfilled the criteria for Clopidogrel resistance.

Soffer D et al (20) showed that after loading dose of Clopidogrel (450mg), those who had less inhibition of Platelets had higher anginal class.

Matetzky S, et al (21) correlated clinical non-response to clopidogrel resistance after primary angioplasty in which patients were given 300 mg aspirin on admission and eptifibatide and heparin during PCI. After stenting they were given clopidogrel 300mg immediately and then 75mg daily for 3 months. Platelet function tests were done with turbidometric analysis after stimulation with ADP (5 ìmol/L) and epinephrine (10 ìmol/L), and also by a cone and plate(let) analyzer.(15) Patients were divided into quartiles of inhibition of platelet aggregation (platelet aggregation compared with baseline platelet aggregation). First quartile was of non-responders (day 6 aggregation 103±8% compared with baseline). Quartiles 2 through 4 had varying levels of response, with platelet aggregation of 69%, 58%, and 33% of baseline values. During 6-month follow-up, 7 patients (40%) in quartile 1 (non-responders) had 8 clinical events, including stent thrombosis, myocardial infarction, recurrent ACS, and peripheral arterial occlusion. One patient in the second quartile (6.7%) and no patients in quartiles 3 or 4 had recurrent events. Although the study population was small, this data strongly suggest that there is individual variability in response to clopidogrel in the setting of PCI after STEMI and more broadly that clopidogrel resistance may be a marker for increased risk of recurrent cardiovascular events.

Matetzky S, et al (21) (6 months follow up), reported that event rate in the non-responder group was 40% versus 25% in responders. However, not all non-responders had anginal symptoms. Our study had a small sample size and a short follow up period; due to this reason, possibly, we did not observe such finding.

In addition to variability in clopidogrel response, presently available tests to evaluate efficacy of antiplatelet drugs have limitations and hence they cannot predict a long term out come in clinical terms.

Main limitation of the present study is small sample size and too short follow-up to see clinical events

CONCLUSION

About 40% of our patients using Clopidogrel, especially those who have undergone PCI, are likely to be resistant or hypo-responsive to this antiplatelet agent. However, they are not bound to have ischemic symptoms or adverse cardiac events.

ACKNOWLEDGEMENT

We are indebted to Prof. Akhtar Sohail Chughtai, CEO Chughtai's Lahore Lab. who provided this test on nonprofit basis. All authors declare no conflict of interest.

REFERENCES

1. Bhatt DL, Bertrand ME, Berger PB , L'Allier PL, Moussa I, Moses JW, et al. Meta-analysis of randomized and regis try comparisons of ticlopidine with clopidogrel after stenting. J Am Coll Cardiol. 2002; 39:9-14.
2. Yusuf S., Zhao F, Mehta SR, Chrolavicius S, Tognoni G, Fox KK. Effects of clopidogrel in addition to aspirin in pa tients with acute coronary syndromes without ST-segment elevation. N Engl J Med. 2001;345:494-502.
3. Mak KH, Belli G, Ellis SG, Moliterno DJ. Subacute stent thrombosis. Evolving issues and current concepts. J Am Coll Cardiol 1996; 27:494-503.
4. Nguyen TA, Diodati JG, Pharand C. Resistance to clopidogrel: A review of the evidence, Am Coll Cardiol 2005;45(8):1157-64.
5. Gurbel PA, Bliden KP, Hiatt BL, O'Connor CM. Clopidogrel for coronary stenting. response variabil ity, drug resistance, and the effect of pretreatment platelet reactivity. Circulation 2003;107:2908-13.
6. Gurbel P.A., Bliden K.P. Durability of platelet inhibition by clopidogrel. Am J Cardiol 2003;91:1123-5
7. Wiviott SD, Braunwald E, McCabe CH, Montalescot G, Ruzyllo W, Gottlieb S, et al. Prasugrel versus Clopidogrel in Patients with Acute Coronary Syndromes. N Engl J Med 2007;357:2001-15.
8. Stein B, Fuster V, Israel DH, Cohen M, Badimon L, Badimon JJ, et al. Platelet inhibitor agents in cardiovascu lar disease: an update. J Am Coll Cardiol 1989; 14:813-836.
9. Savage MP, Goldberg S, Bove AA, Deutsch E, Vetrovec G, Macdonald RG, et al. Effect of thromboxane A2 blockade on clinical outcome and restenosis after successful coro nary angioplasty. Multi-Hospital Eastern Atlantic Restenosis Trial (M-HEART II). Circulation 1995; 92: 3194–3200.
10. Clarke TA, Waskell LA. The metabolism of clopidogrel is catalyzed by human cytochrome P450 3A and is inhibited by atorvastatin. Drug Metab Dispos 2003; 31:53-59.
11. CAPRIE Steering Committee, A randomised, blinded, trial of clopidogrel versus aspirin in patients at risk of ischaemic events (CAPRIE). Lancet 1996;348:1329- 39.
12. Steinhubl SR, Berger PB, Mann JT 3rd, Fry ET, DeLago A, Wilmer C, et al. Early and sustained dual oral antiplatelet therapy following percutaneous coronary intervention: a randomized controlled trial. JAMA 2002;288:2411-20. [Erratum, JAMA 2003;289:987]
13. Grotemeyer KH, Scharafinski HW, Husstedt IW. Two-year follow-up of aspirin responder and aspirin non responder: a pilot-study including 180 post-stroke patients. Thromb Res 1993;71: 397-403.
14. Rand ML, Leung R, Packham MA. Platelet function assays. Transfus Apheresis Sci 2003; 28:307–17.
15. Varon D, Lashevski I, Brenner B, Beyar R, Lanir N, Tamarin I, et al. Cone and plate(let) analyzer: monitoring glycopro tein IIb/IIIa antagonists and von Willebrand disease re placement therapy by testing platelet deposition under flow conditions. Am Heart J 1998;135: S187–93
16. Muller I, Besta F, Schulz C, Massberg S, Schönig A, Gawaz M. Prevalence of clopidogrel non-responders among pa tients with stable angina pectoris scheduled for elective coronary stent placement. Thromb Haemost 2003;89: 783–7.
17. Saw J, Steinhubl SR, Berger PB, Kereiakes DJ, Serebruany VL, Brennan D,et al. Lack of adverse clopidogrel-atorvastatin clinical interaction from secondary analysis of a random ized, placebo-controlled clopidogrel trial. Circulation 2003;108:921–4.
18. Lau WC, Waskell LA, Watkins PB, Neer CJ, Horowitz K, Hopp AS, et al. Atorvastatin reduces the ability of clopidogrel to inhibit platelet aggregation: a new drug–drug interac tion. Circulation 2003;107:32–7.
19. Lau WC, Gurbel PA, Watkins PB, Neer CJ, Hopp AS, Carville DGM, et al. Contribution of hepatic cytochrome P450 3A4 metabolic activity to the phenomenon of clopidogrel resis tance. Circulation 2004;109:166–71.
20. Soffer D, Moussa I, Harjai KJ, Boura JA, Dixon SR, Grines CL, et al. Impact of angina class on inhibition of platelet aggregation following clopidogrel loading in patients un dergoing coronary intervention: Do we need more aggres sive dosing regimens in unstable angina? Catheter Cardiovasc Interv 2003;59:21–5.
21. Matetzky S, Shenkman B, Guetta V, Shechter M, Beinart R, Goldenberg I, et al. Clopidogrel resistance is associated with increased risk of recurrent atherothrombotic events in patients with acute myocardial infarction. Circulation 2004;109:3171–5.