Original Article

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Pharmacology Section

Assessment of Clopidogrel Resistance in Post Myocardial Infarction Patients after 24 to 48 Hours of Initiation of Treatment: A Cross-sectional Study

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ABSTRACT

Introduction: Clopidogrel is an antiplatelet agent used to prevent platelet aggregation and further blockage of coronary arteries in Acute Coronary Syndrome (ACS) patients. Inadequate response to clopidogrel has been demonstrated in some patients that might lead to re-infarction even when receiving standard doses of clopidogrel.

Aim: To demonstrate the presence of resistance to standard oral doses of clopidogrel in a tertiary care hospital located in eastern India.

Materials and Methods: It was a descriptive cross-sectional study conducted from January 2015 to June 2016, in Medical College Kolkata, (previously known as Calcutta Medical College), India. Total 32 patients, previously not on any antiplatelet therapy, presenting with biomarker positive ACS were evaluated. The patients were given clopidogrel (300 mg) along with aspirin (325 mg) on presentation followed by clopidogrel (75 mg) and aspirin (75 mg) once daily. Blood samples were collected after 24-48 hours of administering the above mentioned doses orally. A 3.2% citrate was used as anti-coagulant. Platelet Rich Plasma (PRP) and Platelet Poor Plasma (PPP) were prepared from this blood samples by centrifugation. Platelet aggregation was studied by adding 10 µM Adenosine Diphosphate (ADP) in that PRP and it was compared with PPP in Light Transmittance Aggregometer (LTA). Platelet aggregation ≥50% in presence of 10 µM ADP was termed as Clopidogrel Resistance (CR). Differences between groups were assessed with Chi-square test and Fisher-exact test for categorical variables. The p-value of <0.05 was considered to be statistically significant.

Results: Mean age of the study participants was 60.7 years, and 23 (71.8%) out of 32 patients were male while 9 (28.2%) were female. Total 7 (21.8%) of the patients were found to be resistant to standard doses of clopidogrel. A 3 (60%) out of 5 patients with positive family history of Cardiovascular Diseases (CVD) showed CR (p-value=0.025). Incidences of CR was higher among women 3 (33.3%) and in patients receiving thrombolysis 4 (28.5%). Though these percentages were high but not statistically significant.

Conclusion: In this study, 21.8% ACS patients showed resistance to the antiplatelet effects of clopidogrel in the conventional dose. A long term prospective Randomised Controlled Trials (RCT) with larger sample size is required to give an insight into this problem.

Keywords: Antiplatelet, Re-infarction, Stent thrombosis

INTRODUCTION

Clopidogrel is one of the most commonly used antiplatelet agents used in patients with ACS. Even after timely treatment with antiplatelet drugs many patients suffer from reinfarction [1]. This has raised concern among the cardiologists throughout the world about a new phenomenon called antiplatelet resistance [2]. The antiplatelet drugs, namely aspirin and clopidogrel, fail to prevent platelet aggregation despite administration of standard doses.

Clopidogrel is administered orally and only 50% of it is absorbed. It is a pro-drug and is partially converted into active metabolite by CYP2C19 enzyme in liver. It acts on P2Y12 receptor and irreversibly inhibits platelet function [3]. But this antiplatelet action takes nearly four hours to start and develops over days. Moreover, due to genetic polymorphism, clopidogrel activation shows high inter individual variability [4]. Sometimes, drugs like omeprazole which inhibit CYP2C19 may also be responsible for poor antiplatelet action of clopidogrel [5].

Some studies, done all over the world, showed varied presence of CR from 5-44% [6,7]. Studies done in India are few in number [6,8]. Ray S, found that CR is a problem here as well, but methods to identify it were not standardised and not freely available [6]. Moreover, Kar R et al., showed that CR may be multifactorial but was not associated with single gene polymorphism [8]. A large number of patients suffering from Acute Myocardial Infarction (AMI) are treated with clopidogrel and some of them develop adverse cardiovascular event like re-infarction or stent thrombosis within six months as a result of CR [7]. The study was done to know the prevalence of CR among AMI patients and factors associated with it.

MATERIALS AND METHODS

It was a descriptive cross-sectional study conducted from January 2015 to June 2016, in Medical College Kolkata, (previously known as Calcutta Medical College), India. After obtaining Institutional Ethics Committee clearance (memo no. MC/KOL/IEC/NON-SPON/421/11-2014) 32 consenting patients were recruited in the study.

Inclusion criteria: Patients admitted with history of AMI or biomarker positive ACS in the last 24-48 hours were included in the study.

Exclusion criteria: Patients who were on Nonsteroidal Anti-inflammatory Drugs (NSAIDs) (other than Aspirin), receiving drugs like omeprazole which inhibit CYP2C19, had known personal or family history of bleeding diathesis or had platelet counts of $<150\times10^3$/mL or $>450\times10^3$/mL were excluded from the study.

On admission, patients were given 300 mg clopidogrel orally along with other medications. It was followed by oral dose of 75 mg clopidogrel/day. In this study, 32 patients, admitted in Intensive Coronary Care Unit (ICCU) following AMI, were recruited.

Collection of Blood Sample

After following proper aseptic technique, 21 gauge needles were used to draw blood from antecubital vein within 24-48 hours of initiation of treatment. Initial 3-4 mL of blood was used for other routine tests to avoid spontaneous activation of platelets. 3.2% citrate solution was used as anticoagulant while collecting blood.

Analysis of Platelet Function

Platelet-Rich Plasma (PRP) was prepared from this blood sample by centrifugation at 200 gm for 15 minutes. PPP, required for comparison in LTA, was prepared by centrifugation of blood at 1500 gm for 15 minutes. Platelet aggregation was studied by adding 10 µm ADP in that PRP and it was compared with PPP in LTA. Platelet aggregation ≥50% in presence of 10 µm ADP was termed as CR [9,10].

STATISTICAL ANALYSIS

Windows Microsoft Excel 2010 was used for tabulation of data and statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) software (version 22.0). Data were found to be normally distributed by using Kolmogorov-Smirnov test. Normally distributed continuous variables were presented as Mean±SD. Categorical variables were expressed as frequencies and percentages.

RESULTS

All recruited patients were ≥45 years of age and mean age was 60.7 years. Out of 32 recruited patients, 23 (71.8%) were male and 9 (28.2%) were female. The study revealed that 7 (21.8%) patients were clopidogrel-resistant; most of them belonged to 51-60 years age group. The [Table/Fig-1] shows that more females were resistant to clopidogrel 3 (33.3%) than males 4 (17.3%) (p-value=0.327).

Parameters	Clopidogrel resistance absent	Clopidogrel resistance present	p-value (Chi-square test)
Sex			
Male	19 (82.61%)	4 (17.39%)	0.327
Femal	6 (66.67%)	3 (33.33%)	
ST Elevation			
No	2 (100%)	0 (0%)	0.44
Yes	23 (76.67%)	7 (23.33%)	
Thrombolysis			
No	15 (83.33%)	3 (16.67%)	0.419
Yes	10 (71.43%)	4 (28.57%)	
Pathological Q wave			
No	20 (76.92%)	6 (23.08%)	0.732
Yes	5 (83.33%)	1 (16.67%)	
T2DM			
No	17 (77.27%)	5 (22.73%)	0.863
Yes	8 (80%)	2 (20%)	
Family history of CVD			
No	23 (85.19%)	4 (14.81%)	**0.025**
Yes	2 (40%)	3 (60%)	
Past history of AMI			
No	23 (76.67%)	7 (23.33%)	0.44
Yes	2 (100%)	0 (0%)	
Angina			
No	11 (73.33%)	4 (26.67%)	0.538
Yes	14 (82.35%)	3 (17.65%)	
Dyslipidaemia			
No	23 (76.67%)	7 (23.33%)	0.44
Yes	2 (100%)	0 (0%)	
Hypertension			
No	11 (64.71%)	6 (35.29%)	0.051
Yes	14 (93.33%)	1 (6.67%)	
Alcoholism			
No	21 (77.78%)	6 (22.22%)	0.912
Yes	4 (80%)	1 (20%)	
Tobacco chewing			
No	21 (75%)	7 (25%)	0.258
Yes	4 (100%)	0 (0%)	
Smoking			
No	13 (76.47%)	4 (23.53%)	0.81
Yes	12 (80%)	3 (20%)	

[Table/Fig-1]: Clopidogrel resistance vs various factors.
T2DM: Type 2 diabetes mellitus; CVD: CardioVascular disease; AMI: Acute myocardial infarction

Thirty (93.75%) patients presented with STEMI and all seven CR cases belonged to this group (p-value 0.44). CR was more common in patients treated by thrombolysis though, it was not statistically significant (p-value 0.419). No statistically significant association was found between pathological Q wave and CR (p-value=0.732).

Among 10 diabetic patients, 2 (20%) were clopidogrel-resistant; whereas in non diabetic group, 5 (22.73%) patients were clopidogrel-resistant (p-value=0.863). Patients having positive family history of CVD showed statistically significant association with CR (p-value 0.025). Three patients (60%) with family history of CVD showed CR. Those having no such history showed clopidogrel-resistance in 4 (14.81%) cases. Seven patients (23.33%) with no history of AMI showed CR.

A 3 (17.6%) out of 17 patients having history of angina showed CR. Only two patients had known history of dyslipidaemia. All 7 (23.33%) clopidogrel-resistant patients had no known history of dyslipidaemia (p-value=0.44). One (6.67%) hypertensive patient showed CR and in the non hypertensive group it was 6 (35.29%) (p-value=0.051).

DISCUSSION

Recurrent ischaemic event following AMI is an important cause of mortality among ACS patients. 'Antiplatelet Drug Resistance' is an emerging problem that is compelling researchers and cardiologist alike to look into it further as causal relationship between it and increased mortality in ACS patients is not established yet. Even after timely medical intervention, some patients do not respond to standard treatment and suffer from recurrent myocardial infarction and reinfarction. There is probability that the antiplatelet drug resistance might play a role in the mentioned scenario.

In literature, CR has been reported and it varies from 4.3-63% [11]. In this study, 7 (21.8%) patients were CR. This variation may be due to the fact that the definition of CR is not clearly coined yet [12]. In this study, aggregation of 50% or more platelets despite standard dose of clopidogrel, after 24-48 hours of initiation of treatment, was considered as CR.

According to a paper published by Nguyen TA et al., about 4-30% of patients treated with conventional doses of clopidogrel did not display adequate antiplatelet response [12]. Guha S et al., in 2009 found CR in 42.3% patients presenting after first episode of ACS [13]. The resistance was as high as 72.5% when recurrent ACS group was considered. Another study by Guha S et al., done in 2009 showed 19.44% patients to be Clopidogrel resistant [14].

In this study, 33.33% female patients were seen to be Clopidogrel-resistant compared with 17.39% male. But this difference was not statistically significant. Kumar S et al., reported a similar trend [15]. CR increases the chance of adverse cardiovascular event like re-infarction or stent thrombosis [7]. Contrarily, Shaya FT et al., found no significant relationship between re-infarction and sex, age, race of the patients [16].

Every participant was ≥45 years of age in this study and most of them belonged to 51-60 years and 61-70 years age group. Highest CR was seen in 51-60 years age group (27.27%) followed by >70 years age group (25%). Ahumada M et al., showed that there is increased chance of re-infarction with advancement of age [17]. Contrary to this Guha S et al., found that recurrent ACS is common in younger patients [13]. Shaya FT et al., found no such relationship between the two [16]. Thus, there exists varied finding based on the association between age and resistance to clopidogrel. Larger studies are required to know whether there is actually any relation between age and CR.

Thirty out of total 32 patients presented with STEMI and all seven Clopidogrel-resistant cases belonged to this group. There were only two patients in NSTEMI group. In a population based study McManus DD et al., showed that incidence of NSTEMI is more (132 per 100,000) compared to STEMI (77 per 100,000) [18]. This difference in findings was may be due to the fact that in the index study smaller number of patients was recruited from a tertiary care hospital.

Among 14 patients treated with thrombolytic agents, 4 (28.5%) showed CR whereas in the other group it was 3 (16.6%). Though, this data was not statistically significant, increased platelet activity following thrombolysis has been reported in literature. Rasmanis G et al., reported that increased thromboxane formation is seen following thrombolysis leading to increased platelet activity [19].

Total 16.67% patients with pathological Q wave in ECG showed CR. Total 23.08% patients with no pathological Q wave in ECG were resistant to standard doses of clopidogrel. This finding, however, showed no statistical significance. Ahumada M et al., found that the presence of pathological Q wave in ECG significantly increases the risk of re-infarction [17]. Further studies are required to find out if there is any association between pathological Q wave and CR so that re-infarction can be prevented.

In this study, 10 (31.25%) patients were diabetic. Out of them 25% were Clopidogrel resistant. In the non diabetic group, 22.72% were Clopidogrel resistant (statistically not significant). Ahumada M et al., showed greater risk of re-infarction in diabetic patients [17]. Paul R et al., has shown that metabolic syndrome can increase CR [20]. Guha S et al., also found increased chance of recurrent ACS in T2DM patients [14].

Those having positive family history of CVD showed CR in 60% cases. It was 14.81% when the family history of CVD was negative. This finding was statistically significant at p-value =0.025. Su F et al., however found no relation between positive family history of CVD with CR [21]. On the contrary, Wallentin L et al., described that positive family history of CVD is related to Clopidogrel-resistance [22]. Idrissi H et al., found that CYP2C19 alleles are inherited in autosomal co-dominant manner [23]. This pattern of inheritance might be responsible for the fact that all patients, having family history of CVD, were not poor responders to clopidogrel.

Among 32 recruited patients, only two had history of AMI. All 7 (23.33%) Clopidogrel-resistant patients had no history of AMI. This data carried no statistical significance. Ahumada M et al., found that history of AMI independently associated with re-infarction. It may be due to decreased response to antiplatelet agents in these patients [17].

In this study, among the patients with positive history of angina, 17.65% showed CR while it was 26.67% for those with no history of angina. No similar comparison could be found in literature despite thorough search. The two patients with dyslipidaemia did not show CR while 23.33% with dyslipidaemia showed CR. In 2009, Guha S et al., found that raised low-density lipoprotein level in serum is associated with recurrent ACS [14]. Shaya FT et al., showed that statins used to treat dyslipidaemia can actually decrease event of re-infarction or in other words hyperlipidaemia increases chance of re-infarction [16]. The two patients with dyslipidaemia in the index study were also on statin.

Total 35.29% normotensive patients were Clopidogrel-resistant where as 6.67% hypertensives had that attribute. This data was not statistically significant though Shaya FT et al., found hypertension and heart disease to be the most prevalent comorbidities associated with re-infarction [16]. Guha S et al., also observed that hypertension is related to recurrent ACS [13]. Among the hypertensive group many patients were receiving Angiotensin Receptor Blocker (ARB) and Angiotensin Converting Enzyme Inhibitor (ACEI) for control of blood pressure. Anti-aggregatory effect of ARB and ACEI may be responsible for this finding in the present study [24,25].

Among non alcoholic patients CR was 22.22% and it was 20% among alcoholics. Su J et al., found that lower P2Y12 gene promoter DNA methylation increases the risk of CR in alcohol abusers but it depends on other extrinsic factors as well [26].

Only four patients had history of tobacco chewing and none of them were found to be Clopidogrel resistant. On the other hand, 25% patients belonging to non tobacco chewer group were Clopidogrel resistant. 23.53% non smokers in this study were Clopidogrel-resistant whereas 20% smoker showed the same feature. There was no statistical significance in this observation though Hung et al. had shown that smoking increases platelet activity in patients taking aspirin [27]. On the other hand, smoking was found to positively modify the beneficial effect of clopidogrel on angiographic and clinical outcomes, in a study by Desai NR et al., [28].

Limitation(s)

Clopidogrel is a prodrug. It is converted into active metabolite in the body by CYP2C19 enzyme. In some individuals antiplatelet action develops slowly due to genetic polymorphism of metabolising enzyme [4,29]. Analysis of this inter-individual variability due to genetic factors was beyond the scope of this study. It was found that many patients were ignorant about their health conditions. Blood pressure, blood sugar, lipid profile of many patients was never checked before. The study was done in a tertiary care institution, so the sample may not be representative of the population. Whether the platelet function is affected by any other drugs, being used for treating other comorbidities could not be ascertained in this study. AMI patients treated by primary percutaneous coronary intervention were excluded from the study because they received different dose of clopidogrel.

CONCLUSION(S)

Clopidogrel resistance may be responsible for re-infarction in some patients presenting with ACS. The study found 21.86% patients to be Clopidogrel-resistant. Patients having positive family history for CVD were more likely to be clopidogrel-resistant. Further studies are required involving large number of patients in multiple centres to ascertain these findings.

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