A Study of Platelet Aggregation in Patients with Acute Myocardial Infarction at Presentation and after 48 hrs of Initiating Standard Anti Platelet Therapy.

Santanu Guha[1], Soura Mookerjee[1], P. Lahiri[2], Sidhartha Mani[1], Jayanta Saha[3], Sharmila Guha[4], Debdatta Majumdar[1], Md. Miraj Mandal[1], Raja Bhattacharya[5]

[1]Dept. of Cardiology, Medical College, Kolkata, West Bengal
[2]Institute of Haematology & Transfusion Medicine, Medical College, Kolkata, West Bengal
[3]Dept. of Cardiology, NRS Medical College, Kolkata, West Bengal
[4]Dept. of Microbiology, Medical College, Kolkata, West Bengal
[5]Dept. of Medicine, Medical College, Kolkata, West Bengal

Abstract

Aims & Objectives: *Platelet aggregation is a key factor behind coronary artery disease. Various complications after an attack of acute coronary syndrome are often related to the platelet hyperactivity in the early hours following the event. There is a growing concern regarding aspirin & clopidogrel resistance, which has put the time-tested therapies under scrutiny. Time has come to address the issue of platelet hyperactivity in the early hours & whether to individualize therapy and drug doses in different patients.*

Materials & Methods: *We prospectively enrolled 41 patients with a diagnosis of acute myocardial infarction (AMI) between July 2009 and July 2010 admitted to the cardiology ward and ICCU of Medical College , Kolkata, after fulfillment of inclusion & exclusion criteria. The study was reviewed and approved by the Institutional Ethical Committee. Platelet Aggregation (PA) with 10μM epinephrine, 2μg/ml collagen and 10μM ADP was performed with light transmittance aggregometry in all patients according to the standard protocol. Tests were done within 3 hours of sampling with platelet-rich plasma (PRP) by the turbidometric method in a 2-channel aggregometer (Chrono-Log 490 Model, Chrono-Log Corp, Havertown, Pa). Aspirin & clopidogrel resistance were defined as per ACC/AHA guidelines. Platelet aggregation studies were done at presentation (zero hour) and 48 hours after instituting dual antiplatelet therapy in standard doses.*

Results: *Patients with first attack of AMI showed a high mean platelet aggregation at 0 hours of 77.4% ± 18.8% with ADP, 77.5% ± 26% with Epinephrine & 73.5% ± 24.9% with Collagen. With all three agonists, the initial hyperactivity of platelets at 0 hours was significantly higher among diabetics & obese. Though reduced , significant platelet hyperactivity remained at 48 hours after initiating standard antiplatelet therapy; 50.3% ± 14.3% with ADP, 56.5% ± 21.6% with epinephrine & 38.4%± 22% with collagen.*

Conclusion: *In the early hours after AMI there is a fairly high degree of platelet aggregation . Even after 48 hours of standard antiplatelet therapy the platelet aggregation though reduced, still remains significantly high. Since recurrent ischemic episodes frequently occur in this vulnerable period , time has come to assess platelet aggregation status in high risk groups, if not in all patients of acute coronary syndrome during this period so that therapy may be individualized. Further researches are required in this area.*

INTRODUCTION

"Platelets"- the small anuclear cell fragments in blood act as the major culprit behind the pandemic of this era -"non-communicable diseases" - Coronary artery disease (CAD), cerebrovascular disease & metabolic syndrome. Even with well established therapies to prevent platelets from producing complications, it has not yet been possible to prevent such adverse events. There is wide inter-individual variability in the response of platelets to different agonists. The reason behind this is probably the multiplicity of receptors, ligands, signaling pathways and responses. The growing concern regarding Aspirin & Clopidogrel resistance with regards to anti-platelet therapy led researchers to probe into this important issue through a series of studies. The results of The Antithrombotic Trialists' Collaboration, Clopidogrel in Unstable Angina to Prevent Recurrent Events (CURE) and

Correspondence: Prof. Santanu Guha, *Professor & Head, Medical College, Kolkata, Dept. of Cardiology, Medical College, Kolkata, West Bengal, India*
E-mail: *guhas55@hotmail.com*

The Clopidogrel for the Reduction of Events During Observation (CREDO) trials led to universal use of Aspirin & Clopidogrel to prevent vascular events in Coronary artery diseases. But perhaps time has come to be more specific and individualize therapy and drug doses in different patients depending on individual responses.

METHODS

We prospectively enrolled 41 patients with a diagnosis of Acute Myocardial Infarction (AMI) (first attack) between July 2009 and July 2010 after obtaining their written consent. The consecutive patients admitted to the cardiology ward and ICCU with AMI were recruited for the study. The study was reviewed and approved by the Institutional Review Board. Patients who were >21 years old were only included. Exclusion criteria were use of nonsteroidal anti-inflammatory drugs, family or personal history of bleeding disorders, platelet count $<150x10^3$/L or $> 450 x10^3$/ L.

Blood samples

Blood samples for platelet function assays were collected from an antecubital vein using a 21-gauge needle. The first 2 to 4 mL of blood was discarded to avoid spontaneous platelet activation. Blood samples were collected in 3.2% citrated plasma.

Antiplatelet therapy

Antiplatelet therapy was given in standard doses (aspirin 325mg loading and 150 mg daily maintenance; clopidogrel 300 mg loading and 75 mg daily maintenance)

Platelet Function Analysis

Platelet Aggregation (PA) with 10 µM epinephrine, 2µg/ml collagen and 10 µM adenosine dipliosphate (ADP) was performed with light transmittance aggregometry in all patients according to the standard protocol[1,2]. Tests were done within 3 hours of sampling with platelet-rich plasma (PRP) by the turbidometric method in a 2-channel aggregometer (Chrono-Log 490 Model, Chrono-Log Corp, Havertown, Pa). PRP was obtained as a supernatant after centrifugation of citrated blood at 800 rpm for 10 minutes. The isolated PRP was kept at 37°C before use. Curves were recorded for 6 minutes. For optical platelet aggregation, optical density changes were detected photoelectrically as platelets began to aggregate. .

RESULTS

Platelet function at presentation (0 hr) & 48 hours after dual antiplatelet therapy with aspirin and clopidogrel was analyzed in all 41 patients with first attack of AMI, by conventional aggregometry. Demographic parameters, risk factors, medications used and left ventricular function of the patients are depicted in Table 1. Platelet activity in patients done at 0 hour showed a high mean platelet aggregation of 77.4 ± 18.8% with ADP, 77.5 ± 26% with Epinephrine & 73.5 ± 24.9% with Collagen. 4 patients (9.8%) showed spontaneous aggregation. Though reduced, significant platelet hyperactivity remained at 48 hours, the corresponding figures being 50.3% ± 14.3% for ADP 56.5% ± 21.6% for epinephrine & 38.4% ± 22% for Collagen (p value <0.001 for all) (Figure1).

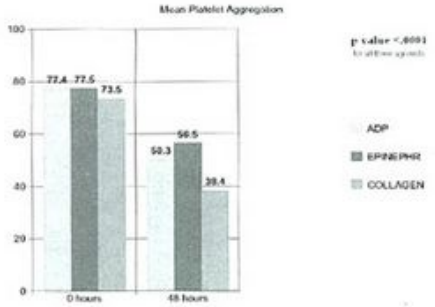

Figure 1: *Mean Platelet Aggregation with ADP, Epinephrine, Collagen at 0 hours & 48 hours*

Table 1: *Demographics of study population.*

VARIABLE	n=41
Age, years (+/- SD)	59.78 ± 12.25
BMI(Kg/m2)	23.59 ± 2.72
Male Gender, n (%)	29(70.73%)
Statin usage	41(100%)
ACE inhibitors/ARB	36(87.8%)
Beta blockers	31(75.61%)
Calcium channel blockers	4(9.76%)
LVEF(%)	49.4 ± 4.6
Total cholesterol(mg/dl)	203.14 ± 35.54
HDL(mg/dl)	46.91 ± 16.3
LDL(mg/dl)	128.82 ± 34.11
TG(mg/dl)	133.32 ± 38.65
FBS(mg/dl)	121.59 ± 48.13
Hb(gm/dl)	12.59 ± 1.2
TC	10400 ± 2900
ESR	49.9 ± 20.06
Platelet (lacs)	1.89 ± .86
PULSE	76.4 ± 15.58
MAP	91.76 ± 6.81
Urea	29.85 ± 9.2
Creat	.98 ± .2

The initial hyperactivity of platelets at 0 hour was significantly higher among diabetics compared with non-diabetics (85.7% ± 22.3% vs 69.4% ± 18.7% with ADP; p value= 0.00286 , 87.7% ± 21.5% vs 67.4% ± 16.1% with Epinephrine; p value= 0 .013, 84.1% ± 23% vs 62.9% ± 19.2% with collagen; p value= 0.006) (Figure 2). With ADP this higher trend in diabetics remains no longer significant at 48 hours (52.1% ± 13.9% vs 48.6% ± 14.1%) (Figure 3). At zero hour, statistically significant difference was also observed in subgroup analysis among obese vs non-obese (84.8% ± 20.7% vs 72.6% ± 21.4% with ADP; p value= 0.012, 89.1% ± 21.8% vs 69.8% ± 19.6% with Epinephrine; p value=

0.004, 86.2% ± 24.3% vs 65.1% ± 19.4% with collagen; p value=0.004) (Figure 4.). No such significant difference was observed among hypertensives or dyslipidemics.

At 48 hours of therapy , 24.39% of patients were dual drug resistant. Additionally 17.07% of patients were resistant to aspirin alone while 21.95 % of patients were resistant to clopidogrel only.

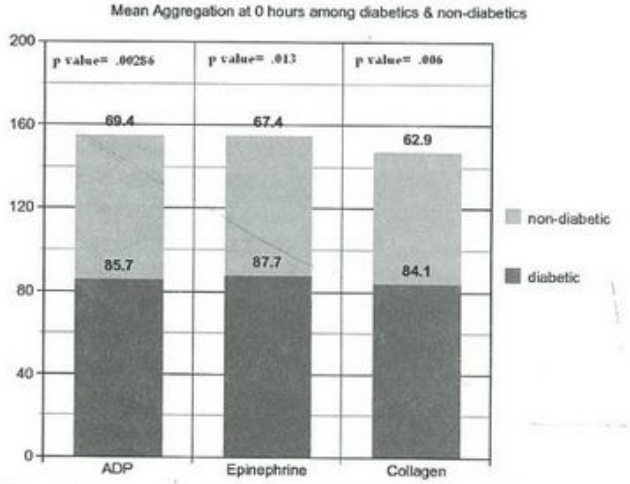

Figure 2: Comparison of mean platelet Aggregation at 0 hours among diabetics & non-diabetics.

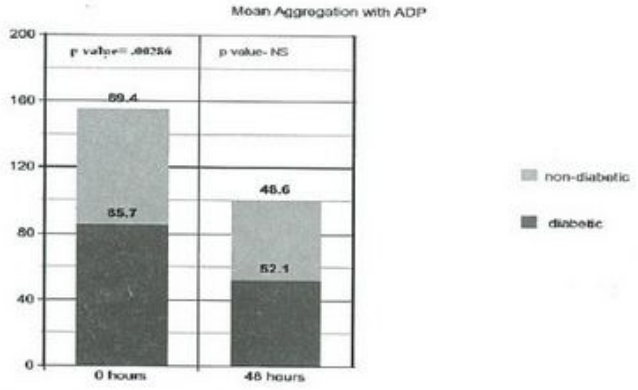

Figure 3: Comparison of mean platelet Aggregation at 0 hours ,48 hours (with ADP) among diabetics & non-diabetics.

The patients showing single or dual drug resistance at 48 hours had a higher mean platelet aggregation (84.77% with ADP) at presentation in comparison to those without drug resistance (59.67% with ADP).

DISCUSSION

The entire array of platelet surface receptors , interactions with ligands, signal transductions leading to ultimate platelet activation, degranulation, aggregation & thrombosis is complex. A number of agonists bind to their respective

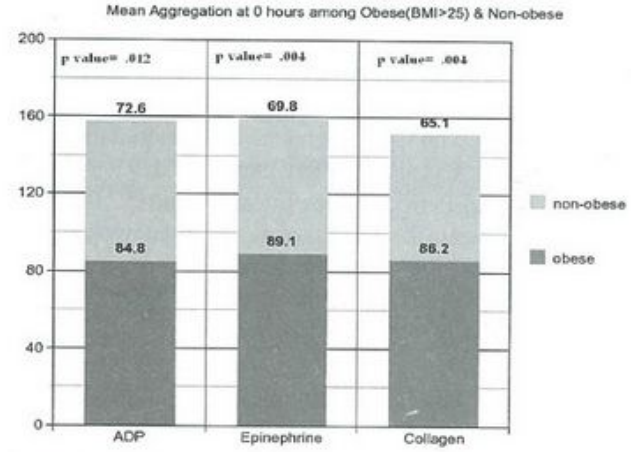

Figure 4: Comparison of mean platelet Aggregation at 0 hours among obese & non-obese.

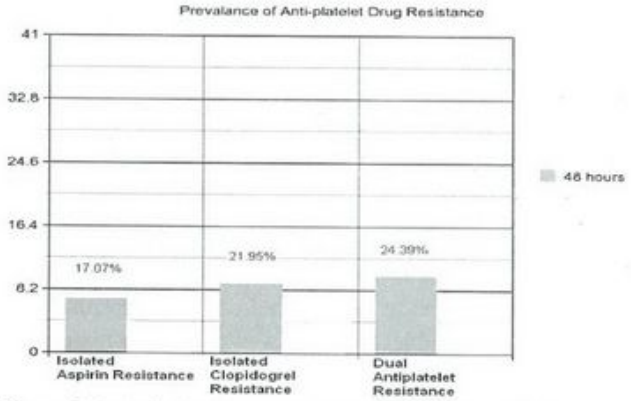

Figure 5: Anti-platelet drug resistance in study group at 48 hours

membrane receptors on adherent platelets which includes humoral mediators in plasma (e.g Thromboxane A_2, Thrombin), mediators released from activated cells- (ADP, Serotonin) and vessel wall extracellular matrix constituents (Collagen, vWF). All these processes ultimately lead to conversion of alpha IIb/beta 3 (or glycoprotein IIb/IIIa) from its resting state to active state where it binds fibrinogen and allows cross linking of platelets culminating in platelet aggregation[3,4,5,6,7].

Several of these stimuli can synergistically activate platelets and amplify response to other mediators. Thus a sudden surge in the level of catecholamines can potentiate the aggregation mediated by other agonists like ADP and arachidonic acid by increasing the sensitivity of the platelets to such agonists. The presence of even a small concentration of ADP and/or arachidonic acid may activate platelets in such situations.

Hence therapy to inhibit platelet activation may be targeted at different levels[8,9] e.g. Thromboxane A_2, ADP, Glycoprotein

IIb/IIIa inhibitor, Thrombin, Epinephrine[8].

Clinical trials have shown the efficacy of aspirin and clopidogrel in prevention of Acute Coronary Syndrome & cardiovascular death e.g. The Antithrombotic Trialists Collaboration[9,10], The Clopidogrel vs. Aspirin in Patients at Risk of Ischaemic Events (CAPRIE) study [11], CURE trial [10,12]. CREDO trial and Per-cutaneous Intervention (PCI)-CURE substudy[13,14]. Despite the benefits of dual antiplatelet therapy, many patients continue to suffer adverse consequences of cardiovascular disease[15]. Prevalence of aspirin and clopidogrel resistance has been reported to be between 5 and 30% in world literature[16].

There is marked inter-individual variation in platelet hyperactivity[18].The enhanced platelet function is correlated with the degree of myocardial damage in MI patients, and this is observed well before peak levels of CK-MB and Troponin T are reached[17]. The platelet hyperactivity persists mostly in early hours and days following AMI & gradually normalizes over next 48 hours to 7 days. This is also evident in our present study. On sub-group analysis, this high platelet aggregability at presentation was found to be significantly higher among diabetics & obese. Such difference among sub-groups was however not observed with other risk factors e.g. Dyslipidemia, Hypertension and Smoking. Studies have shown that diabetic patients are more susceptible to platelet aggregation[19]. Even in unstimulated state, platelets from diabetic subjects release more noradrenaline than platelets from non-diabetic subjects[20]. The in-vitro documentation of increased platelet aggregation in diabetics in the present study reconfirms the fact, that, they are truly at higher risk as far as platelet activation and aggregation is concerned, and require a risk adapted therapy.

Though there is no clearcut definition of aspirin resistance. Patients on aspirin and clopidogrel with ≥50% aggregation with collagen (2 µg/ml) but ≤ 50% aggregation with ADP (10µM) were labeled as aspirin resistant and ≥50% aggregation with ADP (10µM) but ≤50% aggregation with collagen (2 µg/ml) were labeled as resistant to clopidogrel. Dual resistance was defined as ≥50% aggregation with both collagen (2 µg/ml) and ADP (10µM), as published in recent literatures and according to the recommendation of ACC/AHA 2005 guideline[21].

At 48 hours of therapy, 24.39% of patients were dual drug resistant. Additional 17.07% of patients were resistant to asprin alone, and 21.95% of patients were resistant to clopidogrel alone. The demonstration of increased platelet aggregation even after 48 hours of dual antiplatelet therapy, highlights the altered milieu in the early hours after the event which takes more time to settle down. A study by Gum et al demonstrated that aspirin resistance was associated with an increased risk of death, MI, or CVA. Stratified multivariate analyses identified platelet count, age, heart failure, and aspirin resistance to be independently associated with major adverse long-term outcomes[22].

The patients showing single or dual drug resistance at 48 hours had a higher mean platelet aggregation at presentation in comparison to those without drug resistance . Hence initial platelet hyperaggregability at 0 hours may serve as a marker of future drug resistance. Thus there remains a scope for altering the therapy and to prevent drug resistance from the beginning by assessing platelet aggregability at 0 hours. However the treatment for antiplatelet drug resistance is as yet undefined. Various studies have shown superiority of using 600 mg of clopidogrel prior to PCI when compared with 300 mg of clopidogrel as pretreatment or loading dose[23,24]. In an earlier study, we observed that the resistance can be overcome in many patients by doubling the maintenance dose of the respective drug[25]. With the availability of increasing number of potent antiplatelet drugs, more research is required to look for whether individualized approach in anti platelet drug therapy is needed.

CONCLUSION

Our study shows that in the early hours after AMI there is a fairly high degree of platelet aggregation. After 48 hours of standard antiplatelet therapy the platelet aggregation though reduced, still remains significantly high. As this early vulnerable period is frequently associated with recurrent ischemic episodes, it may be worthwhile to assess platelet aggregation status in high risk groups, if not all patients of Acute Coronary Syndrome during this period so that therapy may be individualized. Further researches are required in this area.

REFERENCES

1) *Prabir Lahiri, Utpal Chaudhuri, Arnab Chattopadhyay, etal. Platelet responsiveness to yohimbine hydrochloride and MRS2179 in the context of the interaction between collagen and epinephrine in acute coronary syndrome . Blood Cells, Molecules, and Diseases; Volume 34, Issue 3, May-June 2005, Pages 248-252*

2) *Antiplatelet Trialists' Collaboration: Collaborative overview of randomised trials of antiplatelet therapy. I. Prevention of death, myocardial infarction, and stroke by prolonged antiplatelet therapy in various categories of patients. BMJ 1994; 308:81 106.*

3) *Löwenberg EC, Meijers JC, Levi MNeth J Med. Platelet-vessel wall interaction in health and disease. 2010 Jun;68(6):242-51.*

4) *F. Adam , T. J. Verbeuren, J.-L. Fauchère, etal. Thrombin-induced platelet PAR4 activation: role of glycoprotein Ib and ADP. Journal of Thrombosis and Haemostasis. Volume 1 Issue 4, Pages 798 – 804.*

5) *Heinrich Patscheke. Role of activation in epinephrine-induced aggregation of platelets. Thrombosis Research. Volume 17, Issues 1-2, 1 January 1980-15 January 1980, Pages 133-142.*

6) *Robert T. Dorsam and Satya P. Kunapuli. Central role of the P2Y12 receptor in platelet activation .J Clin Invest. 2004 February 1; 113(3): 340–345.*

7) *P Mangina1, C Nonnea, A Ecklya, etal. A PLCß2-independent platelet collagen aggregation*

requiring functional association of GPVI and integrin á2â1. Volume 542, Issue 1, Pages 53-59 (8 May 2003).

8) *David P. Faxon. Antiplatelet therapy: Cangrelor for ACS—lessons from the CHAMPION trials. Nature Reviews Cardiology 7, 124-125 (March 2010).*

9) *Gregg W Stone .Ticagrelor in ACS: redefining a new standard of care? The Lancet, Volume 375, Issue 9711, Pages 263 - 265, 23 January 2010 .*

10) *Thomas H. Wang, Deepak L. Bhatt and Eric J. Topol Aspirin and clopidogrel resistance: an emerging clinical entity Eur. Heart J. 2006; 27:647-654.*

11) *CAPRIE Steering Committee. A randomised, blinded, trial of clopidogrel versus aspirin in patients at risk of ischaemic events (CAPRIE). Lancet 1996;348:13291339.*

12) *The Clopidogrel in Unstable Angina to Prevent Recurrent Events Trial Investigators. Effects of clopidogrel in addition to aspirin in patients with acute coronary syndromes without ST-segment elevation. N Engl J Med 2001;345:494502.*

13) *Steinhubl SR, Berger PB, Mann III JT, et al, for the CREDO Investigators. Early and sustained dual oral antiplatelet therapy following percutaneous coronary intervention: a randomized controlled trial. JAMA 2002;288:24112420.*

14) *Mehta SR, Yusuf S, Peters RJG, et al. Effects of pretreatment with clopidogrel and aspirin followed by long-term therapy in patients undergoing percutaneous coronary intervention: the PCI-CURE study. Lancet 2001;358:527533.*

15) *Geisler T, Langer H, Wydymus M, et al. Low response to clopidogrel is associated with cardiovascular outcome after coronary stent implantation. Eur Heart J. 2006;27(20):2420-5.*

16) *Geisler T, Gawaz M. Variable response to clopidogrel in patients with coronary artery disease. Semin Thromb Hemost. 2007 ;33(2):196-202.*

17) *Paul F. Bray. Platelet Hyperreactivity: Predictive and intrinsic properties. Hematol Oncol Clin North Am. 2007 August; 21(4): 633–vi .*

18) *Martin Frossard, Ingrid Fuchs, Judith M. Leitner, etal. Platelet Function Predicts Myocardial Damage in Patients With Acute Myocardial Infarction. Circulation. 2004;110:1392-1397.*

19) *Lahiri P, Chaudhuri U, Dasgupta AK, et al. Insensitivity to the alpha2-adrenergic receptor blocker yohimbine hydrochloride and occurrence of spontaneous platelet macroaggregation (SPMA) in diabetes. Platelets 2005;16:111-5.*

20) *Smith CC, Wilson AP, Prichard BN, Betteridge DJ. Platelet efflux of noradrenaline in patients with type 1 diabetes mellitus. Clin Sci (Lond) 1989;76:603-7.*

21) *Angiolillo DJ, Shoemaker SB, Desai B et al. Randomized comparison of a high clopidogrel maintenance dose in patients with diabetes mellitus and coronary artery disease: results of the Optimizing Antiplatelet Therapy in Diabetes Mellitus (OPTIMUS) study. Circulation. 2007 ; 115(6):708-16.*

22) *Gum PA, Kottke MK, Paggio ED etal. Profile and prevalence of aspirin resistance in patientswith cardiovascular disease. American Journal Cardiology 2001; 88: 230-35.*

23) *Thomas H. Wang, Deepak L. Bhatt and Eric J. Topol Aspirin and clopidogrel resistance: an emerging clinical entityEur. Heart J. 27:647-654, 2006*

24) *Lotrionte M, Biondi-Zoccai GG, Agostoni P etal: Meta-analysis appraising high clopidogrel loading in patients undergoing percutaneous coronary intervention.Am J Cardiol. 2007 Oct 15;100(8):1199-206*

25) *Dual Antiplatelet Drug Resistance in Patients with Acute Coronary Syndrome. Guha S, Sardar P, Guha P, Roy S, Mookerjee S, Chakarabarti P et al. Indian Heart J.2009;61:68-73.*